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THE ECOLOGICAL SIGNIFICANCE OF SEED SIZE IN MEDITERRANEAN ANNUAL PASTURE LEGUMES

by

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ABSTRACT

The thesis examines the ecological significance of seed size of some common annual pasture legumes of the genera *Medicago* and *Trifolium* used in ley farming production systems (crop-pasture rotations) and in permanent pastures. There are four main sections:

1. The first section concerns laboratory research and investigates the influence of seed size, temperature and moisture status on the uptake of water and germination by *M. scutellata* cv. Sava and *T. subterraneum* cv. Trikkala (hereafter referred to as Sava and Trikkala respectively) seed populations.

   (1) The first study examines the trend in the allocation of dry matter of seed components with changes in seed size. Both species produce seeds in which the embryonic component (cotyledons + embryonic axis) increases with seed size.

   (2) The second study examines the germination of contrasting seed-size categories at fourteen different temperatures. Trikkala was more sensitive to higher temperatures than Sava, but had greater tolerance to lower temperatures. Temperature significantly affected germination in both Sava and Trikkala: however, the effect of seed size was only significant in Sava. The rate of germination of Sava tended to decline with increasing seed size; this effect was less evident with Trikkala.

   (3) The third study examines the uptake of water by germinating seeds incubated under fourteen different temperature regimes. The length of the imbibitional phase was also temperature- and seed-size-dependent for both species. This suggests that if small seed imbibes water at higher rates than large seed, especially under unfavourable temperatures, it will cause earlier emergence of the radicle and seedling establishment. In terms of plant competition in a dense pasture sward, earlier emergence is of great ecological significance.

   (4) The fourth study examines the germination of seeds subjected to thirteen wetting periods before drying and re-wetting. The viability of seed is not affected and the rate of germination increased by longer periods of soaking prior to dehydration. The increased rates of germination are due to initiation of processes essential for germination during the soaking period. The subsequent drying of the seed does not alter the metabolic stage achieved. The highest rates of germination were associated with smaller seeds and were most apparent in Sava

medic. This state of pre-germination of seed facilitates rapid germination in response to adequate moisture conditions.

(5) The fifth study examines the germination of seeds subjected to simulated drought conditions. The germination of seeds of both species was influenced by the water potential of the germinating seed medium and the size of the seed. Differences in the rate of germination of both species between seed-size categories, indicate that small seed has greater tolerance to moisture stress and suggest that under natural conditions, better seedling establishment should be obtained.

2. The second section considers glasshouse and raised-bed studies to examine the interrelations and effects of seed size, soil texture, depth of sowing, cereal straw residues and plant density on emergence and early vegetative growth of Sava and Trikkala seedlings.

(1) The first study examines the emergence and early growth of seedlings when seed is sown at five depths in sand and loam in a glasshouse by using three seed-size categories. Seed size, depth of sowing and soil type may all delay emergence and reduce potential emergence. Both responses, especially the rate of emergence, are of critical importance under natural conditions as they influence the rate of seedling establishment and subsequent early forage production. Some evidence suggests that small seeds have higher initial rates of emergence than large seeds. The experiments also demonstrated the critical effects of delayed emergence on seedling vigour, which in turn reduced early production of plant material. Trikkala seedlings were more affected than seedlings of Sava: however, under some circumstances the large size of Sava cotyledons restricted seedling emergence. Under sub-optimal soil moisture conditions, the optimum depth of seed placement in relation to the soil type, did not represent the best strategy to ensure maximum emergence of seedlings.

(2) The second study examines the emergence and early growth of Sava medic seedlings from whole pods placed at four depths in a loamy soil in a glasshouse. The pods contained small, medium and large seed-size categories. In order to examine the additional impedance of cereal straw residues to seedling emergence, unweathered wheaten straw treatments were used. Final emergence was most affected by depth of sowing, but was also reduced at the higher straw

concentrations. Seedlings from large pods were able to emerge from more restricted situations.

(3) The third study examines the early growth of seedlings sown in raised-beds by using three seed-size categories and four plant densities. The potential forage production was influenced by both seed size and plant density. This has considerable practical importance, especially early in the growing season when serious deficiencies of forage occur. High levels of forage availability early in the season can be achieved only at higher plant densities, especially those derived from larger seeds.

3. The third section relates to grazing surveys and investigated the role of contrasting seed sizes provided by annual clover pasture species, such as *T. subterraneum*, *T. balansae* and *T. glomeratum* (hereafter referred to as subterranean clover, balansa clover and cluster clover respectively) on seed survival and pasture establishment under the impact of grazing sheep.

(1) The first study was made in a sown subterranean clover-balansa clover pasture containing some naturalized cluster clover. The seed-seedling dynamics in the soil-pasture-animal complex was studied throughout the summer-autumn period. During the 10 week grazing period, 95.4% of the subterranean clover seed disappeared. This reduction in seed was aggravated by the fact that 94.4% of the burrs were produced above ground on the hard-setting, red-brown earth soil. Furthermore, the sheep initially selected the heavier burrs containing a higher number of heavier seeds. There was also a 56.6% decline of balansa clover and 70.6% of cluster clover seed during the grazing period. Only 1.8% of subterranean clover, 14.9% of balansa clover and 75.6% of cluster clover seed that disappeared from the grazing area were recovered in sheep faeces. Because most of the germinating seed voided in faeces did not survive the desiccation process during the summer-autumn period, lower survival values of 1.1%, 9.0% and 58.7% were ultimately recorded for the three species. This shows that small, hard seed of clover pasture legumes better avoid the impact of grazing and passage through the digestive tract of sheep.

(2) The second study examines the ecological significance of small-seeded naturalized cluster clover, in a subterranean clover-perennial ryegrass (*Lolium perenne*) pasture mixture, established on a sandy loam soil under two contrasting

management practices. Paddock I had been heavily set-stocked permanently during the last fifteen years with about 20 crossbred ewes/ha. Paddock II had been cut for hay over the last twelve years and stocked with about 13.8 crossbred ewes/ha. The seed-seedling dynamics in the soil-pasture-animal complex were studied for part of two growing seasons (1990 and 1991). Subterranean clover cultivars were affected more severely by heavy set stocking, than by regular fodder conservation practices. However, adequate reserves of seed were set in both paddocks which were strategically conserved *in situ* through the summer-autumn period. This ensured a potentially high regeneration of the pasture because a large proportion of the seed bank was soft-seeded. Seedling mortality was high during the early stages of growth under grazing. Defoliation and treading cause a severe reduction in plant density of subterranean clover. Despite the disappearance of cluster clover seeds after seed set and a lower rate of break down of hard-seededness, the small seedlings persisted better than subterranean clover seedlings during the critical period of establishment. This may partially explain the dominance of cluster clover under heavy stocking. Another important component in the success and persistence of cluster clover in pasture, is the ability of the small-seeded clover better to survive the passage through the digestive tract of sheep. This favours the dissemination of cluster clover while subterranean clover seeds suffer higher mortality.

4. The fourth section describes pen-feeding experiments, which examine the survival of seed of different sizes of *M. truncatula* cv. Paraggio (hereafter referred to as Paraggio medic) fed as whole pods to sheep, as well as clean seeds of subterranean clover, balansa clover, cluster clover, Paraggio medic and Sava medic exposed in the rumen of sheep.

(1) The first study examines the effects of *in sacco* digestion in the rumen on the survival of subterranean clover, balansa clover and cluster clover seeds collected from the summer-autumn grazing study [Section **3**, (1)]. The exposure of seeds in the rumen, significantly decreased the germination and viability of the seeds tested. This was due to the complete mortality of soft seeds and some minor changes in hard-seededness caused by digestion. There was little difference between the level of dead seed in the hard seed component before and after digestion. No further changes in germination and viability were observed after the seeds were placed under simulated abomasal conditions. This indicates the

importance of hard-seededness associated principally with the small-seeded clovers for the survival of seed. Despite further damage that may occur later during the passage through the intestine and even after defaecation, hard seed will only survive if it avoids the chewing processes of digestion.

(2) The second study examines the effects of different periods of incubation in the rumen of the same seed species as before and collected prior to the start of the grazing period. This study confirmed the resistance of hard seeds to the digestion in the rumen, as well as under the simulated conditions of the abomasum, where again no further changes in germinability and viability were observed.

(3) The third study examines the effects of ruminal digestion on the survival of small and large seeds of Paraggio medic and Sava medic selected for 100% soft and 100% hard seeds. The hard seed is resistant to digestion in the rumen while the soft seed is susceptible. This suggests that much of the damage to the hard seed ingested by sheep occurs during the chewing process. It is during intake that the size of the hard seed plays a critical role in seed survival.

(4) The fourth study examines the effect of the digestive tract of sheep, when seed of Paraggio medic of two sizes (from small and large pods) and levels of hard-seededness (low and high) were fed in whole pods to sheep. The average recovery of viable seed was 6.6% and 2.0% for small pods fed with high and low levels of hard-seededness, and 4.2% and 1.1% for large pods fed with high and low levels of hard-seededness, respectively. This indicates that the use of pasture species with small seed and high levels of hard-seededness may ensure a better survival of seed and better survival of the species under grazing.

The results of experiments described in this thesis, show that the seed size of self-regenerating pasture legume species has different effects depending on the stage of plant development. Small seed size is a more efficient trait in germination and survival under grazing. In contrast, large seeds are more efficient at emergence and produce more vigourous seedlings with higher initial pasture productivity.

STATEMENT

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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1 GENERAL INTRODUCTION

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1 GENERAL INTRODUCTION

In a period of approximately two hundred years, Australia has developed from an environment without agricultural practices to one of the world's most efficient producers of agricultural commodities (Begg and Peacock 1990). It comprises a total area of about 768.3 million hectares. Of this, 60.8% (467.1 million hectares) was used for agricultural purposes in the 1988-1989 period, including 8.8 million hectares of wheat, 8.7 million hectares of other crops and 30.2 million hectares of sown pastures and grasses. The remaining 419.4 million hectares comprises native grassland, shrub steppe etc. used for extensive grazing of sheep and beef cattle (Australian Bureau of Statistics 1991). Today, agriculture is dominated by farming systems which combine livestock with extensive cropping at different intensity levels (Ockwell 1990), with the animals normally fed entirely on pasture and pasture products such as hay or silage (Wheeler and Freer 1986).

Historically, the most relevant feature of Australian agriculture has been the development, evolution and expansion of sown pasture technologies (Donald 1982). In southern Australia, these changes started with the widespread sowing of annual self-regenerating species such as subterranean clover in the 1920's and medics in the 1930's. Pasture species were integrated in ley farming production systems, in rotation with cereal crops, as well as permanent pastures, causing a reduction in soil erosion and an improvement of soil fertility. This aids the control of weeds and cereal diseases and increases yields of crops. Also, the increase in forage availability and quality improves animal performance, and in consequence stabilises farm income (Carter 1975, 1978; Reed and Cocks 1982).

However, the sustained decline in farmer's trade conditions since 1970 and the rapid evolution in the relative profitability of livestock and cropping activities have directly or indirectly affected the persistence of annual pasture legumes. The decreased application of phosphorus fertilizer to pasture, overgrazing and poor fodder conservation practices

have directly reduced pasture legume persistence. Increased cropping intensity, the introduction of new cropping technologies (e.g. increased use of herbicides and nitrogenous fertilizers) and the appearance of new diseases and insect pests, or the increased prevalence and severity of those previously known, are the direct effects of the cropping phase on the decline of these species in pastures (Carter *et al.* 1982; Gramshaw *et al.* 1989).

The urgent need to restore the legume status of these pastures was stressed by Carter (1982) and at the Leura Conference of pasture specialists sponsored by the CSIRO and the Australian Wool Corporation in December 1985 there was unanimous agreement and concern about this decline of annual legumes in both the cereal zone and higher-rainfall zones of Australia.

During the last thirty years, efforts have been made to develop temperate and Mediterranean pasture plant breeding programs. The goals of these programs include high seasonal forage production, seed production, persistence, competitive ability, drought tolerance, disease resistance and reductions in animal toxicity. Despite these efforts some problems of legume persistence in pasture still remain. Proposals for the improvement of the performance of these species and/or cultivars in existing areas and expanding their use into new production systems will be made by developing new plants better adapted to a wide range of environmental and management situations. Furthermore, there is agreement on the need to consider the improvement of feeding value, especially the mature dry pasture residues (Collins and Stern 1987; Hutchinson *et al.* 1987; Purser *et al.* 1987). This will be an important factor in the regulation of the impact of grazing livestock on seed survival.

The key requirements for persistence of an annual pasture legume, irrespective of the type of production system in which it is grown, are that enough seed must be set and survive the grazing conditions imposed. There should also be sufficient breakdown of hard seed component to ensure an adequate seedling density after effective rainfall occurs in the following season. Plant density is especially important early in the

growing season to provide early feed and ensure livestock performance. Some of these seedlings must also survive to maturity and produce seed. It is also important that an adequate reserve of hard seed remain for more than one growing season, to allow regeneration of the pasture following cropping or after poor seasonal conditions (Jones and Carter 1989).

Several studies have concluded that an inadequate legume seed reserve represents the main constraint to commercial success of annual self-regenerating pastures (Carter *et al.* 1982; Carter and Cochrane 1985; Dear and Loveland 1985; Carter 1987; Jones and Carter 1989). A greater use of legume mixtures, comprising several cultivars and/or species is proposed by Jones and Carter (1989) as a reasonable alternative to reduce the adverse influence of enviromental factors and management practices on pasture persistence. A combination of diverse seed and plant traits (e.g. adaptation to specific soil and/or moisture conditions, species and/or cultivar maturity, levels of forage and seed production, disease and pest resistance, seed size, level of hard-seededness, burr burial capacity, etc.) represents the most suitable strategy to ensure more-sustainable pasture production within a production system.

The effects of seed size in annual pasture legumes have been reported mainly in relation to pasture establishment and early growth (e.g. Black 1956, 1957a, 1957b, 1958, 1959; Lawson 1957; Lawson and Rossiter 1958; Rossiter 1966b; Lawson 1969; Taylor 1972; Adem 1977; Carter and Challis 1987; Ababneh 1991), as well as on seed survival under sheep utilization (e.g. Carter 1966; Playne 1969; Carter 1980; Carter and Lake 1985; Thomson *et al.* 1987; Carter *et al.* 1989; de Koning 1990) but few attemps has been made to test under what conditions legume seed size is an important trait in the persistence of a pasture, especially when it is subjected to grazing by sheep.

This thesis aims to improve the knowledge and understanding of the ecological significance of seed size of some common annual pasture legumes of the genera *Medicago* and *Trifolium* used in ley farming production systems, in rotation with crops, as well as in permanent pastures under Mediterranean conditions in South Australia.

The research work has been organized in four sections. The first concerns laboratory research and shows how seed size and some environmental factors (temperature and moisture status) influence the water uptake and germination of the seed. Secondly, glasshouse and raised-bed studies were done to examine the interrelations of seed size with some environmental and management factors such as soil texture, depth of sowing, cereal straw residues and plant density on emergence and early vegetative growth of pasture seedlings. Thirdly, studies examined the role of seed size on seed survival under the impact of grazing by sheep. The last section describes pen-feeding experiments, in which the survival of legume seeds fed in whole pods or as clean seeds exposed in the rumen of sheep was studied.

2 LITERATURE REVIEW

2 LITERATURE REVIEW

2.1 Introduction

The literature review has been organized in three sections. The first concerns the effect of environmental factors, such as temperature and moisture status, on water uptake and germination of seed. Secondly, the review examines the influence of seed size and some environmental and management factors (depth of sowing, soil texture and physical impedance of cereal straw residues) on emergence and early growth of pasture legumes. The main aspects of seedling growth and inter-plant competition influenced by seed size and plant density are also considered. The last section principally describes specific research work carried out to test the survival of pasture seeds ingested by livestock and the ecological implications of this process.

2.2 Seed size and some environmental factors influencing the germination of seed

2.2.1 Temperature

According to Bewley and Black (1982), the capacity of a seed to germinate will depend mostly on factors such as temperature and availability of water, and in some circumstances on light, oxygen, carbon dioxide and other substances. Seeds adapted to the Mediterranean environment in South Australia, normally germinate and seedlings became established after the first effective rain that falls at any time from April to June, but in some years summer rains allow an early germination of non-dormant seeds (Puckridge and French 1983). The emerging seedlings may or may not survive, depending on temperature and soil moisture.

Annual pasture legume seeds can be ready for germination under a wide range of temperatures. For example at Adelaide, South Australia, (Lat. 34° 56'; Long. 138° 35'; Height above sea level, 43 m) mean air temperatures of 21.0°C , 17.7°C and 14.5°C for

March, April and May respectively have been recorded over a period of 125 years. However, temperatures as high as 43.6°C (1934) and as low as 6.6 °C (1933), as high as 37.0 °C (1938) and as low as 4.2°C (1859), and as high as 31.9°C (1921) and as low as 1.5°C (1985) have been recorded in March, April and May respectively (Australian Bureau of Statistics 1991).

Restriction of germination to a relatively narrow range of temperature may be considered as an important adaptation trait that limits the time in which the seed is able to germinate. Thus, it would be of particular importance under conditions where temperature is the most variable environmental factor and where moisture is either not a critical factor throughout the year, or is not strictly seasonal. Wherever the range of temperature in which germination occurs is wide, control by temperature may seem ineffective. However, under natural conditions, the range of temperature may be importantly narrowed by the adverse effects produced by other environmental factors involved (Koller 1964).

When seeds are germinated over a range of temperatures, an optimum temperature for germination soon becomes apparent (Bewley and Black 1982). Normally, over a short period of time, it is possible to observe three cardinal temperatures for germination (minimum, optimum and maximum). Later, an increasing number of seeds reach their potentials at each side of the initial optimum until the potentials are obtained over a wide temperature range. Then, the narrow optimum temperature disappears and the minimum and maximum temperatures shift to lower and higher values respectively, indicating the absolute temperature limits for germination. Thus time becomes of prime importance and optimum temperatures are not necessarily those giving the highest levels of germination, but those that reduce the time between the seed stage and seedling establishment. Then an early start, a more rapid rate, or a higher final germination may have a different relative value for the survival of seed under dissimilar environmental conditions. These three aspects may be differently affected by temperature.

In relation to Mediterranean and cool temperate pasture legume species, Trumble (1937) found an optimum temperature of germination of 24°C for lucerne (*Medicago sativa*), 14°C for burr trefoil (*M. denticulata*), 18°C for subterranean clover (*Trifolium subterraneum*), 24°C for strawberry clover (*T. fragiferum*) and 15°C for cluster clover (*T. glomeratum*). All species germinated in an optimum range of temperatures between 7°C and 23°C. The widest range of temperature which allowed germination was obtained for subterranean clover (1°C - 33°C), followed by lucerne (4°C - 34°C) and burr trefoil (6°C - 23°C).

Toole and Hollowell (1939) further tested the effects of different temperatures ranging from 5°C to 35°C on the germination of some winter annual species of *Trifolium* and revealed that subterranean clover and low hop clover (*Trifolium procumbens*) showed the sharpest differences in germination at different temperatures. Both species germinated well at 20°C or lower, but at 25°C or higher the germination was reduced. Conversely, buffalo clover (*T. reflexum*) germinated as well at 25°C as at 20°C and even at 30°C. The response of cluster clover was somewhat similar. Persian clover (*T. resupinatum*) was least affected by temperature and, like subterranean clover, produced fairly normal seedlings at the lowest temperatures (5°C).

In annual medic species, a study by Andrew (1965) revealed that the relative ability of *Medicago minima*, *M. truncatula* and *M. polymorpha* to germinate at high temperatures within the range of 15°C to 30°C was species-dependent. This suggested that differences in germination abilities might be one of the factors exerting a selective influence on their distribution in a semi-arid environment. *M. minima* germinated best at high temperatures, *M. truncatula* at intermediate temperatures and *M. polymorpha* at low temperatures.

An interesting example of the dependence on temperature for germination by ten cool-season range plants from temperature was provided by Ellern and Tadmor (1966). When the data was analyzed on the day on which 10% of final germination was reached, bur medic (*Medicago hispida*), barrel medic (*M. truncatula*) and purple clover

(*Trifolium purpureum*) were relatively unaffected by temperatures between 4°C and 30°C. By full germination, defined as the day on which the 80% of the final germination was reached at any one temperature, medic seeds were retarded in their germination by the higher temperatures. Purple clover was least affected. Finally, they stated that low temperatures delayed germination rather than depressing it. In contrast, high temperatures tended to depress germination rather than delay it.

Smith (1968) reported a number of studies of germination and establishment of barley grass (*Hordeum leporinum*) in comparison with some associated annual pasture species, such as Wimmera ryegrass (*Lolium rigidum*), subterranean clover and barrel medic. The annual pasture legume species were much affected by temperature regimes over 32.2°C and some failed completely under conditions where prevalent temperatures were 40°C or over.

According to Young *et al.* (1970 a) a rapid germination at a wide range of temperatures should be an advantage in establishing a legume under rangeland conditions. They studied the effects of a range of temperatures between 0.5°C and 30.0°C on germination of cultivars of legume species such as subterranean clover, rose clover (*Trifolium hirtum*), cup clover (*T. cherleri*), bur medic (*Medicago hispida*), barrel medic (*M. lupulina*), lucerne (*M. sativa*), woolly pod vetch (*Vicia dasycarpa*), milkvetch (*Astragalus cicer*) and sainfoin (*Onobrychis viciaefolia*). The subterranean clover cultivars, lucerne and cup clover germinated in one week at all temperatures imposed. The rose clover cultivars and medic species required at least 5°C to initiate germination within one week. Finally, the germination of seeds of all legume species was markedly reduced at the highest temperature (30°C).

At the same time, McWilliam *et al.* (1970) reported the results of a number of studies dealing with germination and early seedling development of temperate pasture legumes and grasses. In one of these studies the influence of temperature on the rate of germination as well as on final germination was estimated over a range of temperatures from 5°C to 29°C. The legume pasture species showed a faster rate of germination in

comparison with the non-legume species, especially at the lower temperatures. Within the group of legume species, subterranean clover differed from lucerne and white clover (*Trifolium repens*) in that the germination rate of the annual clover declined markedly above 20°C.

A more recent study considered the effects of temperature on germination of several subterranean clover cultivars (Young *et al.* 1978). They grouped the response of germination of cultivars subjected to a range of temperatures from 0.5°C to 20°C in four categories: I. Composed of cultivars Woogenellup and Blackwood: these were shown to be temperature-neutral with a small reduction in total germination obtained at both extreme temperatures. II. Consisted of cultivars Clare, Geraldton, Dinninup and Howard: these were very depressed in germination at 20 °C and the highest germinations were recorded between 5°C and 10°C. Depression of germination at the lowest temperature was negligible in Clare and only slight in the other cultivars. III. In this category, Tallarook, Mount Barker, Bacchus Marsh and Yarloop had depressed germination at lower temperatures and an optimum germination was obtained at 20°C. IV. This was represented by cultivar Dwalganup: germination was markedly reduced between 0.5°C to 10°C, but some improvement was obtained at higher temperatures.

Later work by Evers (1980) comparing the germination of major cool-season annual clover species over a wide range of alternating temperatures ratified the susceptibility of the subterranean clover cultivars used to germinate properly at high temperatures (35/25°C). However, in contrast to the results of Young *et al.* (1978), cultivar Howard was not affected in its germination when day maximum temperature was about 20°C and inclusive up to 30°C occurred.

A study by Silsbury *et al.* (1984) provides further information on the effects of temperature on the germination of seed of the subterranean clover cultivar Mount Barker. Germination tests carried out at constant temperatures ranging between 10°C and 30°C showed an optimum temperature for germination of about 20°C. At 25°C germination was reduced whilst at 30°C germination was almost completely arrested.

These observations indicate that, despite the varying experimental conditions, each species and its cultivars have different temperature requirements to achieve optimum germination. Medic species germinate better at higher temperatures than subterranean clover species. Conversely, the clover species are best suited to germinate at lower temperatures.

2.2.2 Water uptake

The development of a seed and its subsequent germination represent different physiological stages of the plant life cycle. Development of seed is characterized by a rapid accumulation of storage reserves, such as proteins, lipids and carbohydrates and ends with the desiccation of the seed tissues and the corresponding reduction of physiological activity. In contrast, during the germination process, catabolic reactions favor the mobilization of stored reserves. The products of these degradative processes are used as substrates and energy source for the early growth of the seedling (Dure 1975; Kermode *et al.* 1989 ; Leopold and Vertucci 1989).

Seeds have been classified into two categories according to their response to water, orthodox or recalcitrant. The orthodox seeds can be dried to 5% moisture content (0.05 g of water/g of fresh weight) or less and tolerate freezing temperatures (Roberts 1973). A large proportion of Angiosperm seeds, including the seeds of annual legume pasture species, are orthodox and can then survive desiccation (Vertucci and Leopold 1987).

Water plays an essential role in the rehydration of seeds. It acts as substrate or as a solvent for most biochemical reactions, affecting the intramolecular motion of proteins involved in catalytic activities and modifying the phospholipid structure that represents an important step for seed membrane permeability (Acker 1969; Jaenicke 1981; Crowe and Crowe 1986; Leopold and Vertucci 1989). However, water may also influence orthodox seed survival during imbibition. In the early stages of imbibition a rapid uptake of water by dry seeds can result in imbibition injury, due to the leaking of solutes

such as organic and inorganic ions, sugars, aminoacids, and even proteins into the surrounding medium (Simon and Raja Harun 1972; Powel and Matthews 1978; Leopold 1980; McKersie and Stinson 1980; Murphy and Noland 1982). The imbibition injury is particularly effective in seeds immersed in water, as in laboratory germination tests (Ellis *et al.* 1982 a). Seeds are more susceptible to damage the lower their moisture content (Klingmüller and Lane 1960; Pollock 1969; Ellis *et al.* 1982 b) and the lower exposure to lower temperatures (Pollock 1969; Powell and Matthews 1978) although the effect is most severe in the large-seeded legume species. More recent studies by Murphy and Noland (1982) and Vertucci and Leopold (1983) have shown that the temperature effect on imbibition rate is correlated with the viscosity of water. The slower imbibition rate at lower temperatures has been attributed to increased viscosity of water.

The amount of water absorbed by imbibing seeds depends on different seed factors, such as size and hydratability of contents, but in absolute terms it is relatively small and often may not exceed two or three times the dry weight of the seed (Bewley and Black 1978). The rate at which the water is taken up by the seed is controlled by a gradient induced by the water potential of the surrounding external medium (Vertucci 1989). The water potential is an expression of the energy status of the water and the diffusion of water occurs down an energy gradient from high to low potential. The water potential in the cell is influenced by three factors: the osmotic potential that is related to the concentration of dissolved solutes in the cell (the greater the concentration, the greater the attractive force to water); the matrix potential that is given by the ability of the seed contents (e.g. cell wall, proteins, etc.) to be hydrated and bind water, and the turgor pressure that occurs as a consequence of the entry of water into the cell, swelling its contents and exerting a force upon the cell wall (Bewley and Black 1978).

When the seed is incorporated into the soil a more complex situation occurs. The rate of water uptake by seed may be determined by the water potentials, the hydraulic conductivity of the soil matrix and the degree of seed-soil moisture contact (Sedgley 1963; Harper and Benton 1966; Hadas and Russo 1974). However, under laboratory

conditions using moist filter paper in Petri dishes, the seeds are in contact with the aqueous medium and so the external impedance (seed-substrate contact) and the internal impedance of the matrix are more or less eliminated (Bewley and Black 1978).

According to Bewley and Black (1978), Karssen *et al.* (1989) and Vertucci (1989) the water uptake by seeds, under optimal conditions of water supply, shows a triphasic pattern. Phase I or imbibition, is a physical process as a consequence of the matric forces of the seed (cell walls, cell contents) and the availability of water. Manohar (1966 b) and Shaykewich and Williams (1971) reported that dry seeds may have an initial water potential as low as -10,000 joules/kg given their enormous matric potential. Phase II is the lag period of water uptake in which the water potential of the seed is in balance with the surrounding medium and high matric and osmotic potentials are present. Phase III is associated with the radicle elongation. It is characterized by an increase in water uptake which is initially associated with a decrease in the water potential products of unknown changes related to the germination process, and then by decreases in osmotic potential due to post-germination reserve hydrolysis. The length of each of these phases depends on seed properties such as seed coat permeability, oxygen uptake, seed size, etc. and characteristics of the aqueous medium (moisture level, composition of substrate, temperature, etc.).

An earlier study by Brown and Worley (1912) on the effects of temperature on the rate of water uptake by barley seeds (*Hordeum vulgare)* revealed that the response in water uptake was almost an exponential function of the temperature, suggesting that imbibition represents a chemical or physiological process. Alternatively, Shull (1920), working with *Xanthium* seeds and pea cotyledons (*Pisum sativum*), found that the water uptake at any given time was approximately an inverse exponential function of the amount of water previously imbibed. A later study carried out by Shull and Shull (1924) using corn seed, supported the postulate that the rate of water uptake is represented by an inverse exponential function and depends of the previous absorption. The rate of water absorption at 55°C was eight times greater than at 5°C, suggesting that the water uptake

by seeds is merely physical in nature with no change in the surrounding aqueous medium.

A subsequent study by Stiles (1948) in relation to water absorption by varieties of corn and cotton seeds concluded that not all the seeds involved required the same percentage of water for germination: seeds differed in the total amount of water imbibed and rates of imbibition. Allerup (1958) studying the effects of temperature in a range between 5°C and 35°C on the uptake of water by wheat, pea and rape seeds, found a decrease in the rate of water uptake with time, concurrent with an increase in the water content of the seeds. The phenomenon was described by an inverse logarithmic function of the amount of water already absorbed. For all species there was an increased rate of water uptake as temperature increased and only for rape seed was the total amount of water absorbed independent of the temperature regime involved.

These results are in accordance with previous studies, where the total amount of water taken up by seeds is independent of the temperature. According to the author, the contradictory responses obtained in wheat and pea seeds may be explained by short experimental periods at the low temperatures, starting of visible signs of germination (Phase III) and the use of the wet weight of the seed, that in calculations done in previous studies, have concealed important differences especially at high levels of water content in the seed. More recent results obtained by Murphy and Noland (1982), are in complete agreement with this theory, confirming that the rate and the total water uptake by seed is dependent and independent of temperature, respectively.

Calero *et al.* (1981) working with soybean seeds found a negative correlation between seed size and the percentage of water uptake in some tested materials, but this relationship did not hold for all cultivars. Apparently other factors are also involved, such as the amount of seed coat on each seed (Smith and Circle 1978). Results obtained by Calero *et al.* (1981) indicate that large seeds have a small percentage of seed coat. Electron microscope studies revealed that the configuration of the coat pores in the

seed having a high percentage of seed coat was superior for water uptake. The percentage of seed coat decreased as the seed weight increased.

Martin (1946) stated that differences in the capacity of a seed to imbibe water are related to internal characteristics of the seed, such as the greater size of the embryo in legumes. However, it is the embryonic axis of the seed that has greatest water absorption capacity compared with other parts of the seed including the endosperm, cotyledons and seed coat (Stiles 1948).

More specific studies on the rate of water uptake by pasture legume seeds have been done. Andrew (1965) studied the moisture and temperature requirements for germination in *Medicago truncatula, M. polymorpha* and *M. minima.* All species showed a rapid uptake of water during the first two hours, the fastest being *M. minima, M. truncatula* was intermediate and the slowest was *M. polymorpha.* The faster the rate of water uptake for each species, the greater the decline in the water uptake after this period, reaching after approximately five, seven and nine hours respectively, the lag phase of water absorption, at which the water potential of the seed is in balance with the surrounding medium. The total amount of water taken up by seeds was estimated to be 217%, 295% and 203% of the original weight of the seed, respectively. No relationship ascribed to the size of the seed could be observed. Qualls and Cooper (1968) also found no differences in the rate of water uptake by contrasting seed sizes of birdsfoot trefoil (*Lotus corniculatus*) varieties.

McWilliam *et al.* (1970) studied some factors influencing the germination and early seedling development of temperate pasture species. In relation to water imbibition by seeds, they found that the legume seeds absorbed substantially higher amounts of water than grasses. Legume seeds absorbed most of the water required to initiate germination after four to eight hours. All three species of legumes (subterranean clover, lucerne and white clover) exhibited similar rates of water uptake during the first four hours of incubation, then differed, in that subterranean clover reached the lag phase of water uptake at about eight hours. In contrast, lucerne and white clover were still imbibing

water after 16 hours. The total amount of water (based on dry seed weight) absorbed by seeds, was lowest in subterranean clover (125-150 %), intermediate in lucerne (150-175 %) and highest in white clover (175-200 %).

These authors, also studied the establishment and survival of pasture species from seeds sown on the soil surface. They found a beneficial effect of lime coating on the germination of seed due to improved water relations in the vicinity of the seed. The uptake of water by subterranean clover and perennial ryegrass seeds was about 120% and 70% on lime-coated seed, and 50% and 40% on non-lime-coated seeds respectively (Dowling *et al.* 1971).

Finally, Bowman and King (1987) studied the rate of water imbibition by seeds of the subterranean clover cultivars Woogenellup, Larisa, Enfield and Dalkeith, that had been size-graded into small and big classes. The results showed that the smallest seeds of each cultivar exhibited a more rapid rate of imbibition and a higher total amount of water absorbtion (based on dry seed weight) than the largest seeds, causing an early germination of small seed.

These various studies indicate that each species differs in the amount of water imbibed and in the rates of imbibition. Temperature plays an important role by influencing the rate at which a seed takes up water but is not responsible for the potential amount of water taken up by the seed. Specific characteristics of seeds, associated with their sizes, seem to be of critical importance.

2.2.3 Water uptake and subsequent drying

Bewley and Black (1978) describe the different phases of water uptake and their relationship with changes in the metabolic status of the seed. In Phase I the imbibition of water by seed occurs on living and dead tissue and is, therefore, independent of the metabolic activity of the seed, although metabolism commences rapidly as a consequence of hydration. Phase II represents a period of active metabolism in preparation for

germination in non-dormant seeds, of active metabolism in dormant seeds, or inertia in dead seeds. Phase III is associated with germination and subsequent growth. Metabolic activity is correlated with the commencement of mobilization of stored reserves. Only viable non-dormant seed is able to initiate this last phase.

Desiccation of seeds from the early stages of their phase of imbibition to the time prior to radicle emergence (cell division and vacuolation in the developing seedling) has no permanent deleterious effects on subsequent germination or seedling vigour (Akalehiywot and Bewley 1977; Hegarty 1978; Bewley 1979; Akalehiywot and Bewley 1980; McKersie and Tomes 1980). In fact, there are many examples in the literature that reveal enhanced rates of germination and increased seedling vigour, in seeds subjected to post-imbibitional drying treatments during the desiccation-tolerant state of germination, due to an advance in the onset of germination (Keller and Bleak 1968; Austin *et al.* 1969; Hegarty 1970; Berrie and Drennan 1971; Hanson 1973; Cocks and Donald 1973; Heydecker *et al.* 1973; Sen and Osborne 1974; Crèvecoeur *et al.* 1976 ; Akalehiywot and Bewley 1977; Vincent and Cavers 1978).

At the same time, it is postulated that dehydration after soaking confers drought-resistance without interfering with germination (Henckel 1964). It is suggested that this effect is produced by physico-chemical changes within the cell, such as an increase in the hydrophilic properties of the protoplasmic colloids, a higher viscosity and elasticity of the protoplasm, and an increase in the temperature required for coagulation of protoplasmic proteins.

The completion of germination and the emergence of the radicle is faster in seeds dehydrated and rehydrated following initial imbibition than seed hydrated only once. The total time that the seeds are hydrated before germination is almost the same under both situations. These effects suggest that early metabolic events of germination occur during the soaking process prior to desiccation and all these events are arrested and not reversed, thus do not have to be repeated in order to complete the germination process (Bewley and Black 1982). Seeds desiccated during germination commence protein

synthesis more quickly, and reform polysomes faster than non-desiccated seeds (Akalehiywot and Bewley 1980). However, if the dehydration treatment is delayed until after radicle emergence, the subsequent germination and seedling vigour is reduced. McKersie and Tomes (1980) studied the effects of dehydration treatments on germination, seedling vigor and cytoplasmic leakage in wild oat (*Avena fatua)* and birdsfoot trefoil (*Lotus corniculatus*). They found that the transition between the desiccation-tolerant to the desiccation-sensitive stage was coincidental with the initiation of radicle elongation. If the seed has entered the latest stage, dehydration prevents subsequent germination or greatly reduces seedling vigour.

The magnitude of the reduced viability is related with a breakdown in membrane integrity as a result of the dehydration that is reflected in an increased cytoplasmic leakage of solutes during the rehydration of the seed (Crèvecoeur *et al.* 1976; McKersie and Stinson 1980; McKersie and Tomes 1980). Alternatively, work done by Chen *et al.* (1968), Sen and Osborne (1974), and Crèvecoeur *et al.* (1976) suggested that a breakdown of DNA in wheat embryos, an irreversible damage of an essential RNA or protein product in rye embryos and a denaturation of DNA in corn embryos respectively, occurred as a consequence of dehydration during the sensitive stage, altering the cellular metabolism and then decreasing viability. This behaviour is most relevant for seed survival under Mediterranean conditions in South Australia, when summer or autumn light rains can produce a "false break" of the season i.e. germination and emergence followed by a resumption of drought conditions, before an effective rain ensures a full germination of annual species (Cocks and Donald 1973).

2.2.4 Moisture stress

Under Mediterranean conditions in South Australia, autumn rains are not always effective in achieving a proper germination, emergence and establishment of annual pasture species. Sometimes heavy rains can be followed by variable periods of drought sufficient to create a wide spectrum of moisture tension in the soil. Thus seeds are

subjected to different moisture conditions, favourable or not, by the intrinsic characteristic of the seed and the surrounding medium (Cocks 1969).

According to Mayer and Poljakoff-Mayber (1989) the water uptake by a seed represents the most important factor influencing germination. Mayer (1986), suggested that the water content or water potential in the seed must be well balanced in all seed parts in order to reach germination status. Any imbalance will determine the inability of the seed to germinate. Well-documented studies have demonstrated how sensitive cell division is to changes in the water content of tissue (Hsiao 1973) and how the water uptake provides the crucial driving force for cell expansion (Boyer 1968; Cleland 1971; Hsiao 1973). There is also abundant evidence that factors influencing the water content of the cell and its turgor pressure play an important role in the magnitude of the metabolic activity in the seed (Hsiao 1973; Zimmermann 1978). However, plant cells have the ability to adjust their internal osmotic pressure and thus their turgor pressure in response to water stress (Zimmermann 1978). Finally, McIntyre (1987) suggested that water plays a critical role in the trend of the various physiological mechanisms that regulate the pattern of plant growth.

An increase in water stress either delays the completion of germination, decreases the rate at which the seed completes its germination or reduces the total amount of germination (Johnston *et al.* 1979a; Bewley and Black 1982). However, improvement in germination may be obtained when seeds are placed in an aqueous medium with slightly reduced water potential. This improvement may be due to slower rates of water imbibition and then reduced imbibition injury (Uhvits 1946; El-Sharkawi and Springuel 1977).

The specific effects differ with species, varieties and cultivars (Koller *et al.* 1962 ; Bewley and Black 1982). Hunter and Erickson (1952), Hadas (1970) and Hadas and Russo (1974) postulated that for each species there is a matric potential value below which the seed is unable to germinate.

An early study done by Harper and Sagar (1953), in relation to the effect of soil moisture on seed germination of *Ranunculus* species, concluded that the level of the water table influenced in different ways the establishment and then the distribution of species, from seed. *R. repens* established poorly to *R. bubosus* well, under a free-draining treatment. In contrast, under water-logged soil conditions, the situation was reversed. The third species, *R. acris* showed an intermediate response with respect to the other species. Lazenby (1955) demonstrated good establishment of *Juncus effusus* from seed when the water table was at the soil surface in comparison with the water table 5 cm below the surface. When the water table was 10 cm below the surface no seedling establishment was observed.

The effect of moisture stress on the uptake of water by a seed and its germination can be estimated physically by the use of instruments that detect moisture suction, or chemically, by placing the seed in solutions prepared with different concentration of solutes that are not absorbed by the seed and that are not toxic or deleterious to the seeds.

The physical effect of water stress on seed germination was tested by Collis-George and Sands (1959) using *Medicago denticulata, M. confinis, M. tribuloides* and *Juncus vaginatus*. Moisture was controlled by the use of a sintered-glass plate. There was no evidence in *Medicago* species, that the total germination of the seed was reduced as the suction was increased. In all species, an increase in suction resulted in a decrease in the rate of germination until 10 atm, where germination practically ceased. *Medicago* species were less affected than *J. vaginatus*.

The soil water requirements for maximum germination rate and total germination have been reported by Collis-George and Sands (1961) for oats and lucerne at zero matric potential. Decreasing germination for both species was observed with increasing suction up to 6 cm of water, while for a third species (perennial ryegrass) an increase in germination was obtained within this range. In a subsidiary experiment, in which pressure membrane equipment was used at a matric potential of 15,000 cm (wilting

point), perennial ryegrass and oat seeds were unable to germinate, whereas lucerne had 38% cumulative germination at four days after which there was no further germination (Collis-George and Sands 1962).

Roberts (1966) tested the emergence of subterranean clover seedlings by using different types of sandy soil under sub-optimal soil water conditions. He revealed an important relationship between the emergence of seedlings and the water-holding capacity of the sandy soils used. When the moisture content of the soil was maintained for at least four days at -10 cm of water potential, the highest seedling emergence was achieved. In contrast, little emergence was obtained at soil moisture content of -100 cm of water potential.

An additional study by Sedgley (1963), using a similar technique previously used by Collis-George and Sands (1959), found that the rate of germination in *Medicago tribuloides* at 1 cm of matric potential was higher than at 200 cm. Irrespective of the water tension used, the rate of germination was improved by better contact between the seed and the water medium. It was concluded that the use of a suction plate and related types of instruments in germination studies may not provide adequate water-seed contact without interfering with aeration. A more recent study carried out by Collis-George and Hector (1966) supported Sedgley's conclusion that the seed area in contact with water played an important role in the germination of the seed, as well as the matric potential of the wetted contact area.

In the same manner, Harper and Benton (1966) studied the germination of seeds of several species differing in size, shape and surface characteristics. They found that the presence and the amount of mucilaginous material in the seed make it less sensitive to water tension than spiny and reticulate seeds. Seeds with smooth testas showed a graded response to tension and its effect became lower with small seeds than with larger ones. These results demonstrate that there is a more complex relationship between germination

and the water tension of the matrix. The mucilage content of the seed, the nature of the seed coat and the size of the seed are important factors in germination.

This is relevant to the annual pasture legume species that self-regenerate under Mediterranean conditions in South Australia. Firstly, because species like subterranean clover have the ability to bury some seed in the soil and this ensures a better contact between the seed and the available soil moisture. Some other seeds, especially those enclosed in fibrous structures such as *Hordeum leporinum* are provided with awns that aid in anchoring the seed to the soil surface, increasing their contact with the surrounding medium (Carter 1968a; Smith 1968). Similar situations occur with pod spininess in annual medics (Crawford *et al.* 1989). Secondly, in broadcast sowing cases, spherical-shaped seeds like subterranean clover have poorer water relationships with the soil surface than flatter seeds like annual medics. Thirdly, large seeds on the soil surface will have less contact with the medium relative to their total surface area than small seeds, which better occupy sites for germination like cracks, crevices and small depressions. Finally, seeds that germinate faster will allow more opportunity for seedlings to succeed, than seeds that germinate more slowly.

Young *et al.* (1970b) tested the germination of seed from cultivars of subterranean clover using different soil textures and matric potentials between 0 and minus 15 bars obtained through the use of a ceramic-plate soil-moisture extractor. The germination of all cultivars was dependent on the texture of the soil and varied according to the soil-water matric potential. The germination was depressed at matric potential of -6 bars and virtually suppressed at higher water tensions. At low matric potentials, cultivars with larger seed size achieved the highest germination: however, at high matric potentials the cultivars showed a wide range of germination independent of seed size.

The effects of soil water and aeration on germination of *Oryzopsis holciformis* seeds support the evidence that the matric potential, under contrasting soil textures, and the area of contact between the seed and the medium, govern the process of germination (Dasberg and Mendel 1971). The optimum matric potential for germination was -0.005

bar and -0.5 bar in coarse sand and sandy loam soils respectively. At the same time, Dasberg (1971) studied the soil water movement to germinating seeds. He found that germination was very dependent upon seed water uptake, which was determined by soil water availability. Seed germination of legume pasture species such as *Medicago truncatula* and *M. hispida* was greatly affected by decreasing the water content of the soil.

Research on the effects of moisture conditions on seed germination by the use of different chemical solutes has been reported for different plant species. Uhvits (1946) tested the effect of solutions of sodium chloride and mannitol at osmotic potentials ranging between -1 and -15 atm on the germination of alfalfa seeds. For both solutes, the lower the water potential used, the higher the rates and total germination obtained. Germination was practically inhibited only by concentrations of NaCl between -12 and -15 atm. A similar response was obtained by Dotzenko and Dean (1959) working with six varieties of alfalfa within a range of osmotic pressures between 0 and minus 12 atm produced by the use of mannitol solutes. The ability of a seed to germinate at high osmotic pressures was a heritable trait. Further information related to the ability of alfalfa seeds to germinate at lower osmotic potential was confirmed as heritable by Dotzenko and Haus (1960).

Younis *et al.* (1963) germinated alfalfa varieties in solutions of mannitol ranging between 0 and minus 12 atm and observed a decrease in germination with an increase in moisture stress. Differences in total germination recorded after seven days were highly significant among varieties, osmotic potentials and the interaction (Variety x Osmotic Potential). Both germination and rate of germination were reduced with an increase in moisture tension. A similar response was found by Rodger *et al.* (1957) working with alfalfa seeds subjected to germination in solutions of sodium chloride having osmotic pressures between -3 and -9 atm, and Heinrichs (1959) studying the germination of alfalfa varieties in sucrose or sodium chloride solutions of minus 3, 6 and 9 atm.

Andrew (1965) tested germination of *Medicago polymorpha* and *M. minima* seeds over a range of 0 to minus 12 atm of osmotic pressure obtained by the use of carbowax polyethylene glycol. Only the germination of *M. minima* was improved by increasing the osmotic pressure from 0 to -3 atm: however, *M. polymorpha* appeared to be more sensitive than *M. minima* at higher osmotic pressures.

McWilliam *et al.* (1970), and McWilliam and Dowling (1970) simulated the influence of soil moisture stress by the use of aqueous solutions of polyethylene glycol (mol. wt. c. 20,000) over a range of water potentials (0 to -16 bars) in pasture species, such as lucerne (*Medicago sativa*), white clover (*Trifolium repens*), subterranean clover (*Trifolium subterraneum*), harding grass (*Phalaris aquatica*), cocksfoot (*Dactylis glomerata*) and perennial ryegrass (*Lolium perenne*). Ryegrass was the least affected by increased water potential in comparison to the other species. Its rate of germination was only affected at osmotic potentials exceeding -8 bars and some seed was able to germinate at potentials as high as the wilting point. The other species were more susceptible to water stress, especially subterranean clover which was markedly affected in total germination even at water potentials down to -6 bars. Young *et al.* (1968) reported similar germination responses for range grasses in relation to germination under osmotic stress. Total germination of grasses was higher at different levels of stress: however, the subterranean clover cultivars maintained considerable germination at osmotic pressures up to -12 bars.

Young *et al.* (1970 b) tested the germination characteristics of several range legumes species, including subterranean clover, between 0 and -16 bars using polyethylene glycol (mol. wt. c. 1,540). All species, except rose clover, showed reduced germination between 0 and -6 bars. Between -8 bars and -12 bars, germination in all species was strongly inhibited, with nil germination at -16 bars. McGinnies (1960) and Chippindale (1949) reported that species that are able to germinate at high osmotic potentials are not necessarily those that can persist better under extreme drought conditions. Normally

seeds from these species are more susceptible to false starts and die before establishment if there are not sufficient follow-up rains.

Despite the variation in the methods used, the analysis of published information demonstrates that the ability of a seed to germinate under moisture stress is species-dependent and seeds of those species that germinate at high osmotic potentials under controlled laboratory conditions are not necessarily those that can persist better under natural extreme moisture situations. The important influence of the seed-water contact on germination has been emphasized, suggesting that the characteristics of seeds, including the size, are important factors in determining both the rate of germination and the final germination percentage, when the seed is subjected to increased levels of moisture stress.

2.3 Seed size and some environmental and management factors influencing the emergence and early growth of pasture legume seedlings

2.3.1 Depth of sowing and soil texture

Annual pasture legumes are important in Australian agriculture because most of the animal and grain production occurs in ley-farming situations or under relatively low-input permanent grazing systems (Gramshaw *et al.* 1989).

Several authors (Carter *et al.* 1982; Gillespie 1983; Dear and Loveland 1985; Carter 1987) have identified the recent deterioration in quantity and quality of annual legume pastures in southern Australia. There is ample evidence to suggest that seed reserves of annual medics and subterranean clover have declined in the annual pastures on many farms.

One of the factors influencing the persistence of legumes in pasture-crop systems is the increased use of more frequent cropping practices or longer cropping sequences. The main problem of annual pastures is frequently regeneration rather than the establishment (Wheeler 1987). However, Carter (1987) emphasized also the importance of a proper

depth of sowing in annual pasture establishment, especially when normal variations in seed size between species and cultivars, and variations in soil texture are present. Field experiments related to land development and pasture establishment carried out by Toms (1958), Carter and Heard (1962) and Amor and Mann (1966) revealed the influence of depth of sowing on proper emergence of subterranean clover and annual medics. Under these circumstances, the regeneration of legume pasture species depends on seed that survived the previous growing season without germination (Taylor 1985).

Cultivation, and the type of equipment used also affects germination and emergence of annual pasture species. Increasing the depth of tillage causes more medic pods and subterranean clover burrs to be buried deeply, decreasing the potential softening of hard seed and seedling emergence (Taylor 1984, 1985; Quigley *et al.* 1987; Fulwood and Carter 1989).

Fulwood and Carter (1989) reported contrasting soil profile distributions of Sava snail medic (*Medicago scutellata*) and Paraggio barrel medic (*M. truncatula*) pods subjected to different tillage treatments imposed on a loamy-sand soil. After deep mouldboard ploughing (w/d 15-18 cm), only 15% and 5% of Sava and Paraggio medic seed was in the top 5 cm of soil, compared with 87% and 81% following scarifying (w/d 6-8 cm). Snail medic (18.1 mg/seed) and barrel medic (3.5 mg/seed) did not emerge from mean depths greater than 5.8 cm and 3.0 cm respectively. Similar results were obtained by Carter *et al.* (1988 b) by using the same two tillage treatments. After deep mouldboard ploughing, 15% of medic seeds were recorded in the top 5 cm of soil. In contrast, 90% of seed was found at that level following scarifying.

A sowing depth of 5 cm in sandy soils and 3 cm in loams is probably the limit for successful seedling emergence of annual medics under normal weather conditions (Carter and Challis 1987). Similar findings were obtained by Ameziane *et al.* (1989) who concluded that seed reserves in the top 5 cm of soil were the best indicator of how well annual medic pasture species would be maintained in a crop-pasture rotation system (Carter 1982; Carter *et al.* 1989b ; Carter and Porter 1992).

The extent of seed burial can be influenced by tillage practices under different rotations. In a rotation of one year pasture and one year crop, 44% of the seed reserves of subterranean clover cultivar Daliak, were buried below 2 cm when minimum tillage practices were used (Taylor 1985). When a conventional tillage treatment was applied, 65% of seeds were found buried below 2 cm. Taylor concluded that repeated tillage operations improved clover establishment because the seed that was able to survive long enough was returned close to the soil surface.

It has been demonstrated at various times for a number of species of economic importance, that large-seeded species can emerge from deeper positions in the soil than small-seeded ones (Herriot 1958; Carter and Challis 1987). According to Yates (1958), the variation of seed size in a population of subterranean clover seeds is due mainly to microenvironmental conditions at the time of seed set, and the susceptibility of a particular cultivar to such conditions. Additionally, within any specific strain there is only a small genetic variation, so the mean weight of any viable seed, is expected to vary principally with the environment.

The extent to which seedlings are able to emerge from various depths of sowing, in contrasting soil types, is related to the force exerted by emerging seedlings which is strongly and positively correlated with seed weight (Williams 1956; Jensen *et al.* 1972; Haskins and Gorz 1975). The largest seeds of subterranean clover were observed by Williams (1956) to exert greatest force during emergence compared to smaller seeds of lucerne that exerted the least force. Most of the variation in emergence force between species (99%) was accounted for by the respective seed weights.

Seedlings appear to emerge by weaving through voids and by moving or deforming some soil obstructions. When the seedling apex is blocked, the stem tends to turn in the direction of least resistance, which is normally towards the soil surface. As the magnitude of the constraints in the soil vary, the method and the difficulties of emergence seem to change with the size of the seedling. When the cross-section area of a

seedling stem increases, the flexibility of the stem decreases whilst the axial force that can be exerted for a given part of the plant increases. However, at the same time greater lifting power tends to be associated with a loss of flexibility (Arndt 1965).

Research work by Moore (1943) who studied the influence of depth of sowing and seed size on emergence within a seed population of crimson clover (*Trifolium incarnatum*), revealed that the rate of emergence was negatively affected by sowing made at 2.5 and 3.8 cm depths compared to shallow sowings. At the same time emergence at 3.8 cm depth was delayed as the size of the seed decreased. The size of seed was therefore important in determining the depth from which a seedling can emerge. Moore also revealed that seedlings produced by extra large seeds were not able to emerge properly, which is possibly due to their large cotyledons. Adem (1977) noted the same phenomenon. Similarly, very large seeds of subterranean clover frequently failed to produce normal seedlings because cotyledons were broken from the embryo axis (Black 1959).

An experiment reported by Erickson (1946) involving the effects of various seed sizes sown at different depths on emergence of lucerne (*Medicago sativa* L.), showed that emergence was influenced more by seed size than by depth of sowing. At shallow sowing depth the medium- and small-sized seeds were about 3% and 13% lower in emergence than the large seeds. However with deep sowings the medium- and the small-sized seeds were 10% and 21% lower in emergence than the large seeds. He further revealed that shallow depth of sowing of about 0.6 cm was importantly beneficial for the emergence of seedlings from small seeds but it reduced seedling emergence from large seeds. This inverse relationship was observed when contrasting seed sizes were sown at about 1.9 cm depth. Additional studies have been reported for lucerne. Beveridge and Wilsie (1959) found a positive relationship between seed size and emergence. In contrast, Nel and Burger (1968) revealed that emergence of alfalfa seedlings decreased as depth of sowing increased, but the relation between seed size and emergence was

inconsistent. In the latter study, it was concluded that seed size was not a priority factor of significance.

Both depth of sowing and temperature influence pre-emergence weight changes in subterranean clover (Black 1955). He showed that both factors were importantly involved and suggested that the subsequent growth of the seedlings after emergence could be affected by the level of depletion of cotyledons during emergence. However, later work (Black 1956) showed that this was not the case and rather than a depletion of cotyledonary reserves, a limitation of extension growth in the hypocotyl occurred because a linear relationship existed between seed size and potential elongation of the hypocotyl. Also at the same pre-emergence stage, Tadmor and Cohen (1968) revealed that root elongation of Mediterranean grasses and legumes was generally positively correlated with seed weight.

The effects of seed size and depth of sowing on emergence has also been studied in birdsfoot trefoil (*Lotus corniculatus* L.) by Henson and Tayman (1961) and Stickler and Wassom (1963) as well as in milkvetch (*Astragalus cicer* L. and *A. falcatus* Lam.) by Townsend (1972) and in sweetclover (*Melilotus officinalis* L.) by Haskins and Gorz (1975). In general, the larger seeds gave better seedling emergence from the greater depths of sowing.

A more recent study made by Carter and Challis (1987), on the effects of depth of sowing and soil type on emergence of annual pasture medic species, ranging in individual seed weight between 17.8 mg (*Medicago scutellata* cv. Robinson) and 2.7 mg (*M. littoralis* cv. Harbinger), revealed that as depth of sowing increased (1, 3 and 5 cm) emergence of seedlings decreased. The impact of depth of sowing was greatest in a loamy soil compared with a sandy soil. Emergence of the larger-seeded cultivars was reduced more than for medium-seeded ones at 1 cm and 3 cm depth, principally in a loam soil. It was concluded that under ley-farming systems it is imperative to avoid deep burial of medic seed by ploughing, and not to sow small-seeded cultivars below 1 cm in loamy textured soils.

These findings with annual pasture medics were confirmed by Ababneh (1991) who concluded that, under natural conditions, seedlings produced by seeds placed at greater depths will be constrained compared with those emerging from shallower sowings, especially in heavy-textured soils. This study confirmed that seed size was the dominant influence on emergence from various depths and soil textures.

From the evidence presented, it appears that legume seed size determines the depth from which a seedling can emerge in a particular soil. However, under natural conditions, the optimum depth of sowing, determined under controlled environmental conditions, will not necessarily be the best to ensure optimum emergence. This is especially true when rapid drying of the seedbed occurs. Root development is also generally correlated with seed weight, but the deepest root penetration occurs at the greatest sowing depth from which a seedling can emerge. Hence, the optimal sowing depth must be a compromise between seed size, soil texture and soil moisture and may well be the deepest from which a seedling is able to emerge satisfactorily (Tadmor and Cohen 1968).

2.3.2 Physical impedance of cereal straw residues

The development and expansion in the use of stubble mulching started more than 60 years ago in the United States (USA) in order to control soil erosion in the Great Plains of USA and Canada. This farming system was originally developed for arid and semiarid areas where wheat is grown as monoculture and has been also adopted to some extent in more humid areas (McCalla and Army 1961). Positive and negative implications of stubble retention in cropping systems have been reported by Parr and Papendick (1978). In Australia, the effects of a stubble retention on soil properties is, in general, beneficial. However, longer-term solutions are required to minimize inhibitory effects of stubble retention, especially if a diversification of crop species and their management is to be continued in Australian agriculture (Lovett *et al.* 1982).

The effects of cereal residues on the growth of the following crop have been well described (e.g. Kimber 1967; Cochran *et al.* 1977; Lynch and Cannel 1980; Lovett and Jessop 1982). However, the influence of cereal straw on the regeneration of annual legume pastures has received little attention (Quigley and Carter 1985). The potentially-negative effects that cereal crop residues have on natural regeneration of annual legume pasture species in crop pasture ley-farming systems in southern Australia have been noted for some time, especially in response to the increased use of practices such as stubble retention and minimal cultivation. Further, cereal straw walker rows, created by harvesting machinery during reaping, influence the breakdown of hard-seededness, as well as interfering with the emerged legume seedlings by shading them (Webber *et al.* 1976, 1977; Quigley and Carter 1985; Quigley 1988).

Reviews on dry land agriculture in southern Australia (Carter 1975; Puckridge and Carter 1980) and on the role of annual legume pastures grown in ley-farming systems in southern Australia (Carter 1978; Puckridge and French 1983), have proposed the control of the level of cereal stubble in order to favor the self-regeneration of pasture species. The relationships between cereal straw residues on germination, emergence and productivity of annual medics were well described by Quigley (1988). This study represents one of the few attempts to integrate the whole problem by taking account of both the annual medics and the corresponding production systems where cereal stubble is retained.

Quigley's results from field surveys, designed to quantify the adverse effects of cereal straw on pasture productivity, indicated that, as in previous reports, high concentrations of straw, especially in the walker rows, reduced seedling emergence and consequently herbage yield. By removing cereal residues which pass out of the harvesting equipment from the paddock, it was estimated that the availability of medic dry matter would increase 16%. This was ascribed to the concentration of straw which prevented emergence of medic seedlings and also to the straw cover (mulch) over the summer-autumn period which reduced seedling density by retarding the breakdown of hard seed.

In a field experiment Quigley (1988) used concentrations of barley straw between 0 and 16 ton/ha and confirmed the survey findings: at the highest straw concentration the amount of mulch caused physical impedance to seedling emergence. There was no shortage of soft seed: parallel treatments of emergence recorded from soil cores revealed the presence of a relatively high density of soft seed at the highest straw treatment. In contrast, a second field experiment using an equivalent of 3,250 seeds/m^2 of *Medicago truncatula* cv. Paraggio sown at 1.0 cm depth and covered with chaffed barley straw ranging between 0 and 8 ton/ha demonstrated that seedling establishment was unaffected by the imposed straw treatments. The higher levels of mulch physically retarded the emergence of seedlings but did not inhibit their development so there were no lasting effects on the yield of medic estimated seven weeks after sowing.

Physical impedance produced by mulch may affect the normal mechanism utilized by seedlings to emerge, delaying their normal development (Chambers 1962; Sedgley and Barley 1963; Arndt 1965). According to Quigley (1988), in common crop-pasture ley-farming systems in South Australia, normal concentration of straw residues in walker rows, ranges between 5 to 9 tonne/ha. Over the summer-autumn period, residues in these rows became a dense and compact mulch due to trampling by livestock and weathering.

In order to eliminate the inhibitory influence of straw residues on seedling emergence and in an attempt to evaluate the single effect of physical impedance, Quigley (1988) reported an experiment that examined the influence of stress produced by cereal mulch (300 g/m^2) when seeds of cultivar Paraggio, as before, were sown at depths ranging between 0.5 to 3.5 cm. A normal inverse relationship between sowing depth and seedling emergence was observed. Unfortunately only a single low concentration of straw was considered and the plant response to the imposed treatments only was estimated as the number of seedling emerged per area.

These studies suggest that physical impedance by straw mulch *per se* had variable effects on seedling emergence depending on concentration. However, it should be emphasized

that further research could be considered to evaluate the response of medic seedlings to straw mulch under more stressful conditions, including factors such as depth of sowing, waterlogging and low temperatures.

2.3.3 Seed size and seedling growth

Seedling growth has been related to seed size in a number of studies. In a review by Morley (1961) some aspects related to seed size in subterranean clover were discussed. He commented that the ecological significance of variations in seed size found between strains is still obscure. Although large seed size represents an advantage in closed communities, small seed sizes are preferable in open communities. In subterranean clover there appears to be a tendency for larger seeds to be better represented in the warmer areas (e.g. Morocco and Israel) than in the cooler ones (e.g. southern Australia) within the naturalized area of distribution. These responses may reflect intensity of competition but it seems more probable that they are a consequence of selection for rate of root growth. Seedlings produced by large seeds have more root growth and are better able to cope with frequent drying of surface layers of the soil than seedlings produced by small ones. However, large seed size did not confer any overwhelming advantage.

There is little information in annual pasture legume seed populations about the allocation of dry matter at different morphological structures in the seed and its relevance to seedling growth. Black (1956, 1957a) studied the influence of seed size and depth of sowing on pre-emergence and early vegetative growth in a Bacchus Marsh seed population of subterranean clover, and the early vegetative growth in relation to seed size in three seed populations (Bacchus Marsh, Clare and Mount Barker) of subterranean clover. In his experiments, he used seeds in which the proportion by weight of the embryo (cotyledons plus embryonic axis) was practically constant for the seed size classes involved. A more recent and complete examination of seed populations in subterranean clover has been reported by Taylor (1972). By studying seed populations of strains Daliak and Bacchus Marsh, he found an increase in the proportion of the

embryo with increased seed sizes, while in others the proportion of the embryo remained constant. It seems to be an important factor in defining when a precise relationship between seed size and early seedling growth is required.

According to Black (1959) the period of early growth in a pasture ends when increased inter-plant competition starts to reduce the individual growth rates of plants.

Early superiority of individual seedlings of crimson clover coming from large seed compared to those coming from small seed has been reported by Schmidt (1922). This was attributed to a faster onset in leaf development. However, there was an early decline in the growth rate of plants produced by large seeds, due to an early onset of intraspecific competition. At the end of the experimental period, seedlings produced by medium-sized seeds reached similar yields.

Seedling vigour in alfalfa plants was found by Erickson (1946) to be directly related to seed size and inversely associated with depth of sowing. He stated that lack of competition between small plants generated by small seeds, may have been a relevant factor influencing the increased growth rates of small plants. However, this conclusion does not take into account the principle of onset of competition, because it compares the growth rate of plants that are not subjected to competition with those that are under competition (Black 1959).

Later results reported by Black (1956) for (Bacchus Marsh) subterranean clover showed a direct relationship between dry weight obtained until the eighth trifoliate leaf stage and initial cotyledon area. Black concluded that the significance of seed size lay in the direct relationship between the size of seed and the cotyledon area that influences seedling growth. At the same time, he postulated that the relative growth rate of seedlings of different sizes is the same and that their absolute growth depends on embryo size.

Photosynthesis in legumes having epigeal cotyledons has been reported as a factor of prime importance for proper seedling growth (Williams *et al.* 1968; Lovell and Moore 1970; Cooper and Fransen 1974; Machado *et al.* 1974). Photosynthetic rate of

cotyledons was estimated by Shibles and MacDonald (1962) as important as leaves in birdsfoot trefoil, demonstrating the importance of the photosynthetic area of cotyledons for early seedling development. A higher rate of cotyledon expansion, earlier onset of leaf production and earlier leaf development are all consequences of larger seeds with greater cotyledon area (Hur and Nelson 1985). All these factors were critical in determining the rate of initial seedling growth. This can also be demonstrated (Rossiter 1992) by studying the effects of early defoliation, such as removal of cotyledons, on seedling growth of subterranean clover. When 40% to 60% of leaf area was removed a decrease between 15% to 30% in subsequent shoot weight occurred, whereas at almost complete defoliation (>90% leaf area removal) a 60 % to 70% of reduction in shoot weight was achieved.

Further work by Black (1957a) with cultivars of subterranean clover, showed that dry weight and leaf area per plant were linearly correlated with embryo weight, but independent of strain at any time during the early vegetative stage of plant growth. Although the relative growth rates were variable, the fact that there were no differences can be attributable to embryo size for any cultivar involved. Thus, there was no evidence to suggest that there were any differences between the relative growth rates of the three cultivars.

These conclusions from experiments on the effect of seed size on seedling growth of subterranean clover (Black 1956, 1957a) agree with earlier investigations by Ashby (1930, 1932). In these studies hybrid vigour in the cultivars of maize were a result of the maintenance of an initial advantage in embryo size. However, these conclusions were criticised by Sprague (1936) who claimed that there was no clear advantage for large seeds of corn in subsequent reciprocal crosses. More recently, Bremmer *et al.* (1963) showed that embryo size in wheat had a negligible effect on growth, whilst endosperm (that had been claimed by Black (1956) as absent in subterranean clover cultivars) had a considerable effect. It was suggested that the relationship between seed size and plant size is derived by the amount of seed reserve in the seed.

Further contradictory data is provided by Taylor (1972). In subterranean clover cultivar Daliak, the proportion of embryo increased with seed size, and the relative growth rate of seedlings declined with increasing seed size. This is in agreement with Evans et al. (1992) but in contrast with the conclusions of Black (1956, 1957 a), in which the relative growth rate of seedlings was constant for any given seed size. However, the proportion by weight of the embryo in seed populations used by Black was constant also for all seed-size classes involved, unlike Taylor's study.

Lawson and Rossiter (1958) reported the results of a field experiment in which small and large seeds of two cultivars of subterranean clover (Dwalganup and Mount Barker) were sown at equal weights of viable seed per unit area. They concluded that seed size had no influence on the growth rate when the sowing rate was held constant. It is possible from the results of this experiment and those of Black (1956, 1957 a, 1957 b) and Lawson (1957) to conclude that seed size has no influence on the growth of subterranean clover cultivars under sward conditions, when small and large seed are sown at the same rate in weight of seed per unit area. The initial cotyledon area of the pasture sward, determines the pattern of growth, independent of whether the sowing rate used is composed of many small seeds or fewer large ones (Black 1959).

However, when small and large seeds of the subterranean clover cultivars Dwalganup and Mount Barker were sown at equal densities of viable seed per unit area, higher yields were obtained from large-seed sowing treatments, not because of any effect of seed size, but because it had been sown at a higher rate. The sward produced from small seeds was more leafy than that obtained from large seeds (Lawson 1957).

Finally, Silsbury and Hancock (1990), using six cultivars of subterranean clover grown as undefoliated swards under controlled conditions, demonstrated the importance of seed size as a factor influencing the early growth of the sward. Where swards of cultivar Mount Barker were established from large seed, they grew faster than where small seed was used.

From this evidence the relative advantages of larger seeds is most important during the early growth stage of plants and may not last until plant maturity. It has been suggested that normally a decrease of this advantage is produced by the gradual onset of plant competition, which would be expected to start early in those seedlings that reached more advanced stages of development more quickly. However, it is important to note that these results also demonstrated that the early vegetative growth in swards is strongly dependent on embryo weight per unit area of ground, and that factor can be managed for practical applications either by modifying seed size or plant density.

2.3.4 Seed size and plant density

In Mediterranean-type climates, availability of forage in rainfed production systems is not usually a limiting factor in spring and summer. However, a marked reduction in body weight of livestock due to a decline in the availability of forage in combination with lower nutritive values is normal in autumn, and winter, where it is probable that feed availability will be the major determinant of animal production (Purser *et al.* 1987).

It is well recognized that a large seed bank is one of the major requirements to obtain productive legume pasture swards and consequently a high population of seedlings must become established after the opening of the rainfall season (Puckridge and French 1983). For early vegetative growth there is a linear relationship between yield and plant density and the greater value of a dense annual sward compared with a sparse one lies in the early production of forage (Donald 1951).

It has long been accepted that competition between plants occurs for environmental factors, such as water, nutrients and light. These are of major significance in natural plant communities, as well as under agricultural situations (Donald and Black 1958). If there is sufficient water and nutrients, light becomes the factor that limits yield in a community or in a population of plants. Therefore, the ultimate capacity of a sward to produce dry matter is dependent on the ability of the sward to utilize the incident light (Donald 1951).

An early study of intraspecific competition between annual pasture plants, including subterranean clover, carried out by Donald (1951) suggested that under any given environmental conditions, plant density and dry matter production follow a clearly defined trend. Starting from a positive linear relationship between density and embryo weight, competition was initiated first at the highest densities and subsequently appeared over time with more advanced stages of plant development at lower densities. Over time, the yield obtained at the lower densities approaches the yield obtained at the higher densities, where yield per plant was obviously affected and became inversely proportional to plant density. For subterranean clover, it was suggested that competition for light was the main factor involved. In a dense sward, horizontally disposed leaves are arranged as a continuous canopy. During the development of this canopy, individual leaves progressively shade neighbouring leaves so that at the full development stage the rate of growth is restricted to those leaves able to share the light falling on the canopy sward. It was concluded that only swards with high plant densities are able to give satisfactory early winter yields of forage. Further work by Donald (1954) on competition among pasture plants ratified these findings and emphasized that only dense pasture swards had most of the attributes needed for grazing purposes.

A later study reported by Lawson (1957), and Lawson and Rossiter (1958), on the influence of seed size and sowing rate on the growth of two cultivars of subterranean clover (Dwalganup and Mount Barker) showed that yields obtained from high density swards were greater than those obtained from low density swards regardless of seed size. The difference in dry matter was most marked early during the growing season and the growth rates of swards produced by high plant densities were initially greater than those obtained from low plant densities. However, by the end of the experiment there were no differences in growth rate due to plant density. It was concluded that there was a tendency for the contrasting sowing rate treatments used to give similar final yields and, in agreement with Donald (1951), that early vegetative growth in

subterranean clover swards was dependent on embryo weight per unit area, which varies with seed size or plant number.

The effect of seed size as a factor in the growth of subterranean clover becomes more apparent if plants are grown under spaced than under sward conditions (Black 1957b). Seeds of contrasting sizes were sown under sward conditions of about 618 plants/m^2. The swards showed similar early relative growth rates. However, a critical leaf area index (LAI) of 4 was reached first by the swards growing from large seed size, indicating the initiation of inter-plant competition leading to decreases in growth rate. Later swards growing from medium seed sizes and finally swards growing from small seed sizes also reached the same ceiling LAI. At the final harvest, all swards produced the same amount of dry matter according to their common capacity of light utilization. It was concluded that at a LAI about 4, the interception of incident light by pasture swards was complete.

Under similar conditions, Black (1958) examined the competition between plants of different initial seed sizes in swards of subterranean clover. The position of leaves in the profile of the sward determined the level of incident light intercepted and the growth achieved. Seedlings produced by large seeds had an initial advantage over those produced by small seeds because their leaves are located higher in the profile, having priority in light interception from the early stages of growth and after the onset of competition when conditions of reduced light intensity occurred. It was hypothesized that the disappearance of the small-seeded plants (Davidson 1954; Davidson and Donald 1958) from the mixed swards sown with the same number of small and large seed sizes, was due to shading effect produced by the larger-seeded plants. This confirms the importance of the spatial distribution of leaves in subterranean clover swards in plant competition. Therefore when defoliation is absent, the success of a cultivar under competition can be related to its potential ability for petiole elongation (Black 1960 b, 1961).

Growth rate and plant density of subterranean clover swards can be related to LAI (Davidson and Donald 1958). Swards composed of densities of about 25, 100, 350 and 1,250 plants/m^2 tended towards a common ceiling of LAI about 8 by the end of the growing season. The higher plant densities reached that point first followed in succession by lower plant densities. A maximum rate of growth obtained during the final month (October) for shoot dry matter and plant components was obtained at about LAI 4-5, then falling by about 30 % when LAI increased up to 8.7. At that point, an equilibrium between the death of leaves and the growth of new ones occurred. Hence the decline in the rate of forage production occurred as the leaf area index reached high levels (Donald and Black 1958). Thus in grazing management, an optimum LAI rather than a maximum LAI represents the best option for maximising sward productivity.

Based on previously reported studies, Davidson and Philip (1956) developed a quantitative theory of the light-foliage-growth relationship for subterranean clover. In this model the interception of light by leaves is completed when LAI is about 4.5. At a higher LAI some leaves received low intensity light causing senescense. Respiration was assumed to be constant in all leaves and the gross photosynthetic rate of the individual leaf was calculated as a function of the light intensity from a maximum value in the leaves located in the upper part of the canopy to a minimum value given by the leaves located in the lowest part of the canopy. The model indicated that the maximum rate of dry matter production is obtained at intermediate LAI whilst a maximum LAI is associated with a constant rate of dry matter production. For any given amount of radiation there is an optimal LAI at which plant growth rate reaches the maximum level and only subsequent increases in radiation give increased values of optimum LAI (Stern and Donald 1961; Black 1963).

According to Black (1960a), studies on competition of two or more pasture species growing together in mixed swards have normally been made only at one sowing density, or with one species varying while the other remains at constant density. These results cannot be applied to field conditions where differences in plant densities between species

occur, and where differences between the yield:plant density relationship of the species should be considered. To test the role of plant density in competition between pasture species, red clover (*Trifolium pratense* L.) and lucerne (*Medicago sativa* L.) were grown in pure swards and mixed swards with plant densities ranging between 50 and 12,500 plants/m^2. Both species showed a marked decline in plant numbers at higher densities, with lucerne most affected. The normal trend of decline in the relative growth rate as density increases occurred as a consequence of inter-plant competition. However, there was a surprising unexplained decline in relative growth rates also at low densities very early during the growing season.

Research complementary to that carried out by Black (1960 b) and Black and Wilkinson (1963) on the effects of individual plant growth and the importance of light distribution within the canopy in subterranean clover respectively, was reported by Stern (1965). On the basis of previous studies with subterranean clover (Donald 1951; Davidson 1954), densities of 4, 16 and 36 plant/dm^2 were used, in order to prevent mortality at the lowest density and ensure plant mortality at the highest density. It was shown that small differences between members of a plant community determine the pattern of dominance or suppression, whether the initial difference has a genetic origin (Black 1960b), produced by differences in seed size (Black 1958) or given by microenvironmental conditions (Black and Wilkinson 1963).

Silsbury and Fukai (1977) studied the effects of sowing time and sowing density on the growth of subterranean clover swards, by using established plant densities of 1,000, 2,000 and 4,000 plants/m^2 sown in May, June and August. The maximum sward growth rate ranged between 10 and 15 g/m^2/day. High-density swards were initially able to reach high growth rates soon after emergence but the rate declined later over the growing period. Low-density swards showed increased growth rates during the entire growing season so that the final yields were similar at contrasting plant densities. This result is in agreement with that of Silsbury *et al.* (1979) with *Medicago truncatula* cv. Jemalong. This may be explained either by differences in photosynthetic capacity and

canopy structure, or by differences in respiratory losses. In order to examine these explanations, Prioul and Silsbury (1982) estimated the daily carbon influx and efflux of subterranean clover swards sown at two contrasting plant densities of 428 and 4,760 plants/m^2, and 2,330 and 47,600 plants/m^2. The results indicated that subterranean clover is extremely well adapted to succeed under sward conditions. A higher growth rate at a lower density compared with a higher density was explained by lower respiratory losses. The higher respiration rate of a high density sward may be due to plant mortality which occurred when dry matter was over 400 g/m^2.

The relationship between plant density and yield of total dry matter has been also reported by de Koning (1984). Swards of subterranean clover cultivars Clare, Larisa, Mount Barker, Trikkala and Yarloop were grown in pure stands at three densities; 100, 1,000 and 10,000 plants/m^2. Some decline in plant numbers occurred at the highest density throughout the study. A linear relationship between sowing rate and yield was obtained. The performance of individual plants was markedly reduced as plant density increased and time progressed, due to inter-plant competition as observed in other previous studies.

Based on his earlier data, Black (1964) estimated the potential productivity of subterranean clover swards at Adelaide in southern Australia. Utilizing subterranean clover swards grown with no limiting environmental factors obtained for a range of sowing densities (50, 500 and 2,500 plants/m^2), seed sizes (4, 8 and 12 mg/seed) and regular sowing times, he estimated a potential seasonal production of 27 t DM/ha. However, 3 t DM/ha are lost in the period before the sward reaches the optimum LAI and 16 t DM/ha are estimated to be lost when LAI is above the optimum. The production in an average year, therefore, was estimated to be about 8 t DM/ha, which is similar to normal sward yields obtained under agricultural conditions. Black assumed a 3.9% conversion of photosynthetically-active radiation throughout a normal growing season. According to Cocks (1974), higher rates of conversion (4.5%) are possible in southern Australia where there is adequate provision of nutrients. After only five

months of growth, total yield up to 16.4 t DM/ha was obtained by Cocks. This study indicated that the potential yields of subterranean clover swards would be reduced at the low leaf area index values associated with efficient pasture utilization.

The early vegetative growth of a pasture sward is dependent on the magnitude of embryo weight per unit area. At farm level where grazing systems occur, the relative importance of possible losses of productivity produced by a leaf area index above the optimum, is minor compared with those that occur before that time. It is there when management of factors, such as seed size and plant density have important practical implications to ensure an early production of forage, to meet the requirements of grazing livestock. Seed size and plant density positively influenced the rate of growth soon after seedling emergence but also, with advanced stages of plant development, there is an early onset of inter-plant competition. For this reason, it is strongly recommended that grazing management practices be planned to maximize forage production rather to maximize LAI.

2.4 Some factors influencing the survival of pasture seeds ingested by animals

2.4.1 Implications of seed-dispersal by livestock

Seeds produced in grazed pastures are distributed by several means, but birds (Krach 1959) and domesticated livestock (Heady 1954; Lehrer and Tisdale 1956; Yamada and Kawaguchi 1971, 1972) are the main agents carrying seed over long distances in a short period of time. The use of domestic animals for disseminating seed of pasture species has been discussed in an early study by Burton and Andrews (1948), and has been noted by several authors (e.g. Hessberg 1933; Suckling 1950; Yamada and Kawaguchi 1971; Mitamura *et al.* 1982; Simao Neto *et al.* 1987) as an important means of colonizing large areas where normal cultivation practices are impossible or are not recommended, or for recolonization of previously-sown areas where the legume component of the pasture has been partially or completely lost (Suckling 1950; Carter

1981; Mitamura *et al.* 1982; Bishop *et al.* 1985). However, where legumes are well represented in the pasture swards, dispersion of seeds by animals does not provide any advantage (Watkin and Clements 1978).

Herbivorous livestock grazing pasture species during or after the stage of plant development at which seed is set may ingest a significant amount of seed (Dore and Raymond 1942; Suckling 1950; Carter 1980; Carter 1981). The proportion of ingested seed that survives the passage through the digestive tract of animals is highly variable and is dependent on the plant species involved, seed characteristcs (seed size, shape, hard-seededness and dormancy), animal species, the amount of seed consumed and the digestibility of the associated diet (Simao Neto *et al.* 1987).

Daily output of seed voided in faeces by grazing animals sometimes can be very high. Sheep can excrete about 3,000 seeds/head/day (Suckling 1950) and cattle about 300,000 seeds/head/day (Jones and Bunch 1977), when grazed on white clover (*Trifolium repens*) pasture swards. Similarly, a concentration of about 100,000 seeds/kg of dung of cattle steers has been estimated for *T. repens* (Jones 1982). Carter (1981) working with Mediterranean medic cultivars (*Medicago truncatula)* showed a daily average output of viable seed equivalent to 6,155 and 3,251 seeds/sheep obtained during the first and the eighth week of autumn grazing respectively. In subterranean clover, de Koning (1990) found that up to 1,552 seeds/sheep/day were voided in faeces when a dry pasture was grazed during the summer-autumn period. In tropical pastures, concentrations of up to 38 seeds/g of dry faeces in cattle has been recorded for *Stylosanthes humilis* (Playne 1969), 8,000 seeds/kg of faeces in cattle for *S. scabra* (Kerridge *et al.* 1987), 50 seeds/kg of faeces in heifers for *Macroptilium atropurpureum* (Jones and Bunch 1988) and 15 seeds/kg of faeces in steers for *Desmodium intortum* (Jones 1989). The low concentration of seed in the later two species was suggested to be caused by the growth habit of plants and the pattern of seeding that did not allow the animals to ingest the ripened seeds.

According to Simao Neto *et al.* (1987), in the situation of a programmed dissemination of seed, the animals should graze the selected area for colonization or recolonization 3 to 4 days after the desirable seed has been ingested by the animals. On the other hand, to avoid further contamination of grazing areas with undesirable species the animals should not be relocated on to a new area until at least seven days after they leave the contaminated field. The probability of success with this type of management may be enhanced if the legume seed used is small and has a high level of hard-seededness. It has been experimentally demonstrated (e.g. Frankling and Powning 1942) that sheep are unable to digest efficiently the small seeds of *Trifolium glomeratum*, or the hard seed (e.g. Suckling 1950) of *T. repens*. Therefore, a larger amount of undigested seed of these characteristics is expected to be recovered in faeces. The magnitude of digestion varies according to the animal species. Sheep normally digest seeds more completely than cattle (Harmon and Keim 1934; Harvey 1981; Simao Neto *et al.* 1987). Hard legume seeds are also more resistant to the conditions in moist faeces after dropping (Simao Neto and Jones 1986).

These studies suggest that the dissemination of seeds of pasture species by livestock may have important practical implications in situations where current sowing practices are not physically or economically possible. However, further biological information, complemented by economic analyses (cost/benefit) are required before deciding to adopt management practices likes this.

2.4.2 Significance of seed as fodder

Perhaps the most important trait for the long-term persistence of a legume species in a pasture is the ability to become adapted to defoliation by grazing animals. A complex interaction between the characters of a plant, and the presence of environmental and management factors, are expected to determine the degree of success or failure of a particular species or group of species in a pasture sward (Reed *et al.* 1989).

Several examples show the effects of grazing on the appearance, dominance and disappearance of legume pasture species. In Turkey, the increased appearance of subterranean clover (*Trifolium subterraneum* var. *subterraneum)* on heavily grazed pasture swards established on alkaline soils compared to (*T. subterraneum* var. *brachycalicinum*) has been reported by Francis *et al.* (1975). Observations on the environment and the ecology of some Mediterranean annual legumes in southern Italy by Gladstones (1973) indicated that under heavy grazing pressure, cupped clover (*T. cherleri*) became a naturalized common species. In contrast, rose clover (*T. hirtum*) had a low frequency in the pasture.

In Australia, the increase of volunteer clovers, such as *T. dubium*, *T. cernuum*, *T. glomeratum* (cluster clover) and *T. tomentosum* under heavy grazing pressure has been noted (e.g. Carter 1965; Carter and Day 1970; Morley *et al.* 1969; Cameron and Cannon 1970; Reed and Cocks 1982; Carter and Lake 1985; de Koning 1990). In contrast, subterranean clover seems to be more affected than favoured by higher grazing pressures (Reed and Cocks 1982). There is ample evidence to suggest a direct effect of grazing of plant reproductive structures and the persistence of legume species in pasture swards.

The ability of a seed to escape from grazing represents a desirable trait in a legume pasture species because this favours its persistence under grazing. Legume species are provided with different mechanisms that permit them to protect the seed from grazing animals. A good example is the burr burial (grazing-escape) mechanism of subterranean clover which is the most important specialization (Katznelson and Morley 1965). It also protects the seed from irregular patterns of rainfall. Seeds that germinate after inadequate light rains are more likely to survive if the seed is slightly covered by soil (Hagon 1974). However, the burr-burial capacity varies to some extent in the different intraspecific taxa of *T. subterraneum* (Katznelson and Morley 1965) as well as between cultivars (Francis *et al.* 1972). Under hard-setting soil conditions, where burr burial is almost prevented, grazing during the dry pasture stage can greatly reduce seed survival (de Koning and Carter 1989; de Koning 1990). Another adaptation is the extreme hairiness

of surface-laid burrs of some annual clovers (e.g. *T. batmanicum*, *T. globosum* and *T. pauciflorum*) which may reduce their acceptability to grazing animals (Bolland 1987b). In annual medics, pod spininess (e.g. *Medicago minima* and *M. polymorpha*), acts as an efficient seed dispersal agent because it favours the adherence of pods to the sheep fleece and helps the establishment of medic seedlings by anchoring the pods to the soil and thus discourages grazing animals (Crawford *et al.* 1989). Despite that, medic pods (*M. truncatula*) may be severely depleted by grazing sheep during the summer-autumn period especially on hard-setting soils (Carter 1981). In some *Trifolium* spp., seed is set in heads mainly produced in the upper part of the canopy and accessible to grazing animals. This is the case for white clover (*T. repens*), where the accessible ripe and unripe heads are heavily consumed without discrimination by sheep (Suckling 1950). The growth habit and the seeding pattern of *Macroptilium atropurpureum* (Jones and Bunch 1988) and *Desmodium intortum* (Jones 1989) are possible causes of lower ingestion of seeds of tropical pasture species by grazing cattle. Both factors restrict the free accessibility of seed to the animals.

In Mediterranean-type climates, following an effective rain that normally occurs in autumn, germination, emergence, establishment and the consequent growth of annual self-regenerating legume pasture species is initiated. A gradual increase in dry matter is observed during the winter. Even in farm production systems where cropping activities are infrequent, the body weight gains and wool production of sheep are inadequate in this period. The main cause of this is insufficient levels of pasture, but there is also evidence that the quality of forage may also be limiting. Increases in the growth rate of the pasture swards is observed during spring until seed is set and plant maturity is reached. At this time the amount of dry pasture residues frequently exceeds the requirements of livestock, so management practices aimed at improving pasture composition assume critical importance. In summer, the main limit to animal production is the quality of the dry pasture residues and it is the legume component of the pasture which may be useful in improving the quality of summer grazing. In the autumn, both the low feed availability and the poor nutritive value of dry pasture residues are the main

factors causing poor animal performance (Carter and Day 1970; McKeown and Smith 1970; Purser 1981; Dunlop *et al.* 1984; Purser *et al.* 1987).

There is ample evidence from Australian studies indicating the preference of sheep for vegetative plant parts (e.g. Arnold 1964; Arnold *et al.* 1966; Leigh and Holgate 1978). However, apart from the work of Carter (1981), Jones and Bunch (1988) and de Koning (1990) little information is available that relates the selection and ingestion of reproductive structures like medic pods and clover burrs by sheep to subsequent seed survival under grazing.

Selective grazing of plant species or plant parts is determined mainly by the relative acceptability and accessibility of plant material (Curll and Jones 1989). Grazing sheep select diet according to how easily the forage can be harvested and eaten (Kenney and Black 1984). The chemical and physical characteristics of forage on offer (Kenney and Black 1984), the quantity available, and the spatial distribution of the pasture sward (Black and Kenney 1984) also play an important role in determining how easily the forage can be grazed.

Expression of preferences depends on pasture availability and the heterogeneity of the material on offer. Sheep tend to select first the plant parts that have the higher levels of digestibility (Arnold 1981). There is likely to be a strong relationship between digestibility and accessibility of forage (Black 1987). Consequently, the diet selected by grazing sheep has a higher average nutritive value than the plant material on offer (Meyer *et al.* 1957; Arnold 1960; Arnold 1964; Hamilton *et al.* 1973).

Grazing animals ingest seed with the vegetative parts of forage legume plants (e.g. Suckling 1952 working with temperate species; Carter 1981, Carter and Lake 1985, and de Koning and Carter 1989 with Mediterranean species, and Jones and Bunch 1977, and Jones 1989 with tropical species). In Australia, much of the research related to seed intake by grazing animals has been made on a nutritional basis by using stabled or penned animals, but few attempts have been made under grazing conditions.

Vercoe and Pearce (1960), working with Corriedale wethers fed *ad libitum* with barrel medic pods (*Medicago tribuloides* syn. M. *truncatula*), revealed that sheep ingested an average of 78,340 seeds/head/day. They suggested that sheep grazing under favourable conditions might be able to substantially increase their seed intake. Carter (1981) showed that Merino wethers stocked at a density of about 55.5 sheep/ha on dry pasture residues of barrel medic (*Medicago truncatula)* and burr medic (*M. polymorpha*), ingested about 82,869 seeds/head/day.

In subterranean clover, Wilson and Hindley (1968), working with oesophageally-fistulated sheep, showed that burrs comprised about 10.3% of the diet when sheep were grazed on a mixed ryegrass-subterranean clover dry pasture containing about 500 kg/ha of burrs. Similarly, Hall (1984) using oesophageally-fistulated sheep grazing in a lucerne-subterranean clover pasture, reported higher constributions of burrs to the diet in the summer of 1975 (7.5%) or during the winter of 1976 (15.7%), when a severe drought forced the animals to graze only available dry forage. With the same species, de Koning and Carter (1984) found intakes of about 42,868 seeds/head/day when Merino wethers equivalent to about 30.3 sheep/ha grazed dry pasture residues of subterranean clover during the summer-autumn period.

Playne (1974) studied the contribution of the seed of the legume *Stylosanthes humilis* to the nutrition of cattle grazing mature tropical pastures. The intake of intact pods was up to 500 g/head/day in May just after the seed was formed. However, when the seed was ripened from the mature plants (May-June), the intake of pods markedly declined to a range between 25 and 100 g/head/day for the remainder of the dry season. A further but infrequent increase in the intake of pods (250 g/head/day) may occur later during spring (October-November) as a consequence of a lower availability and nutritive value of pasture companion species, irregular patterns of rainfall and grazing pressures.

A further study of the dietary selection and liveweight performance of oesophageally-fistulated steers on a pasture comprising the legume *Stylosanthes hamata* growing with native grasses was reported by Gardener (1980). Animal preferences for dry legume

residues containing inflorescences and seeds started in June after the wet season ended. At that time (e.g. June, 1976), about 45% of reproductive organs was recorded in the diet of cattle and this was coincident with the year in which the legume was importantly represented in the pasture (4,435 kg DM/ha and 435 kg/ha of seeds).

The legume component of annual pastures is important not only for its feed value when it is actively growing, normally between autumn and spring, but also to improve the quality of forage on offer during the summer-autumn dry period. Dry tops including seeds enclosed in burrs (e.g. subterranean clover), pods (e.g. annual medics), heads (e.g. balansa clover) etc. may become the potentially-largest source of nutrients for the grazing animals before new germination occurs (Wilson and Hindley 1968; Malcolm 1969; Siebert and Kennedy 1972).

Body weight changes and wool growth obtained through sheep grazing experiments carried out in a barrel medic (*Medicago truncatula*) based pasture, indicated that pods, often available in excess of 1,000 kg/ha, did not provide the required amounts of nutrients to allow the maintenance of body weight (Brownlee 1973). In contrast, Cocks (1988) revealed that grazing of dry medic pasture residues in summer resulted in significant gains in body weight by ewes. In fact when the pods (*M. rigidula*, *M. rotata*, *M. polymorpha* and *M. truncatula*) were grazed out, the sheep started to loose liveweight. It was suggested that the pod fraction of the dry pasture residues was the most nutritious component of the forage on offer. In subterranean clover, Squires (1978) recorded the changes in liveweight of Merino wethers related to a estimated intake of subterranean clover seed over a 60-day period on irrigated clover pastures at Deniliquin (N.S.W.). All the animals lost weight over the grazing period but the amount varied between 2.3 and 10.2 kg/head. However, the least liveweight loss was associated with a clover seed intake of 750 g/day/head. Some pastures gave unsatisfactory liveweight gains when forage was mature and dry mainly because of poor energy and protein contents. Summer periods of imperfect nutrition did not have long-term effects on sheep performance (Donald and Allden 1959). However, more critical responses

could be expected when the processes of weathering of dry pasture residues occurred (Guilbert and Mead 1931).

An early study on the chemical composition of pods, burrs and seeds of certain species of annual medics (*Medicago spp.*), subterranean clover (*Trifolium subterraneum*) and cluster clover (*T. glomeratum*) indicated large differences between species (Franklin and Powning 1942). For example, the crude protein level of medic species for whole pods ranged from 13.1 % (*M. scutellata*) to about 24.8 % (*M. laciniata*), and for pure seeds from 27.7 % (*M. orbicularis*) to about 50.0 % (*M. laciniata*). In subterranean clover, lower crude protein contents ranging from 18.2% to 22.6% in whole burrs and from 38.7% to 41.6% in pure seeds were obtained. A protein content of 37.7% in the seed was estimated for cluster clover. The apparent digestibility of the crude protein of cluster clover seed was 39.7% for uncrushed seed and 89.3% for crushed seed. The results indicated that sheep were unable to digest efficiently the small seeds of cluster clover when they were incorporated as uncrushed seeds in the normal ration.

In sheep nutrition studies, using digestibility cages, Vercoe and Pearce (1960) found that at a mean weekly intake of 6.6 kg/head of whole pods of barrel medic (*M. tribuloides*), the mean digestibility of dry matter (30%) and organic matter (32%) was relatively low. However, the mean apparent digestibility of crude protein (67.5 %) was relatively high, and it was suggested that although the mean crude protein content of the pods was 18.2%, it was probably used inefficiently. Medic seeds are a valuable protein supplement (Franklin *et al.* 1964). Seed of *Medicago* spp., containing 38.5% (DM basis) crude protein, were comparable to linseed meal, as a supplement to low-protein roughage diets for Merino sheep.

A further study, reported by Denney *et al.* (1978) and Denney *et al.* (1979), was conducted to determine the digestion and utilization of barrel medic (*M. truncatula*) pods fed to penned sheep. They revealed that the organic matter of pods contained 23.8% crude protein, 5.2% long-chain fatty acids (LCFA) and 77.5% acid-detergent fibre including 19.9% of lignin. The digestibility of whole pods in the entire tract was 63.3%

for nitrogen, 79.3% for LCFA, 9.7% for fibre and 24.3% for organic matter. However, an important net loss of protein (40%) occurred as a consequence of high levels of rumen ammonia (52 mg N/100 ml of ruminal liquor). Although the pods provided only small quantities of aminoacids and LCFA, mainly derived from the digestion of seed, the nutrients were equivalent to about 20 and 15 g/100 g of digestible organic matter respectively. They concluded that the digestibility of pods was low as a consequence of the poor digestibility of the fibre that clearly limits their nutritional value.

The nutritive value (DM basis) of seeds and burrs of subterranean clover (cv. Yarloop and Woogenellup) have been evaluated. Both had similar values of nitrogen (4.2 and 4.3%), and different levels of acid-detergent-extractable fibre (35.0 and 28.6%) and lignin (11.9 and 8.9%) respectively. The estimated *in vitro* dry matter digestibilities were 46.8% for cultivar Yarloop and 54.7% for cultivar Woogenellup (Hume *et al.* 1968).

Different diets based on ryegrass and subterranean clover mature plant materials offered to penned Merino sheep, indicated apparent digestibilities of organic matter and nitrogen of burrs equivalent to 46.3% and 61.1% respectively. The results of this study concluded that pods of pasture legumes can be important as protein supplements in the summer nutrition of sheep. However, the main factor limiting further intake by sheep was the low organic matter digestibility of the supplements (Wilson and Hindley 1968).

Also, valuable studies have been carried out on tropical legume pastures. Playne (1969) studied the nutritional value of intact pods of Townsville stylo (*Stylosanthes humilis*). Seed enclosed in pods contained higher levels of nitrogen, phosphorus, sulphur and calcium when compared with published values obtained for tropical grasses during the dry season. Whilst the Townsville stylo seed showed higher levels of nitrogen, phosphorus and sulphur than the corresponding pod, the pod was higher in calcium. The pod with enclosed seeds provided sufficient nitrogen to sheep, but the level of phosphorus was inadequate. Adequate and even excessive availability of nitrogen was

reflected by its high level of apparent digestibility ranging from 73.4% to 81.5%, as determined by the positive nitrogen balance and the high blood urea-nitrogen content.

Playne *et al.* (1972) examined the digestion of seed and pods of *Stylosanthes humilis* by immersing the corresponding materials in the rumen fluid (Terylene bags) of cannulated steers. The rates of disappearance of dry matter, nitrogen, phosphorus, sulphur and calcium were estimated. Cell contents constituted 80% of the seed dry matter but only 20% of the pod dry matter. The rate of digestion of seed was very fast within 48 h of immersion in the rumen (77%, DM basis) compared to the pods (14%, DM basis), mainly due to the almost complete digestion of the cell fraction. In the pod, the concentration of nitrogen, phosphorus and sulphur was lower than in the seed, but the concentration of calcium was higher.

A further study by Playne (1974) examined the nutritional value of intact pods of *Stylosanthes humilis* and revealed that seed provided useful levels of energy, protein (48%), sulphur (0.34%) and phosphorus (0.55%) as well as lower levels of fibre. On the other hand, the pod which was 62% seed (DM, basis), was high in fibre (80%) and calcium (1.7%) content. The dry matter digestibility of whole pods ranged from 55% to 68%.

From these studies, it is evident that in order to meet their nutritional requirements, ruminants select, according to the seed/chaff ratio, the reproductive plant structures containing seed. This represents the pasture component with the highest relative nutritive value after plant maturity has been reached. However, pasture legume seeds are normally available for grazing when enclosed in burrs, pods, heads etc. that reduce the nutritive value of whole plant material. This explains the low or negative response in animal performance obtained with this type of forage resource.

2.4.3 Digestion of seed by livestock

The differences in digestibility of seeds ingested by grazing animals has a significant ecological effect on the relative species composition of pastures (Carter 1968). The seed of pasture species subjected to grazing can be classified in the following categories:

1. Unpalatable seed that is avoided by animals.
2. Palatable seed that avoids ingestion by animals.
3. Seed that is resistant to digestion and survives passage through the digestive tract of animals.
4. Seed that is partially digested and may survive depending of the magnitude of damage.
5. Seed that is completely digested (Ewens 1976).

The initial mastication and rumination, the first mechanism of the digestion process, plays an important role in the degradation of ingested feed by ruminants. Continuous regurgitation of ingested material and grinding by re-mastication are required to accelerate the physical degradation of feed particles and expose fresh surfaces for digestion. Rumen-microflora also help this process by digesting to a lower particle size the ground material, as well as that which escaped uncrushed during chewing (Weston and Hogan 1986). The passage of ingested diet from the rumen to the lower parts of the digestive tract is determined by particle size. Poppi *et al.* (1985) suggested that a particle size of 1.18 mm sieve may be used to divide the rumen contents of both sheep and cattle into particles with high and low relative resistance to passage from the rumen through the reticulo-omasal orifice.

Variable levels of recovery of pasture legume seeds from faeces in relation to the amount of seed fed to animals have been reported by several authors in the literature. According to Carter (1968a), Jones and Simao Neto (1987) and Simao Neto et al. (1987) such differences may have been caused by factors such as plant species, seed characteristics (size, shape, hard-seededness and dormancy), abundance of seed in the diet, quantity and quality of forage on offer, animal species and also the different experimental procedures used. For example, Yamada *et al.* (1972) recovered 0.1% of *Medicago*

sativa seeds in cattle faeces, but as high as 55.5% was recovered by Özer (1979) in sheep faeces. In contrast, Lennartz (1957) recovered 58.0% of *Trifolium repens* in cattle faeces and Özer (1979) only 24.5% in sheep faeces.

Vercoe and Pearce (1960) recovered 1.9% of *Medicago tribuloides* from sheep, while Carter (1980) and Carter *et al.* (1988a), reported a wide variation in percentages of ingested annual medic seeds passed in sheep faeces from 0.0% for *M. murex* to 19.8% for *M. polymorpha.* Thomson *et al.* (1987) tested the survival of annual medic seeds fed to sheep using different methods. Survival values as low as 2.2% for *M. turbinata* using the equal pod method and as high as 27.8% for *M. rotata* using the equal seed method were reported.

Survival of subterranean clover *(Trifolium subterraneum)* seed in sheep, goats and cattle was 6.4% (Wilson and Hindley 1968), 8.0% (Yamada and Kawaguchi 1971) and 0.8% (Yamada and Kawaguchi 1972) respectively. A comparison of the survival of seed from five cultivars of subterranean clover fed to sheep showed a range of recovery from 2.9% for cultivar Nungarin to 5.0% for cultivar Dalkeith (de Koning 1990). However, when a mixture of the same five cultivars previously sown in equal proportions by weight was grazed as a dry pasture during the summer-autumn period only 1.5% of seed was recovered in a viable state (de Koning and Carter 1989; de Koning 1990).

Small-seeded Mediterranean clover species have been reported to have higher percentages of survival than these species reported before. Franklin and Powning (1942) by determining the apparent digestion coefficients for the dry matter and the crude protein for a mixed lucerne (*Medicago sativa*) chaff and *T. glomeratum* seeds ration, recovered 56.7% of undigested seeds in sheep faeces. Other recoveries of small-seeded clover species from pen-feeding experiments by Thomson *et al.* (1987) showed, 23.1% for *T. stellatum*, 36.3% for *T. tomentosum* and 60.0% for *T. campestre*, and by Carter *et al.* (1988a) 37.9% for *T. balansae* and 34.4% for hard-seeded lines of *T. resupinatum.*

In tropical pasture legumes, Playne (1969) reported an average percentage of undigested seed passed through the digestive tract of sheep of 11.6% for *Stylosanthes humilis*, when pods were fed using different proportions of seed in the ration. A more recent study was carried out by Simao Neto *et al.* (1987). They compared, within the legume group of species tested, the survival of two sub-tropical species (*Neonotonia wightii* and *T. semipilosum*) and two tropical species (*S. hamata* and *S. scabra*) seeds ingested by cattle, sheep and goats. From cattle, the total recovery of seed was estimated to be 61.6%, 45.2%, 36.1% and 48.3% respectively, while from sheep, values of 11.3%, 24.3%, 3.5% and 9.4% were obtained respectively. The percentage of undigested seed recovered from goats faeces was intermediate and equivalent to 25.5%, 40.6%, 6.8% and 13.4% respectively.

According to Özer (1979), seeds ingested by animals are importantly damaged during the initial mastication and rumination. However, it seems that rumination represents the main factor of survival (Yamada and Kawaguchi 1971; Piggin 1978; Simao Neto and Jones 1987). Evidence suggests that when a seed is ingested it is usually swallowed with very little initial mastication and is then stored temporarily in the rumen.

If seed is present in the diet, chewing mechanisms would tend to differentiate between seed and fruit structures on the basis of size, hardness, etc. Jones and Carter (1989) explained the low recovery of medic seeds ingested by sheep as a consequence of the chewing effect required to physically degrade the pods so that they could achieve the proper particle size to leave the rumen. Domestic ruminants differ in the extent to which they chew and digest feed. Information provided by Harmon and Keim (1934), Waldo (1973), Harvey (1981) and Simao Neto *et al.* (1987) suggests that cattle digest less seed than sheep or goats.

While the behaviour of the grazing animal is important, the characteristics of the seed also play a significant role in the survival of seed. Early work carried out by Franklin and Powning (1942) showed that sheep were unable to efficiently digest small seeds of *Trifolium glomeratum*, suggesting that seed size should not be overlooked when

considering the establishment of legume pasture species. A further study by Smith (1963), working with *Medicago sativa*, *M. tribuloides* and three cultivars of *T. subterraneum*, found that the relative loss in mean dry weight of seeds after ingestion by sheep seemed to be related to seed size. Larger seeds were digested more than smaller seeds. Later work by Jenkins (1978) showed that larger seeds of gama medic (*M. rugosa*) and snail medic (*M. scutellata*) had lower rates of seed recovery in sheep faeces than smaller-seeded species such as *M. truncatula*, *M. littoralis* and *M. tornata*. Small-seeded species such as *T. glomeratum* and *Poa pratensis* also resisted the effects of digestion by sheep better than larger-seeded species, such as *T. subterraneum* and *Lolium rigidum*. (McArthur 1968).

Dore and Raymond (1942), Lennartz (1957), Piggin (1978) and Özer (1979) all found large variation in the rate and time of excretion for seeds of various plant species. They suggested that the ability of a seed to survive passage through the digestive tract was related to the characteristics of seed, such as size and shape. Total recovery of tropical legume seed from sheep and goats was greatly influenced by the length of seed. Shorter seeds had higher rates of survival than larger seeds (Simao Neto *et al.* (1987). The same trend with respect to seed size was found by Thomson *et al.* (1987). They reported that 80% of the variability in survival of legume seeds ingested by sheep could be explained by the size of seed.

Although the rate of passage is affected by animal species, and the weight and the age of animals (Simao Neto *et al.* 1987), as well as the quality of the associated diet (Jones and Simao Neto 1987), differences in plant species may also be accounted for by the physical form and size of the seeds. Robards (1971) suggested that increasing the size or decreasing the specific gravity of particles, negatively affected the rate of passage from the reticulo-rumen. Fast passage through the rumen may, however, lead to seed being retained in the folds of the omasum (Suckling 1950). This suggests that small seeds could be more easily held than large seeds. The advantages of small seeds has been confirmed by Jones and Simao Neto (1987), using carpet grass (*Axonopus affinis*) and

Safari cultivar (*T. semipilosum*) which have higher survival rates than other large-seeded species tested.

Another important seed characteristic associated with survival is the level of hard-seededness. Suckling (1950, 1952) studied the passage of white clover (*T. repens*) seeds through the body of sheep and its effect on their capacity to germinate. He found that when the animals were fed with hand-harvested, unscarified seed containing 98% hard seed, survival equivalent to 25.3% was obtained compared with commercial seed containing 2.0% hard seed. This indicates that only hard seed is able to survive the digestion process without major changes in viability. Similar trends were reported previously by Atkeson *et al.* (1934) and later by Lennartz (1957).

Few attempts have been made to separate the influence of chewing and ruminal digestion on seed survival. Direct exposure of seeds to the rumen allows examination of the effect of ruminal digestion on legume pasture seeds. Yamada and Kawaguchi (1971) revealed that soft clover seeds (*T. repens* cv. Ladino and *T. subterraneum*) were highly susceptible to digestion when they were soaked in the rumen of goats. Hard seed was not digested and did not show significant changes in viability. Similar observations on the resistance of hard seed and the susceptibility of soft seed to digestion in the rumen of cattle were made by Gardener *et al.* (1983), and Simao Neto and Jones (1987) working with tropical pasture legume species by using the *in sacco* technique. By comparing these *in sacco* results and those obtained in pen-feeding experiments (Jones and Simao Neto 1987; Simao Neto *et al.* 1987) it is suggested that much of the damage to ingested seed will occur in the rumen of animals. However, seeds also lose viability if tested under simulated abomasal conditions, indicating that some seed is damaged at that stage of digestion. Furthermore, Simao Neto and Jones (1986) evidenced seed mortality during retention in faeces indicating that some additional damage occurs during passage of seed through the intestines.

The longer a seed remains in the digestive tract of ruminant animals the greater the probability that the micro-flora and alimentary secretions will act on the seed and reduce

its viability. The extent of digestion and the rate of passage through the digestive tract varies between animals and plant species. However, usually the highest recovery of seeds occurs between 48 and 72 h after ingestion. Then, it decreases rapidly and substantially with negligible recovery by 96 h (Atkeson *et al.* 1934; Burton and Andrews 1948; Yamada and Kawaguchi 1972; Yamada *et al.* 1972; Özer 1979; Simao Neto *et al.* 1987).

Seeds lose viability with increasing time spent in the digestive tract of cattle. For example, Atkeson *et al.* (1934) observed a reduction in viability of 33% for rough pigweed when the time of retention was extended from 47 to 97 h. Similarly, Harmon and Keim (1934) showed that there were only 50% as many weed seeds recovered during the 48-80 hours period after feeding the seeds to cattle, as there were during the first 48 h. McArthur (1968) testing the changes in viability of *L. rigidum*, *Poa pratensis* and *T. glomeratum* seeds fed to penned sheep, noted that there was a decrease in viability with increasing time spent in the gut for the non-legume species. In contrast, no change in germinability was observed for *T. glomeratum*.

A similar pattern to that previously reported was observed by Simao Neto and Jones (1987) when tropical pasture grass and legume seeds were soaked in the rumen of cattle for 24 to 96 h. The viability of seeds was reduced with longer retention periods in the rumen, especially for grass seeds and particularly for signal grass (*Brachiaria decumbens*) seeds which all were dead after 96 h of immersion. These results correlated well with those obtained by Yamada and Kawaguchi (1971) for clover seeds incubated in the rumen of goats.

After passing through the digestive tract of ruminants, legume seeds normally have low levels of germinability but high viability (readily germinable seed + potentially germinable seed), because a high percentage of recovered seeds remain impermeable (i.e. in hard condition) (Simao Neto 1985). Suckling (1950, 1952), Smith (1963), Yamada and Kawaguchi (1972) and Simao Neto *et al.* (1987) revealed that the proportion of hard seed in seed samples prior to ingestion by the animals is lower than in

samples of seed recovered following defaecation, suggesting that soft seeds are more susceptible than hard seeds to the digestion process.

The effects of digestion are reflected in the germinability of seed passed through the digestive tract of ruminant animals. Wilson and Hindley (1968), reported similar levels of germination for subterranean clover (*T. subterraneum*) seeds recovered from sheep faeces to that prior to grazing. However, results obtained by de Koning (1990) in a sheep grazing experiment performed during the summer-autumn period, as well as in sheep pen-feeding studies, showed that the percentage germination of subterranean clover seeds was higher for the seed recovered in faeces than for the original seed on offer for grazing or prior to feeding. Similar findings has been made in other studies by Ewens (1976), Özer (1979), Harvey (1981) and Simao Neto *et al.* (1987). However, the last authors suggested that this trend must be interpreted with care as the seed samples before and after will be different. The higher germination percentage obtained after the seed was recovered, was hypothesized to be due to a breakdown of hard-seededness during passage. Therefore, the proportion of hard seed voided in faeces was lower than before feeding.

Conversely, the percentage germination of some legume species, such as *Neonotonia wightii* and *T. semipilosum* can decline after passage through the digestive tract (Simao Neto *et al.* 1987). It was assumed (Simao Neto and Jones 1987) that most of the soft seed present in the samples prior to ingestion was destroyed in the digestive tract. Consequently, increased levels of hard-seededness and decreased levels of germinability would be expected.

Despite the variation in seed survival caused by using different plant species, animal species, proportion of seed in the diet etc. reported in previous studies, as well as different experimental procedures, it is clear that seed characteristics such as size and hard-seededness play an important role in seed survival. If the chewing effect was avoided, it is apparent that only a small proportion of soft seed is able to survive the

passage through the digestive tract of sheep. In contrast, most of the hard seed was able to retain its viability after passage, especially in small-seeded pasture legume species.

2.4.4 Survival of seed in faeces

Excretions of grazing animals can affect the legume content of a pasture sward by altering the concentration of some nutrients in the soil, by scorching or burning plants, by influencing the grazing behaviour of animals and by disseminating seed voided in faeces (Curll and Jones 1989). According to Marsh and Campling (1970), the processes of change and decomposition of faeces are complex and begin as soon the faecal material is deposited. Small animals, many groups of bacteria and climatic conditions affect the breakdown of dung.

Seedlings of many plant species have been observed emerging from faecal materials voided by grazing animals (e.g. Özer 1979). In pasture legume species, seedlings of *Trifolium repens* (Salzmann 1940; Suckling 1950, 1952), *T. subterraneum* (de Koning and Carter 1989; de Koning 1990), *T. hirtum* (Jones and Carrol 1953), *T. glomeratum* (Carter and Lake 1985), *Medicago truncatula* (Carter 1981) and *Stylosanthes guianensis* (Foster 1961) have been reported emerging from sheep and cattle droppings.

Death of soft legume seeds following prolonged times of storage in faeces has been observed by Suckling (1950), Lennartz (1957), Özer (1979), and Simao Neto and Jones (1986). In contrast, legume seeds that remain hard after passage are resistant to storage in faeces (Suckling 1950; Lennartz 1957; Simao Neto and Jones 1986).

Seed enclosed in sheep or goats faeces, compared with cattle dung, are expected to better survive the process of dung fermentation because the smaller pellets usually have a lower moisture content (Simao Neto and Jones 1986). Despite this, de Koning and Carter (1989) noted that seedlings emerging from soft seed of subterranean clover (*T. subterraneum*) voided in sheep pellets were unlikely to survive the hot and dry summer conditions of southern Australia. The percentage of emergence of seedlings from

droppings under natural conditions was only 0.13% of the seed ingested, suggesting that most of damage at that stage was due to desiccation of any viable soft seed or seedlings deposited in faeces. These results were not in agreement with those reported by Simao Neto *et al.* (1987), who found that seedling emergence from faecal pellets was similar to germination values obtained by testing seed enclosed in washed-out samples of faeces. However, Simao Neto *et al.* used different legume species and kept the faeces moist.

It appears that the survival of pasture legume seeds in faeces after defaecation and the subsequent ability of seed to germinate, emerge and became established has not yet been examined in adequate detail.

2.4.5 Harvest of seed by ants

Ants feed mainly on live insects, nests of other social insects, vegetative parts of plants, dead animal matter and seeds (Carrol and Janzen 1973). In some instances, seed predators are also the main dispersal agent of the seeds upon which they feed (Janzen 1971). Briese (1974) describing the trophic structure of a natural ant community suggested that the seed stored by harvester ants and the energy path represent an important link in the system. All the ant species involved in the community show a similar foraging behaviour, collecting the seed mainly from the ground surface by the worker ants, which travel along trails to recognized foraging areas. Then, the seeds are brought back to the nest either husked or unhusked and stored in underground granaries. According to Janzen (1971) and Baker (1972) seeds are an important food resource because they can be stored in the nest in reserve for a long period, and also have a high nutrient content of lipid, protein and starch.

Considering the ant species as a whole, there is a general trend of reduced harvesting activity over winter with increased activity with warmer weather. The seed-harvesting species seem to be more sensitive to climatic extremes and they show the most irregular patterns of foraging. Biotic factors, such as the presence of larvae, seed stores and

availability of food, are also important factors in the regulation of ant foraging activities (Briese 1974). Ants utilize many species of seed. Field studies carried out under natural conditions show that harvester ants do not significantly affect the total supply of seed. The harvesting activities of ants, therefore have little influence on the absolute abundance and distribution of plant species (Tevis 1958; Whitford 1973; Briese 1974; Hocking 1975).

Smith (1975), in an attempt to classify the selective forces affecting seeds and their predators, describes five general ways in which seed predators can interact with seeds. The first three are based on predator discrimination between size as follows:

(1) Larger seeds are taken first. This option could often increase the harvesting rate of an animal that needed more than one seed, or increase the food supply of an animal that only fed on one seed in a life time.

(2) Smaller seeds are taken first. This choice might be an advantage for an insect that needed less than the contents of one seed or preferred thinner seed coats to chew through.

(3) It can have no discrimination other than to select the correct species of plants irrespective of the nature of the individual seed. Size discrimination favours plants with smaller and more numerous seeds. In two other foraging patterns, seed size is not a determinant of the action of harvesters.

(4) The harvester selects only the correct species of plants, but a specific characteristic of the seed other than seed size will determine the success (eg. thickness or texture of seed coat)

(5) The predator selects which seeds to attack only on the basis of some characteristic other than seed size.

Briese (1974) stated that the basic food strategy of the seed harvester ants is to maximize harvesting activities when the periodic pulses of seed production occur, through a rapid accumulation of seeds in their granary nests. This pattern allows them to ensure available food during periods of reduced or nil production. All the ant species involved in this study, show some degree of trail forming, which seems to be associated with an efficient harvesting capacity of seed which occurs in dense patches, such as newly-fallen seed. All ant species, appear to forage in a manner which optimizes energy expenditure in seed collection that result in narrow food niches and high overlaps between species.

This supports the proposition that ants collect seed for storage largely on the basis of availability (Hocking 1975).

The optimal diet is defined as one which gives the greatest energy yield per unit time expended in feeding (Shoener 1969). Large species of ants optimize their energy intake by having relatively few foragers which select mainly large seeds. Conversely, small-sized ants use a larger number of smaller foragers, and their strategy of collecting many small, easily-carried seeds is probably a more efficient strategy than expending large amounts of energy in attempting to harvest large seed.

Culver (1972) and Briese (1974) also reported that harvester species of ants show some size differentiation. As ant size increases there is an increase in the proportion of larger seeds collected. However, size differences in seeds coincide with species differences and therefore chemical or other stimuli may be determining their favourability to the ants. Seed size or shape may not be as important as chemical stimuli in selection criteria (Nickle and Neal 1972).

Sharp and Barr (1960) and Nagel and Rettenmeyer (1973) under rangeland conditions, and Hutchinson and King (1980) under a pasture conditions, reported an increase in ant populations when an overgrazing pattern is present.

References in the literature about pasture seed harvesting by ant species in Australian agriculture have been made for different regions. In the dry areas of central northern Australia, Greaves (1959) reported that the development of pasture improvement techniques by the sowing of pellets containing seeds of grasses, such as buffel and rhodes grass, has highlighted the important losses that can be caused by ants. Normally, the pelleted seeds are sown from aeroplanes and the ants are able to dispose of them. The use of seeds treated with insecticide has been proposed to overcame this problem. However, the erratic pulses of rainfall and the persistent presence of ants, reduces the effectiveness of the insecticides under such arid conditions. Frank (1968), studying the establishment of pastures in tropical savannah areas of the Northern Territory, observed

that seed-harvesting ants, reduced the potential seedling density of Townsville stylo (*Stylosanthes humilis).*

In Queensland, Champ and Sillar (1961) and Champ *et al.* (1961) recommended the control of seed-harvesting ants as an important factor allowing the success of establishment of buffel grass and other sown pastures. Russell and Coaldrake (1965), in experiments on the establishment of mixed pastures from seed broadcast after burning of fallen brigalow forests, indicated important but variable losses of seed due to removal by ants. Further work by Russell *et al.* (1967) confirmed previous findings and suggested that ants showed marked preferences for collecting seed apparently based on seed size.

Campbell (1966, 1969) noted the serious effect of seed-harvesting ants in central New South Wales. Seeds of many pasture species, such as red clover (*Trifolium pratense*), white clover (*T. repens*), subterranean clover (*T. subterraneum*), perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and phalaris (*Phalaris tuberosa* syn. *aquatica*) were taken by ants. They seemed to have little preference between pasture species, except that larger seeds (e.g. subterranean clover) were taken more slowly, or to a lesser extent, by small ants of the Pheidole family (Campbell 1966).

In northern New South Wales, the seasonal pattern of theft by ants of the genus Pheidole of both bare and coated oversown seed of white clover, lucerne, demeter fescue (*Festuca arundinacea)* and phalaris was examined. It was demonstrated that ants could forage enough seed from the ground surface potentially to influence the success of oversowing practices. Small phalaris seeds were taken more than the seeds of other species (Johns and Greenup 1976 a). Also, a relationship was established between the decline in harvesting rate and the quantity of seed available (Johns and Greenup 1976b).

In north-eastern Victoria, McGowan (1969) tested the effects of seed harvesting ants on the persistence of Wimmera ryegrass (*Lolium rigidum*), barley grass (*Hordeum leporinum*), silver grass (*Vulpia bromoides*), soft brome grass (*Bromus mollis*),

phalaris, cockfoot and subterranean clover. Ryegrass was harvested by ants in preference to the other grass species. At the same time, little selection of barley grass and subterranean clover seeds was observed, explaining the persistence of both species in the area of study. Seed size and seed weight appeared to affect the selection of seed by ants. The seeds of small-seeded species, such as cocksfoot, phalaris and ryegrass were collected most frequently. Again, the heavier subterranean clover seeds were rarely selected.

In order to meet their requirements, seed-harvester ants, try to maximize harvesting activities when periodic pulses of seed production occur, or when agronomic practices favour the accumulation of readily-accessible seed at ground level. Despite the fact that ants showed some evidence of size differentation, it is suggested that preferences for foraging pasture seeds are apparently based on seed characteristics such as seed size and seed weight. In general, small seeds are selected by ants before heavy or large seeds.

FIRST SECTION

3 LABORATORY TEST 1

AND

LABORATORY EXPERIMENTS 1-4

3 SEED SIZE AND SOME ENVIRONMENTAL FACTORS INFLUENCING THE GERMINATION OF SEEDS OF AN ANNUAL MEDIC AND A SUBTERRANEAN CLOVER

3.1 Introduction

The agricultural region of southern Australian lies in a broad zone south of latitude 29° in the east and at 27° 30' in the west (Donald 1970). The climate is of Mediterranean type, with winter-dominant rainfall pattern and lower temperatures, and the presence of a dry period with higher temperatures during the summer (Rossiter 1966a; Puckridge and French 1983). Seeds adapted to this environment normally germinate and seedlings became established after the first effective rain that falls at any time from April to June. Summer rainfall is erratic and normally ineffective for plant growth but on some occasions it is sufficient to produce a false opening to the growing season i.e. an early germination of readily-germinable (soft) seeds that are not constrained by physiological embryo dormancy and subsequent death of seedlings through lack of moisture (Morley 1961; Puckridge and French 1983).

Seeds of annual Mediterranean pasture species can be ready for germination under a wide range of environmental conditions and the success of the germination, emergence and establishment sequence can be assessed in terms of the plant density, because high-density pastures produce more early (autumn-winter) feed. Higher levels of pasture availability are especially important early in the growing season and can lead to higher year-round carrying capacity of livestock.

This chapter reports the results of several studies that examined the imbibition, germination and survival of various seed sizes within populations of snail medic (*Medicago scutellata*) cv. Sava and subterranean clover (*Trifolium subterraneum*) cv. Trikkala under a range of moisture and temperature regimes. The treatments were chosen to simulate the conditions likely to be experienced by germinating seeds in southern Australia, where both species are used extensively. For convenience, hereafter

the Sava snail medic will be abbreviated to Sava and the Trikkala subterranean clover will be abbreviated to Trikkala.

3.2 Laboratory Test 1: The effects of seed size on the allocation of dry matter in the seed

3.2.1 Introduction

Previous studies have shown that the allocation of dry matter to different morphological structures in a seed (e.g. subterranean clover) varies between seed populations. Furthermore, the specific roles of different parts of a seed (e.g. seed coat, endosperm, cotyledons, embryonic axis, etc.) in the natural process of germination, emergence and early growth of a seedling vary considerably. For this reason, each seed population has been defined.

3.2.2 Materials and methods

In order to determine, characterize and select the different seed-size categories involved in the seed populations for use in the Laboratory Experiments 1-4, Glasshouse Experiment 1 and Raised-bed Experiment[1], a 50 kg sack of certified seed of both *Medicago scutellata* cv. Sava and *Trifolium subterraneum* cv. Trikkala was obtained from a commercial source and stored in a room at 12.7 ± 0.6°C (± s.d.).

A representative sample (c. 3kg) of each legume species was obtained from the top, middle and bottom positions in the sack by using a 0.75 cm diameter tube sampler. After removal by hand of all abnormally shaped, coloured and damaged pasture legume seed as well as other pasture, crop and weed seeds and inert material, the samples of normal and pure pasture seeds remaining were sieved using an Endecott® shaker provided with a set of 20 cm diameter round brass laboratory test sieves to obtain a range of seed-size categories within each seed population (Tables 3.1-3.2).

To estimate the influence of seed size on the proportional change in the seed components, four replicate lots of 25 seeds were selected from each category, weighed individually by the use of an AND® FR-200 scale (210 g x 0.0001g) and immersed in distilled water at 20°C until almost full seed imbibition was achieved (6-7 h). Then by use of a scalpel, the testa and the endosperm were peeled from the seed, the cotyledons were separated from the base and the embryonic axis removed. Each lot of seed and the corresponding seed components were oven-dried at 80°C over a 48 h period, reweighed and the individual moisture content of the seed determined (Tables 3.1-3.2). To describe the relationship between the seed components and the whole seed values a regression analysis was performed.

3.2.3 Results

A positive trend in the moisture content of the seed with increasing seed size was obtained in both species (Tables 3.1-3.2) The changes in moisture content ranged between 5.00% and 5.70% (0.05 and 0.06 mg of water/mg of dry seed weight) in Sava seeds, and between 6.11% and 6.57% (0.06 and 0.07 mg of water/mg of dry seed weight) in Trikkala seeds between the smallest and the largest seed size, respectively.

On all occasions, a highly significant positive correlation ($P<0.001$) was found when the dry weight of the seed components was used in the regression analysis against the dry weight of the seed. When each component was considered as a proportion of the total dry weight of the seed, the values of the correlation coefficient were significant at 5% for testa + endosperm and embryonic axis in Sava seeds, and at 1% for embryonic axis in Trikkala seeds. The other seed components had highly significant positive correlation coefficients ($P<0.001$) for both cultivars. The results showed that the cotyledon weight, as a proportion of the total seed weight, increased with increasing seed size. In contrast, the testa + endosperm and the embryonic axis decreased with increasing seed size (Figures 3.1-3.2).

It is concluded that the medic and the subterranean clover seeds used in this study are representative of seed populations in which the proportion of the embryo (cotyledon + embryonic axis) increase with seed size. In this way, they are similar to Daliak

subterranean clover seeds used by Taylor (1972) and different from seed populations of the same species used by Black (1956, 1957 a).

Table 3.1 Characteristics of the seed-size categories obtained from a commercial sample of certified seed of Sava snail medic.

Seed-size category		Mean data on individual seeds				
Sieve aperture Min. - Max. (mm)		Fresh weight as stored (mg)	ratio	Oven dry weight (mg)	ratio	Moisture content (%)
1.41	1.70	5.44	1.00	5.17	1.00	5.00
1.71	2.00	9.87	1.81	9.36	1.81	5.17
2.01	2.36	16.26	2.99	15.39	2.98	5.35
2.37	2.80	23.85	4.38	22.55	4.36	5.45
2.81	3.35	29.67	5.45	27.98	5.41	5.70

Table 3.2 Characteristics of the seed-size categories obtained from a commercial sample of certified seed of Trikkala subterranean clover.

Seed-size category		Mean data on individual seeds				
Sieve aperture Min. - Max. (mm)		Fresh weight as stored (mg)	ratio	Oven dry weight (mg)	ratio	Moisture content (%)
1.41	1.70	4.09	1.00	3.84	1.00	6.11
1.71	2.00	7.19	1.76	6.74	1.76	6.26
2.01	2.36	11.08	2.71	10.37	2.70	6.41
2.37	2.80	13.39	3.27	12.51	3.26	6.57

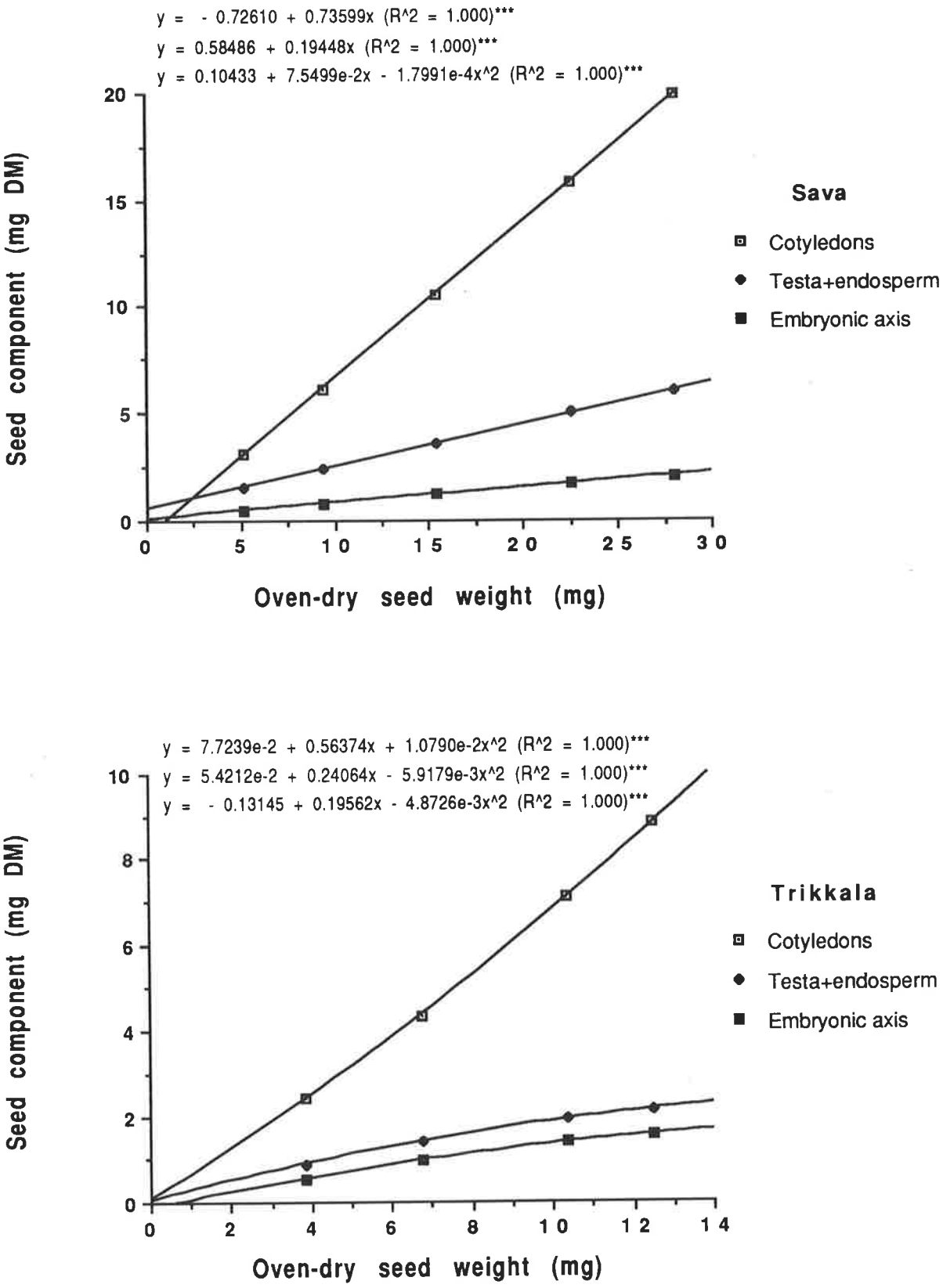


Figure 3.1 The influence of seed size on the actual allocation of dry matter in seeds of Sava snail medic and Trikkala subterranean clover.

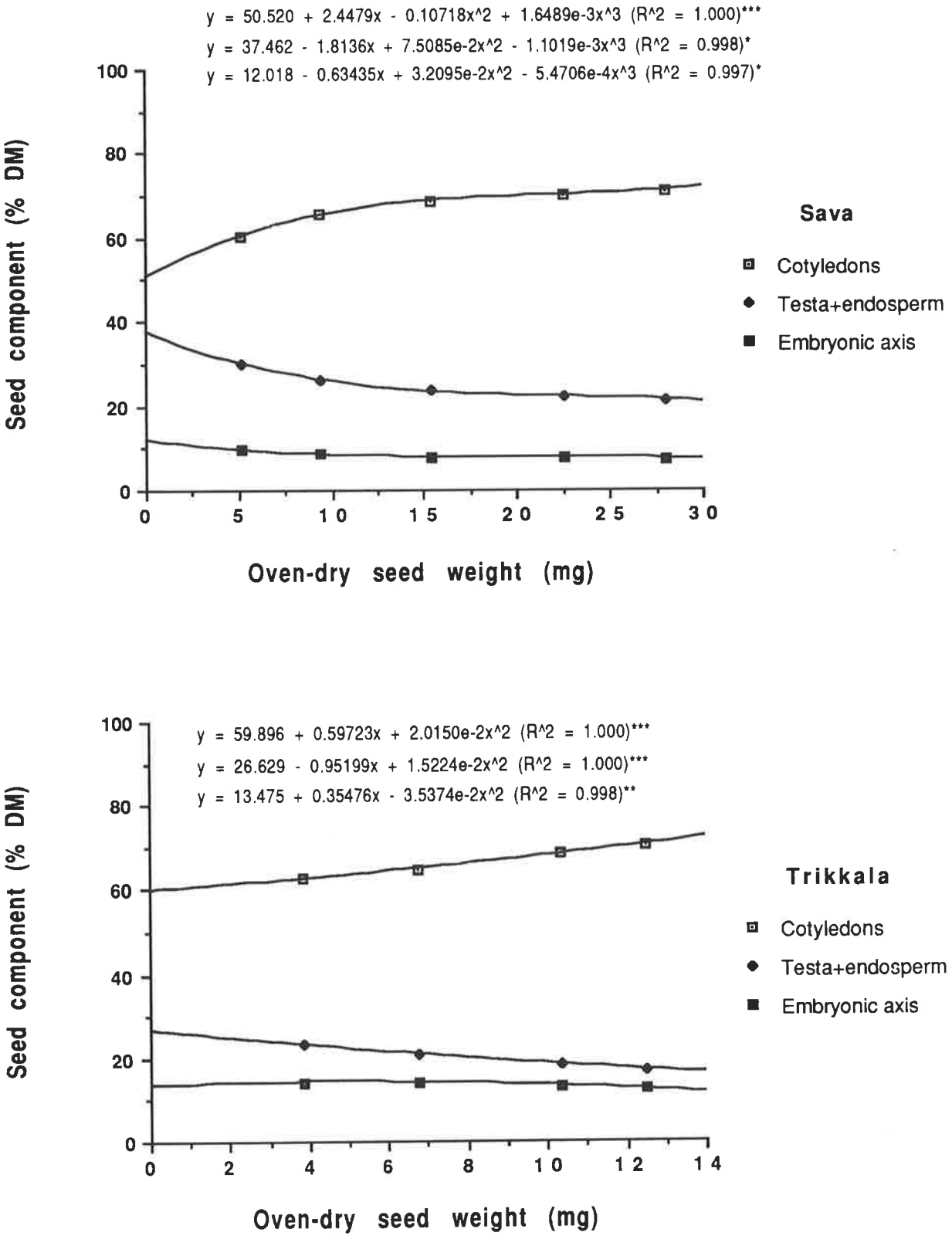


Figure 3.2 The influence of seed size on the proportional allocation of dry matter in seeds of Sava snail medic and Trikkala subterranean clover.

3.3 Laboratory Experiment 1: The effects of seed size and temperature on germination

3.3.1 Materials and methods

A multiple-temperature incubator located in a relatively constant temperature room (22.5 ± 2.5 °C) was used to test the effects of seed size and temperature on germination of selected species. The incubator comprised fourteen contiguous compartments (20 cm high x 15 cm wide x 10 cm deep divided into two sections, upper and lower) each well insulated and fitted with a hermetic door. The range of temperatures is given by three steel bars that finish in a hot water bath at one side and a refrigerator at the other. The temperature range may be changed by adjusting the temperature of the heating device or the cooling system.

After the system was started, ten days were required to stabilize the range of desired temperatures. Then, during the following ten days and before the germination test, temperatures were recorded twice each day by use of a Mercury bulb thermometer in each compartment to obtain the mean temperature (Tables 3.3-3.4).

Seeds were sieved by using an Endecott® shaker to give five seed-size categories (A, B, C, D and E) for Sava and three (A, B and C) for Trikkala. Table 3.5 shows the characteristics of seed used. The fresh weight of the individual seed stored (12.7 ± 0.6°C) was measured for all one hundred seed lots involved in each seed-size category for each legume. The mean values of oven-dry weight of the individual seed were obtained by selecting four replicates of 100 seeds from each seed-size category per cultivar and dried in the same conditions described before (3.2 Laboratory Test 1).

Four replicate lots of one hundred seeds for each seed-size category were placed in plastic Petri dishes (90 mm in diameter x 14 mm deep) each containing a pad of three Whatman Nº 1 filter papers at the bottom and one at the top and moistened with a

solution of 8 ml of Thiram (800 g/kg) prepared with 1.6 g of fungicide/1000 ml of distilled water. During the germination test, the dishes were remoistened as required.

The total capacity of each compartment in the multiple-temperature incubator was ten Petri dishes so it was necessary to divide each dish internally in two equal sections by use of a wooden stick in order to meet all the seed size treatments and replicates. The moistened Petri dishes were placed in the assigned compartment 24 hours before starting the test so that the temperature of the media could come into equilibrium with the imposed temperature regime. The experiments were arranged in a completely randomized block design with factorial treatment structure (5 seed sizes x 14 temperatures x 4 replicates) for Sava and (3 seed sizes x 14 temperatures x 4 replicates) for Trikkala. Each species test was run independently in time.

Seed was recorded as germinated when the length of the radicle exceeded at least the smallest width of the seed in Sava and the smallest diameter of the seed in Trikkala. The germinated seeds were counted and removed every twelve hours over a period of fourteen days. At the completion of the experiments, total germination was calculated for each treatment and adjusted for the potentially-germinable seed, on the basis of a previous germination test (100 seeds replicated four times) carried out in a incubator in the dark, except for the daily germination counts made in daylight at 18.9 ± 0.4°C (± s.d.) under the same conditions described previously before (Table 3.6). The germination rate, represents another important index in characterizing the seed responses to environmental factors. It was calculated as the reciprocal value of the time (days) required to obtain 90% germination. This level of germination was selected because it represents, according to the germination values obtained, a more suitable index in comparison with others normally used (e.g. 50% and 100%).

The data on germination values were analysed using GENSTAT V. Firstly, the partial increases of germination recorded at each time of evaluation were used to separate the cumulative effect over time. Orthogonal polynomials were used to partition the degrees of freedom and sum squares of the interactions into linear, quadratic, cubic and quartic

components. Finally, the analyses of variance were run independently for each interval of time of evaluation and based on cumulative values of germination. No further transformation of the data was required.

3.3.2 Results

In the analysis of variance made on increments of germination values recorded every 12 hours, a significant effect of temperature, seed size and their corresponding interactions was found for Sava and Trikkala, with the exception that the effect of seed size and interaction of Seed size x Time were not significant for the last species (Table 3.7). Only for Sava was a significant quadratic component, Temperature x Seed size found when the degrees of freedom and sum of squares of the interaction were partitioned by the use of orthogonal polynomials (Appendix 3.1). Analyses of variance were also carried out on cumulative values of germination at each time of evaluation, in order to estimate the magnitude of the effect of treatments involved. For Sava species, significant effects of temperature, seed size and their interactions were obtained through the testing period. The effect of temperature was always highly significant in cultivar Trikkala. The interaction of Seed size x Temperature was significant most of the time through the testing period (Tables 3.8 and 3.9).

The seeds of species Sava and Trikkala started germination after 12 h of incubation of the corresponding test. Final germination of Sava occurred in a broader optimum range of temperatures between 2.1°C and 33.6°C. At the minimum temperature, the smallest seed size germinated normally but the final germination became more and more affected as the size of the seed increased. Within a range of temperatures between 6.4°C and 30.1°C germination was relatively unaffected. At 33.6°C some depression in the germination values was observed in the two largest seed size categories and a complete suppression of the germination occurred at the highest temperature (Table 3.10).

The germination in Sava seeds was very fast within the first twelve-hour period for temperatures between 17.2°C and 25.1°C (Figure 3.3) with an optimum temperature of

19.1°C. In this period, the lower temperatures seemed to retard the rate of germination more strongly than the higher ones, with the exception of the highest temperature that killed the seed embryos. After the first day, germination progressively increased at lower and higher temperatures (Figures 3.3-3.4) and the germination at that range of temperatures was almost completed within a two day period. At 4.5 days, most of the germination had occurred and continued only at the extreme temperatures where there remained germinating seed (Figure 3.5). This response was particularly noticeable at the lowest temperature, where germination started after 4.5 days and became progressively greater up to 6.5 days (Appendices 3.2 - 3.4).

The interaction between seed size and temperature on the rate of germination is shown in Figures 3.6-3.7. The highest rates of germination in the two smallest seed sizes occurred over a narrow optimum range of temperatures between 17.2°C and 25.1°C. Also, the rate of germination tended to decline with increased seed size through the range of temperatures used and especially at the extremes.

In the case of Trikkala seeds, germination occurred in a range of temperatures between 2.5°C and 32.9°C. At the lowest temperature, the final germination became affected to some extent in the two larger seed size categories. Values of germination obtained between 6.8°C and 23.8°C were unaffected. At 26.5°C, the germination of the different seed-size categories became very sensitive to temperature, especially the smallest one. At higher temperatures, the germination is almost completely arrested and ceased at 37.9°C (Table 3.10).

Within the first twelve-hour period, the germination of Trikkala seeds was faster between 18.2°C and 22.0°C with a peak at 20.0°C. Germination during that period was very temperature-sensitive. The completion of germination in the optimum range was almost achieved after 2.0 days. Germination then proceeded at the extreme values of temperature, especially under lower ones where the smallest seed-size category had some apparent advantage over the others. Finally, the response in germination almost

ceased within 5.0 days of initiation of the experiment (Figures 3.8 - 3.10 and Appendices 3.5 - 3.6).

The three seed-size categories, had their highest rate of germination between the temperatures of 20.0°C and 22.0°C (Figure 3.11). The progressive rates of germination at extreme temperatures, especially to lower temperatures, show how interactive is the effect of seed size and temperature, and reveals the advantage of the speed of germination of small seed in comparison with large seed. Trikkala subterranean clover shows greater sensitivity to higher temperatures than Sava snail medic, but greater tolerance to low temperatures.

3.3.3 Discussion

The germination of a seed under a wide range of environmental conditions represents a strategic factor for success in the natural regeneration of an annual legume pasture under a Mediterranean-type climate. Very small differences in the ability of a seed to germinate may influence the success or failure of an individual in a population or a group of individuals in a community of emerged seedlings (Cocks and Donald 1973).

It is apparent that both species have different temperature requirements for germination which depends of the size of seed. Although the rate of germination is dependent on the factors involved, there is no evidence that temperatures within the range of 6.4°C and 30.1°C in cultivar Sava and 6.8°C and 23.8°C in cultivar Trikkala are a limiting factor in the final germination of these pasture species. This finding is consistent with the suggestion of Ellern and Tadmor (1966), that low temperatures (4°C - 10°C) are more important in delaying germination rather than depressing final germination. In contrast, high temperatures (25°C - 30°C) seem to depress germination rather than delay germination. In *Medicago truncatula* and *M. hispida*, final germination of both species was reduced by the high temperatures, with *M. truncatula* less affected by the imposed temperature regime. The germination of *M. hispida* was depressed at the highest temperature (Ellern and Tadmor 1966). This difference in behaviour between two

species was also found by Andrew (1965). In three annual species of *Medicago* seeds, germinating in a range of temperatures between 15°C and 30°C, he found that *M. polymorpha* germinated best at low temperatures, *M. truncatula* at intermediate temperatures and *M. minima* at high temperatures.

The results obtained with Trikkala subterranean clover in this experiment agree with the findings of Toole and Hollowell (1939), McWilliam *et al.* (1970) and Evers (1980), where the germination of subterranean clover seeds was greatly affected as temperatures rose from 20°C to 30°C. However, important differences in germination response to temperature had been reported for various cultivars (Young *et al.* 1978).

In the present study, the most rapid germination was at 19.1°C for Sava and 20.0°C for Trikkala. However, the Sava medic germinated more rapidly than the Trikkala subterranean clover at all temperatures where germination occurred. This represents an important trait associated with plant competition, especially under a mixed pasture sward, because seeds from species germinating at higher rates tend to become dominant in comparison with seeds from species that germinate at lower rates (Black and Wilkinson 1963).

The combined effects of seed size and temperature on germination within a population of annual pasture legume seeds has not been reported before. Despite the artificial control of the environmental conditions of the study, particularly the water relationship, the size of the seed persists as an important factor in germination under the non-limiting moisture conditions of the test.

A study by Harper and Benton (1966) reveals the importance of the size of the seed in the efficiency of the seed-surface moisture contact. A small seed has a larger contact/surface area ratio with the surrounding media than a large seed, even when there is no water tension. This aspect is more obvious when a combination of small and flat seeds is given, because flat seeds, like medics, have a better moisture relationship with the surface than round seeds as in subterranean clover.

Under agricultural conditions, the size and shape of seeds becomes important in the establishment of annual pasture species, when they interact with the microtopography of the soil where small seed has the advantage of occupying more favourable sites for germination (Harper *et al.* 1965). An exception is the ability of subterranean clovers to bury a proportion of their seeds, protecting them from unfavourable conditions. Buried burrs have heavy seeds (Yates 1958), more viable (Tennant 1965) and with a higher level of hard-seededness (Quinlivan and Francis 1971).

Because of the size of the seed is a trait related to germination and is associated with environmental factors such as temperature and especially to the ability of seed to take up water, a further and more complete analysis of this topic will be considered in the following test, in which, the response of imbibing seeds of contrasting sizes were tested under a range of temperatures.

Table 3.3 **Mean daily temperatures recorded for compartments of the multiple-temperature incubator used to test the effects of seed size and temperature on the germination of Sava snail medic seeds.**

Day	Compartment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	(°C)													
1	38.0	35.0	31.0	29.0	26.0	24.0	22.0	20.0	19.0	15.0	12.0	11.0	6.5	2.5
2	38.3	33.5	30.0	28.0	25.0	22.5	20.8	19.0	17.0	15.0	12.0	10.0	6.5	2.0
3	38.3	34.0	30.0	28.0	25.0	22.5	21.0	19.0	17.0	15.0	12.5	9.5	6.0	2.0
4	38.5	34.0	30.0	27.0	25.0	22.5	20.8	18.8	17.0	15.0	12.0	10.0	6.0	2.0
5	38.0	33.0	30.0	27.0	25.0	22.5	20.8	19.0	17.0	15.0	13.0	10.0	6.5	2.0
6	38.0	33.3	30.0	27.0	25.0	23.0	21.0	19.3	17.3	15.0	13.0	10.0	6.5	2.0
7	38.0	33.5	30.0	27.0	25.0	22.8	20.5	19.0	17.0	15.0	12.8	10.2	6.5	2.0
8	38.0	33.5	30.0	27.0	25.0	22.5	20.5	19.0	17.0	15.0	12.8	10.0	6.5	2.0
9	38.0	33.0	30.0	27.0	25.0	22.5	20.5	19.0	17.0	15.0	13.0	10.0	6.5	2.0
10	38.0	33.5	30.0	27.0	25.0	23.0	21.0	19.0	17.0	15.0	13.0	10.0	6.5	2.0
Sum (n=10)	381.1	336.3	301.0	274.0	251.0	227.8	208.9	191.1	172.3	150.0	126.1	100.7	64.0	20.5
Mean	38.1	33.6	30.1	27.4	25.1	22.8	20.9	19.1	17.2	15.0	12.6	10.1	6.4	2.1
S.D.	0.176	0.559	0.300	0.663	0.300	0.453	0.418	0.318	0.597	0.000	0.425	0.352	0.200	0.150
C.V. (%)	0.46	1.66	1.00	2.42	1.20	1.99	2.00	1.66	3.47	0.00	3.37	3.49	3.13	7.1
Temp. diff.†		4.5	3.5	2.7	2.3	2.3	1.9	1.8	1.9	2.2	2.4	2.5	3.7	4.3

† Mean temperature difference between adjacent compartments.

Table 3.4 **Mean daily temperatures recorded for compartments of the multiple-temperature incubator used to test the effects of seed size and temperature on the germination of Trikkala subterranean clover seeds.**

Day	Compartment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	(°C)													
1	38.5	33.5	30.0	27.0	25.0	23.0	21.0	19.2	17.5	15.0	12.0	9.3	6.0	2.0
2	38.0	33.0	30.0	26.5	24.0	22.0	21.0	19.0	17.0	15.0	12.0	9.5	6.5	2.0
3	37.0	32.5	29.0	26.0	23.5	21.5	20.0	18.0	16.0	14.0	12.0	9.5	7.0	2.0
4	37.5	33.0	28.5	26.0	24.0	22.0	20.0	18.5	16.0	14.0	12.0	9.0	7.0	2.0
5	38.0	33.0	29.0	26.5	24.0	22.5	20.0	18.0	16.0	14.0	12.0	9.5	7.0	3.0
6	38.0	33.0	29.0	26.5	24.0	22.0	20.0	18.0	16.0	14.0	12.0	9.0	7.0	3.0
7	37.5	32.5	28.5	26.0	22.0	21.0	19.0	17.0	15.5	14.0	11.5	9.0	6.5	2.5
8	38.0	32.5	28.5	26.0	23.0	21.0	19.0	17.5	15.5	14.0	11.5	9.5	6.5	3.0
9	38.0	33.0	30.0	27.0	24.0	22.0	20.0	18.5	16.5	15.0	12.0	9.5	7.0	2.5
10	38.0	33.0	29.5	27.0	24.0	22.5	20.0	18.5	16.0	15.0	12.0	9.5	7.0	2.5
Sum (n=10)	378.5	329.0	292.0	264.5	237.5	219.5	200.0	182.2	162.0	144.0	119.0	93.3	67.5	24.5
Mean	37.9	32.9	29.2	26.5	23.8	22.0	20.0	18.2	16.2	14.4	11.9	9.3	6.8	2.5
S.D.	0.391	0.300	0.600	0.415	0.750	0.610	0.632	0.629	0.600	0.490	0.200	0.224	0.335	0.415
C.V. (%)	1.03	0.91	2.05	1.57	3.15	2.77	3.16	3.46	3.70	3.40	1.68	2.41	4.93	16.60
Temp. diff. †		5.0	3.7	2.7	2.7	1.8	2.0	1.8	2.0	1.8	2.5	2.6	2.5	4.3

† Mean temperature difference between adjacent compartments.

Table 3.5 Characteristics of the seed-size categories used to test the effects of seed size and temperature on the germination of Sava snail medic and Trikkala subterranean clover seeds.

Sieve aperture Min. - Max. (mm)		Seed-size category	Fresh seed weight (mg) Mean	ratio	s.d.	c.v. (%)	Oven dry seed weight (mg) Mean	ratio	Moisture content (%)
		SAVA							
1.41	1.70	A	6.46	1.00	0.335	5.19	6.12	1.00	5.26
1.71	2.00	B	10.57	1.64	0.363	3.43	10.02	1.64	5.20
2.01	2.36	C	15.61	2.42	0.438	2.81	14.85	2.43	4.87
2.37	2.80	D	23.76	3.68	0.517	2.18	22.70	3.71	4.46
2.81	3.35	E	28.14	4.36	0.612	2.17	26.94	4.40	4.26
		TRIKKALA							
1.71	2.00	A	6.63	1.00	0.320	4.81	6.30	1.00	4.98
2.01	2.36	B	9.55	1.44	0.270	2.82	9.09	1.44	4.82
2.37	2.80	C	13.17	1.99	0.320	2.43	12.53	1.99	4.86

Table 3.6 **Mean cumulative germination obtained for different seed-size categories in Sava snail medic and Trikkala subterranean clover.**

Sieve aperture Min.	Max. (mm)	Seed size category	Cumulative germination (%) at various times (days) after initial watering: 1	2	3	4	5	6	7	8	9	10†	Seed Dead	Hard (%)
		SAVA												
1.41	1.70	A	1	13	53	74	78	78	78	78	78	78	1	21
1.71	2.00	B	4	30	73	76	76	76	76	76	76	76	0	24
2.01	2.36	C	2	10	68	76	78	78	79	79	79	79	0	21
2.37	2.80	D	7	60	74	75	77	78	78	78	79	79	0	21
2.81	3.35	E	20	73	85	86	88	89	89	90	90	90	0	10
		TRIKKALA												
1.71	2.00	A	0	66	75	77	78	79	80	81	82	83	1	16
2.01	2.36	B	0	71	76	76	78	79	79	80	81	82	0	18
2.37	2.80	C	0	60	74	77	78	79	80	80	81	82	1	17

† No additional germination was recorded after the ten-day test period.

Table 3.7 **Summary of analyses of variance made on increased values of germination in time for Sava snail medic and Trikkala subterranean clover.**

Treatment	Species	
	Sava	Trikkala
	(Significance levels)†	
Temperature	***	***
Seed size	***	NS
Temperature x Seed size	***	*
Time	***	***
Temperature x Time	***	***
Seed size x Time	***	NS
Temperature x Seed size x Time	***	***

† NS = Not significant; * = P<0.05; *** = P<0.001

Table 3.8 Summary of analyses of variance made on cumulative values of germination in time for Sava snail medic.

Treatment	Cumulative values of germination (%) recorded at: 1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0 days
	(Significance levels)†								
Temperature	***	***	***	***	***	***	***	***	***
Seed size	***	**	*	***	***	***	*	***	**
Interaction	***	***	***	***	***	***	***	**	***

Treatment	Cumulative values of germination (%) recorded at: 5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5 days
	(Significance levels)†								
Temperature	***	***	***	***	***	***	***	***	***
Seed size	***	***	***	***	***	***	***	***	***
Interaction	***	***	***	***	***	***	***	***	***

Treatment	Cumulative values of germination (%) recorded at: 10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0 days
	(Significance levels)†								
Temperature	***	***	***	***	***	***	***	***	***
Seed size	***	***	***	***	***	***	***	***	***
Interaction	***	***	***	***	***	***	***	***	***

† * = P<0.05; ** = P<0.01; *** = P<0.001

Table 3.9 Summary of analyses of variance made on cumulative values of germination in time for Trikkala subterranean clover.

	Cumulative values of germination (%) recorded at:								
Treatment	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**5.0 days**
	(Significance levels)†								
Temperature	***	***	***	***	***	***	***	***	***
Seed size	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	*	*	*	NS	NS	NS	NS	NS

	Cumulative values of germination (%) recorded at:								
Treatment	**5.5**	**6.0**	**6.5**	**7.0**	**7.5**	**8.0**	**8.5**	**9.0**	**9.5 days**
	(Significance levels)†								
Temperature	***	***	***	***	***	***	***	***	***
Seed size	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	NS	NS	*	*	**	*	**	**	**

	Cumulative values of germination (%) recorded at:								
Treatment	**10.0**	**10.5**	**11.0**	**11.5**	**12.0**	**12.5**	**13.0**	**13.5**	**14.0 days**
	(Significance levels)†								
Temperature	***	***	***	***	***	***	***	***	***
Seed size	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction	**	*	*	*	*	*	*	*	*

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Table 3.10 Mean final germination of seed-size categories of Sava snail medic and Trikkala subterranean clover held at different temperatures and recorded after 14 days of testing.

Seed-size category	Temperature (°C)													
	2.1	6.4	10.1	12.6	15.0	17.2	19.1	20.9	22.8	25.1	27.4	30.1	33.6	38.1
	(%)													
SAVA														
A	97.8	98.0	92.5	97.8	95.5	99.0	94.3	94.3	95.5	94.0	95.3	91.8	91.8	0.0
B	84.5	99.8	99.0	97.0	95.8	98.3	97.5	98.8	98.0	99.8	100.0	99.3	95.8	0.0
C	49.0	94.8	98.8	98.3	97.8	97.3	100.0	100.0	98.8	98.8	99.0	95.8	91.0	0.0
D	16.3	99.8	100.0	100.0	99.3	99.3	100.0	100.0	100.0	100.0	99.8	98.5	87.0	0.0
E	14.0	97.8	96.5	96.5	99.0	98.0	100.0	99.5	97.5	96.3	96.5	96.8	80.5	0.0

Seed-size category	Temperature (°C)													
	2.5	6.8	9.3	11.9	14.4	16.2	18.2	20.0	22.0	23.8	26.5	29.2	32.9	37.9
	(%)													
TRIKKALA														
A	93.0	96.5	99.8	94.5	97.5	98.5	98.0	97.8	97.8	93.5	45.3	4.0	5.5	0.0
B	83.3	98.3	94.8	95.5	95.3	97.5	99.0	99.5	98.5	96.3	60.0	5.8	3.5	0.0
C	73.8	94.5	100.0	99.8	100.0	100.0	99.8	99.8	100.0	100.0	78.3	6.5	2.0	0.0

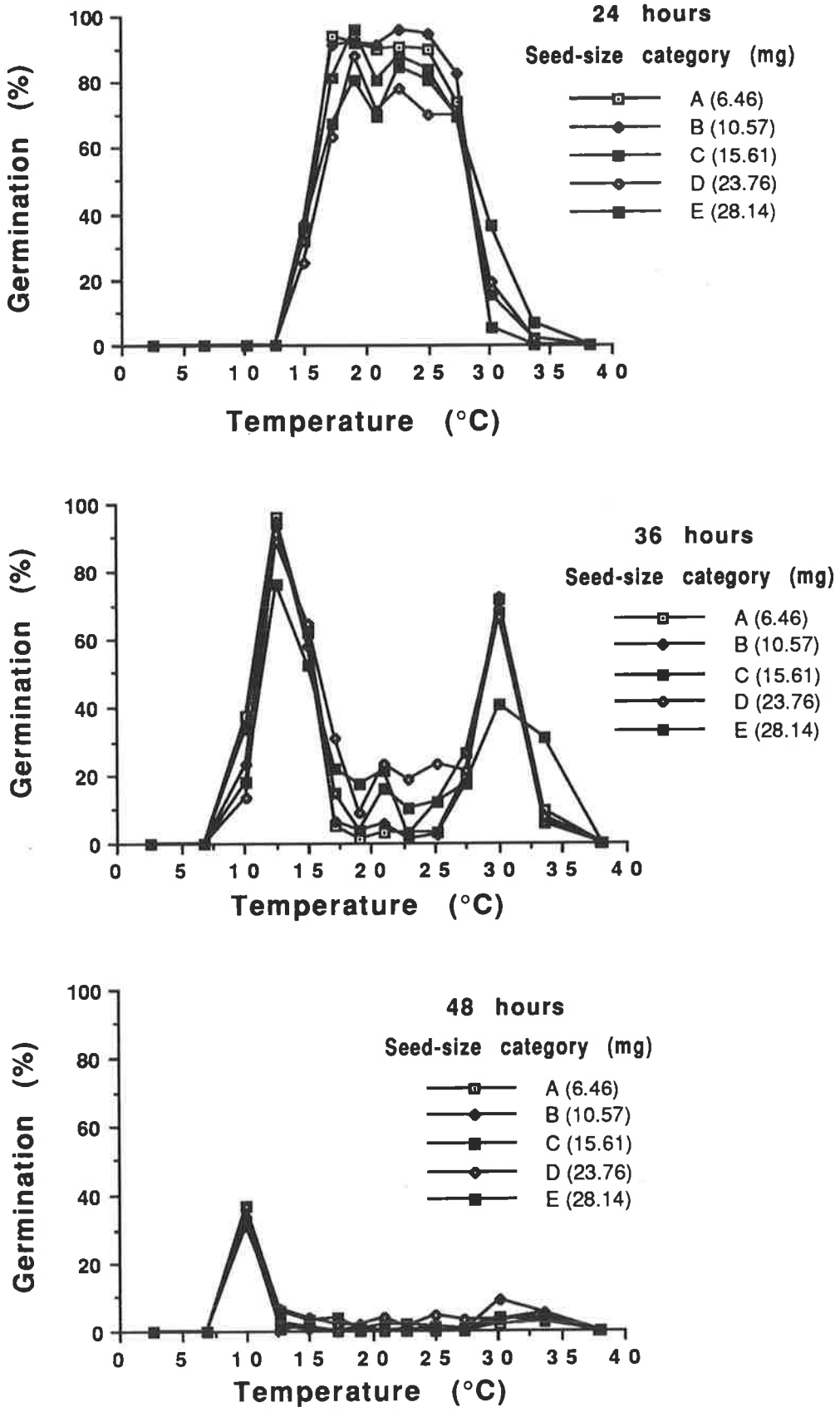


Figure 3.3 The increments in germination of five seed-size categories of Sava snail medic estimated after 24, 36 and 48 hours.

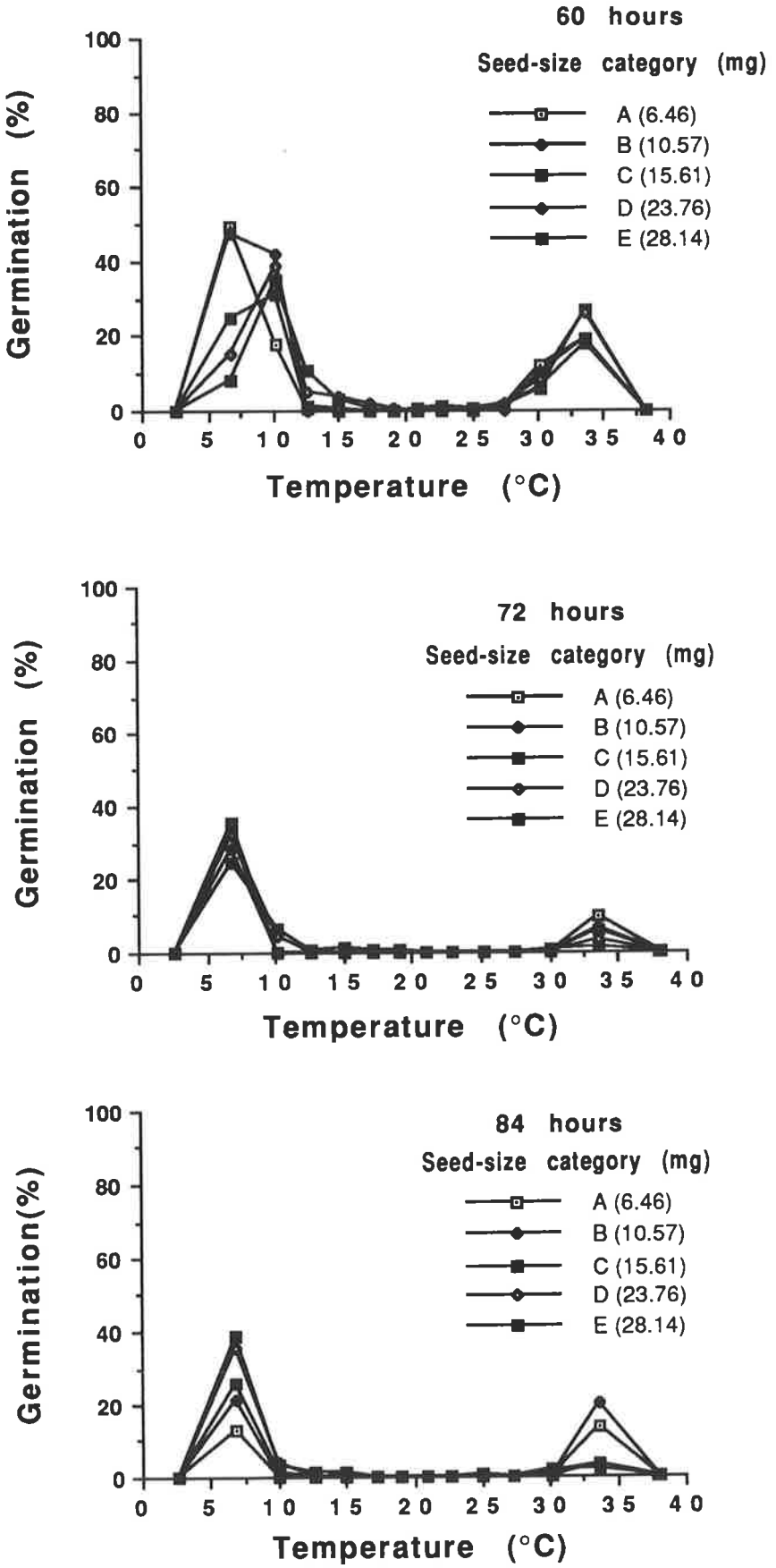


Figure 3.4 The increments in germination of five seed-size categories of Sava snail medic estimated after 60, 72 and 84 hours.

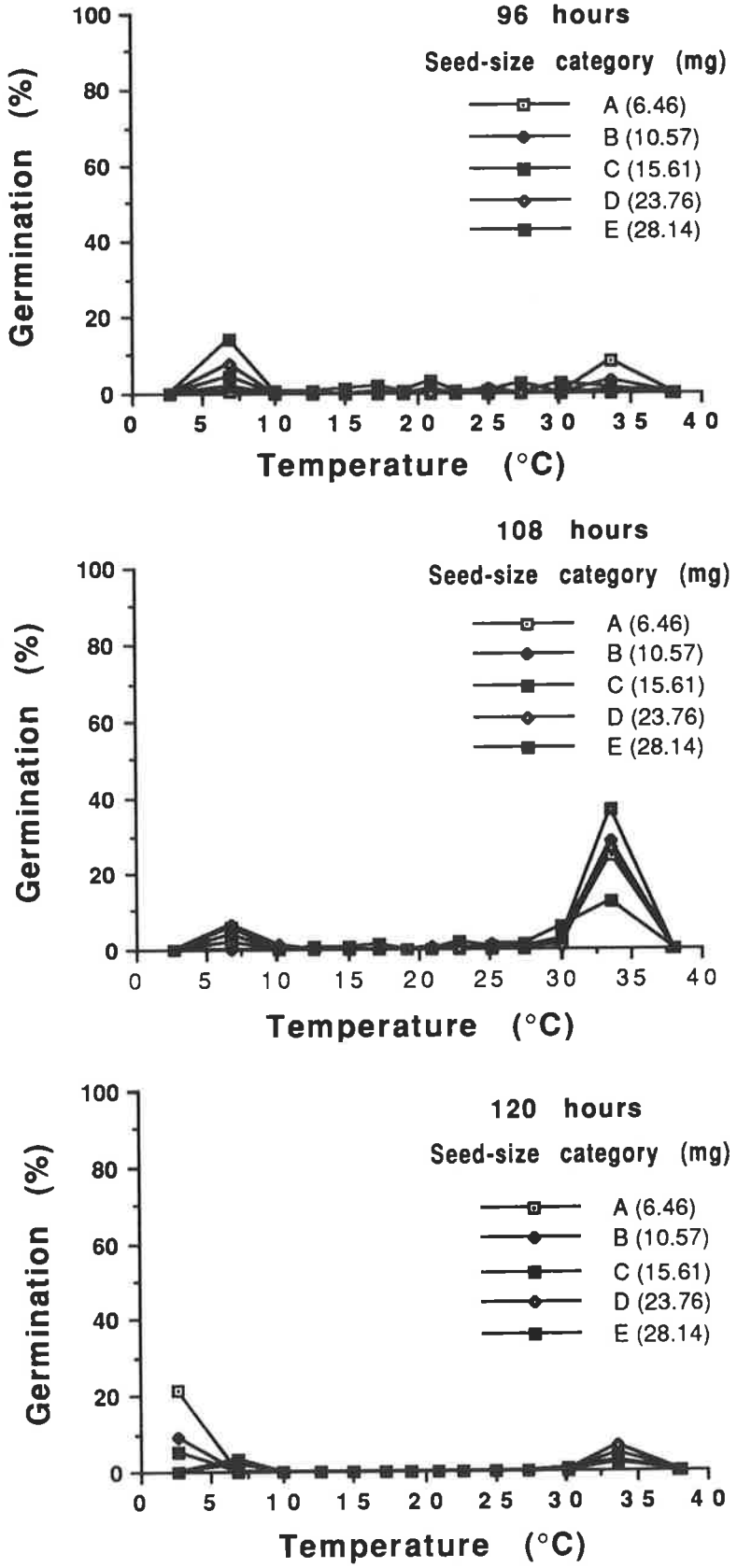


Figure 3.5 The increments in germination of five seed-size categories of Sava snail medic estimated after 96, 108 and 120 hours.

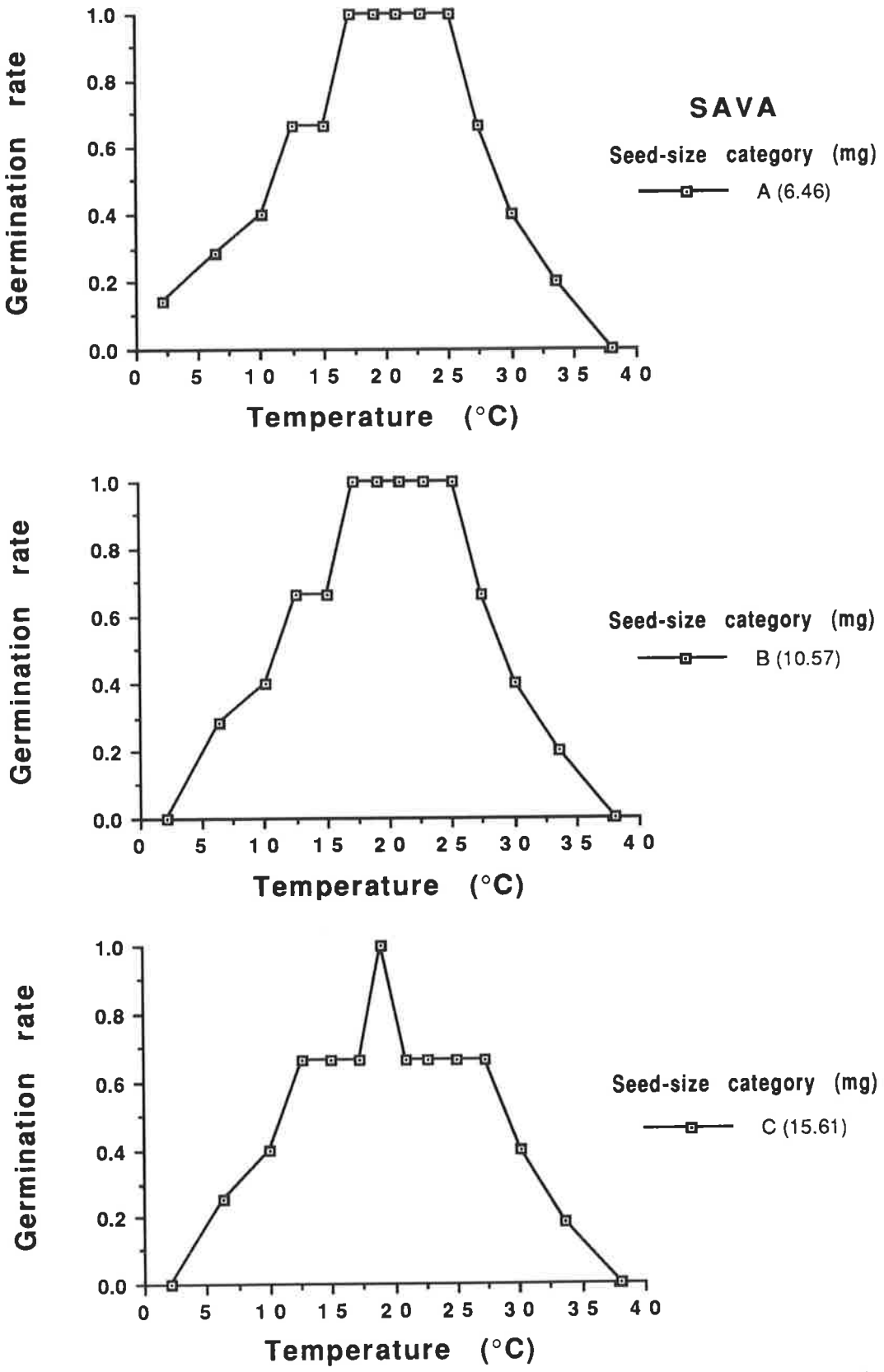
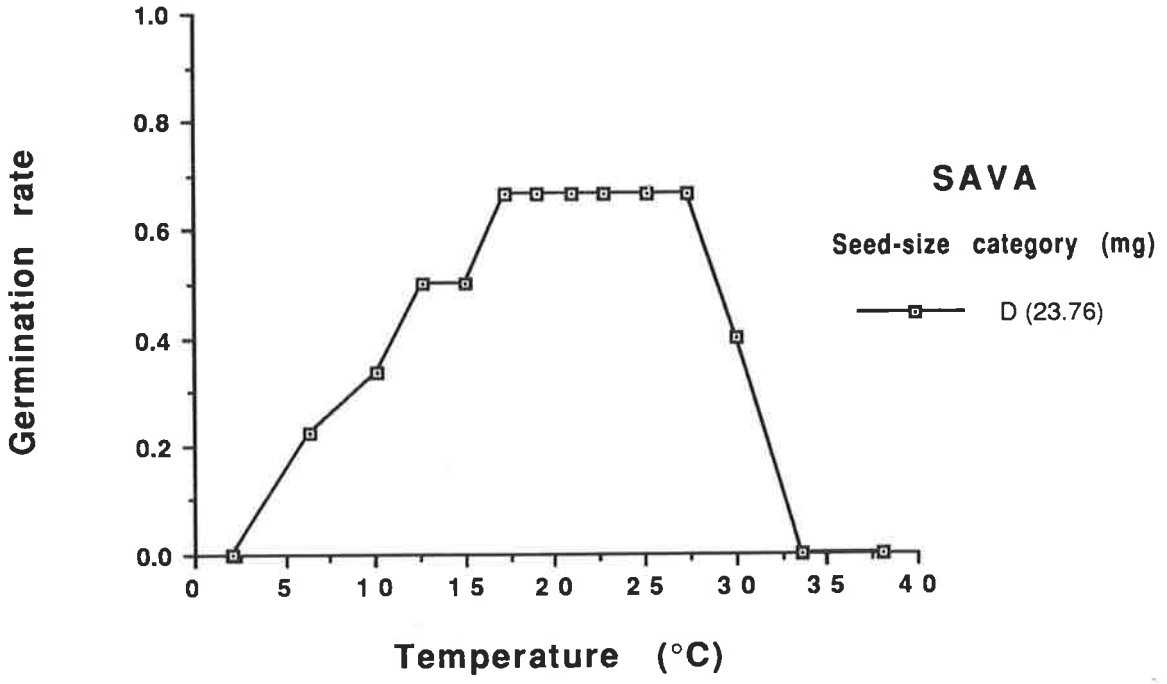


Figure 3.6 The germination rate (1/days, 90% germination) of Sava snail medic seed-size categories A, B and C.



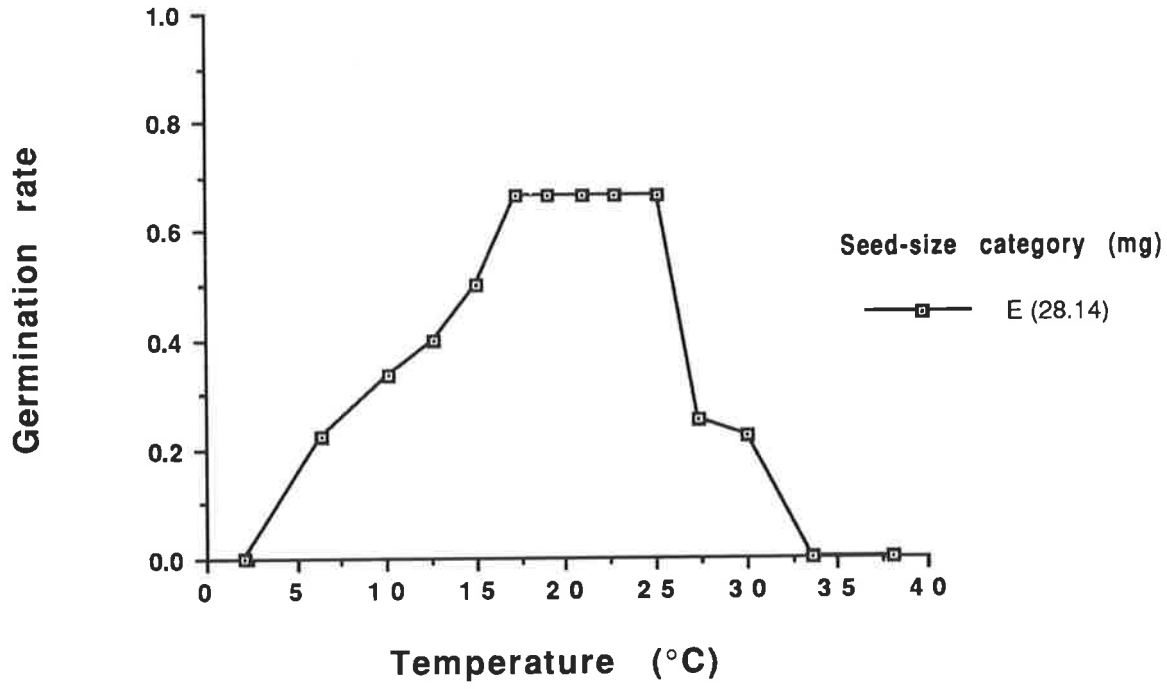


Figure 3.7 The germination rate (1/days, 90% germination) of Sava snail medic seed-size categories D and E.

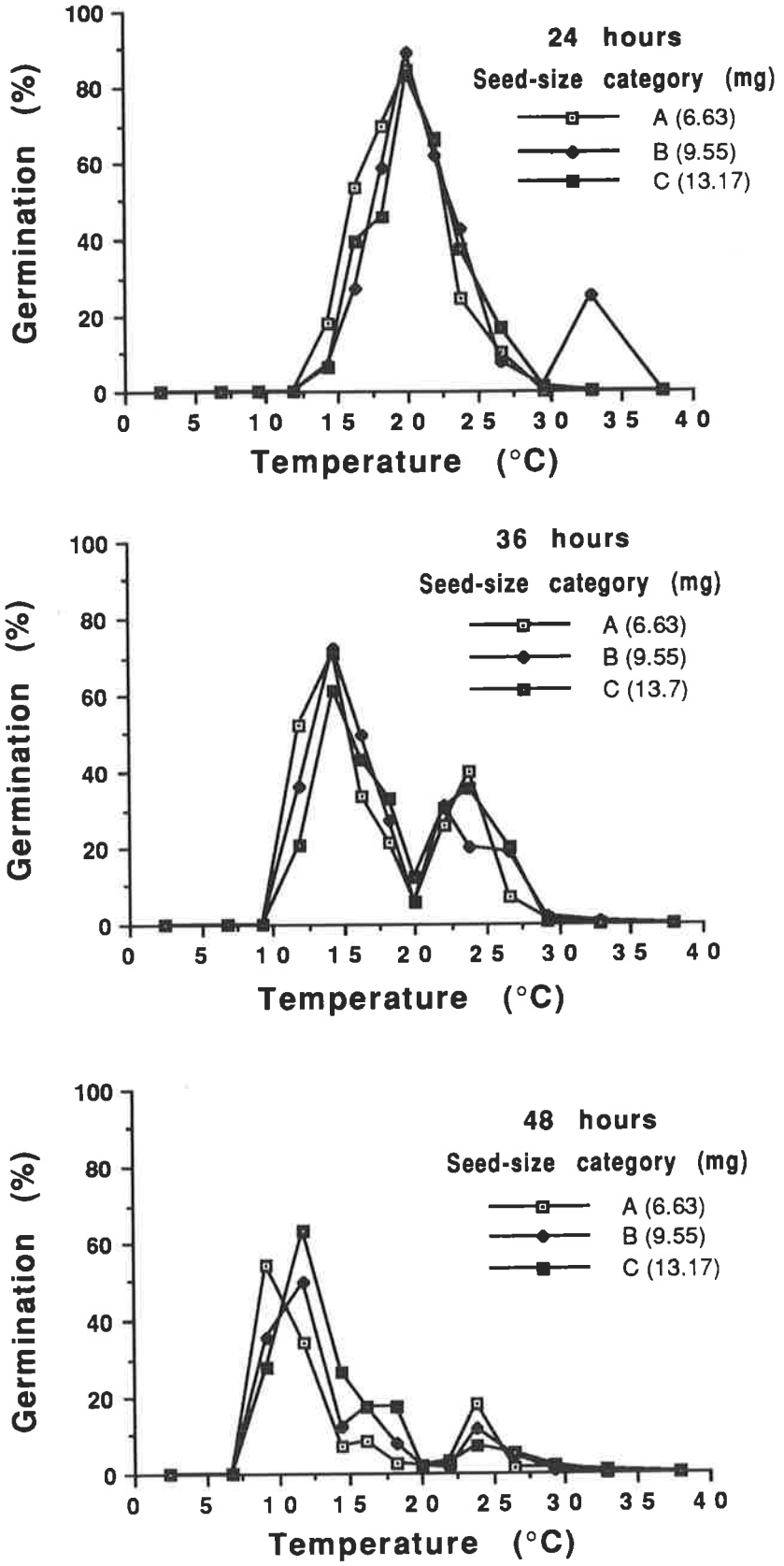


Figure 3.8 The increments in germination of three seed-size categories of Trikkala subterranean clover estimated after 24, 36 and 48 hours.

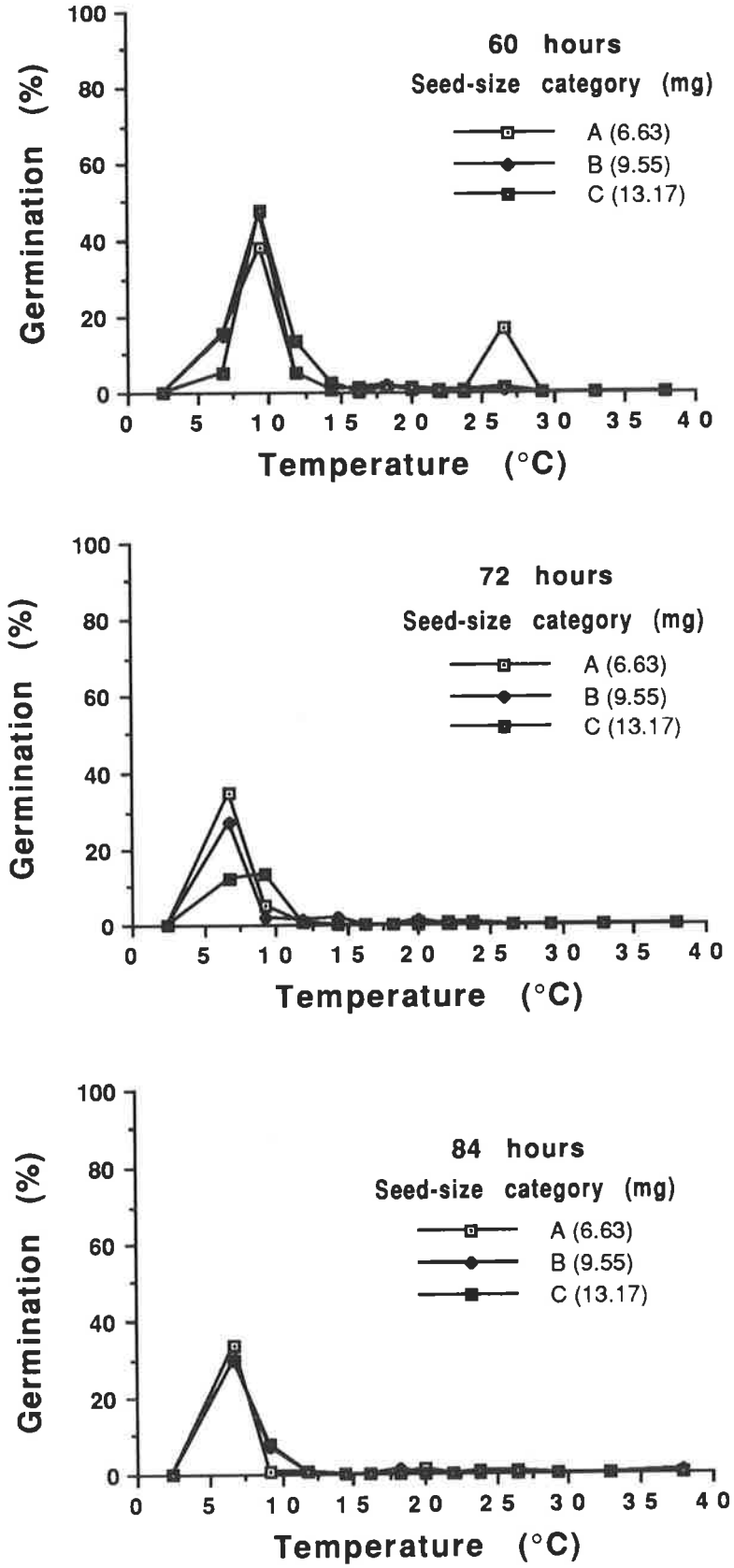


Figure 3.9 The increments in germination of three seed-size categories of Trikkala subterranean clover estimated after 60, 72 and 84 hours.

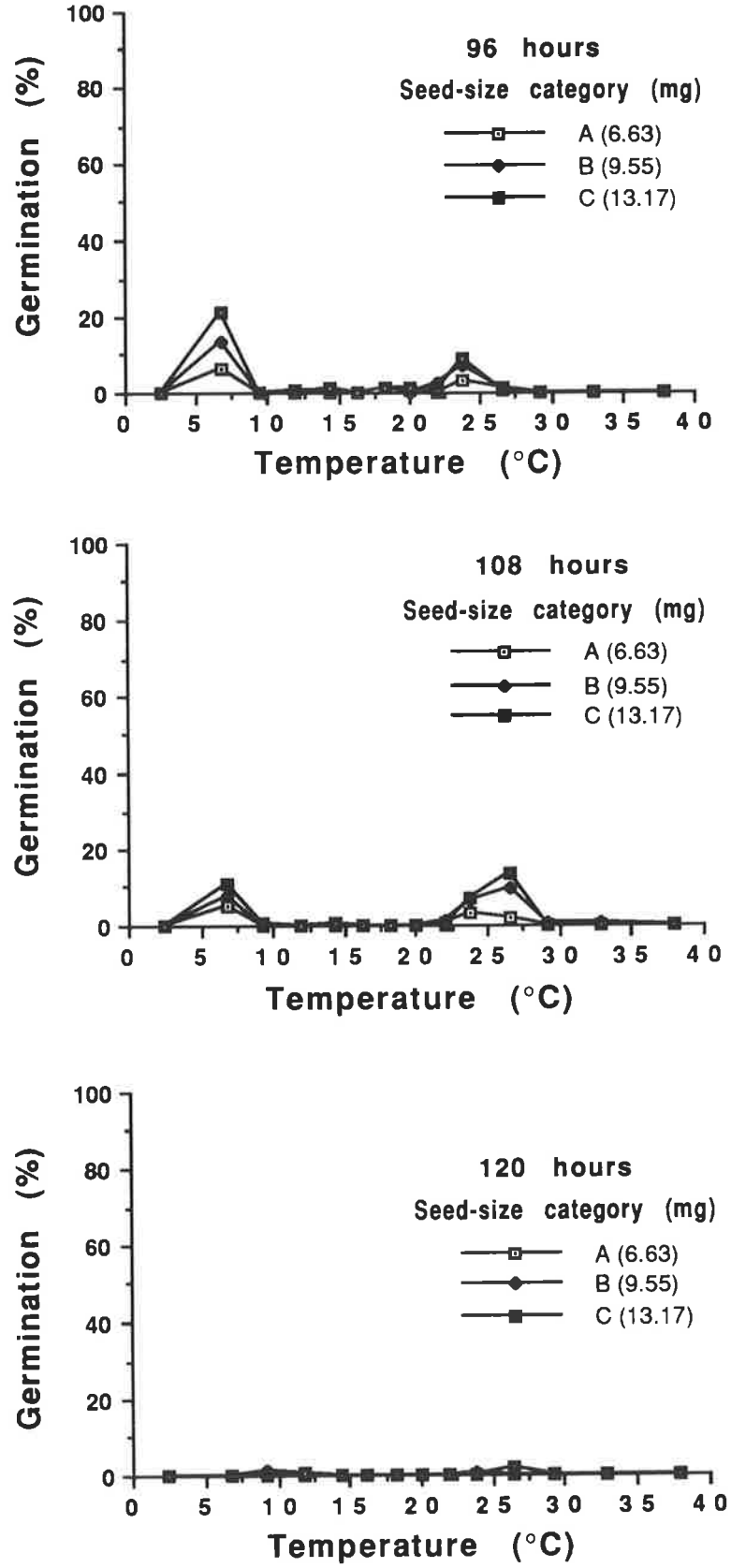


Figure 3.10 The increments in germination of three seed-size categories of Trikkala subterranean clover estimated after 96, 108 and 120 hours.

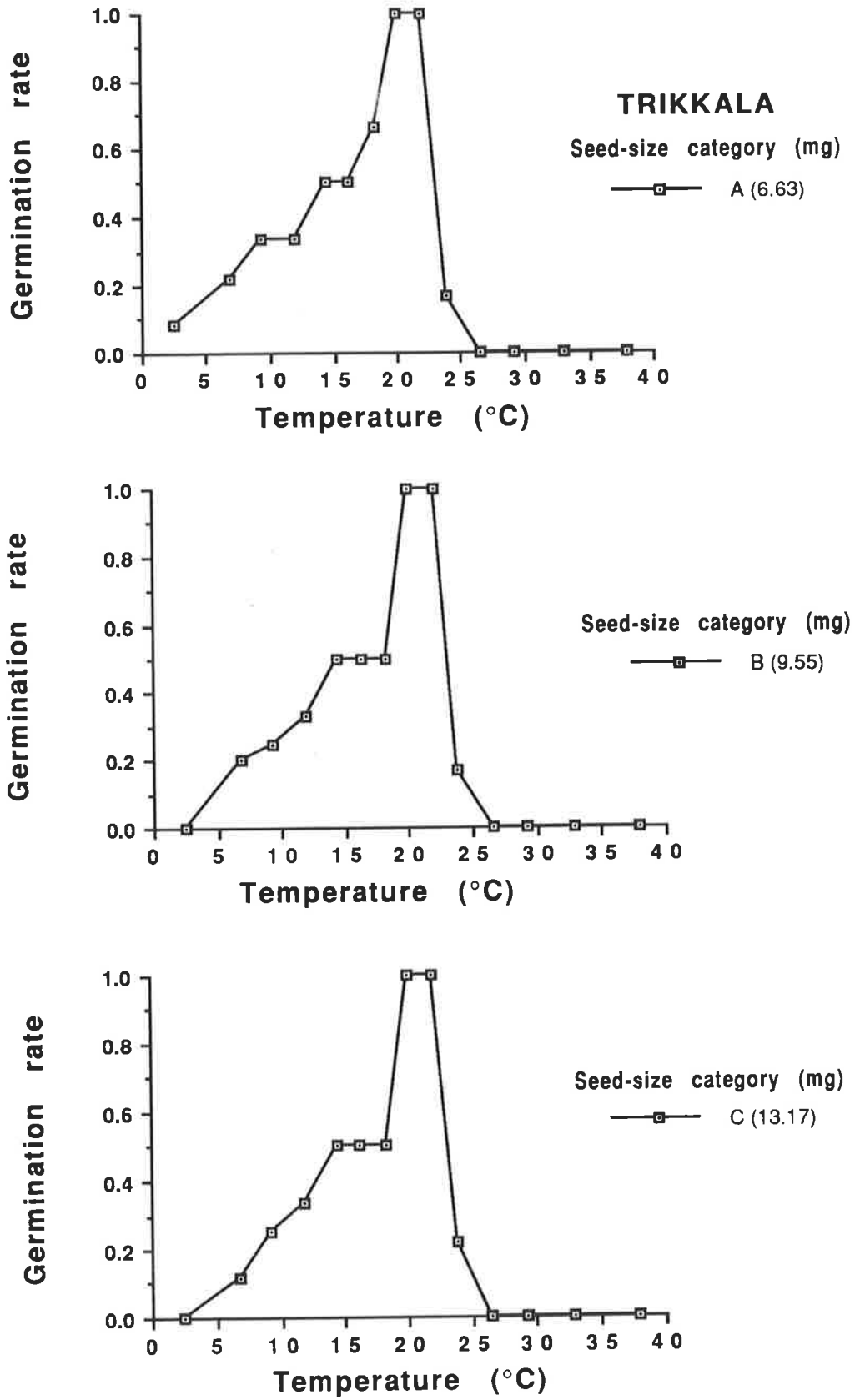


Figure 3.11 The germination rate (1/days, 90% germination) of Trikkala subterranean clover seed-size categories A, B and C.

3.4 Laboratory Experiment 2: The effects of seed size and temperature on the uptake of water by seeds

3.4.1 Introduction

It has previously been shown that both seed size and temperature influence the germination of Sava snail medic and Trikkala subterranean clover. In order to extend the scope of the study it was decided to examine the effects of seed size and temperature on uptake of water by seeds because this represents an essential prerequisite for germination.

3.4.2 Materials and methods

Four seed-size categories (A, B, C, and D) for Sava snail medic and three seed-size categories (A, B, and C) for Trikkala subterranean clover were selected (Table 3.11), to determine the water uptake by seeds submitted to fourteen different temperatures ranging between 2.0°C and 34.5°C (Table 3.12). The procedures used for the selection of the seed-size categories and the temperature regimes achieved were the same as described before (3.3 Laboratory Experiment 1).

The selected seed-size categories were kept at room temperature at 12.7 ± 0.6°C (±s.d.) before the studies. The number of seeds used per treatment was adjusted according to the level of hard-seededness in each seed-size category (Table 3.6) so that equal numbers of readily swollen seed were used in each treatment. In order to achieve more equivalent seed moisture contents across all treatments at the start of the experiment, the seed was stored in a desiccator over silica gel (2-4 mm in size) for 48 h prior to the commencement of the experiments. The moisture contents within each species were subsequently determined to be within 1% of each other (Table 3.11).

The mean individual fresh weight of the seed was calculated by using the corresponding values obtained for each lot of seed in each seed-size category as stored

at room temperature. The mean individual stored weight of the seed refers to the seed placed above silica gel for 48 h. The oven-dry weight and the moisture content of the seed were determined under the same conditions described in 3.3 Laboratory Experiment 1 (Table 3.11).

Seed lots were placed in Petri dishes, as before, previously moistened with 15 ml of distilled water and kept at the assigned temperatures for 48 h. The different treatments were located at random in a factorial arrangement by the use of a complete randomized block design as follows: Sava: Seed size (4) x Temperature (14) x Replicates (3)= 168 Petri dishes. Trikkala: Seed size (3) x Temperature (14) x Replicates (3)= 126 Petri dishes. Each test per cultivar at each replicate was run independently in time.

The changes in seed weight due to the uptake of water by seeds were obtained by removing from the Petri dishes sub-samples of 25 seeds per treatment. These seeds were blotted with absorbent tissues, placed in plastic hermetic phials and weighed at one-hour intervals for the first 12 hours and then at two-hour intervals until 24 hours. Because an initial discrimination between soft and hard seed was sometimes difficult, especially at lower temperatures, a magnified electric lamp was used to select the swollen seeds. The changes in the moisture content of the seed were expressed as a percentage of the corresponding oven-dry weight and the rates of water imbibition, calculated as the reciprocal value in time (hours) required by the seed to double its original oven-dry weight.

The data were analysed using GENSTAT V. Primary analyses of variance were performed on partial increment values of hydration in time, with and without partitioning of the degrees of freedom and sum of squares of the interactions. Finally, the analyses of variance were carried out independently for each interval of time of evaluation and based on cumulative values of uptake of water throughout the testing period. No further transformation of the data was required.

3.4.3 Results

The whole increases in water uptake by the seeds between evaluations, indicated a significant effect of temperature, seed size and the corresponding interactions for both Sava and Trikkala. The interaction of Temperature x Seed size x Time, however, was not significant for Trikkala (Table 3.13). When the analysis of variance considered the partition of the interactions into linear, and quadratic components, non-significant effects were obtained (Appendix 3.7). Analyses of variance made by the use of cumulative values of hydration of seeds, at each interval of time of evaluation, reveal significant effects of temperature and seed size for both cultivars throughout the testing period. A significant interaction Temperature x Seed size were more common in Sava than in Trikkala during the study (Tables 3.14-3.15).

A two-phase pattern of water uptake was observed in germinating Sava and Trikkala seeds during the test. In the early phase, the initial imbibition of water by seeds was important during the first hour of testing. The changes in seed weight were less dependent on the treatments involved, especially the size of the seed and more related to the high water potential gradient between the seed and the surrounding aqueous media (Figures 3.12 and 3.20; Appendices 3.8 and 3.10). Then a progressive differentiation in imbibition response by the seeds became more apparent, and was related to seed size and the imbibitional temperature, especially at higher temperatures. This effect was most noticeable up to four hours of hydration, during which the smallest seed size had the highest values of water absorption in the range of temperatures used. The effect persisted most clearly until twelve hours of testing: however, it remained until the end of the test, particularly with Sava. The response reveals that the total amount of water that can be absorbed by the seed is not completely independent of the size of the seed and the temperature after twenty four hours of evaluation (Table 3.16; Figures 3.12-3.17 and 3.20-3.25; Appendices 3.8-3.11).

For Sava seeds, the length of the initial imbibitional phase varied according to the factors involved. At the highest temperature (34.5°C), virtually all the water required to

initiate germination was imbibed in the first five hours for the smallest seed size and in the first eight hours for the largest seed size. In contrast, at the lowest temperature (2.0°C) none of the seed-size categories, with the exception of the smallest seed size, completed the initial phase of imbibition even after twenty four hours of testing. Under the nearest temperature (e.g. 19.6°C) to the optimum temperature of germination reported in the previous experiment (3.3 Laboratory Experiment 1), the time required by the seed to imbibe most of the water sufficient to start a visible process of germination, defined as the extention of the radicle, is about eight hours for all seed-size categories, although it differs in the total amount of water imbibed (Figures 3.12-3.17).

The rate of water imbibition shows a clear advantage to the smaller seed-size categories in comparison with the larger ones. In particular, the smallest seed size required between nine hours at the lowest temperature and two hours at the highest temperature to double its own dry weight. The largest seed, however, required between twelve hours at the lowest temperature and four hours at the highest temperature to double its dry weight (Figures 3.18-3.19).

When Trikkala seeds were wetted at the highest temperature, almost all the water required to initiate the growth of the radicle at all seed-size categories was taken up in the first five hours. However, at the lowest temperature, the smallest and largest seed sizes required about nine and twelve hours to complete the initial phase of water uptake. Under the nearest temperature (e.g. 19.6°C) to the optimum temperature of germination reported before the imbibition phase was finished after about eight hours in all seed sizes (Figures 3.20-3.25).

The rate of water imbibition calculated for Trikkala seeds confirmed the importance of seed size and temperature in governing the capacity of the seed to cope with the initial stage of water uptake in germinating seeds. The small, intermediate and large seed-size categories required about seven, twelve and twelve hours respectively at the lowest

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temperature and about two, three and three hours respectively at the highest temperature to double their original dry weight (Figure 3.26).

In both species, after the initial phase of imbibition was complete, an almost steady state second phase of water uptake occurred in which small variations in seed weight were recorded (Figures 3.12-3.17 and 3.20-3.25).

3.4.4 Discussion

Water uptake by a seed is an essential prerequisite for germination. Failure or delay in germination is reported by Harper and Benton (1966) as a result of insufficient or retarded water imbibition by seeds.

When seeds germinate under optimum environmental conditions (water, temperature, light, etc.) the external impedance produced by the degree of contact between the seed and the aqueous medium, and the internal impedance of the matrix in which the seed is imbibing water are minimized. Thus, the difference in water potential between the seed itself and the imbibitional medium is the main factor driving the rate of water flow into the seed (Bewley and Black 1978). This is especially valid if we assume that the losses of water from the seed and from the water-supplying substrate to the surrounding environment were zero.

Under the laboratory conditions of germination, normally a triphasic pattern of water uptake is observed (Bewley and Black 1978; Vertucci 1989). The initial phase, regarded as a physical process (imbibition), is characterized by a rapid hydration as a consequence of the matrix forces at seed level. The second phase, is the lag period of water uptake, represented by an almost steady state situation, in which, the ability of the seed matrices to be hydrated and bind water (matrix potential) decreases, as well as the concentration of dissolved solutes at cell level (osmotic potential). At this stage, the most important metabolic processes are started in preparation for final germination.

Then, when the visible process of germination starts, another phase of rapid water uptake is initiated and an accelerated elongation of the radicle is observed.

In these studies, both legume cultivars showed two phases of water uptake during the testing period, although some seeds had visible signs of germination at the end of the test. The results of the experiments show that the rate at which the seeds under study became fully-imbibed was influenced mainly by temperature and the size of the seed.

It has been known for some time that imbibition by a seed is a temperature-dependent process and proceeds more slowly at lower temperatures (Brown and Worley 1912; Shull 1920; Shull and Shull 1924; Fayemi 1957; Allerup 1958; Keller and Bleak 1970; Blacklow 1972). More recent reports also supply evidence that temperature affects water bind in seeds (Vertucci and Leopold 1987) through its relationship with the viscosity of the imbibing water (Murphy and Noland 1982; Vertucci and Leopold 1983).

The initial water uptake by seeds of both legume species after one hour of testing, appeared to be more related to the existing water potential gradient between the dry seed and the free water rather than to the temperature or seed size. Vertucci and Leopold (1987) reported a water potential gradient of about 100 M Pa in low-moisture-content seeds, that declined exponentially as the seed was hydrated. This suggests that the permeability of the scarified seed coat present at different seed-size categories used does not influence the physical process of imbibition. However, the seed coat is considered a barrier to the diffusion of water and gases (Mayer and Shain 1974) and in soybean, the amount of seed coat is correlated with the size of the seed. Small seeds have a higher proportion by weight of seed coat with larger, rounded pores. In some cultivars, this leads to higher rates of water uptake in comparison with large seeds (Calero *et al.* 1981).

Following this initial uptake of water, the total amount of water imbibed by the seeds during the testing period is dependent on the treatments involved. It has been shown

that the total amount of water which can be taken up by a seed is independent of the temperature (e.g. Allerup 1958; Murphy and Noland 1982). This phenomenon is not supported by the present study and may be explained by shorter imbibition times at lower temperatures. It could also be influenced by the size of the seed and, in the specific case of cultivar Sava, by progressively advanced stages of water uptake at higher temperatures, due to the initiation of the last phase of hydration. Only soft, non-dormant and viable seed is able to enter that phase (Bewley and Black 1978), so the explanation will be valid only if the highest temperature used did not affect the viability of the seed embryos and their further development. According to the previous experiment (3.3 Laboratory Experiment 1), some mortality of seed embryos was observed at 33.6°C, especially in the higher seed-size categories. In the present study, the highest temperature recorded was 34.5°C. If similar levels of embryo mortality as above were present, it is possible to suggest that the initiation of the last phase of water uptake, principally in smaller seed-size categories, was the principal reason for such dependence.

Yamamoto (1964) has reported important differences in the ability of seeds of several species to take up water, suggesting that higher rates of hydration may be an adaptation to xeric conditions. The differences in water uptake by seeds of various sizes seems to be due both to external factors and to internal seed factors. Despite the controlled moisture conditions during the test, Harper and Benton (1966), working with a wide range of species with different seed sizes, found that the greater the size of the seed, the greater was the dependence of germination on water tension. Species with largest seed did not germinate, even when water tension was low. It appears therefore that the size and the shape of the seed play an important role in the efficiency of the seed-surface moisture contact obtained and its relationship with the substrate and the surrounding atmosphere.

Martin (1946) stated that differences in the capacity of a seed to take up water is related to internal factors such as the greater size of the embryo in legumes. Rather than the

embryo (cotyledons plus embryonic axis), it is the embryonic axis that has a greater and faster water absorption capacity than the other parts of the seed including the endosperm, cotyledons and seed coat (Stiles 1948). This theory is supported by the seed population used in the present studies in which the embryonic axis of the seed as a proportion of the total seed components and independent of the moisture content of the seed, decreases as the seed size increases (3.2 Laboratory Test 1). Bowman and King (1987) also found that the smallest seeds of subterranean clover cultivars had higher efficiencies of hydration than larger seeds, both between and within different seed populations and therefore germinated early. On the other hand, McWilliam *et al.* (1970), confirmed that high rates of water uptake in pasture legumes are also associated with high rates of dehydration and may not represent a clear advantage, particularly in subterranean clover, which requires an abundant supply of moisture to achieve a rapid germination (Roberts 1966). This is especially important when seeds are placed on the soil surface prior to natural regeneration (McWilliam and Dowling 1970).

However, if the dehydration of seeds which had previously imbibed became effective before radicle emergence, the viability of the seed is not damaged and in consequence the germination and the subsequent seedling vigour are not affected (Hegarty 1978; McKersie and Stinson 1980). Another important internal factor to consider is the presence of mucilage tissues in the testa of some seeds, such as medic species, that spreads around the seed and extends the pathway of water-flow from the imbibing medium to the seed (Harper and Benton 1966).

The present study suggests that if a small seed is able to imbibe water at higher rates than a large seed, especially when unfavourable temperatures are involved, an early approach to the lag phase of water uptake, where metabolic events occur in preparation for the final germination, will cause an earlier emergence of the radicle and seedling establishment. In terms of plant competition in a dense pasture sward this should be of great significance.

Table 3.11 Characteristics of the seed-size categories used to test the effects of seed size and temperature on the uptake of water by Sava snail medic and Trikkala subterranean clover seeds.

Sieve aperture Min. - Max. (mm)		Seed-size category	Fresh weight (mg)	ratio	Stored weight (mg)	ratio	Moisture content (%)	Oven dry weight (mg)	ratio	Moisture content (%)
		SAVA								
1.41	1.70	A	6.57	1.00	6.52	1.00	0.76	6.25	1.00	4.14
1.71	2.00	B	10.94	1.66	10.84	1.66	0.91	10.35	1.66	4.52
2.01	2.36	C	15.32	2.33	15.19	2.33	0.85	14.48	2.30	4.67
2.37	2.80	D	23.50	3.57	23.35	3.58	0.64	22.36	3.58	4.24
		TRIKKALA								
1.71	2.00	A	6.72	1.00	6.61	1.00	1.64	6.25	1.00	5.45
2.01	2.36	B	10.14	1.51	10.01	1.51	1.28	9.48	1.52	5.29
2.37	2.80	C	13.43	2.00	13.24	2.00	1.41	12.50	2.00	5.59

Table 3.12 **Mean daily temperatures recorded at various compartments of the multiple-temperature incubator used to test the effects of seed size and temperature on the uptake of water by Sava snail medic and Trikkala subterranean clover seeds.**

Day	Compartment													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
							(°C)							
1	34.0	30.0	26.5	24.0	21.5	19.5	17.5	16.0	14.0	12.0	10.0	7.0	4.8	2.0
2	36.0	31.0	27.0	24.5	22.0	20.0	18.0	16.0	14.0	12.0	10.0	8.0	5.0	2.0
3	35.0	30.0	26.5	24.0	21.5	19.0	17.0	15.5	13.5	11.5	9.5	8.0	4.0	2.0
4	34.5	30.0	26.5	24.0	21.0	19.0	17.0	15.5	14.0	12.0	10.0	8.0	6.0	2.5
5	34.0	29.0	26.0	23.5	21.0	19.0	17.5	16.0	14.0	12.0	10.0	8.0	5.0	1.5
6	34.5	30.0	26.0	24.5	21.0	19.0	17.0	16.0	14.0	12.0	10.0	8.0	5.0	2.0
7	34.0	30.0	27.0	24.0	22.0	20.0	18.0	17.0	15.0	13.0	11.0	8.5	5.5	2.0
8	34.0	30.0	27.0	24.5	22.5	20.0	18.0	16.0	15.0	13.0	11.0	8.5	5.5	2.0
9	34.0	30.0	27.0	24.5	22.0	20.0	18.0	16.0	14.0	12.0	11.0	8.0	5.0	2.0
10	34.5	30.0	27.0	24.5	22.0	20.0	18.0	16.0	14.0	12.0	11.0	8.0	5.0	2.0
Sum (n=10)	344.5	300.0	266.5	242.0	216.5	195.5	176.0	160.0	141.5	121.5	103.5	80.0	50.8	20.0
Mean	34.5	30.0	26.7	24.2	21.7	19.6	17.6	16.0	14.2	12.2	10.4	8.0	5.1	2.0
S.D.	0.610	0.447	0.391	0.332	0.502	0.472	0.436	0.387	0.450	0.450	0.550	0.387	0.498	0.224
C.V. (%)	1.77	1.49	1.46	1.37	2.31	2.41	2.48	2.42	3.17	3.69	5.29	4.84	9.76	11.20
Temp. diff†		4.5	3.3	2.5	2.5	2.1	2.0	1.6	1.8	2.0	1.8	2.4	2.9	3.1

† Mean temperature difference between adjacent compartments.

Table 3.13 Summary of analyses of variance made on increased values of water uptake with time for Sava snail medic and Trikkala subterranean clover seeds.

Treatment	Species	
	Sava	Trikkala
	(Significance levels)†	
Temperature	***	***
Seed size	***	***
Temperature x Seed size	**	***
Time	***	***
Temperature x Time	***	***
Seed size x Time	***	***
Temperature x Seed size x Time	***	NS

† NS = Not significant; ** = P<0.01; *** = P<0.001

Table 3.14 Summary of analyses of variance made on cumulative values of water uptake in time for Sava snail medic seeds.

Treatment	Cumulative values of water uptake recorded at: 1	2	3	4	5	6 hours
	(Significance levels)†					
Temperature	***	***	***	***	***	***
Seed size	***	***	***	***	***	***
Interaction	*	***	***	***	***	***

Treatment	Cumulative values of water uptake recorded at: 7	8	9	10	11	12 hours
	(Significance levels)†					
Temperature	***	***	***	***	***	***
Seed size	***	***	***	***	***	***
Interaction	***	NS	NS	NS	NS	***

Treatment	Cumulative values of water uptake recorded at: 14	16	18	20	22	24 hours
	(Significance levels)†					
Temperature	***	***	***	***	***	***
Seed size	***	***	***	***	***	***
Interaction	***	**	***	**	**	**

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Table 3.15 Summary of analyses of variance made on cumulative values of water uptake in time for Trikkala subterranean clover seeds.

	Cumulative values of water uptake recorded at:						
Treatment	**1**	**2**	**3**	**4**	**5**	**6**	**hours**
			(Significance levels)†				
Temperature	***	***	***	***	***	***	
Seed size	**	***	***	***	***	***	
Interaction	NS	NS	NS	*	NS	NS	

	Cumulative values of water uptake recorded at:						
Treatment	**7**	**8**	**9**	**10**	**11**	**12**	**hours**
			(Significance levels)†				
Temperature	***	***	***	***	***	***	
Seed size	***	***	***	***	***	***	
Interaction	NS	NS	NS	NS	***	***	

	Cumulative values of water uptake recorded at:						
Treatment	**14**	**16**	**18**	**20**	**22**	**24**	**hours**
			(Significance levels)†				
Temperature	***	***	***	***	***	***	
Seed size	***	***	***	***	***	***	
Interaction	NS	**	**	***	*	***	

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Table 3.16 Mean final uptake of water by seed-size categories of Sava snail medic and Trikkala subterranean clover held at different temperatures and recorded after 24 hours of testing.

Seed-size category	Temperature (°C)													
	2.0	5.1	8.0	10.4	12.2	14.2	16.0	17.6	19.6	21.7	24.2	26.7	30.0	34.5
	(% of dry weight)													
SAVA														
A	192	195	199	212	211	210	221	221	220	219	222	231	246	244
B	168	186	180	186	187	198	193	192	199	194	197	196	208	204
C	187	194	195	190	208	194	194	196	211	205	205	208	232	227
D	153	171	174	185	197	208	212	197	199	209	205	211	223	234
TRIKKALA														
A	149	145	147	144	149	147	151	150	154	151	149	152	150	145
B	128	139	146	147	146	147	149	149	149	147	146	149	151	153
C	114	138	142	142	142	141	144	143	144	143	145	145	145	142

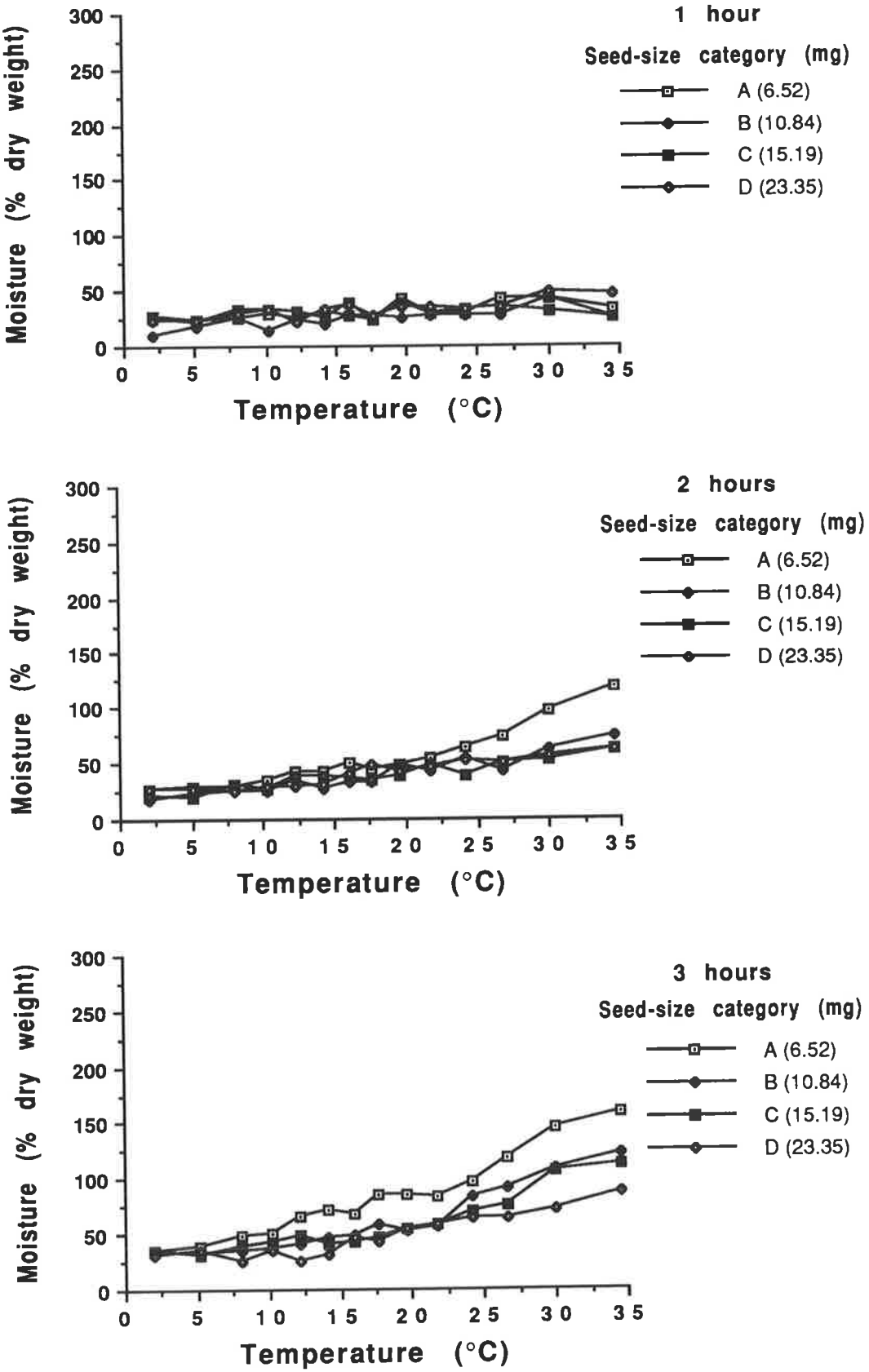


Figure 3.12 The water uptake by Sava snail medic seeds after 1, 2 and 3 hours of testing.

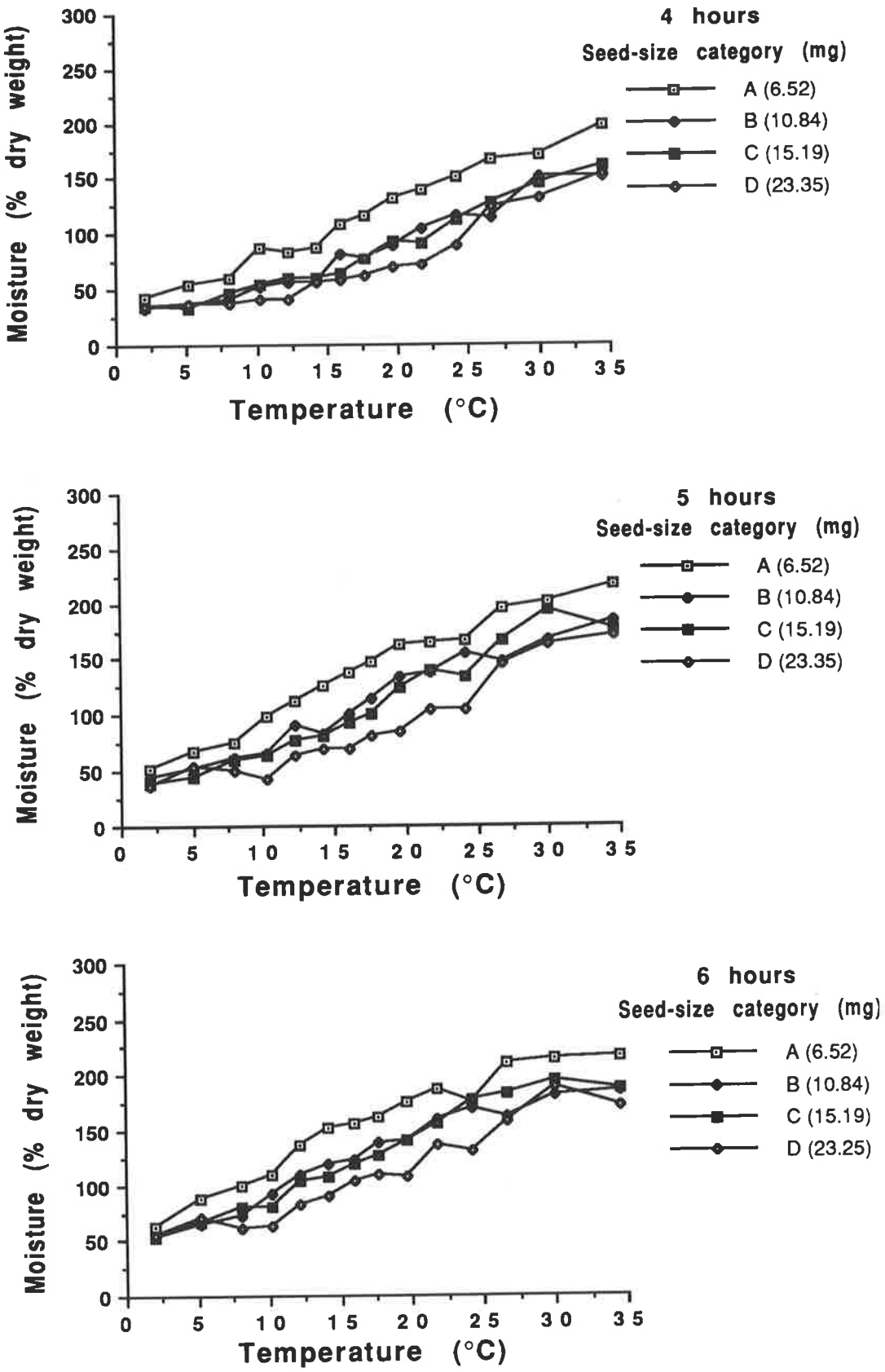


Figure 3.13 The water uptake by Sava snail medic seeds after 4, 5 and 6 hours of testing.

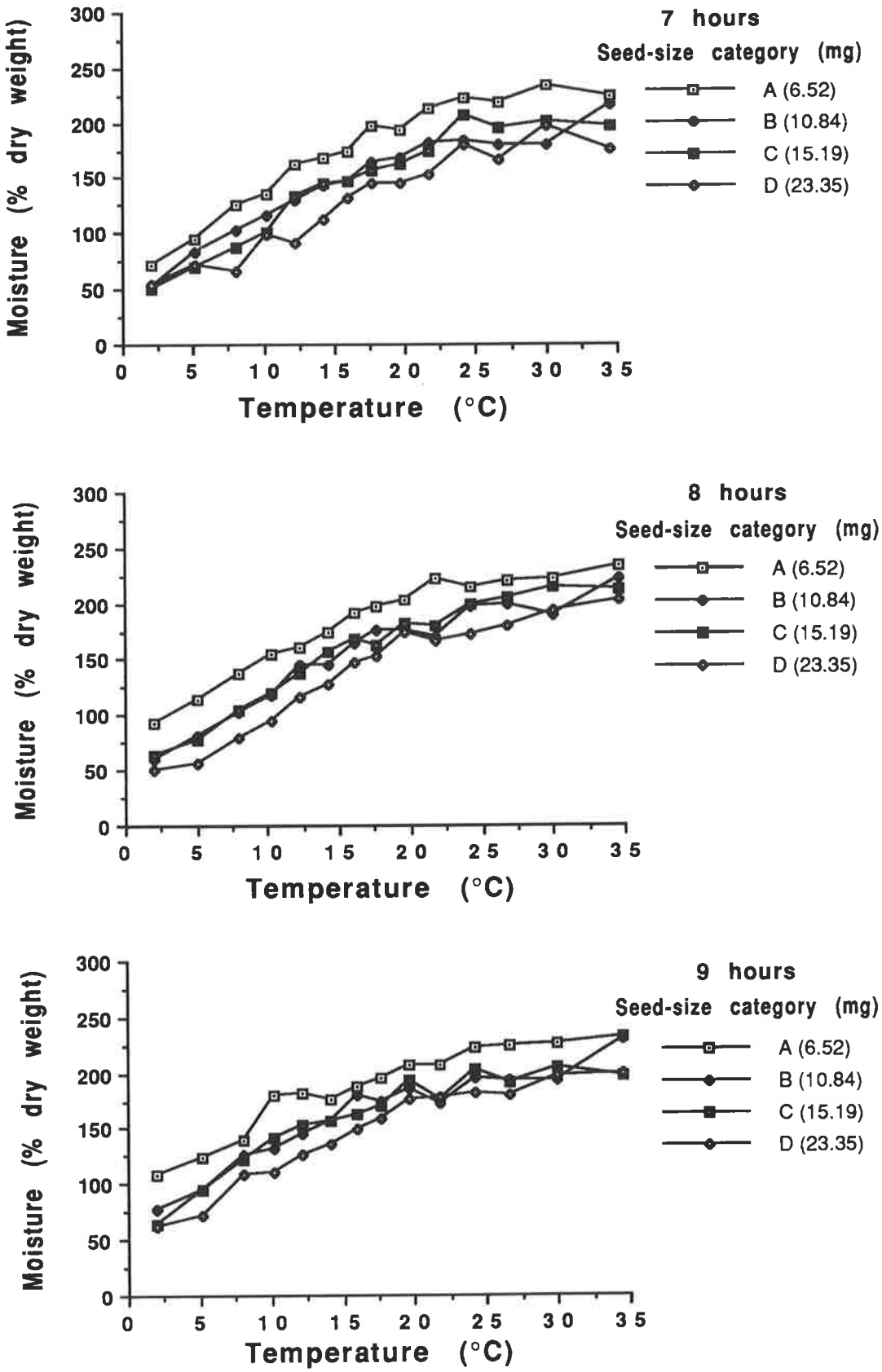


Figure 3.14 The water uptake by Sava snail medic seeds after 7, 8 and 9 hours of testing.

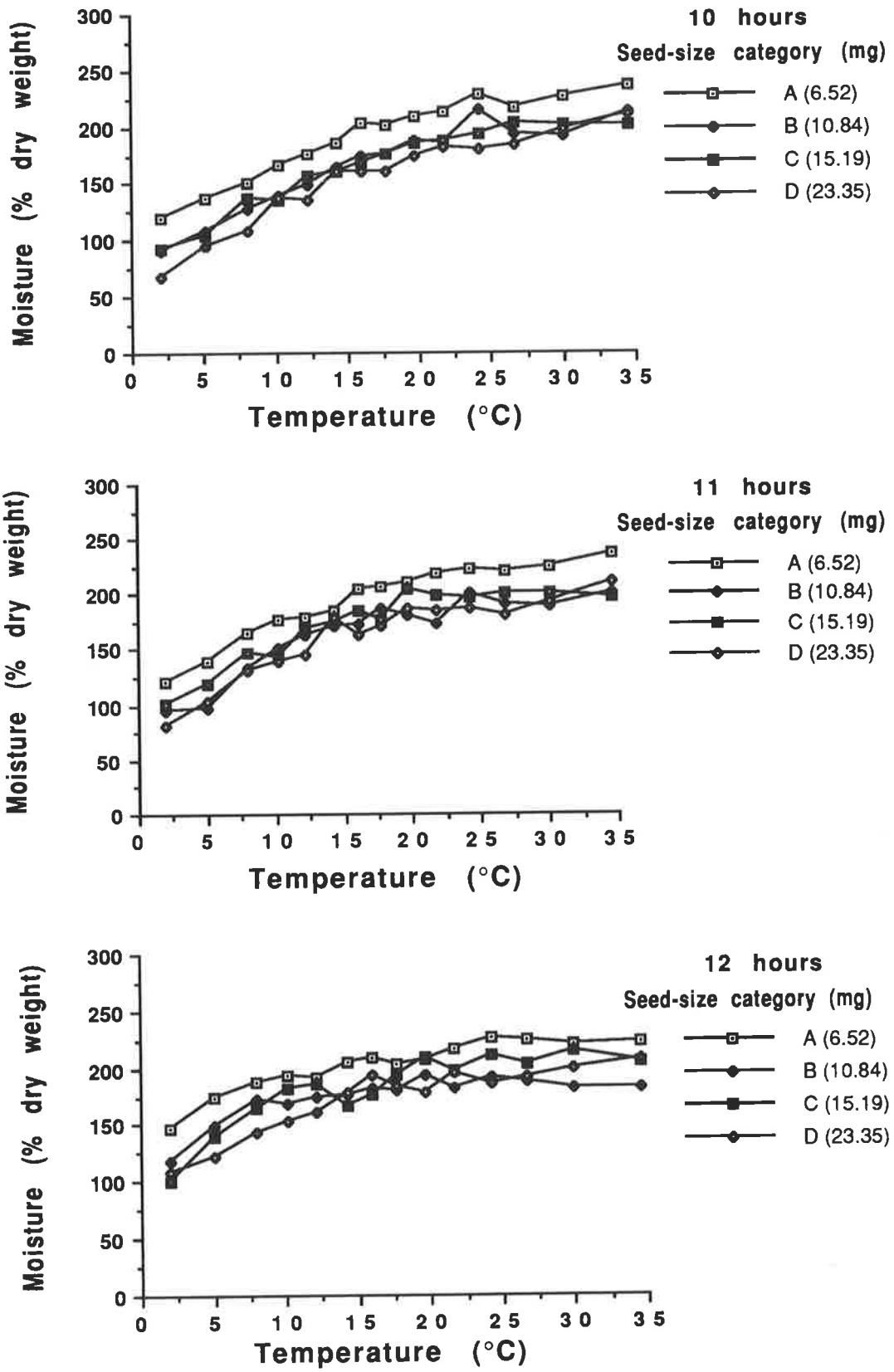


Figure 3.15 The water uptake by Sava snail medic seeds after 10, 11 and 12 hours of testing.

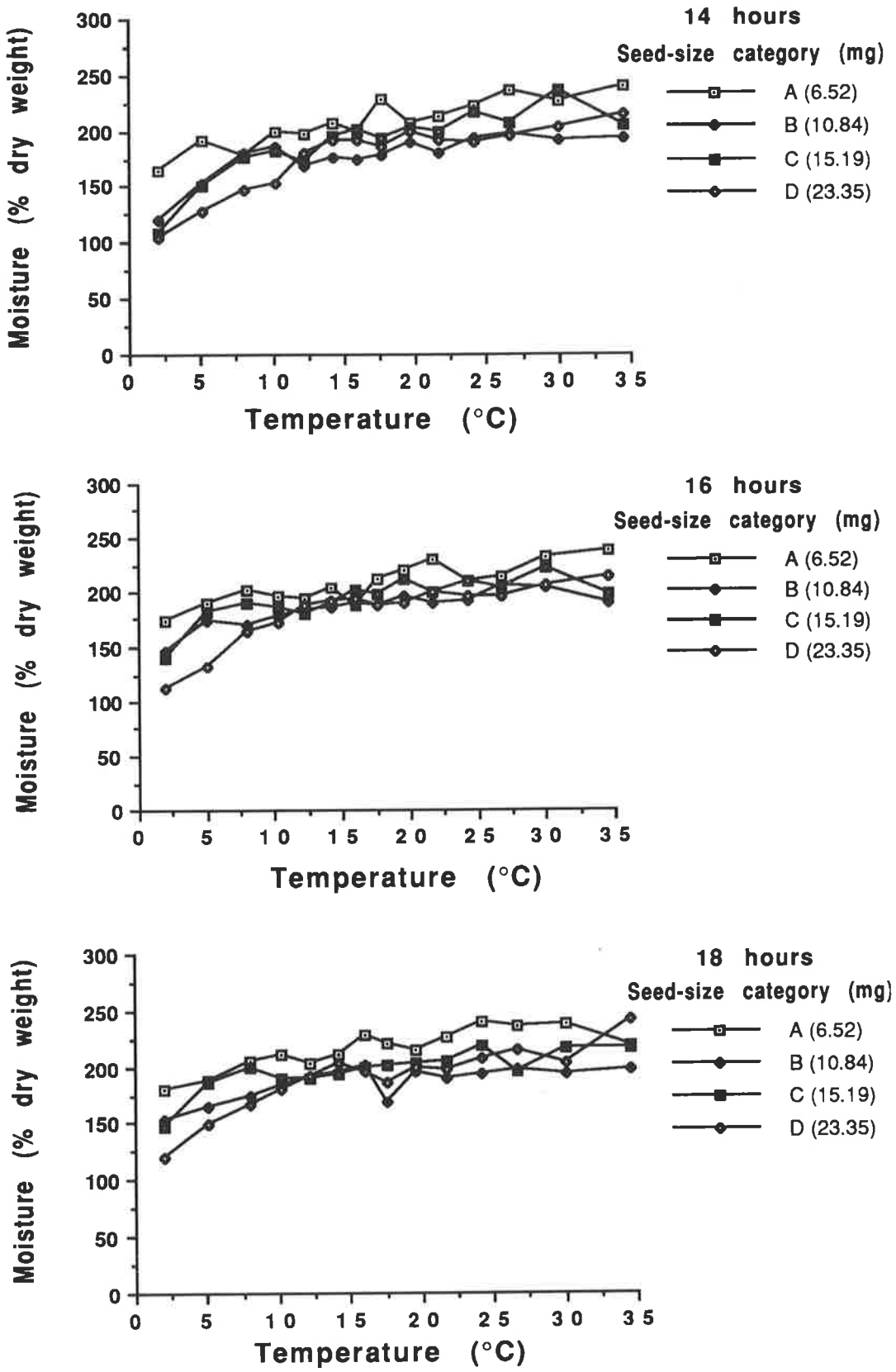


Figure 3.16 The water uptake by Sava snail medic seeds after 14, 16 and 18 hours of testing.

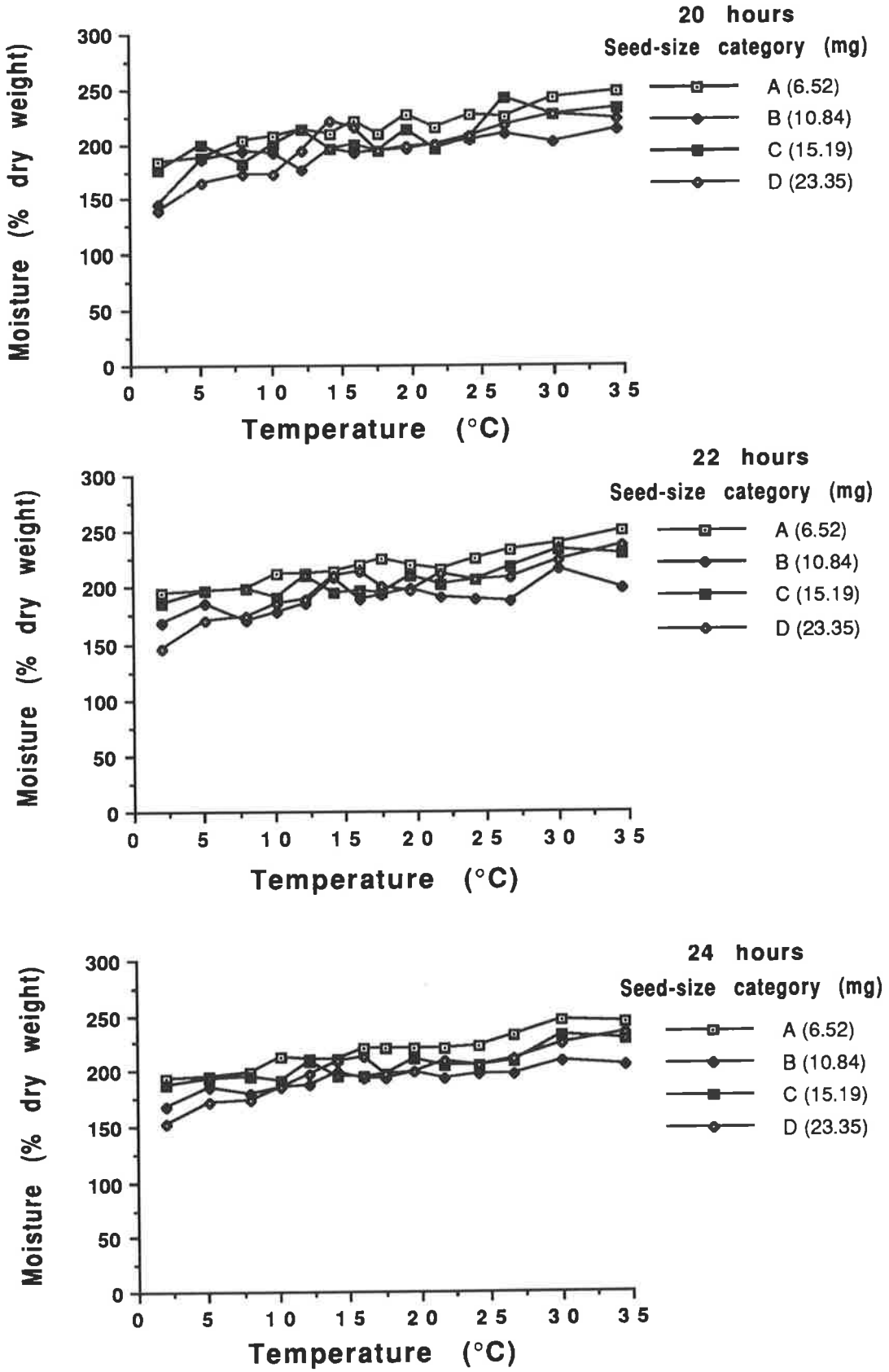


Figure 3.17 The water uptake by Sava snail medic seeds after 20, 22 and 24 hours of testing.

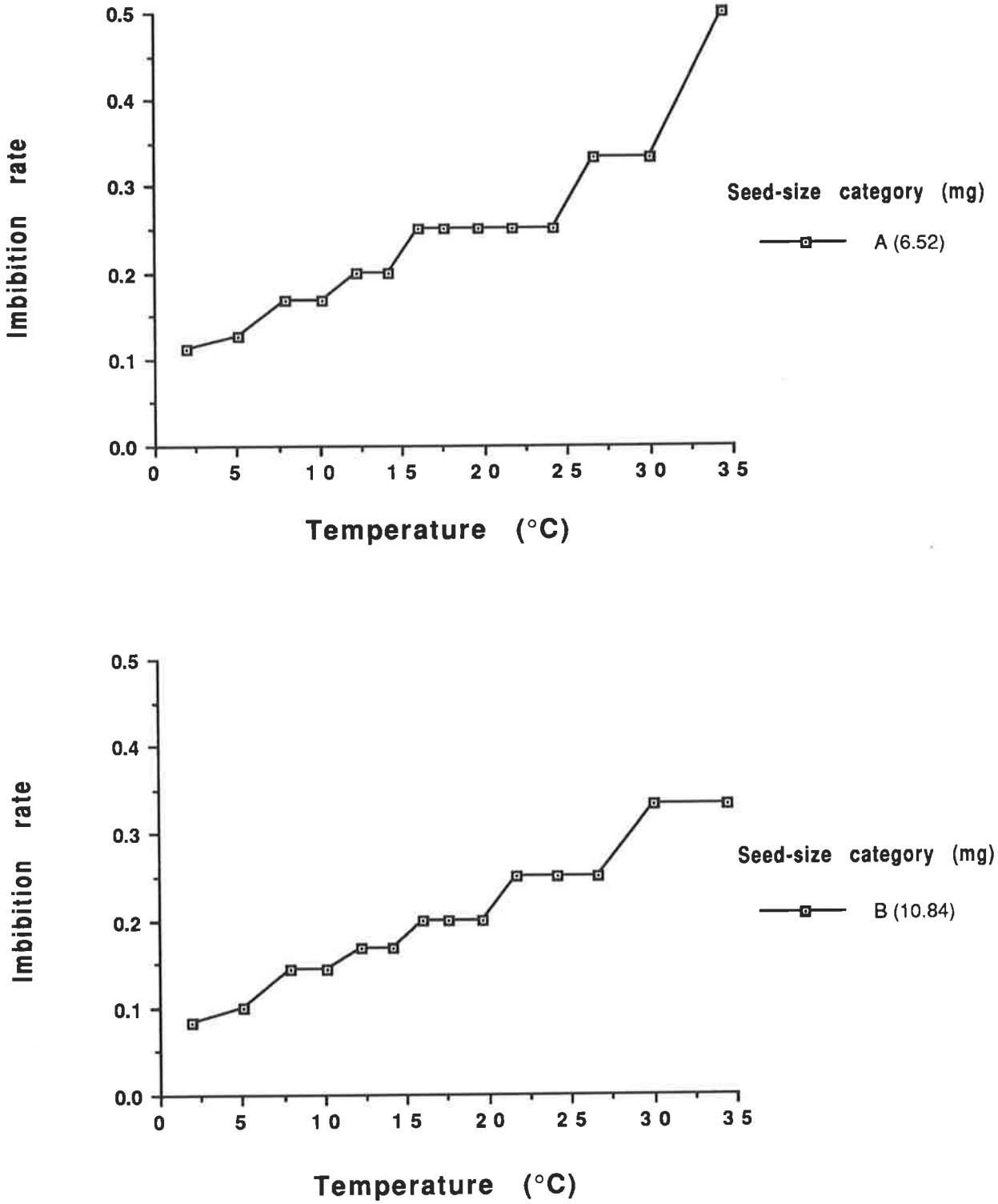
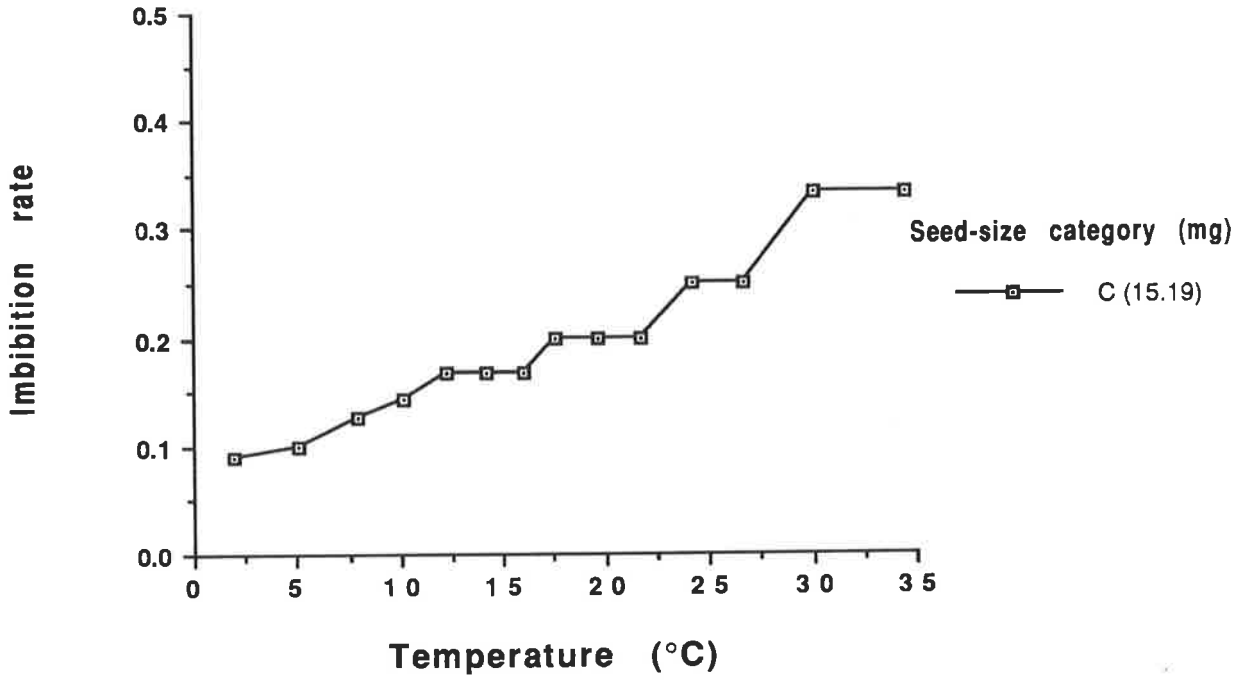


Figure 3.18 The imbibition rate (1/hours, double dry weight) of Sava snail medic seed-size categories A and B.



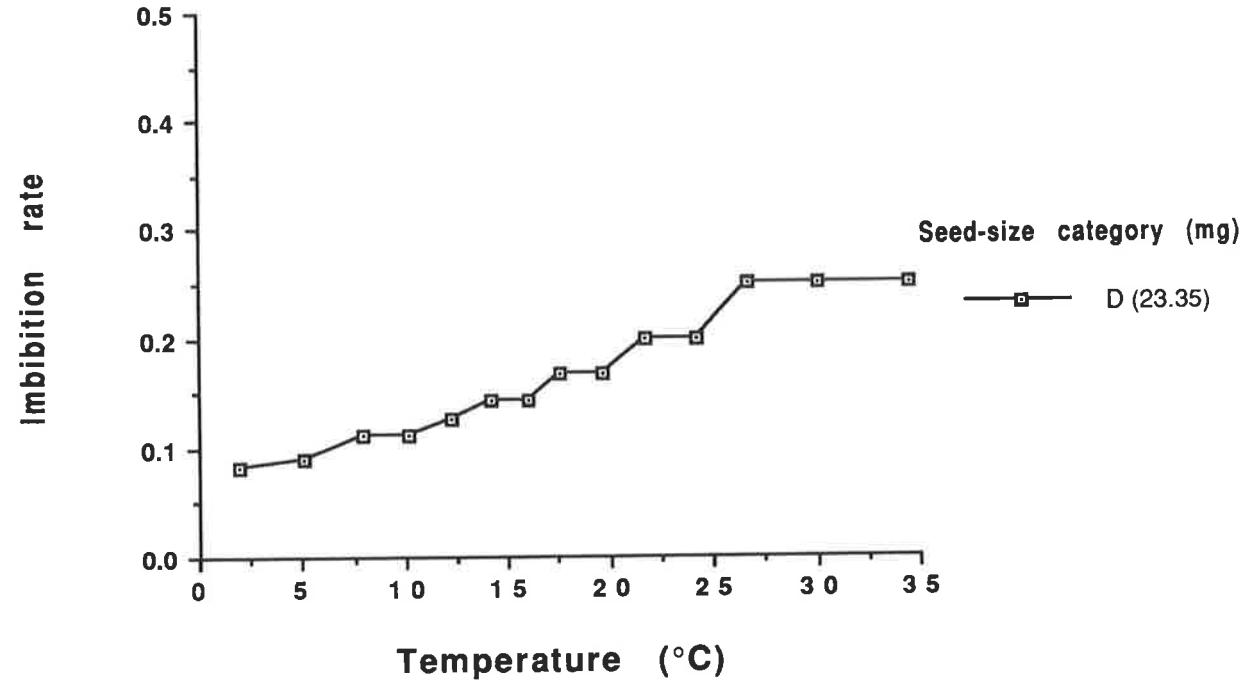


Figure 3.19 The imbibition rate (1/hours, double dry weight) of Sava snail medic seed-size categories C and D.

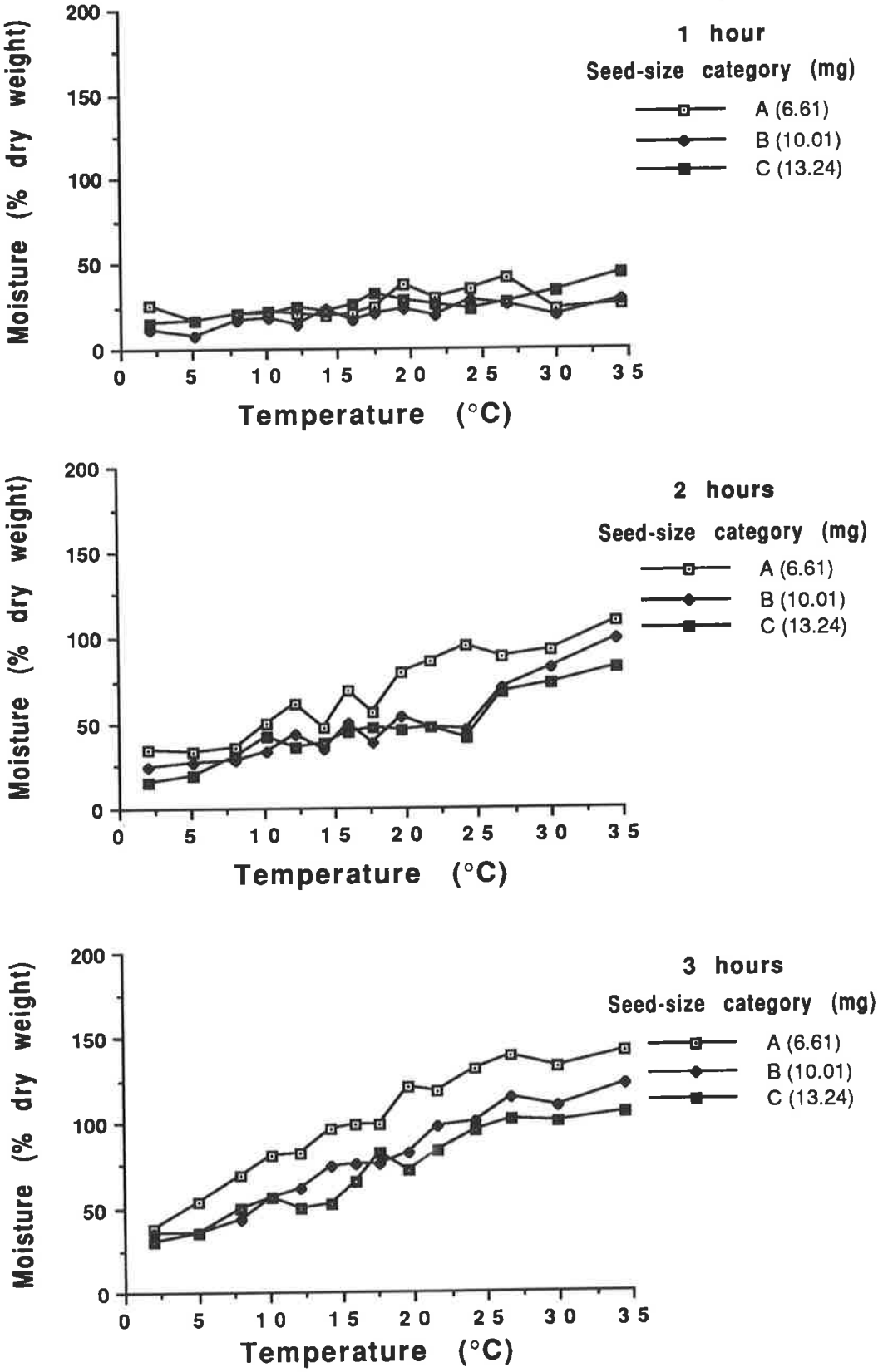


Figure 3.20 The water uptake by Trikkala subterranean clover seeds after 1, 2 and 3 hours of testing.

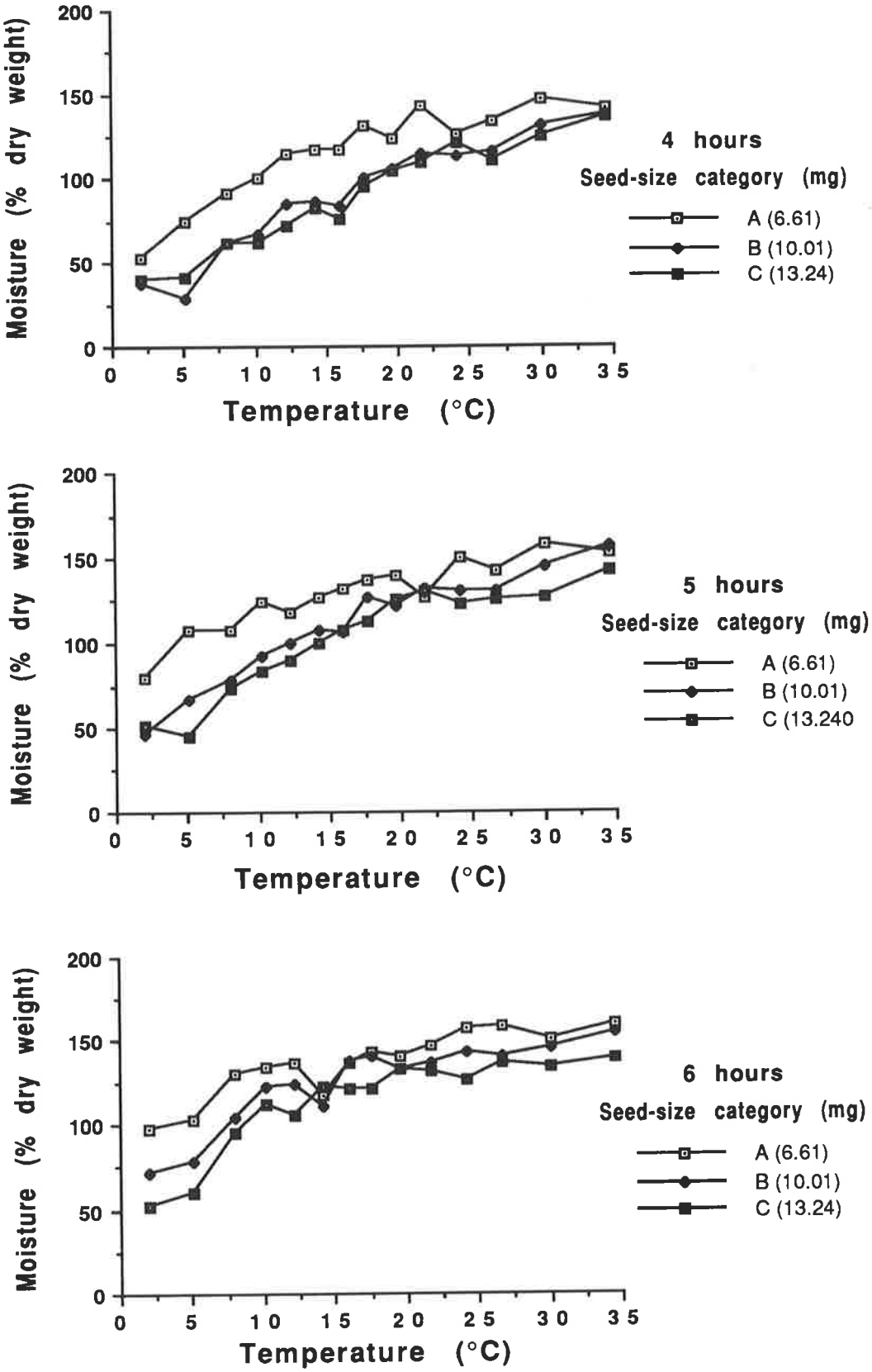


Figure 3.21 The water uptake by Trikkala subterranean clover seeds after 4, 5 and 6 hours of testing.

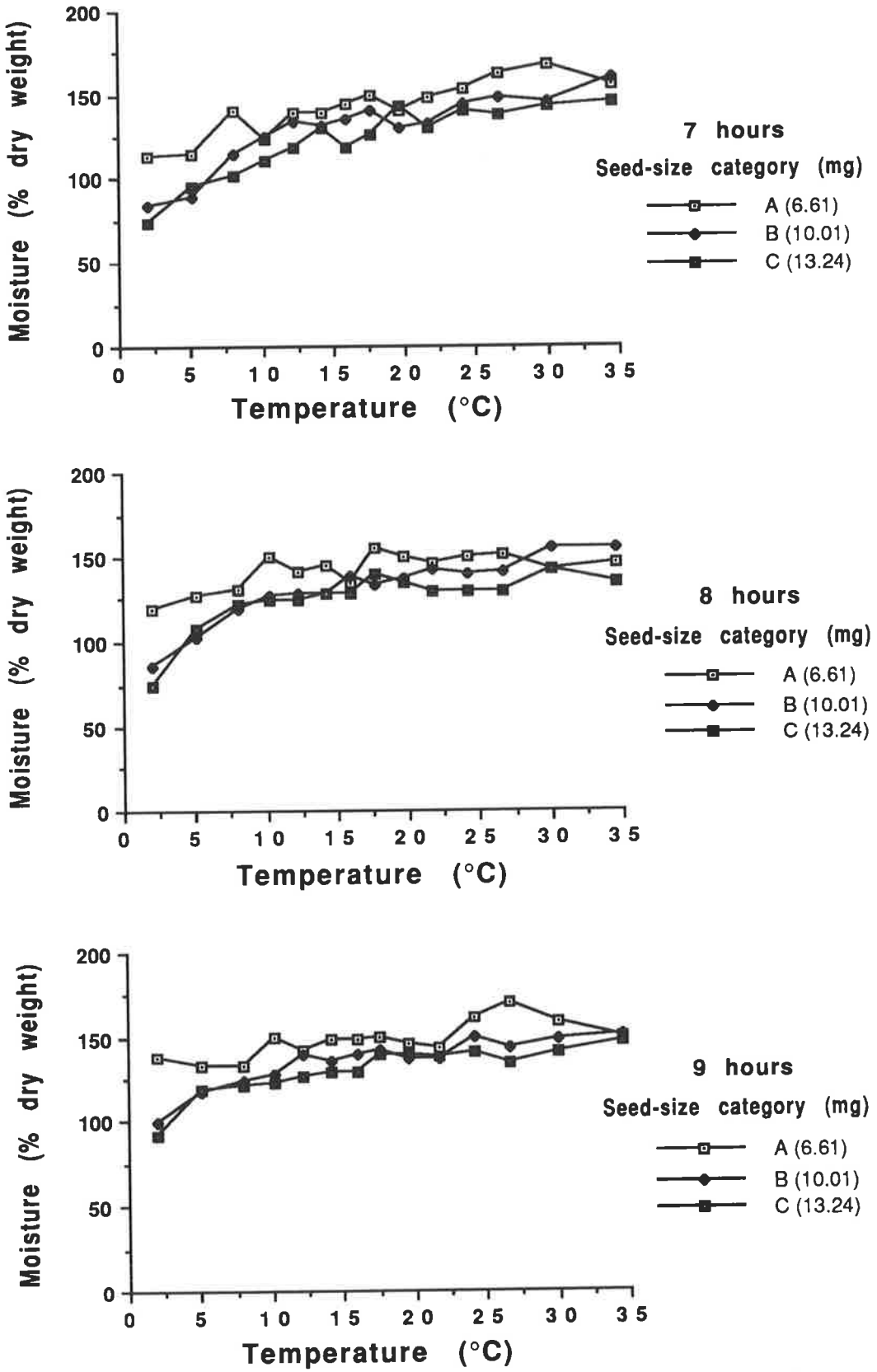


Figure 3.22 The water uptake by Trikkala subterranean clover seeds after 7, 8 and 9 hours of testing.

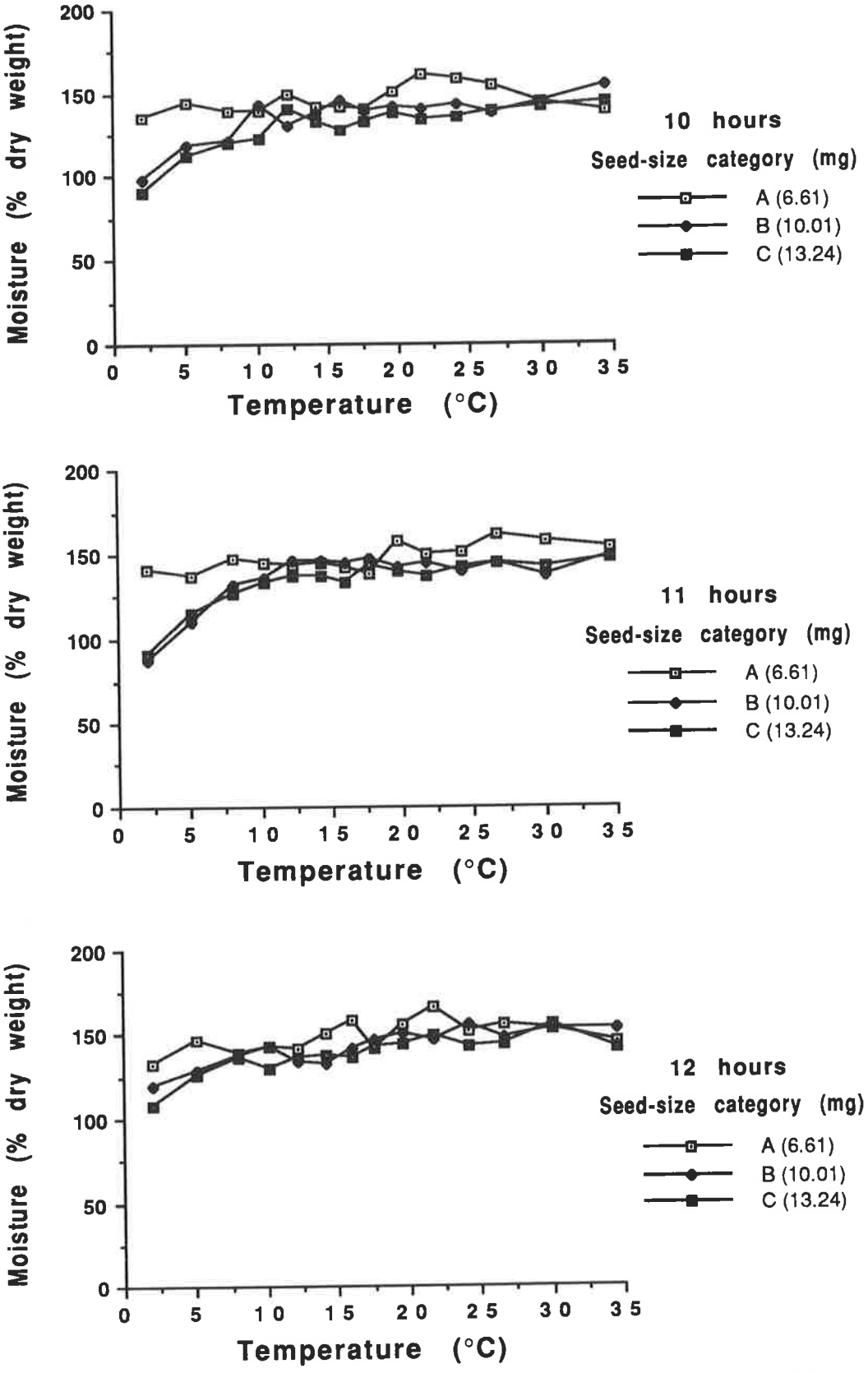


Figure 3.23 The water uptake by Trikkala subterranean clover seeds after 10, 11 and 12 hours of testing.

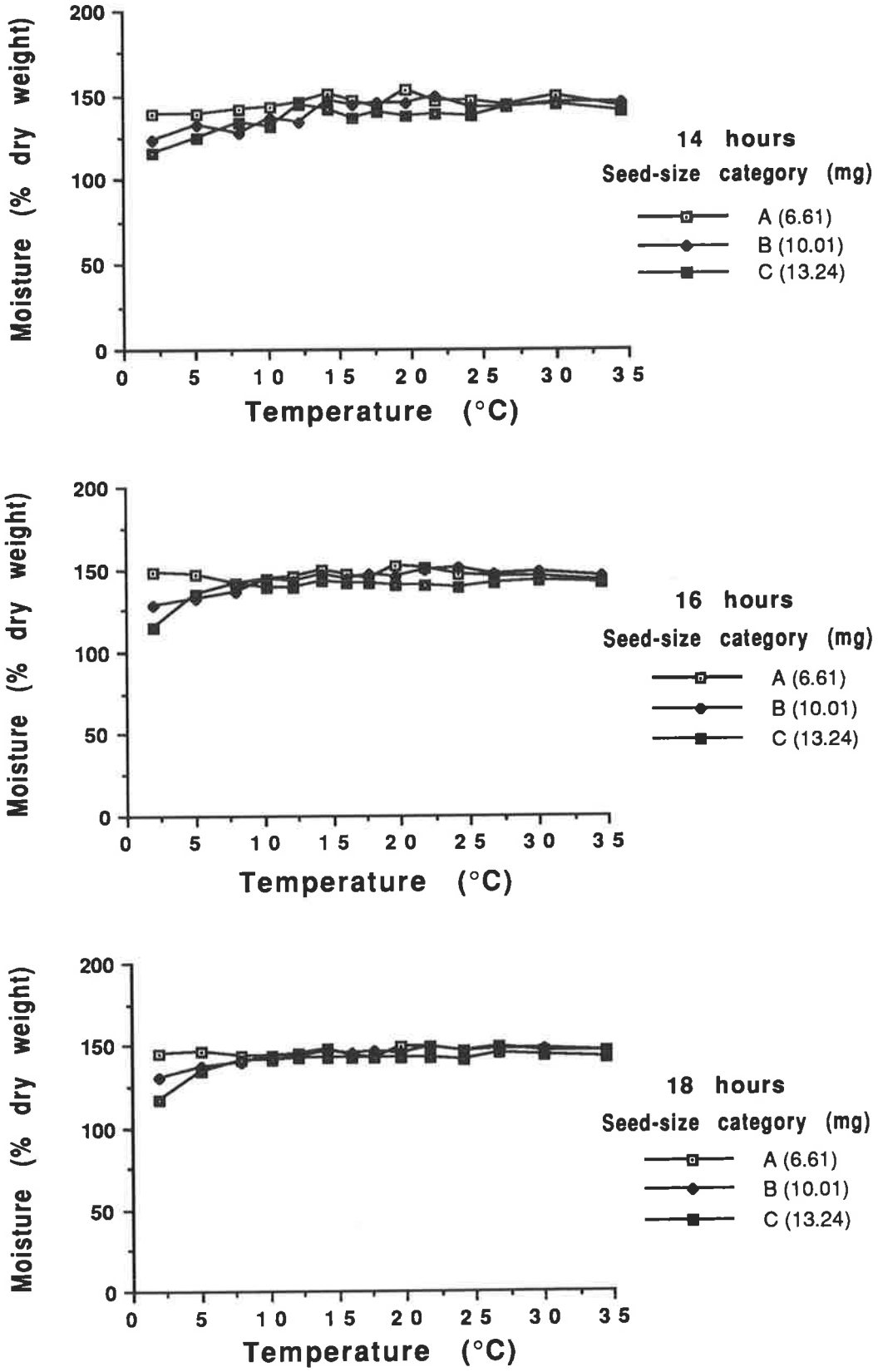


Figure 3.24 The water uptake by Trikkala subterranean clover seeds after 14, 16 and 18 hours of testing.

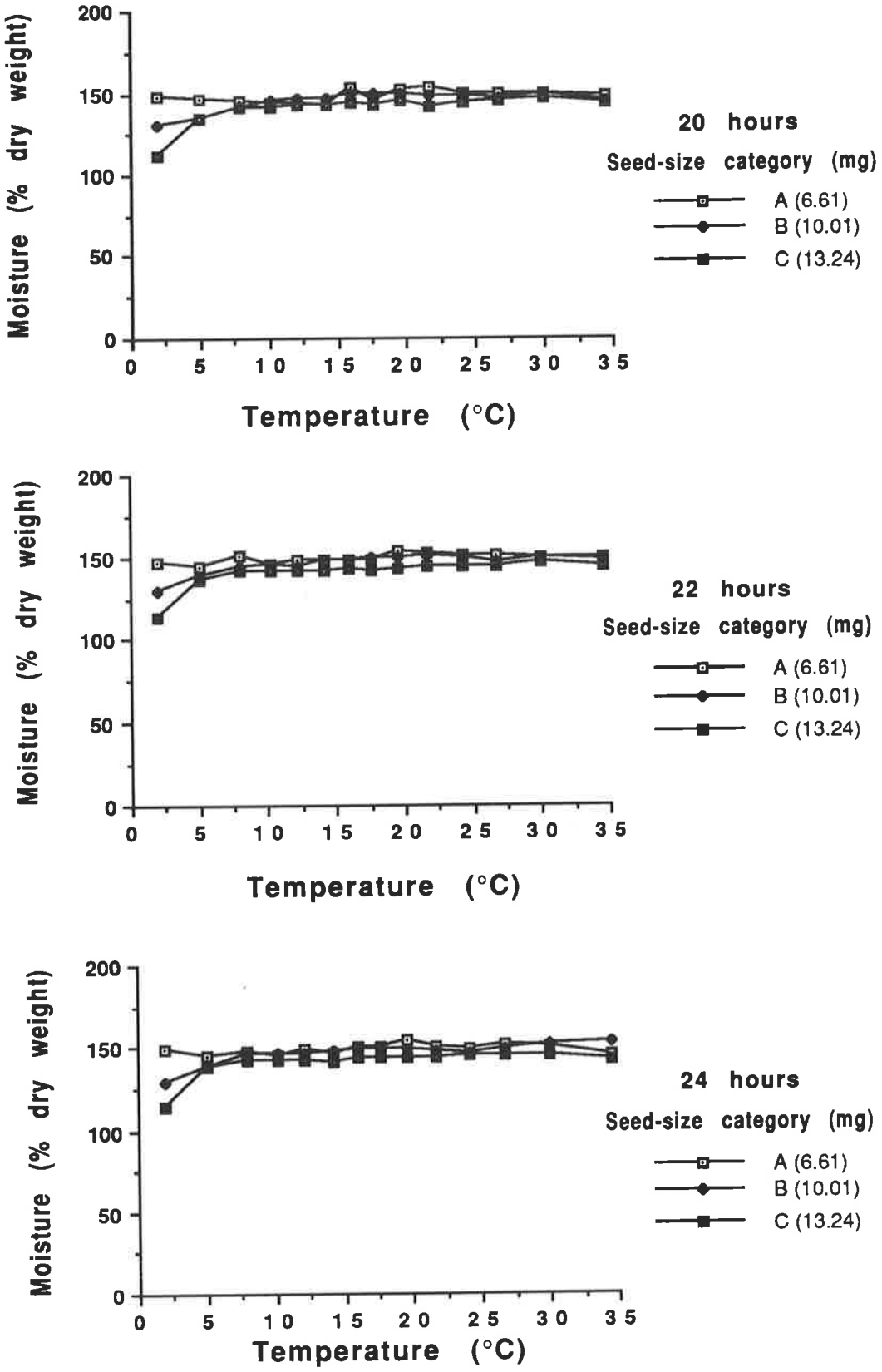


Figure 3.25 The water uptake by Trikkala subterranean clover seeds after 20, 22 and 24 hours of testing.

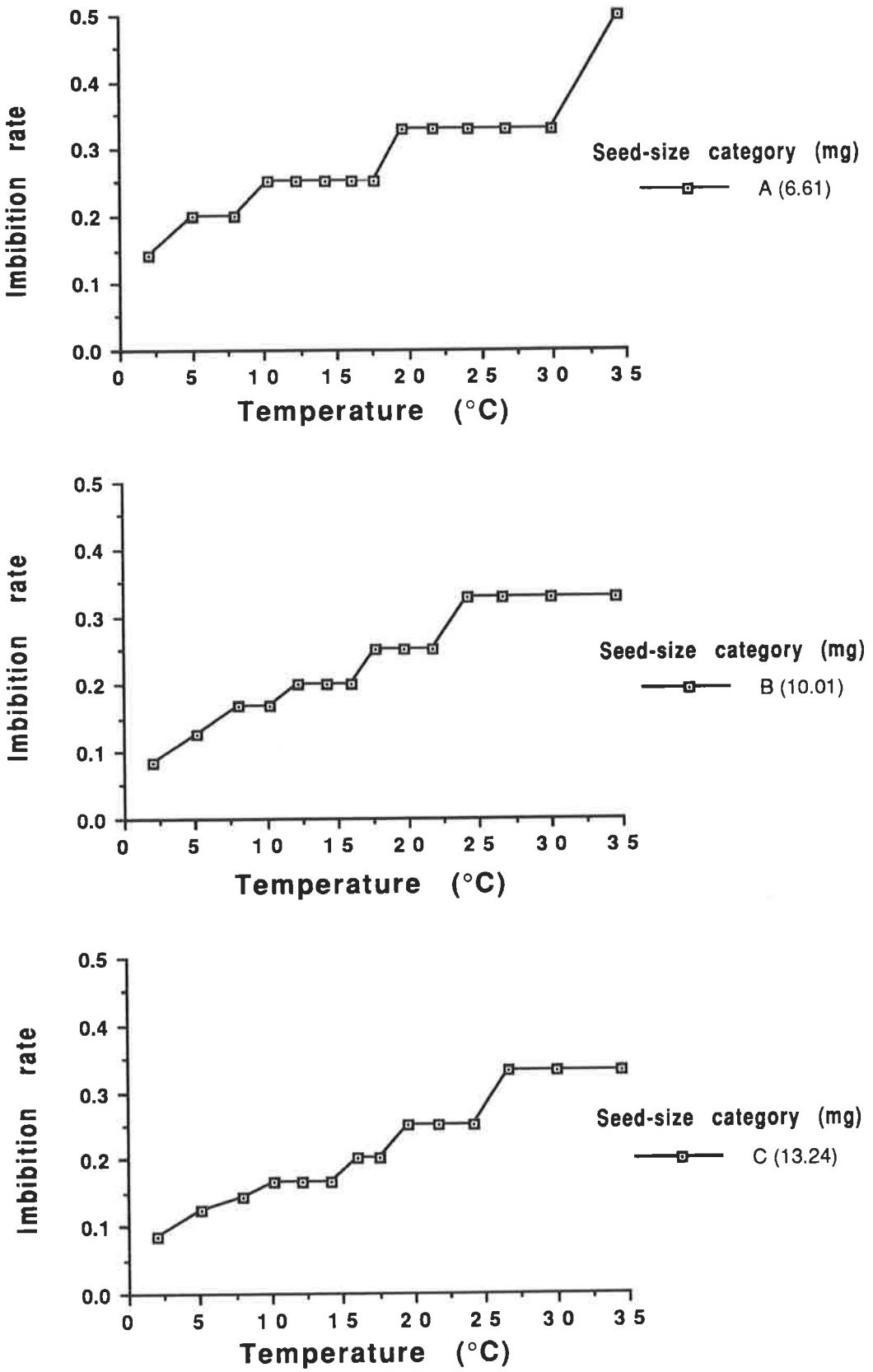


Figure 3.26 The imbibition rate (1/hours, double dry weight) of Trikkala subterranean clover seed-size categories A, B and C.

3.5 Laboratory Experiment 3: The effects of seed size, water uptake and subsequent drying on germination

3.5.1 Introduction

The occurrence of erratic pulses of rainfall during the summer-autumn period could be of particular importance for the survival of non-dormant pasture legume seeds. Because differences in the magnitude of the moisture uptake related to seed size were revealed in the previous experiments, it was decided to examine the ecological significance of wetting and drying treatments to seed germination. Alternate wetting and drying cycles occur naturally with such patterns of rainfall during summer and autumn.

3.5.2 Materials and methods

Three seed-size categories (A, B and C) for Sava and Trikkala were selected as before (Table 3.17). The seed was previously kept at 12.7 ± 0.64 °C (± s.d.) and then placed in a desiccator on active silica gel, 2 to 4 mm in size, for a period of 48 h before commencing the experiment (Appendices 3.12-3.13).

In order to ensure at least 100 readily-germinable seeds per treatment, sub-samples of 150 seeds of each cultivar were selected, based on a previous germination test carried out on each seed-size category under the same conditions described in 3.3 Laboratory Experiment 1 (Table 3.17). The corresponding lots of seed, replicated four times, were placed in Petri dishes, previously moistened with 15 ml of distilled water and placed at random within replicates in an incubator in the dark at 18.9 ± 0.3°C. (± s.d.) The sub-samples remained there for various times (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours). At each interval of time, one hundred swollen seeds were taken out, blotted with sorbent tissues, placed in plastic hermetic phials and weighed. The changes in seed weight due to water uptake (shown in Appendices 3.12-3.13) were calculated and expressed on an oven-dry seed weight basis, according to the oven-dry values observed

in Table 3.17 and obtained by the same method used in the previous test (3.4 Laboratory Experiment 2).

At the end of each 2 hour-period, the selected lots of one hundred seeds were enclosed in Manilla envelopes, dried in a forced draught dehydrator at 30°C for 24 h and weighed (Appendices 3.12-3.13). These pre-wetted lots of seeds and a control treatment with no prior wetting were again placed in Petri dishes that had been maintained at incubator temperature and remoistened as required. The number of germinated seeds, based on the length of the radicle, was recorded every twelve hours for a period of 14 days. The percentage germination was adjusted by the initial percentage of readily-germinable seed and the germination rate calculated as the reciprocal value of time (days) required to obtain a 90% of germination.

In order to analyse the corresponding responses in germination, analyses of variance were carried out by the use of the program GENSTAT V. First, the incremental increases of germination over time were used, then the analyses of variance were run independently for each interval of time of evaluation by using the cumulative values of germination obtained until seven days of testing, at which time most of the response in germination was achieved. No further transformation of the data was required.

3.5.3 Results

The analysis of variance, made on partial increases in germination recorded every 12 h during the testing period, reveals a significant effect of species, wetting period and seed size on final germination. However, non-significant interactions were observed between factors. When time of germination was included in the analysis, it was also highly significant, as well as the corresponding interactions with the variables involved in the study. The only exception was the interaction: Germination time x Wetting period x Seed size (Table 3.18). Analyses of variance, made on cumulative values of germination at each time of evaluation until the seventh day of testing, showed significant effects of the wetting period, seed size, and the interaction: Species x Wetting period and Species

x Seed size. This was largely during the first three days of testing when the most relevant changes in germination were observed (Appendix 3.14).

The pre-wetting period revealed differences between species and among seed-size categories within species in the rate of water uptake by seeds and in the total amount of water that seeds were able to store at the end of the 24 h soaking period. The smallest seeds, as reported before, showed a faster and higher level of hydration than the largest seeds (Figure 3.27).

The seeds of both species started to germinate within the first 12 hours only after pre-wetting periods of at least eight and six hours were imposed in Sava and Trikkala seeds, respectively (Appendices 3.15-3.16). The final germination obtained after 14 days of testing was not affected by the pre-wetting periods, resulting in higher values of germination independent of the species, or the seed-size categories used (Appendix 3.17).

Wetting the seed for increased periods of time before drying and rewetting resulted in, after 12 h of testing, a progressive rise in the rate of germination of both species. Up to 24 h of testing, a sustained trend of increased germination in time occurred through lowered periods of pre-wetting. After that, an irregular pattern of germination was observed (Figures 3.28-3.33; Appendices 3.15-3.16).

When the rate of germination was expressed as the reciprocal value of the time in days required to obtain 90% of germination (Figures 3.34-3.35), higher rates of germination were found in smaller seed of Sava snail medic than in larger ones. In contrast, larger seeds of Trikkala subterranean clover (in both intermediate and large size categories), showed higher germination rates than the smallest seed-size category during most of the pre-wetting period.

3.5.4 Discussion

In a Mediterranean-type climate, during the summer-autumn period, seeds of annual pasture species that are readily-germinable (i.e. not constrained by hard-seededness or dormancy) are sometimes exposed to irregular patterns of rainfall. The natural process of wetting and drying of the seed occurs and some degree of stimulation of the germination rate in a subsequent germination may be expected. This phenomenon is particularly important in the context of available moisture conditions early in the growing season when more non-dormant seed is ready for germination (Cocks and Donald 1973).

In the present study, the drying treatment imposed on Sava snail medic and Trikkala subterranean clover seeds after various periods of pre-wetting was coincident with the desiccation-tolerant state of the embryo when the viability of the seed is not affected and the rate of germination increased by longer periods of soaking prior to dehydration (McKersie and Stinson 1980; McKersie and Tomes 1980).

The increased rates of germination were a consequence of the initiation of the physiological processes for germination during the soaking period; the subsequent drying of the seed does not alter the metabolic stage achieved (Hegarty 1978; Bewley and Black 1982).

Differences in the rate of germination between species and particularly among seed-size categories within species, suggest that at each particular dehydration period, advanced physiological stages were achieved as a consequence of the characteristic of the seed and its ability to become hydrated during the pre-wetting stage. However, it seems that some instances of the drying treatment on each seed-size category are responsible for metabolic changes resulting in increased and decreased rates of germination.

The highest rates of germination were associated with smaller seed sizes and were most apparent in Sava snail medic. This should be an additional advantage for an early germination when the season break is not followed by drought.

Table 3.17 Characteristics of the seed-size categories used to test the effects of seed size and soaking and drying on subsequent germination of Sava snail medic and Trikkala subterranean clover.

Sieve aperture Min. - Max. (mm)	Seed-size category	Fresh seed weight (mg)	ratio	Oven dry seed weight (mg)	ratio	Moisture content (%)	Readily germinable seed (%)
	SAVA						
1.71 - 2.00	A	10.36	1.00	9.75	1.00	5.89	76
2.01 - 2.36	B	15.22	1.47	14.29	1.47	6.11	81
2.37 - 2.80	C	23.53	2.27	22.21	2.28	5.61	84
	TRIKKALA						
1.71 - 2.00	A	6.70	1.00	6.26	1.00	6.57	81
2.01 - 2.36	B	9.97	1.49	9.37	1.50	6.02	82
2.37 - 2.80	C	13.47	2.01	12.57	2.01	6.68	87

Table 3.18 Summary of analysis of variance made on increasing values of germination with time.

Treatment	Significance levels †
Species	***
Wetting period	*
Seed size	*
Species x Wetting period	NS
Species x Seed size	NS
Wetting period x Seed size	NS
Species x Wetting period x Seed size	NS
Germination time	***
Germination time x Species	***
Germination time x Wetting period	***
Germination time x Seed size	***
Germination time x Species x Wetting period	***
Germination time x Species x Seed size	***
Germination time x Wetting period x Seed size	NS
Germination time x Species x Wetting period x Seed size	***

† NS = Not significant; * = $P<0.05$; *** = $P<0.001$

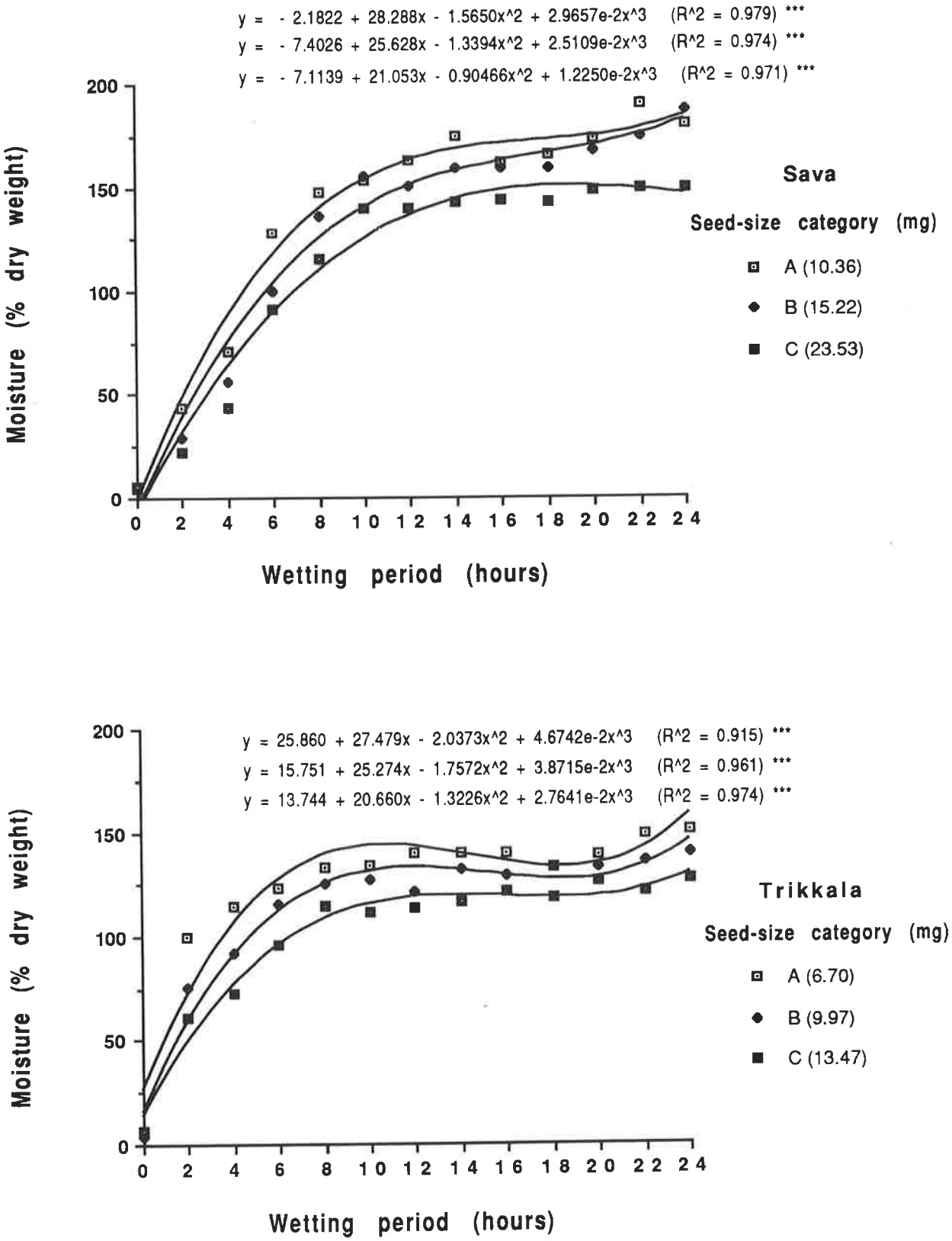


Figure 3.27 The Moisture content of Sava snail medic and Trikkala subterranean clover seed-size categories submitted to different wetting periods.

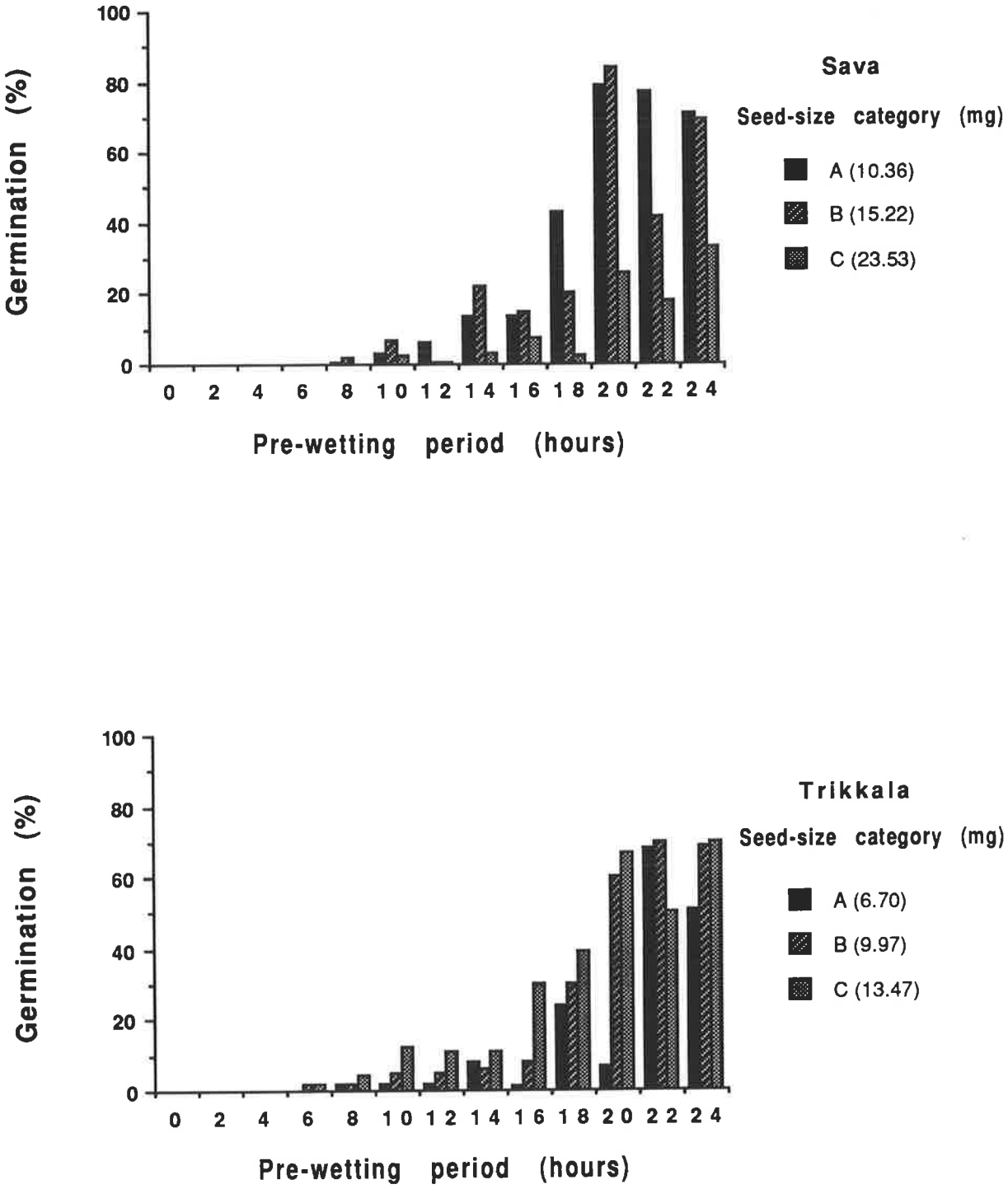


Figure 3.28 Partial germination from 0-12 h of Sava snail medic and Trikkala subterranean clover seeds submitted to different pre-wetting periods.

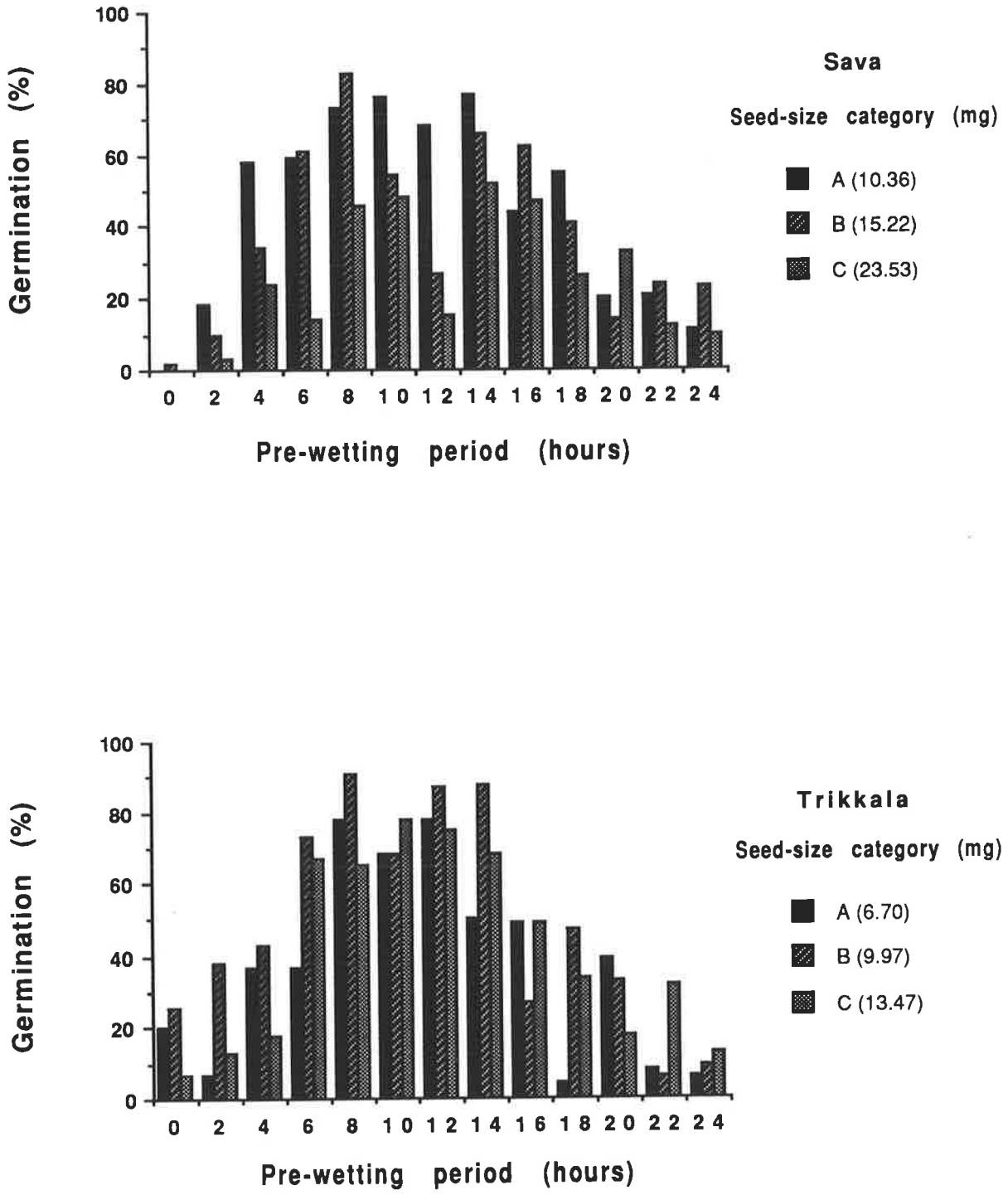
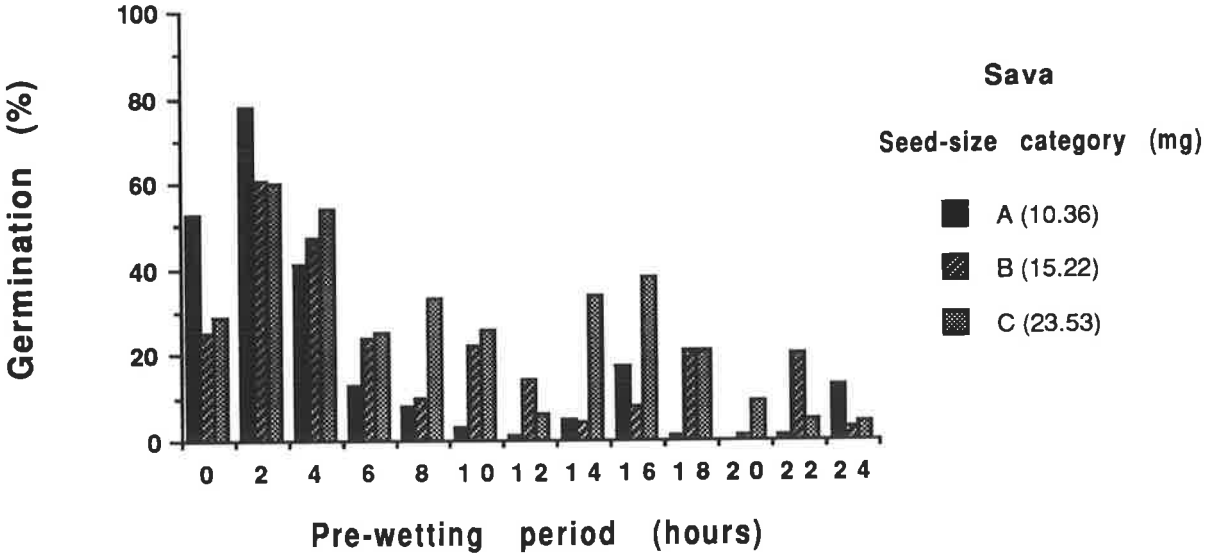


Figure 3.29 Partial germination from 12-24 h of Sava snail medic and Trikkala subterranean clover seeds submitted to different pre-wetting periods.



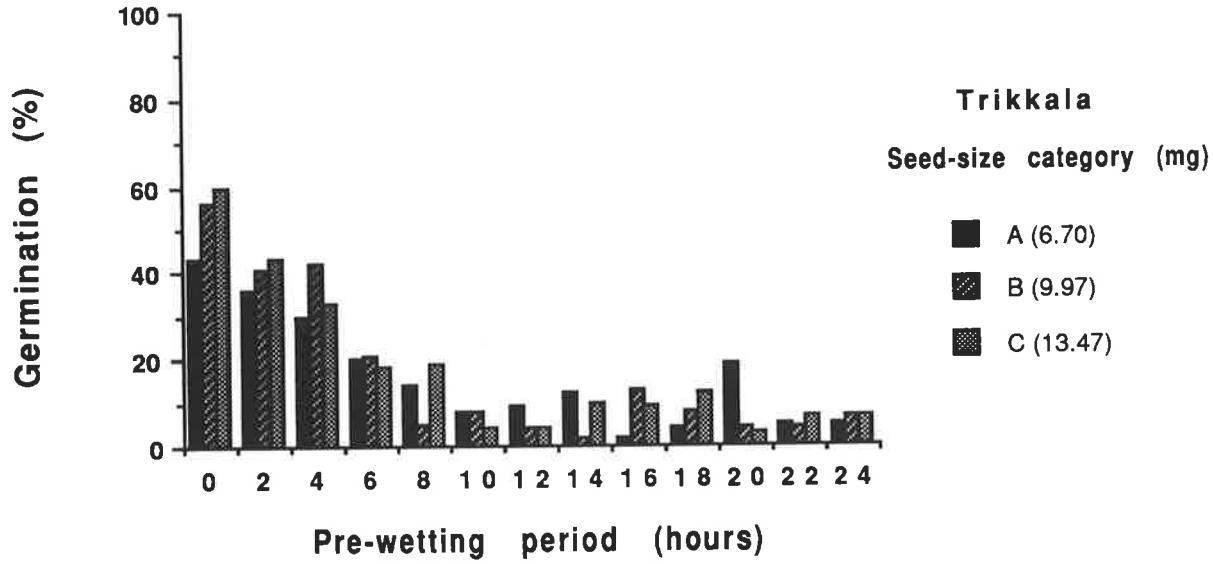


Figure 3.30 Partial germination from 24-36 h of Sava snail medic and Trikkala subterranean clover seeds submitted to different pre-wetting periods.

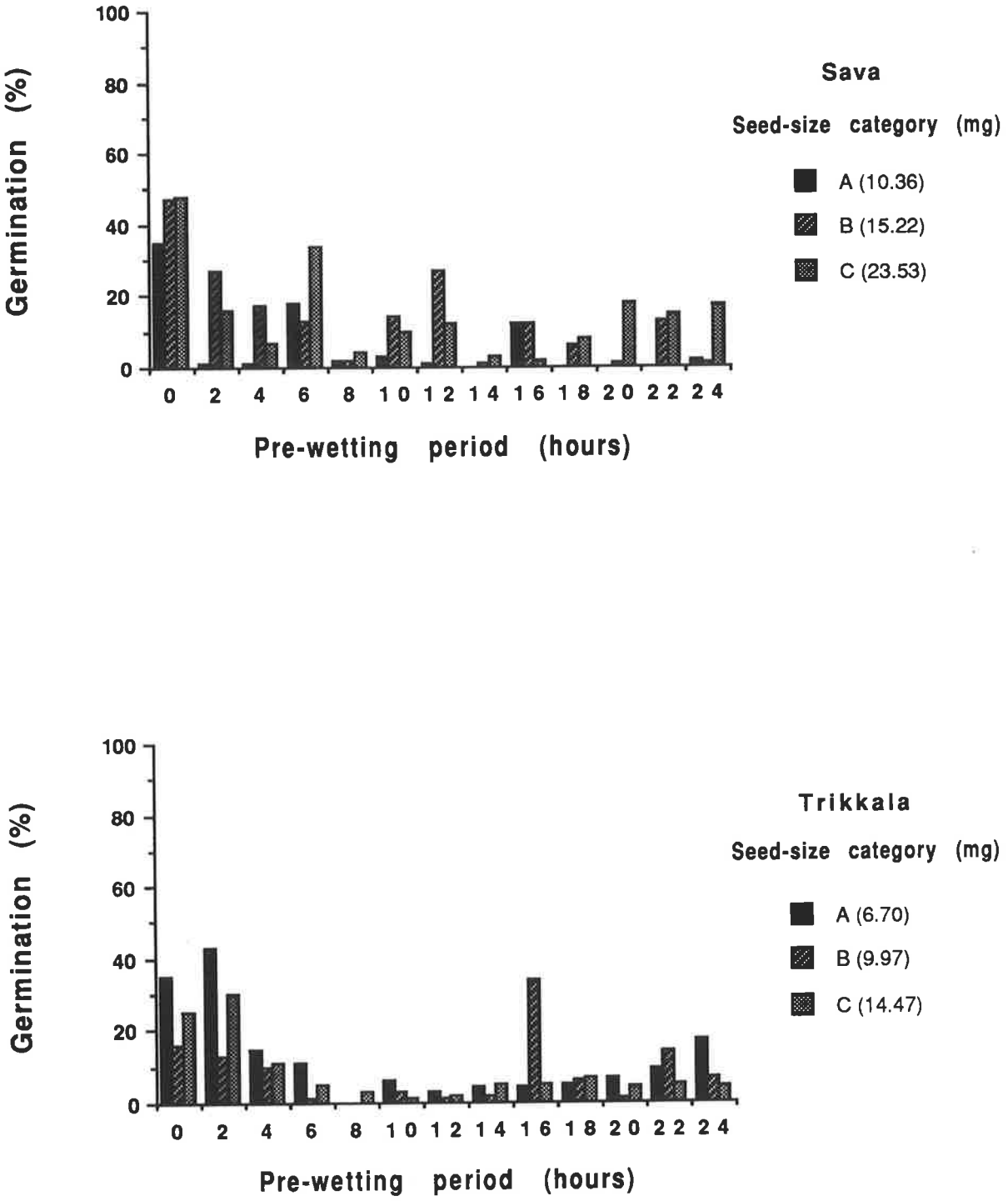


Figure 3.31 Partial germination from 36-48 h of Sava snail medic and Trikkala subterranean clover seeds submitted to different pre-wetting periods.

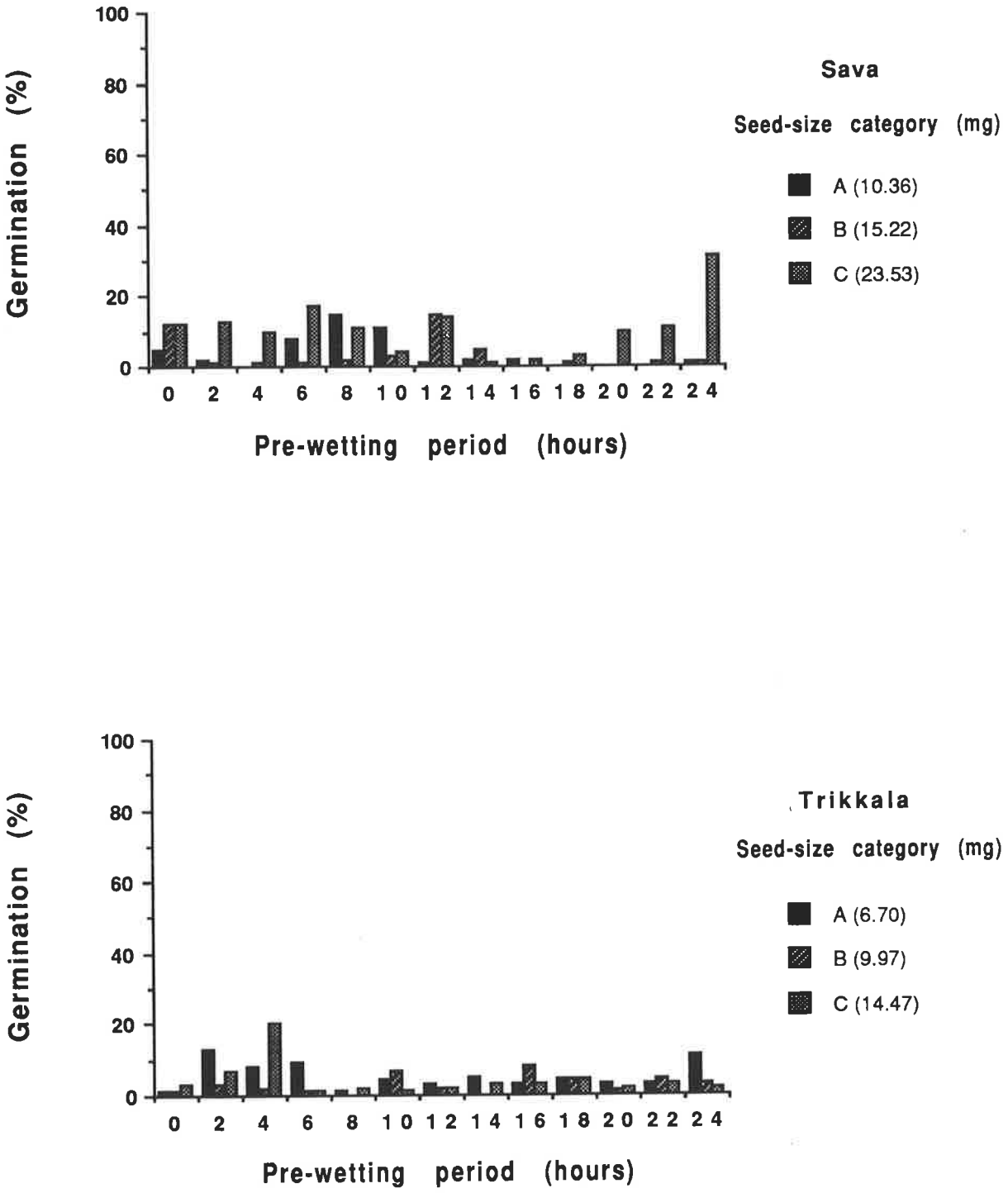


Figure 3.32 Partial germination from 48-60 h of Sava snail medic and Trikkala subterranean clover seeds submitted to different pre-wetting periods.

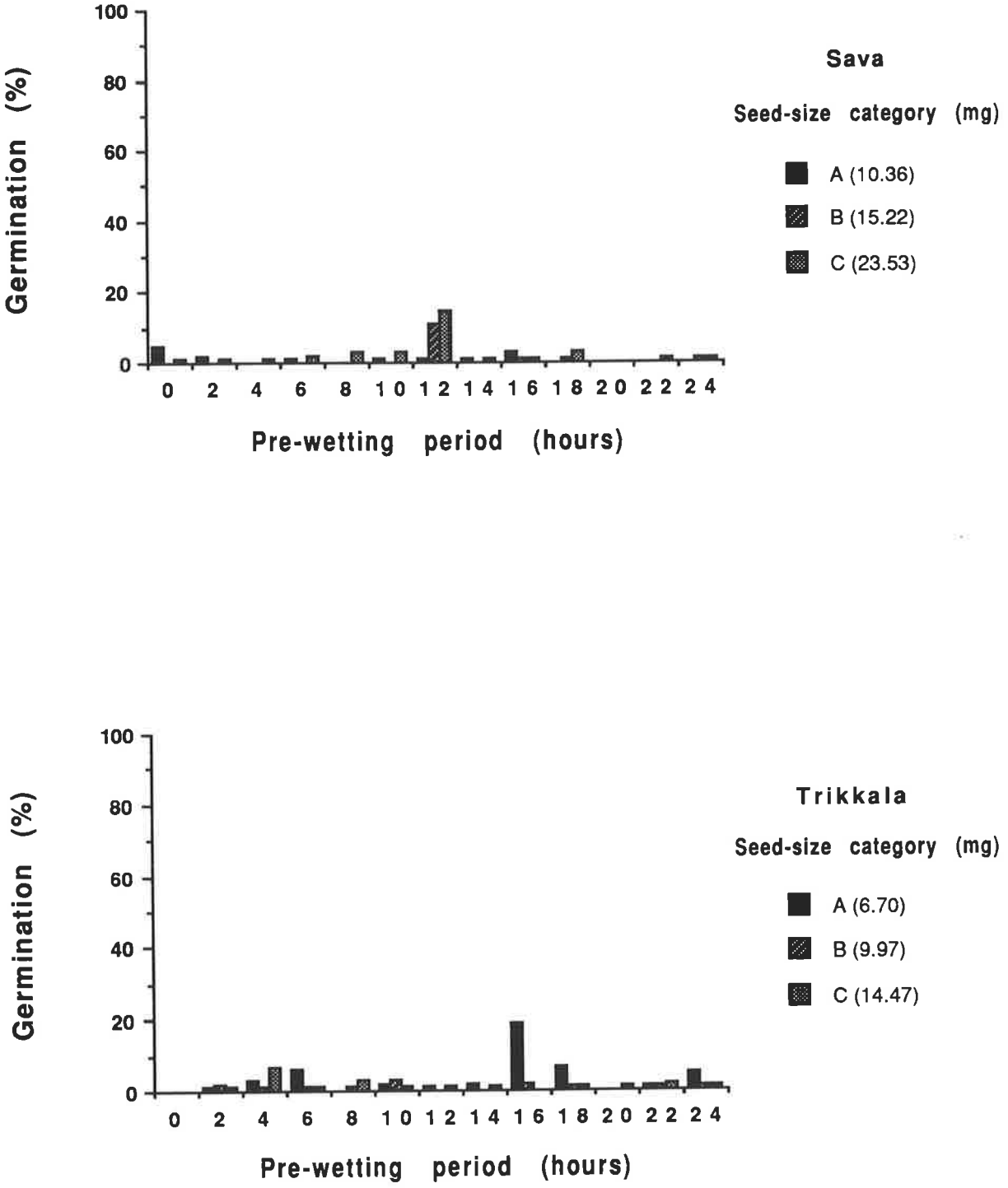


Figure 3.33 Partial germination from 60-72 h of Sava snail medic and Trikkala subterranean clover seeds submitted to different pre-wetting periods.

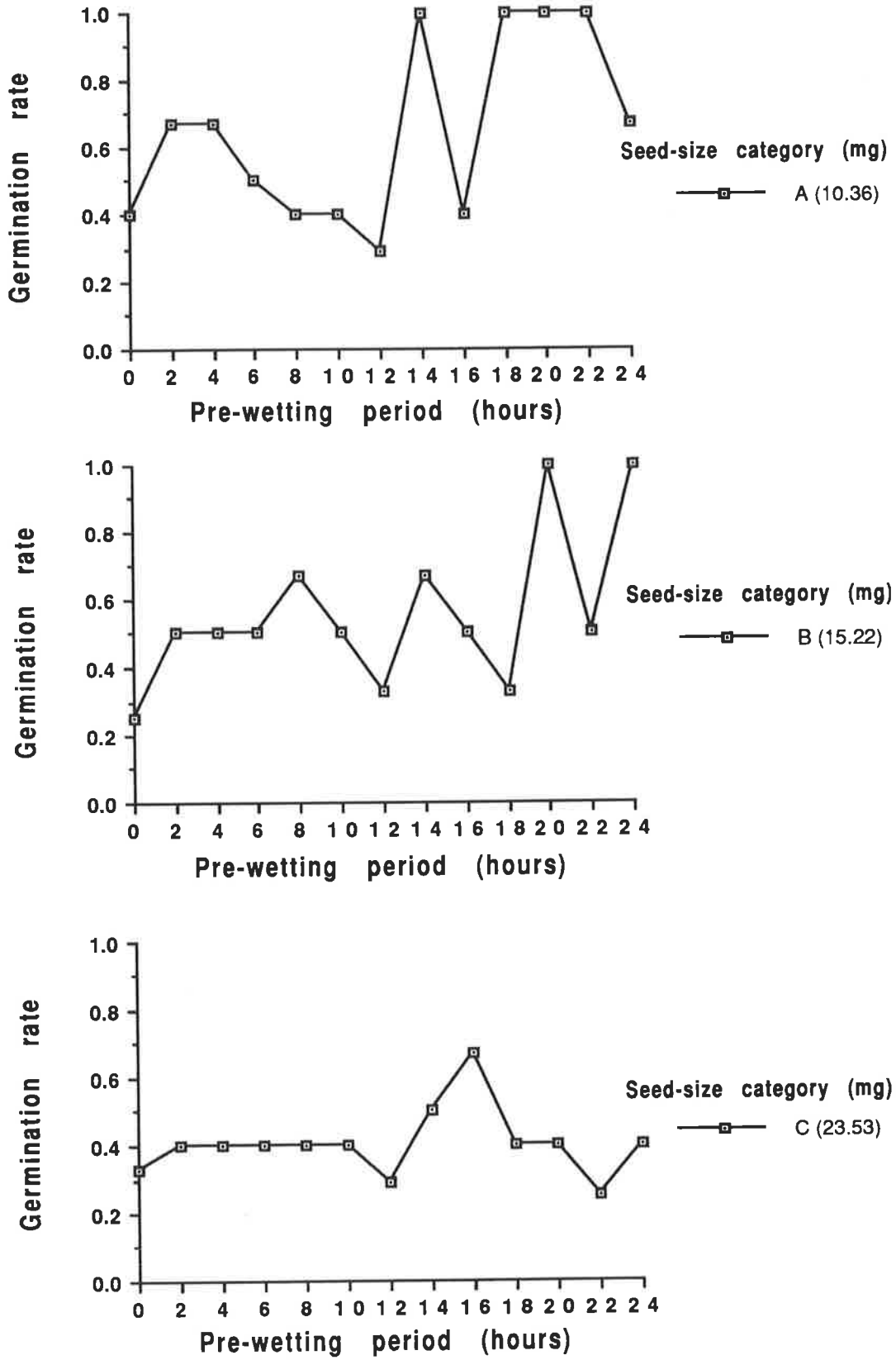


Figure 3.34 The germination rate (1/days, 90% germination) of Sava snail medic seeds submitted to different pre-wetting periods.

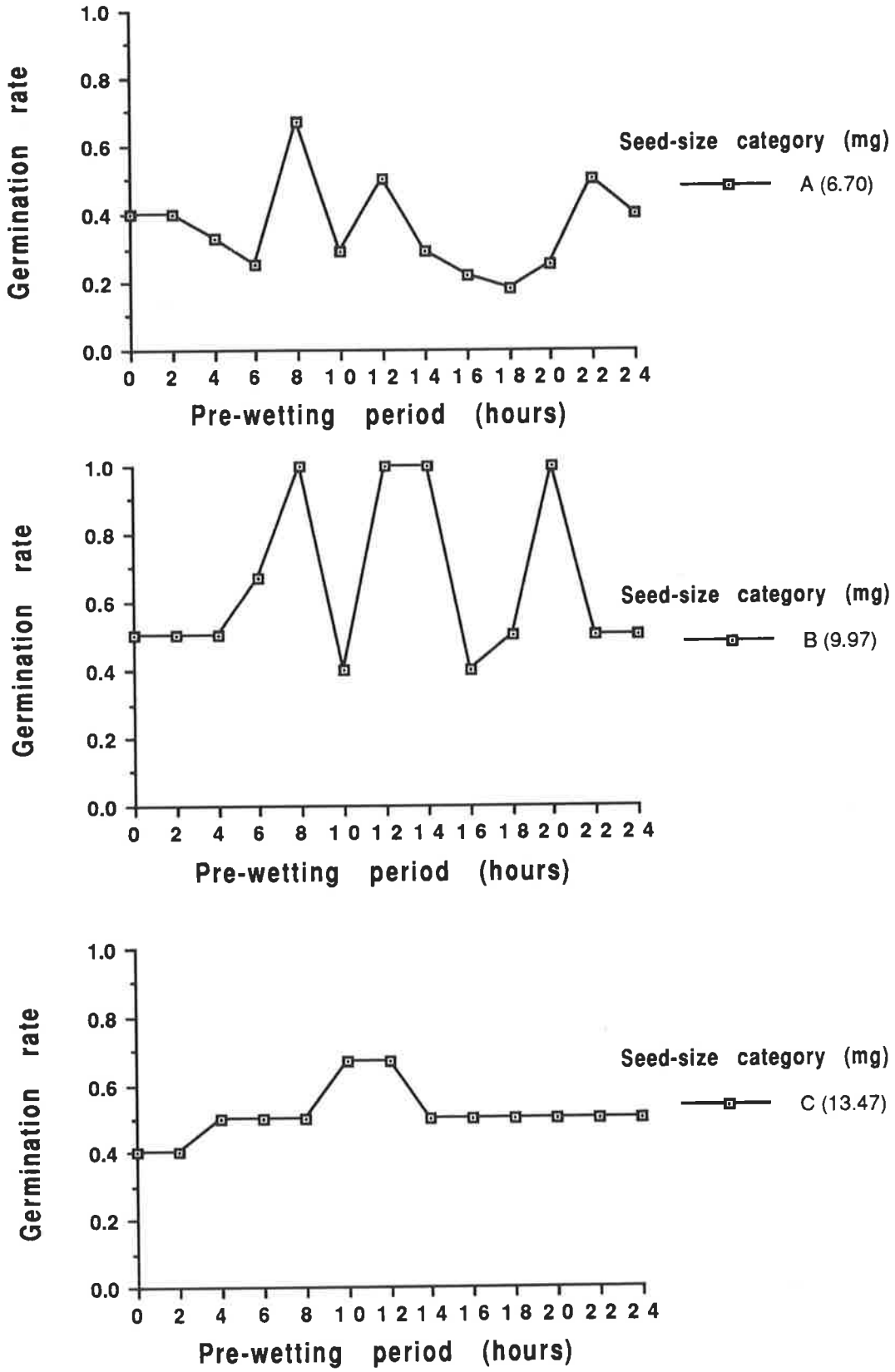


Figure 3.35 The germination rate (1/days, 90% germination) of Trikkala subterranean clover seeds submitted to different pre-wetting periods.

3.6 Laboratory Experiment 4: The effects of seed size and solutions of different water potentials on germination

3.6.1 Introduction

Different types of mechanisms control the potential capacity of a seed to germinate, emerge and become established under agricultural conditions. The ability of a seed to germinate under reduced moisture availability may confer ecological advantage, but it is not a separate factor involved in the success or failure of a seed to become an established seedling. For this reason, it was decided to test the relative ability of different seed-size categories to germinate under the effect of water stress simulated by solutions of different water potentials.

3.6.2 Materials and methods

Three seed-size categories (A, B and C) were selected for both Sava and Trikkala (Table 3.19). The seed-size categories, as well as the estimation of the individual mean fresh weight of the seed, the oven-dry weight of the seed and its moisture content were obtained in the same manner as described in previous experiments (3.3-3.5. Laboratory Experiments 1-3).

Polyethylene glycol (PEG), molecular weight aproximately 20,000, converted to various osmotic potentials (-2, -4, -6, -8, -10, -12, -14 and -16 bars), was used to simulate drought conditions during germination, while distilled water was applied to the controls. High molecular weight PEG was selected because it is chemically inert and non toxic (Parmar and Moore 1966), it is a good simulator of drought by blocking the pathway of water movement (Sharma 1973) and it does not penetrate the seed coat (Manohar 1966 a).

Water potentials were determined using a Wescor model HP-115 thermocouple psychrometer (microvoltmeter) with fifteen channels operating in a dew point mode at

25 ± 0.01 °C (± s.d.). Firstly, to calibrate the thermocouples for each channel, several readings were made on dry thermocouple chambers until similar and steady values were achieved. To each thermocouple chamber, a piece of Whatman N°1 filter paper (3.5 x 1.8 cm) was enclosed and they were hermetically sealed before immersion in a tank filled with vigorously-aerated distilled water, maintained at 25°C. The water level of the tank and the temperature of the water were continuously observed during the process. All the equipment was located in a growth cabinet that maintained the temperature evenly at about 25°C.

Then previously-prepared solutions of sodium chloride (NaCl) of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 molar concentration were used to calibrate the phychrometer. Each concentration of NaCl was run independently by the use of all available channels in a reading time interval of 30 minutes and left overnight generating a number of about thirty replicate values at each channel. Each channel received an aliquot of 150 microliters of NaCl for each replicate and every concentation used. The data expressed in microvolt was transferred to a HP-115 down load disk. Each concentration of NaCl associated to the mean value of the response in microvolts was converted to water potentials (bars) by using Lang's coefficients (Lang 1967). Linear regression analyses were made in order to obtain the corresponding regression equation for each channel (Appendix 3.18).

At this stage, four runs of readings (eight replicates at each PEG concentration at each run) of prepared solutions of PEG in concentrations of 5, 10, 15, 20, 25, 30 and 35 g/100 ml of distilled water dissolved in a water bath at 80 °C for 30 minutes were carried out under the same conditions described before. The mean value of the response in microvolts was calculated for each concentration and included in the corresponding regression equation previously obtained according to the channel used for the reading of a specific PEG concentration. The corresponding water potential at each concentration was obtained. Finally, the various concentrations of the PEG solutions were plotted against the osmotic potential obtained. An exponential model of variation of the water

potential with concentration was derived for use in the preparation of the corresponding drought treatments involved (Figure 3.36).

One hundred seeds per treatment were placed in Petri dishes as reported before (3.3-3.5 Laboratory Experiments 1-3) and moistened with 10 ml of PEG solution or distilled water in the case of the control. The treatments were assigned at random and arranged as a factorial experiment in a complete block design with four replicates: Species (2) x Seed sizes (3) x Osmotic potentials (9) x Replicates (4). The Petri dishes were incubated in a controlled environment incubator in the dark at a constant temperature of 25 °C for a period of fourteen days. The seeds were recorded as germinated according to the length of the radicle and counted and removed from each dish every twelve hours for the duration of the experiment. Germination was expressed as a percentage of the potential readily-germinable seed and calculated according to the level of hard seed recorded for each treatment at the end of the test and corrected by the proportion of soft, dormant and dead seeds obtained from the control treatment. Rates of germination were calculated as the reciprocal value of the time (days) required to obtain 50% germination.

In order to analyse the effects of the imposed treatments, an analysis of variance was carried out using the GENSTAT V Statistical Programme by the use of successive increased arc-sine-transformed values of germination over time. Finally, separate analyses of variance were performed on transformed data for incremental values in sequential assessments of germination.

3.6.3 Results

Statistical analysis of the data showed a highly significant interaction ($P< 0.001$) for Time x Species x Water potential x Seed size (Table 3.20). When the successive incremental increases of germination were used at each time of evaluation (in order to avoid the cumulative germination effect which increased with each succeeding germination record), the interaction of the three variables was significant for most

recordings. Water potential as a single effect was the variable that was significant throughout the period considered in the analyses of variance (Appendix 3.19).

Data on germination calculated within the first six days of testing was selected because most of the response occurred during this time. The lower the water potential of the solution used, the lower the germination, except that the response obtained by the control treatment for Trikkala, was lower than at -2 bars of water potential. At equal water potential the decrease in germination is clearly dependent on the seed-size categories used, especially under the extreme size situations. The total germination was progressively depressed by lower water potentials. In Sava seeds germination was practically inhibited at -6 bars and completely suppressed at -8 bars, while with Trikkala, suppression of germination occurred at -10 bars for the two smaller seed sizes and at -8 bars for the largest one (Table 3.21 ; Figures 3.37-3.40).

Despite the differences in the rate of germination between seed-size categories in the control treatment, the effect of seed size became very relevant at -2 bars and -4 bars of water potential for Sava and at -4 bars for Trikkala. Trikkala subterranean clover was more tolerant to restricted moisture conditions and showed higher percentages and rates of germination than Sava snail medic (Figure 3.41 and Appendices 3.20-3.23).

3.6.4 Discussion

Lower water potential alters the imbibition rate of a seed, either retarding the occurrence of a complete germination by slowing the rate at which the seeds complete germination or reducing the total final germination. The adverse effects of lower water potentials (by use of chemical osmotic agents) on the germination of pasture legume seeds had been reported by several workers (Uhvits 1946; Rodger *et al.* 1957; Dotzenko and Dean 1959; Dotzenko and Haus 1960; Younis *et al.* 1963; Mc William *et al.* 1970; Young *et al.* 1970a; Hadas and Russo 1974)

The results of the present study have shown that the germination of the seeds of both cultivars was influenced by the water potential of the germinating seed medium and the size of the seed. Differences in the ability to germinate at lower osmotic potentials was found between Sava and Trikkala. The subterranean clover seeds showed higher tolerance to lower water potentials, supporting results obtained by Young *et al.* (1970 a). Trikkala was also able to germinate better under the slightly reduced water potential of -2 bars than in the control. Uhvits (1946), and El-Sharkawi and Springuel (1977) have reported improvements in germination when seeds are imbibed in media at slightly reduced water potentials, because the slower rates of imbibition may cause less imbibitional damage to the seed (Vertucci 1989). Under the same circumstances, seeds with damaged seed coats such as scarified seeds used in this present study, perform better under osmotic stress situations than do seeds with intact coats (Johnston *et al.* 1979a; Johnston *et al.* 1979b; Manning and Van Standen 1987).

The ability of a seed to germinate under lower water potentials does not necessarily mean that it will survive and reproduce better under more xeric conditions. Seeds with this ability normally are less adaptable to Mediterranean conditions, where summer rainfall storms, and false breaks during autumn can occur (Chippindale 1949; McGinnies 1960). Thus subterranean clover is in general, less adapted to xeric conditions than annual medic species (Crawford 1981; Collins and Francis 1989).

Despite this, McWilliam *et al.* (1970) postulated that the greater tolerance to moisture stress shown by germinating seeds of ryegrass in comparison with other pasture species, including subterranean clover, may contribute to a better establishment of the seedlings under field conditions. Furthermore, this may be of particular relevance when ryegrass seeds are sown on the surface of the soil, where the moisture conditions for germination are more critical (McWilliam and Dowling 1970).

No attempt has been made to test the ability of seeds, within a population of annual pasture species, to germinate under contrasting osmotic pressures. According to Young *et al.* (1970 b), subterranean clover cultivars vary in their ability to germinate under

various soil-water matric potentials. The larger-seeded cultivars tend to have higher germinations under conditions of lower matric potentials, whereas at high matric potentials, the germination of the cultivars varies greatly and is independent of the size of the seed. These results are not in agreement with the present study. The explanation may be related to the particular ability of each cultivar to germinate under contrasting matric potentials, that may also differ between different seed sizes within the same cultivar population.

It is apparent from the present study, that the ability of the seed to germinate in aqueous solutions with different osmotic pressures differs between species and that the size of the seed is not an independent factor influencing the germination of the seed.

Table 3.19 Characteristics of the seed-size categories used to test the effects of seed size and water stress on germination of Sava snail medic and Trikkala subterranean clover seeds.

Sieve aperture Min. - Max. (mm)		Seed-size category	Fresh seed weight (mg)	ratio	Oven dry seed weight (mg)	ratio	Moisture content (%)
		SAVA					
1.71	2.00	A	10.27	1.00	9.71	1.00	5.45
2.01	2.36	B	15.31	1.49	14.48	1.49	5.42
2.37	2.80	C	23.54	2.29	22.28	2.29	5.35
		TRIKKALA					
1.71	2.00	A	6.53	1.00	6.19	1.00	5.21
2.01	2.36	B	9.97	1.51	9.46	1.53	5.12
2.37	2.80	C	13.66	2.00	12.99	2.10	4.90

Table 3.20 Summary of analysis of variance made on increasing values of germination with time.

Treatment	Significance levels †
Species	***
Water potential	***
Seed size	NS
Species x Water potential	***
Species x Seed size	NS
Water potential x Seed size	NS
Species x Water potential x Seed size	**
Time	***
Time x Species	***
Time x Water potential	***
Time x Seed size	***
Time x Species x Water potential	***
Time x Species x Seed size	**
Time x Water potential x Seed size	***
Time x Species x Water potential x Seed size	***

† NS = Not significant; ** = P<0.01; *** = P<0.001

Table 3.21 Mean final germination of seed-size categories of Sava snail medic and Trikkala subterranean clover held at different water potentials and calculated after 14 days of testing.

Seed-size category	Water potentials (-bars)								
	0	2	4	6	8	10	12	14	16
					(%)				
SAVA									
A	100	100	58	6	0	0	0	0	0
B	100	100	58	3	0	0	0	0	0
C	97	81	28	2	0	0	0	0	0
TRIKKALA									
A	99	100	90	14	1	0	0	0	0
B	96	100	70	14	3	0	0	0	0
C	100	100	80	18	0	0	0	0	0

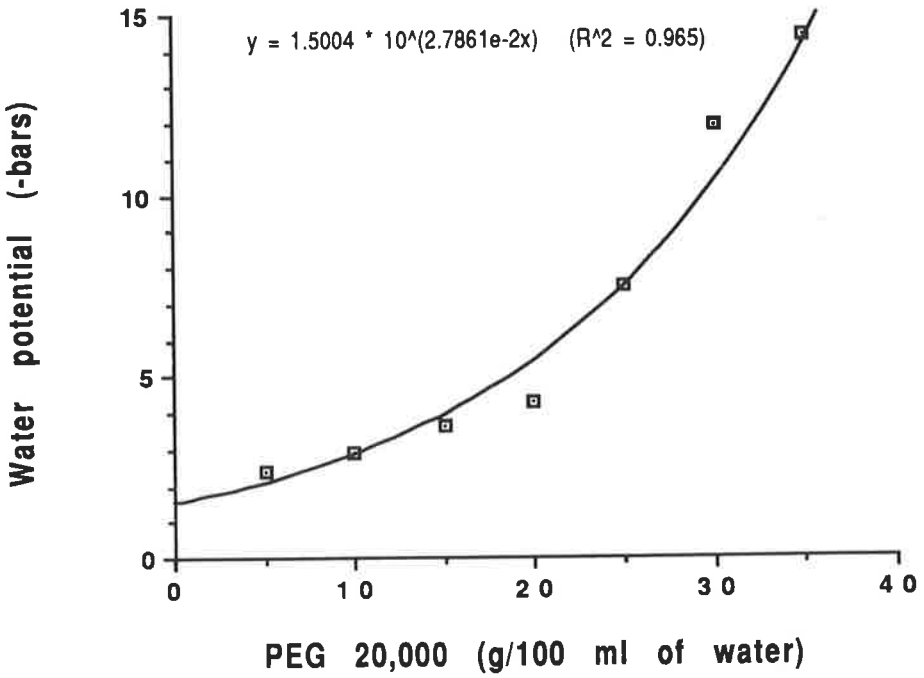


Figure 3.36 The exponential relationship obtained between PEG concentration and water potential.

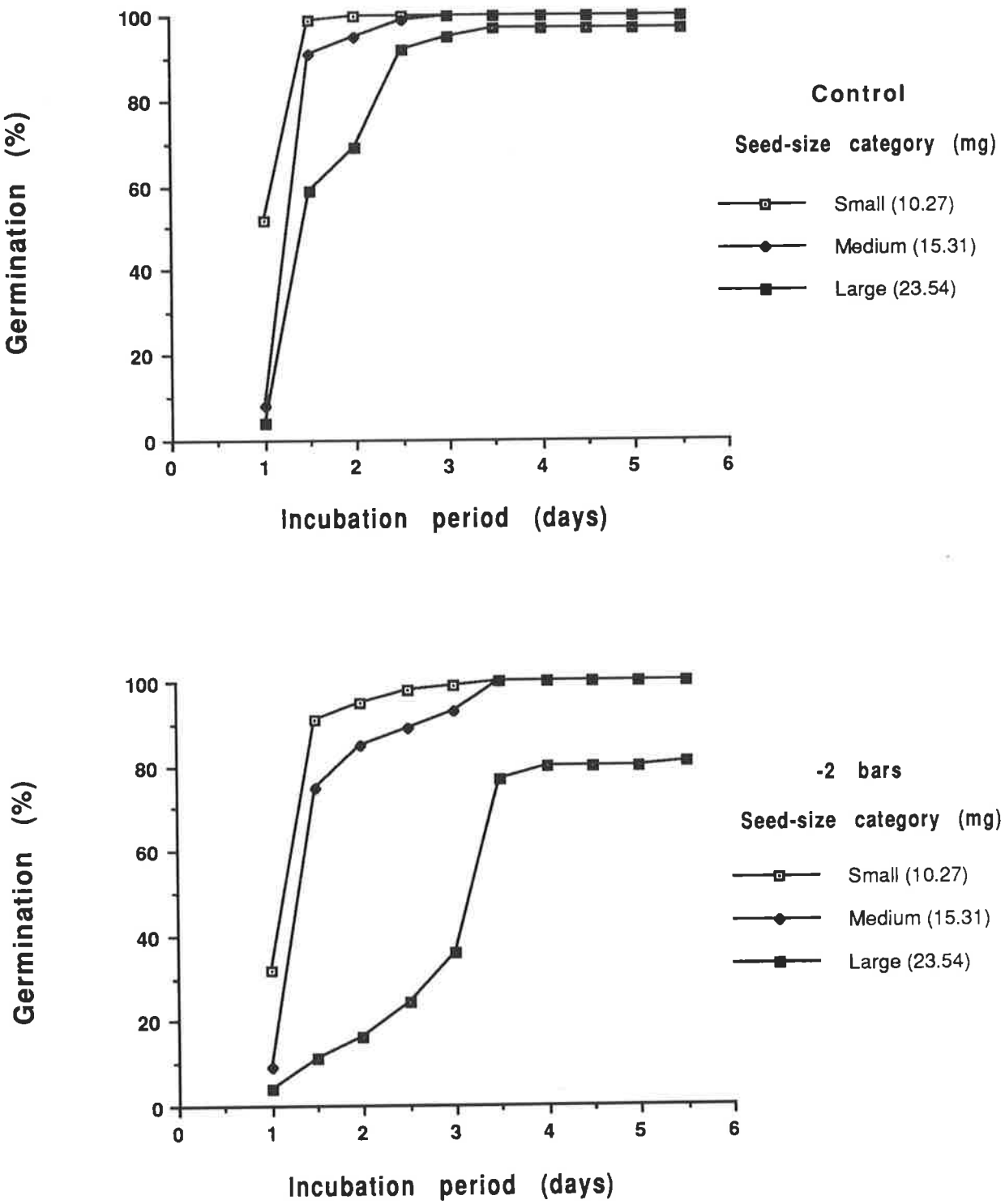


Figure 3.37 The germination of Sava snail medic seed-size categories at control and -2 bars of water potential.

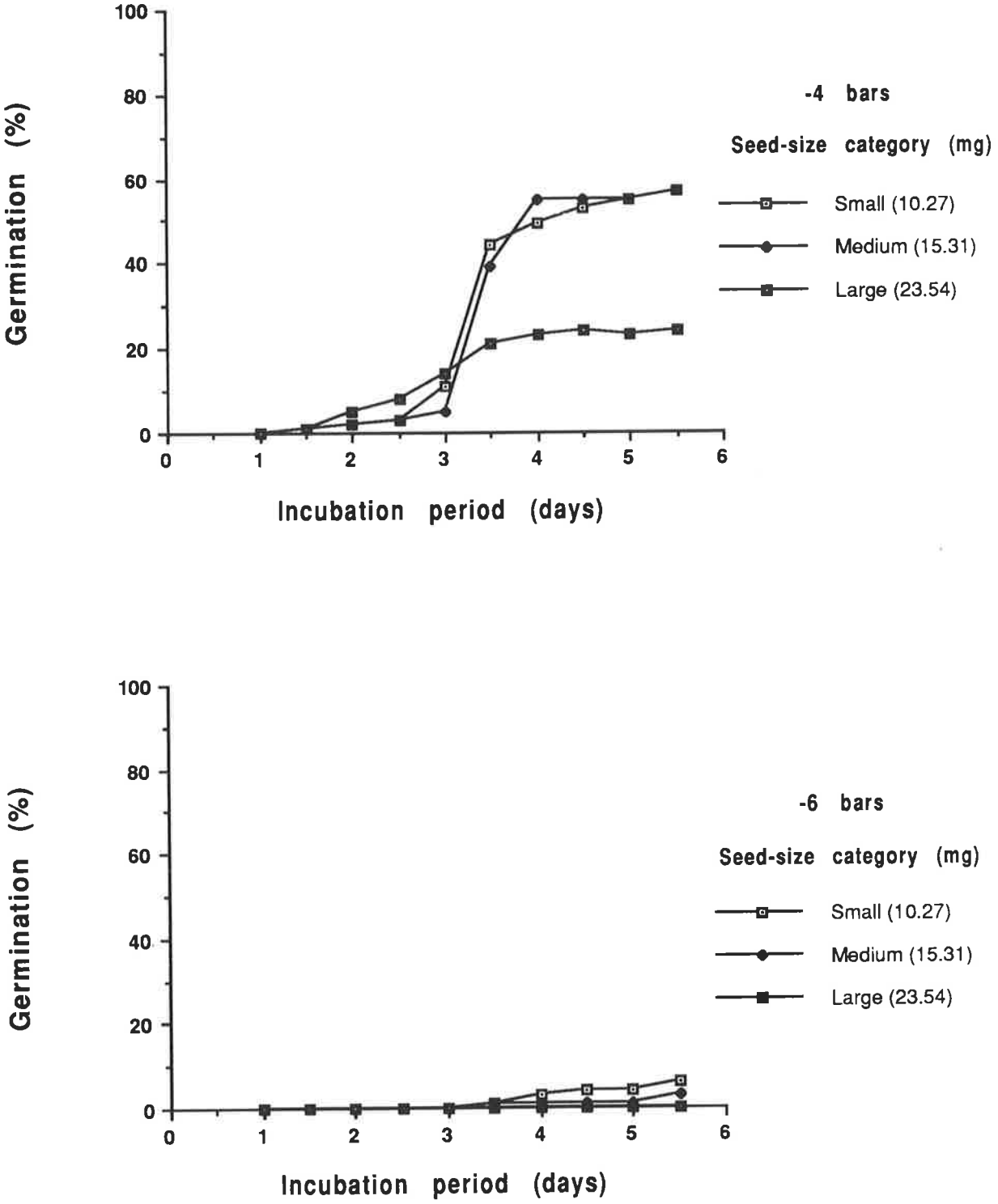


Figure 3.38 The germination of Sava snail medic seed-size categories at -4 bars and -6 bars of water potential.

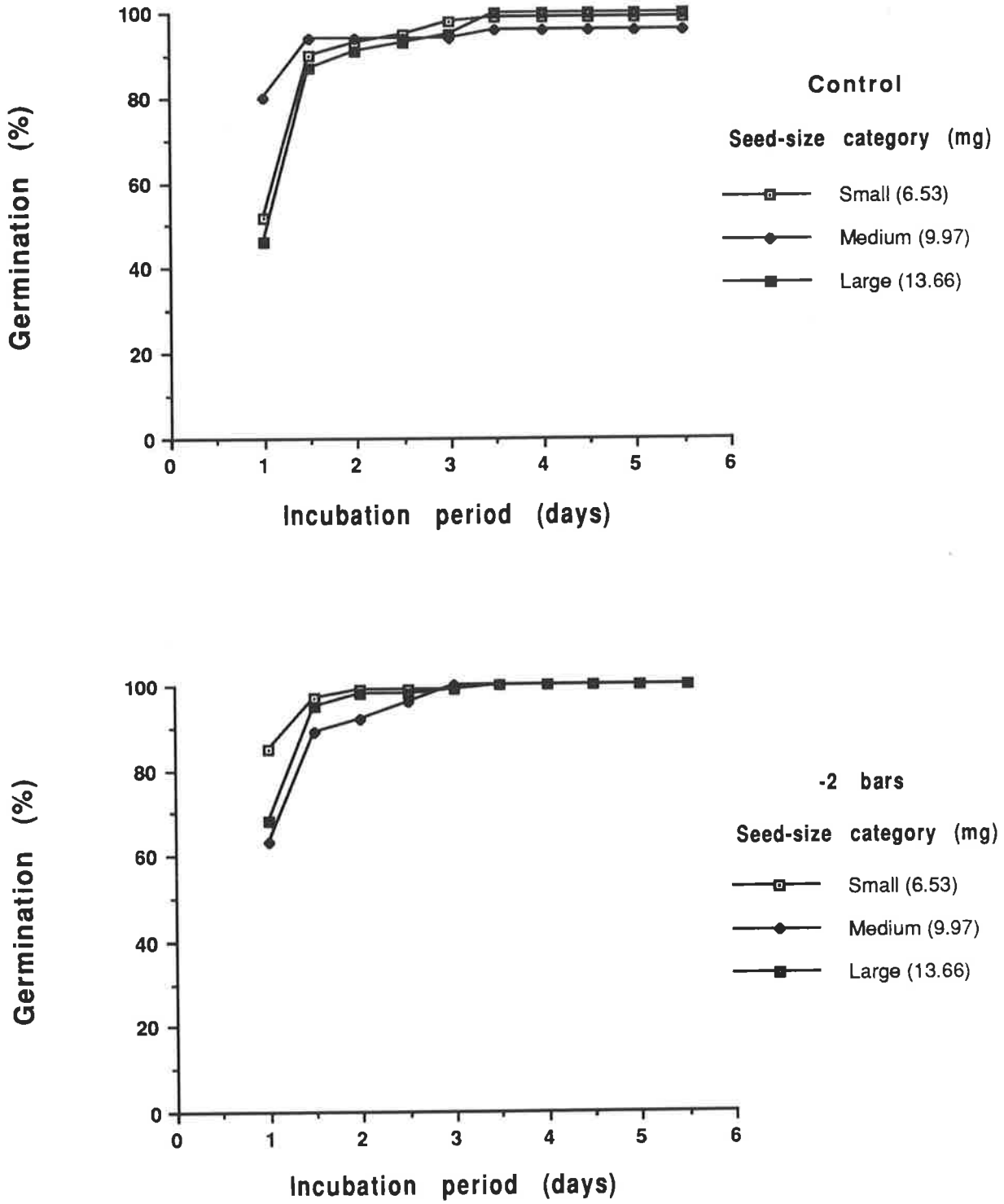


Figure 3.39 The germination of Trikkala subterranean clover seed-size categories at control and -2 bars of water potential.

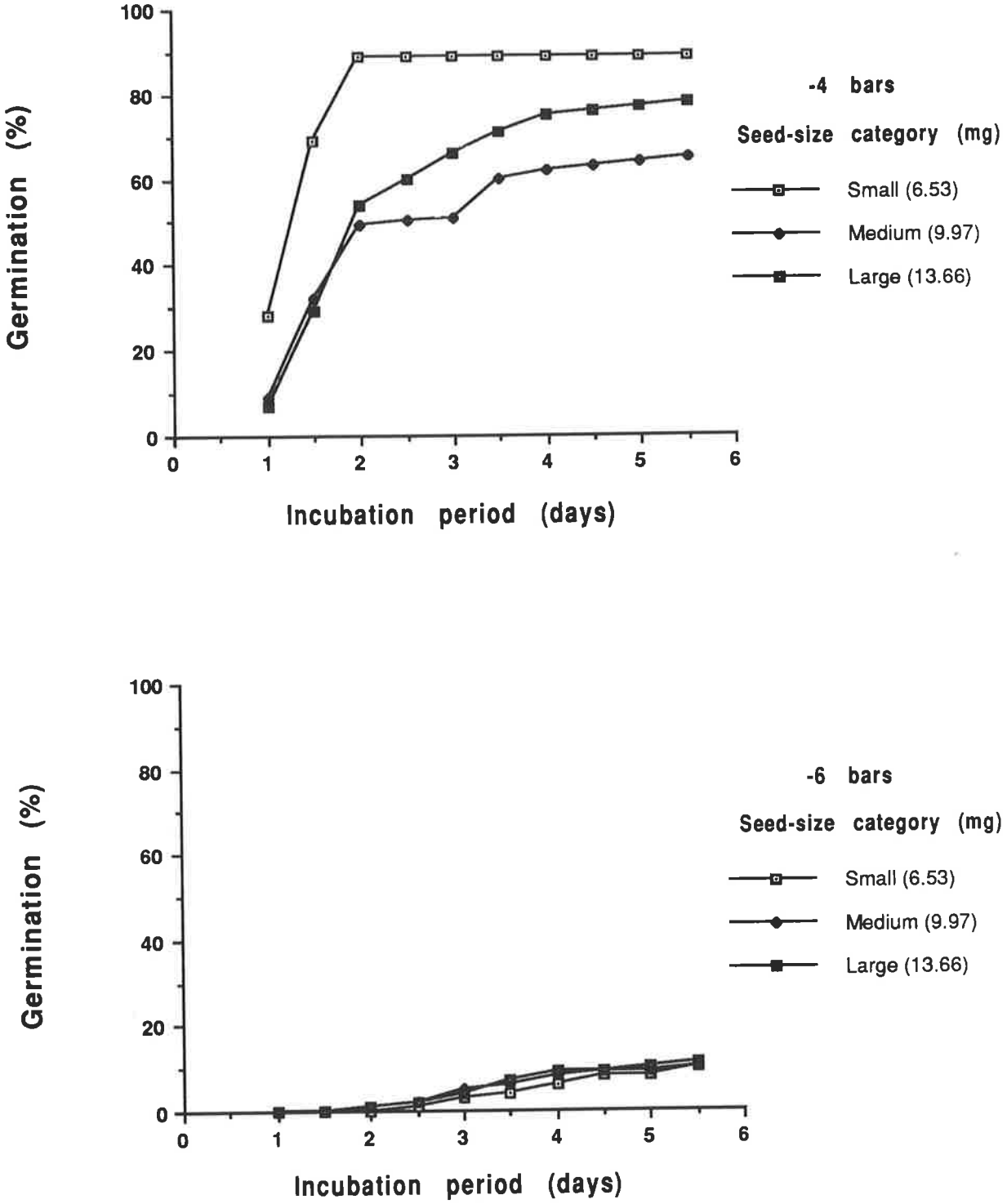


Figure 3.40 The germination of Trikkala subterranean clover seed-size categories at -4 bars and -6 bars of water potential.

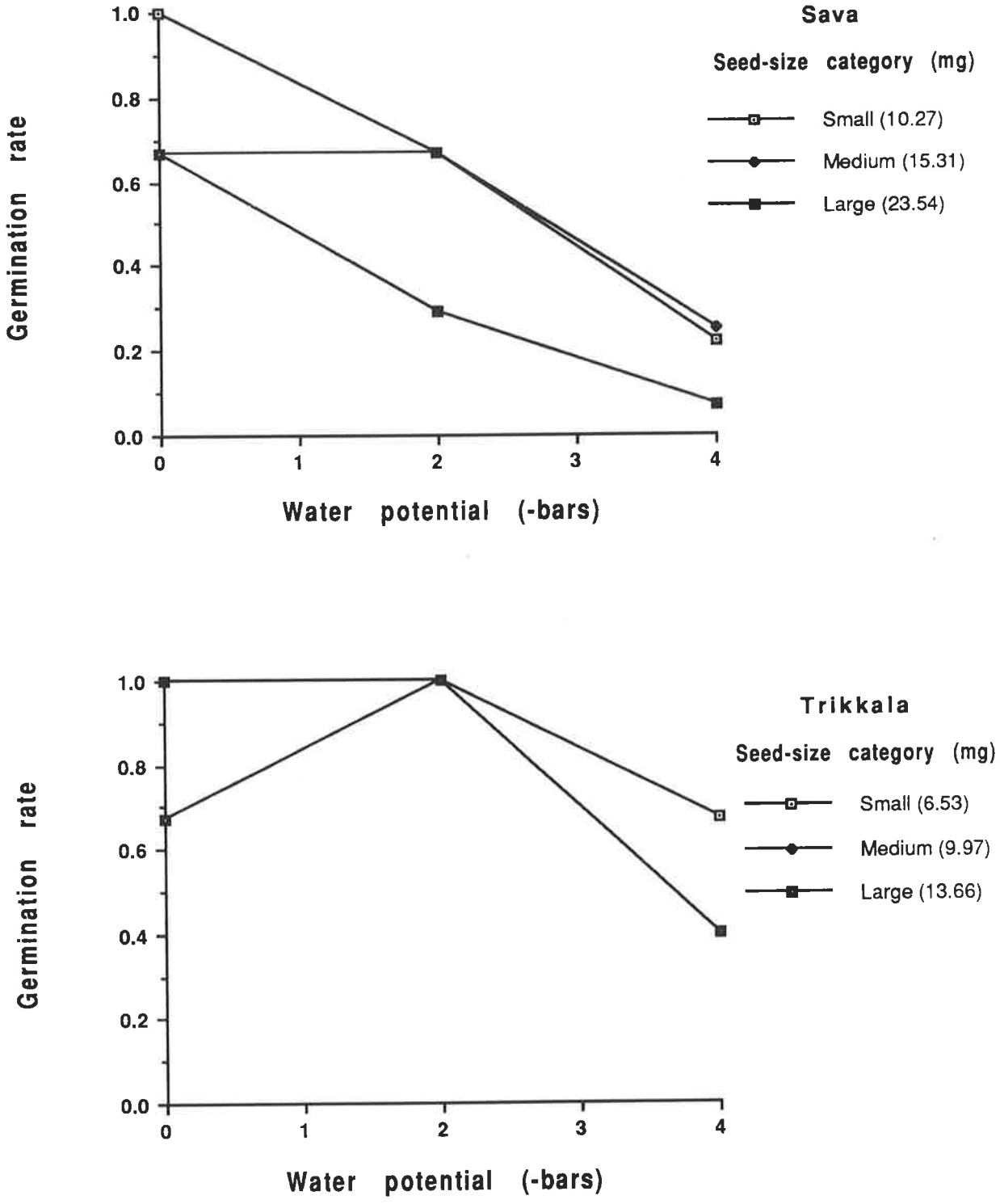


Figure 3.41 The germination rate (1/days, 50% germination) of Sava snail medic and Trikkala subterranean clover seeds held at different water potentials.

3.7 Conclusions from Laboratory Experiments

It is apparent that both species have different requirements of temperature for germination and the size of the seed is an important interactive factor with temperature on seed germination. The rate at which the seed germinates is evidently temperature-dependent and, in accordance with the suggestion of Ellern and Tadmor (1966), low temperatures delay germination rather than reducing germination. In contrast, high temperatures, especially in Trikkala subterranean clover, produce the partial or total death of the seed embryos with the consequent suppression of germination.

The higher rates of germination obtained with smaller seed sizes, seem to be related to a better seed-surface moisture contact of small seeds in comparison to large ones (Collis-George and Sands 1959; Sedgley 1963; Harper and Benton 1966). In the present studies, the rate of water uptake by seeds was consistently dependent on temperature, the size of the seed, and also internal factors in the seed such as the proportion in size of the embryonic axis (Stiles 1948). The presence of mucilaginous material (Harper and Benton 1966) would represent an additional trait favouring the moisture relationship between the seed and the surrounding water substrate.

During the desiccation-tolerant state of the embryo, longer periods of water uptake by seeds before drying leads to more advanced physiological stages in the germination process: hence the rate of germination of both species was enhanced in time and the differences between species and within species of particular seed size, was explained by the different physiological stages achieved in accordance with the ability of the seed to become hydrated. The expected advantage of the small seed size in germinating early was more obvious in the medic species than in the subterranean clover species.

Germination of Trikkala subterranean clover seeds was more tolerant to water stress than Sava snail medic. However, the ability of a seed to germinate at lower water

potentials does not necessarily ensure its survival and reproduction under more xeric conditions (Chippindale 1949; McGinnies 1960). Differences in the rate of germination of both species observed among seed-size categories, produced evidence that small seed has greater tolerance to moisture stress and suggests that under natural conditions, a better seedling establishment should be obtained from small seeds if water potential is a limiting factor.

The results of these laboratory experiments reveal the ecological significance of temperature and moisture on seed germination according to the size of the seed in seed populations of Sava snail medic and Trikkala subterranean clover. The data suggest that further studies are needed under natural conditions where the seed is mostly enclosed in pods and burrs located at or near the soil surface or buried naturally by livestock treading or by means of tillage practices. In consequence, changes in the magnitude of the relationships between the seed and the surrounding medium are expected.

SECOND SECTION

4 GLASSHOUSE EXPERIMENTS 1-2 AND RAISED-BED EXPERIMENT 1

4 SEED SIZE AND SOME ENVIRONMENTAL AND MANAGEMENT FACTORS INFLUENCING THE EMERGENCE AND EARLY GROWTH OF SEEDLINGS OF AN ANNUAL MEDIC AND A SUBTERRANEAN CLOVER

4.1 Introduction

Under a Mediterranean-type climate, livestock production systems based on annual legume pasture species must cope with great variations in the availability and nutritive value of green forage and dry residues through the year. This is particularly so early after the opening rains, when the availability of green feed is low and dry residues have been largely consumed (Purser *et al.* 1987).

The main problem of annual pastures is normally related to regeneration rather than to establishment (Wheeler 1987). There is ample evidence that the legume seed reserves of many farms with annual medic and subterranean clover pastures for sheep and cattle grazing have decreased consistently. This has been exacerbated where an increase in the frequency of cropping in the rotation has occurred and the alternative of resow is not adopted because it is expensive (Carter 1982; Carter *et al.* 1982).

For adequate regeneration of pasture it is essential that seed survive tillage during the cropping phase of rotations, because there is a direct relationship between the depth of seed burial and the level of seedling emergence (Taylor 1985; Fulwood and Carter 1989; Quigley *et al.* 1987). In the same manner, Carter (1987) emphasizes the importance of depth of sowing in pasture establishment, especially given the normal variations in seed size between species and cultivars, and in the characteristics of soils.

Another important component in ley farming systems, that influences the natural regeneration of the pastures is the amount and distribution of cereal straw residues left after the cropping phase (Quigley and Carter 1985; Quigley 1988). The concentration of cereal straw residues present over summer and autumn can influence seedling density by retarding the breakdown of hard seed because of the lower fluctuation of soil

temperatures below the straw (Burton 1964; Quigley 1988). After suitable autumn rains the initiation of pasture regeneration commences with germination but seedling emergence, growth and survival may be affected by residues as a consequence of allelopathic and pathogenic effects as well as by mechanical impedence to emerging seedlings (Arndt 1965; Quigley 1988).

Given the normal restrictions in forage availability at farm level during the critical autumn-winter period, the establishment of a high-density pasture legume sward represents a key factor favouring forage production (Donald 1951). To compete vigorously with naturalized grasses and weed species, a legume pasture requires a density of 1000-2000 established plants/m^2 (Puckridge and French 1983). For some medic cultivars in which only about 10% of seeds germinate, and only 50% of these become established, a seed supply of about 700 kg/ha is required to ensure an adequate stand of seedlings. With subterranean clover, which normally has between 30-40% germination at the start of the rainfall season, a seed bank of 300-550 kg/ha is needed to give a productive pasture (Puckridge and French 1983).

This chapter reports the results obtained from experiments that examined the ability of contrasting seed sizes within seed populations of *Medicago scutellata* cv. Sava and *Trifolium subterraneum* cv. Trikkala to germinate and for seedlings to emerge and grow under a range of conditions related to seed size, depth of sowing, soil type, cereal straw residues and varying plant density.

4.2 Glasshouse Experiment 1: The effects of seed size, depth of sowing and soil type on emergence and early growth of medic and subterranean clover seedlings

4.2.1 Materials and methods

In order to test the effects of seed size, depth of sowing and soil type on the emergence and early growth of Sava snail medic and Trikkala subterranean clover, a factorial pot experiment with the following treatments was used: Species (2) x Seed size (3) x

Sowing depth (5) x Soil types (2) x Blocks (4)= 240 pots. The three seed-size categories (small, medium and large) were selected as before and corresponding to the seed populations described in 3.2 Laboratory Test 1 (Table 4.1).

Two soils with contrasting textures were used: a sandy soil obtained from a mallee sand hill at Mallala about 60 km north of Adelaide and a loamy soil, from a hard-setting red-brown earth soil collected from the Waite Agricultural Research Institute (WARI). The soil materials were obtained from the top-soil (0-15 cm), sieved through a 0.6 cm square-holed rotary sieve, steam sterilized at 100 °C for a period of 90 minutes, air-dried and re-sieved through a 0.4 cm square-holed sieve.

Two hundred and forty square black polypropylene pots (15 x 15 x 14 cm) freely drained, were initially partly-filled with 3 cm of sterilized wood chips. Then, each depth of sowing (1, 3, 5, 7 and 9 cm) was determined by filling the pots at the proper levels with the corresponding soil types. Forty nine seeds were sown in each pot, corresponding to a population of 2205 seeds/m^2. A plastic stamp (10 x 10 cm) with 49 evenly spaced 0.5 cm diameter round holes was centred at each slightly compacted soil level, the seed was located inside each hole mark by the use of a forceps, and then filled with soil to the final level and compacted again in order to ensure an adequate distribution of soil over the sowing line, as well as ensuring proper contact of the seed with the surrounding soil medium.

The seeds were sown on 1 and 2 October, 1989 for replicates I and II, and on 3 and 4 October for replicates III and IV. Then the pots were transferred to a glasshouse and the soils were moistened with rainwater to field capacity on 5 October, based on the water-holding capacity of same soil types reported by Adem (1977). The soils were maintained at field capacity during the experimental period in order to avoid further constraints to the growth of emerging or emerged seedlings.

Emerged seedlings were counted daily until 28 days (2 November) after the initial watering and a single harvest was made on 19 October, 14 days after the initial

watering. The plants were cut with a scalpel then washed, counted, enclosed in sealed plastic bags and stored at 4°C until processing. Ten seedlings from each treatment were dissected into cotyledon lamina, monofoliate-leaf lamina, trifoliate-leaf lamina, petiole and stem, and the corresponding leaf laminae were utilized to determine the leaf area by the use of an electronic planimeter. Plant components, as well as the bulk samples, were oven-dried at 80 °C for 48 h and individually weighed. The emergence of seedlings, and the corresponding values of dry matter, were corrected according to expected levels of emergence, obtained from a previous germination test done at each seed-size category and carried out as before for a period of 14 days (Table 4.1).

The air temperatures in the glasshouse were recorded during the experimental period by using a thermohydrograph located at plant level and protected against the direct influence of sun (Figure 4.1). A soil penetrometer was also used to test the unconfined compressive strengths of soils held at field capacity on emerging seedlings. One hundred readings per soil type were done (Figure 4.2).

Analyses of variance were carried out using GENSTAT V on the daily partial increases in percentage emergence between day 4 and day 15, after the initial watering, when most of the response was achieved. The data were transformed using an arc-sine transformation, because some improvement of the residual plots were noted. Plant variables related to the dry matter values and leaf area values calculated from the harvested plant material were also analysed.

4.2.2 Results

Analyses of variance carried out on daily increases in emergence, show a four-way significant interaction between species, soil type, seed size and depth of sowing on days 4, 5, 7, 13 and 14. On the remaining days, a significant interaction: Species x Soil type x Depth of sowing was always observed. Thus, all the factors have a significant effect on emergence. However, seed size appeared to be of least importance (Table 4.2).

The seedlings of species Sava and Trikkala started emergence, defined as the complete appearance of cotyledons above the soil surface, on day 4 and 5 respectively. The emergence of Sava seedlings in the sandy soil was not greatly affected by sowing depth except at the greatest depth, for seedlings from small and medium-sized seeds. In contrast, there was a progressive decline in emergence with depth in the loamy soil. However, seedlings produced from larger seeds also were able to emerge from deeper positions into the soil. Despite some emergence being observed from 7 and 9 cm, it appears that between 1 and 5 cm, especially at the deeper levels (3 cm and 5 cm), the larger cotyledons of seedlings from large seeds restricted seedling emergence (Figures 4.3-4.4 and Appendix 4.1).

Seedlings of Trikkala subterranean clover were always more affected than seedlings of cultivar Sava. Emergence was reduced by greater depth of sowing, especially in loamy soil, from which no seedling was able to emerge from 7 and 9 cm depth. The only exception occurred at 1 cm depth on loamy soil, where Trikkala seedlings reached higher levels of emergence than Sava, which may be due to the smaller size of their cotyledons (Figures 4.5-4.6 and Appendix 4.1).

When the total dry matter of seedlings harvested per pot and the individual dry matter weight of seedlings were statistically analysed, a significant three-way interaction: Species x Soil type x Seed size was only observed for the total dry matter (per pot) of seedlings harvested (Table 4.3). However, a significant response of species, soil type, seed size and depth of sowing and the corresponding two-way interactions were always observed. The exception was by the Soil type x Seed size interaction when the individual dry matter weight of seedlings was analysed (Table 4.3). At this stage, 14 days after the initial watering, the depth of sowing and the seed size seemed to be the least important factors influencing the total and the individual seedling yield respectively.

Higher yields per pot were related to higher seedling densities (Plates 4.1-4.2). The maximum yield was obtained when the larger Sava seeds were shallow-sown in the sandy soil. However, in the loamy-textured soil, medium-sized seeds of Sava, rather

than large-sized seeds, at 3 and 5 cm in depth gave the highest yield, supporting the previous comment about the influence of cotyledon area on seedling emergence (Figure 4.7 and Appendix 4.1). In contrast, at 1 cm sowing depth, similar densities of cultivar Trikkala recorded in the sandy and the loamy soils, showed differences in yield (Figure 4.8 and Appendix 4.1), revealing that heavier individual seedlings were produced in the loamy soil (Appendix 4.2). This suggests, that when the depth of sowing is not a limiting factor to seedling emergence, Trikkala plants were better suited to perform in the heavier-textured soil. Independent of the species, seedlings growing in loamy soil, especially those emerging from 1 and 3 cm depths, showed more advanced stages of development, through the appearance of trifoliate leaves (Appendix 4.3-4.6). The same trend in the dry matter of individual seedlings was observed. Sava seedlings under all conditions were more vigorous than those of Trikkala, but both lost vigour as depth increased, especially in loamy soil. However, more vigourous plants resulted from seed placed between 1 and 3 cm in depth in the loamy soil compared with the sandy soil (Plates 4.1-4.2; Appendix 4.2). This may well have reflected the superior nutritional status of the loamy soil.

The leaf lamina area of individual cotyledons, monofoliate leaves and total for seedlings, showed a significant four-way interaction for species, soil type, seed size and depth of sowing, demonstrating the importance of the factors involved in determining the photosynthetic area of emerging seedlings (Table 4.3). Despite the contrast in leaf lamina area between species and the corresponding seed-size categories used, both cultivars showed important reductions in cotyledon and monofoliate lamina areas as depth of sowing was increased. This revealed that larger individual areas of laminae were related to shallow depth of sowing primarily in the loamy-textured soil than in the sandy soil. In contrast, when the depth of sowing limited seedling emergence, larger individual areas of laminae occurred at greater depth of sowing and in the sandy soil rather than in the loamy soil (Appendix 4.7).

The total area per pot given by the different plant leaf components was also statistically analysed (Table 4.4). There was a consistent influence of species, depth of sowing and seed size on the magnitude of the Leaf Area Index (LAI) obtained in each situation, which was concordant with the trend observed during the analyses of the previous plant variables (Figures 4.9-4.12).

4.2.3 Discussion

The rate of emergence, as well as the final emergence, of Sava snail medic and Trikkala subterranean clover seedlings was influenced by all the factors considered in the study. Seedlings of cultivar Trikkala were almost always more affected than seedlings of cultivar Sava, revealing the importance of embryo weight (3.2 Laboratory Test 1) in allowing a seedling to avoid constraints to emergence. Williams (1956), evaluating the emergence force exerted by seedlings of small-seeded legumes, including subterranean clover, found that the emergence force was improved as the weight of the seed increased. The same trend was detected within the seed populations used in these studies, confirming that seed size in this epigeal pasture species limits the potential elongation of the hypocotyl and consequently the ability of seedlings to emerge from the soil (Black 1956). However, when the seedlings of cultivar Sava were emerging from between 1 cm and 5 cm in the loamy soil, the emergence was progressively delayed and decreased as seed size increased, suggesting as Moore (1943) and Carter and Challis (1987) indicated, that larger cotyledons associated with larger seeds retard emergence.

Seedlings of snail medic were highly efficient in emerging in a sandy soil. When the depth of sowing varied from 1 cm to 5 cm for small seed size, from 1 cm to 7 cm for medium seed size and from 1 cm to 9 cm for large seed size, final emergence of seedlings was not reduced. This suggests that seed size is important in determining the potential emergence as well as the rate of emergence. This finding is not in complete agreement with Adem (1977) working under similar conditions with an unspecified snail medic cultivar. He found that seed with large cotyledons sown at 5 cm depth in a sandy

soil, lifted the soil crust and did not emerge as well as seedlings with smaller cotyledons.

For Trikkala subterranean clover, a more consistent influence of seed size, soil type and depth of sowing was observed. The results demonstrate the importance of these factors in maximizing emergence and the need to avoid deep burial of seed during the establishment of a pasture as well as during normal cultivation practices within a crop-pasture rotation system (Carter and Challis 1987).

Higher dry matter yields were related to higher seedling densities as a consequence of the emergence trends observed previously. Higher yields from medium seed sizes rather than larger seed sizes were due to the effect of cotyledon size. The emergence of Sava snail medic seedlings growing in the loamy soil, was depressed when sown at depths between 3 cm and 5 cm.

A direct relationship between embryo weight, seed weight, the individual weight of seedling, the individual lamina area of cotyledons and monofoliate leaves, and the corresponding values of Leaf Area Index (LAI) was observed during the early autotrophic growth phase of seedlings. These relationships suggest that competition between plants was not operating at that stage of growth and consequently seedlings from large seeds were still performing with increased rates of growth as well as seedlings from small seeds (Black 1959).

According to Black (1956, 1957 a), the amount of reserves available upon emergence have little or no influence on subsequent seedling growth. The significance of seed size lay in the fact that larger seed developed seedlings with larger cotyledons and greater photosynthetic areas, which increased seedling growth. Taylor (1972), also working with subterranean clover species, described the same relationship for the monofoliate leaf and the first trifoliate leaf in cultivar Daliak.

In the present study, the size of the cotyledon and the monofoliate leaf decreased more gradually with depth in the sandy soil rather than in the loamy one. Cotyledon area in the

sandy soil did not change greatly for Sava snail medic between 1 cm and 5 cm depth and for Trikkala subterranean clover between 1 cm and 3 cm depth, supporting the results of Black (1956), where seedlings emerging from 1 cm and from 5 cm did not differ in cotyledon area for any given seed size considered. This contrasts with the results of Adem (1977) who found a progressive decline in cotyledon area when the depth of sowing was increased between 1 cm and 5 cm. However, a further increase in depth of sowing, markedly reduced the cotyledon area, suggesting that 5 cm in Sava and 3 cm in Trikkala represents the limit of sowing depth where the area of cotyledon was not affected. In contrast, under loamy soil conditions, where the mechanical resistance of soil more strongly influenced the emergence of seedlings, a progressive depression of leaf area with depth was observed, thus further reducing the photosynthetic area and consequently the seedling vigour of annual legume pasture species.

Shibles and MacDonald (1962) demonstrated that photosynthetic area of birdsfoot trefoil cotyledons was as important as that of leaves. Larger seeds produced greater cotyledon areas and the speed of cotyledon expansion after emergence was closely associated with seedling vigour (Hur and Nelson 1985).

Despite the higher rates of emergence achieved in the sandy soil conditions, individual cotyledon and monofoliate leaf areas were greater with shallow sowing (especially at 1 cm depth) in the loamy soil than in the sandy one. There was also an earlier onset of trifoliate leaf production, particularly in subterranean clover. These results suggest that when adverse conditions (such as those retarding emergence and delaying the onset of the autotrophic growth), were not present, contrasting levels of fertility between soils could play an important role in seedling growth. Taylor (1972) reported changes in weight of subterranean clover seedlings, as early as the end of the heterotrophic growth phase compared with the weight of the seed embryo, which were partly explained by mineral uptake. A similar pattern was described by McWilliam *et al.* (1970) for lucerne and subterranean clover, where the differences in weight of seedlings growing with and without nutrients became apparent after five days of imbibition. This indicates that young plants are able to utilize external nutrients, such as nitrogen and phosphorus within days of emergence when favourable conditions are present.

Table 4.1 Characteristics of seed-size categories used to test the effects of seed size, depth of sowing and soil type on emergence and early vegetative growth of Sava snail medic and Trikkala subterranean clover seedlings.

Sieve aperture Min. - Max. (mm)	Stored seed weight (mg/seed)	(ratio)	Oven-dried seed weight (mg/seed)	(ratio)	Moisture content (%)	Cumulative germination (%)
	SAVA					
1.71 - 2.00	10.94	1.00	10.35	1.00	5.39	79
2.01 - 2.36	15.32	1.40	14.38	1.39	6.14	80
2.37 - 2.80	23.50	2.15	22.36	2.16	4.85	80
	TRIKKALA					
1.71 - 2.00	6.72	1.00	6.25	1.00	6.99	82
2.01 - 2.36	10.14	1.51	9.48	1.52	6.51	81
2.37 - 2.80	13.43	2.00	12.50	2.00	6.92	82

Table 4.2 Summary of analyses of variance carried out on consecutive daily increases in percentage emergence which occurred during the period of greatest response.

Source of variation	Days after inital watering											
	4	5	6	7	8	9	10	11	12	13	14	15
	(Significance levels) †											
Species	***	***	***	*	*	***	NS	**	***	**	NS	NS
Soil	**	***	***	***	***	***	***	*	***	**	*	***
Seed size	***	NS	*	NS	*	NS	NS	NS	NS	**	NS	NS
Depth	***	***	***	***	***	***	***	***	NS	NS	NS	NS
Species x Soil	**	***	***	NS	NS	NS	***	NS	NS	NS	NS	NS
Species x Seed size	***	NS	NS	NS	NS	NS	NS	NS	NS	*	**	NS
Species x Depth	***	***	***	***	***	***	***	***	NS	***	**	**
Soil x Seed size	***	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Soil x Depth	***	***	***	NS	NS	**	**	**	*	***	**	***
Seed size x Depth	***	**	NS	*	NS	NS	NS	NS	NS	NS	NS	NS
Species x Soil x Seed size	***	NS	**	NS	NS	**	NS	NS	NS	*	*	**
Species x Soil x Depth	***	***	***	***	***	***	***	***	***	***	***	***
Species x Seed size x Depth	***	NS	NS	***	NS	NS	NS	NS	NS	NS	*	NS
Soil x Seed size x Depth	***	NS	NS	***	NS	NS	NS	NS	NS	NS	**	*
Species x Soil x Seed size x Depth	***	**	NS	*	NS	NS	NS	NS	NS	**	**	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Table 4.3 Summary of analyses of variance carried out on yield and individual leaf lamina area of harvested seedlings.

Source of variation	Yield		Individual leaf area (cm^2)		
	(mgDM/pot)	(mgDM/sdlg)	Cotyledon	Monofoliate	Seedling
	(Significance levels) †				
Species	***	***	***	***	***
Soil	***	***	NS	***	*
Seed size	***	***	***	***	***
Depth	***	***	***	***	***
Species x Soil	***	***	**	***	***
Species x Seed size	***	***	***	***	***
Species x Depth	***	**	***	***	***
Soil x Seed size	***	NS	NS	NS	NS
Soil x Depth	***	***	***	***	***
Seed size x Depth	***	***	NS	*	***
Species x Soil x Seed size	***	NS	NS	*	NS
Species x Soil x Depth	NS	NS	**	***	NS
Species x Seed size x Depth	NS	NS	NS	NS	*
Soil x Seed size x Depth	NS	NS	NS	*	NS
Species x Soil x Seed size x Depth	NS	NS	**	*	*

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Table 4.4 Summary of analyses of variance carried out on components of leaf lamina area of harvested seedlings.

Source of variation	Components of leaf lamina area (cm^2)			
	Cotyledon	Monofoliate	Trifoliate	Total
	(Significance levels) †			
Species	***	***	***	***
Soil	***	***	***	***
Seed size	***	***	***	***
Depth	***	***	***	***
Species x Soil	***	***	NS	***
Species x Seed size	***	***	***	***
Species x Depth	***	***	***	***
Soil x Seed size	***	***	NS	***
Soil x Depth	***	***	***	***
Seed size x Depth	***	*	***	***
Species x Soil x Seed size	***	***	NS	***
Species x Soil x Depth	***	***	***	NS
Species x Seed size x Depth	NS	*	***	NS
Soil x Seed size x Depth	NS	NS	**	NS
Species x Soil x Seed size x Depth	NS	NS	NS	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

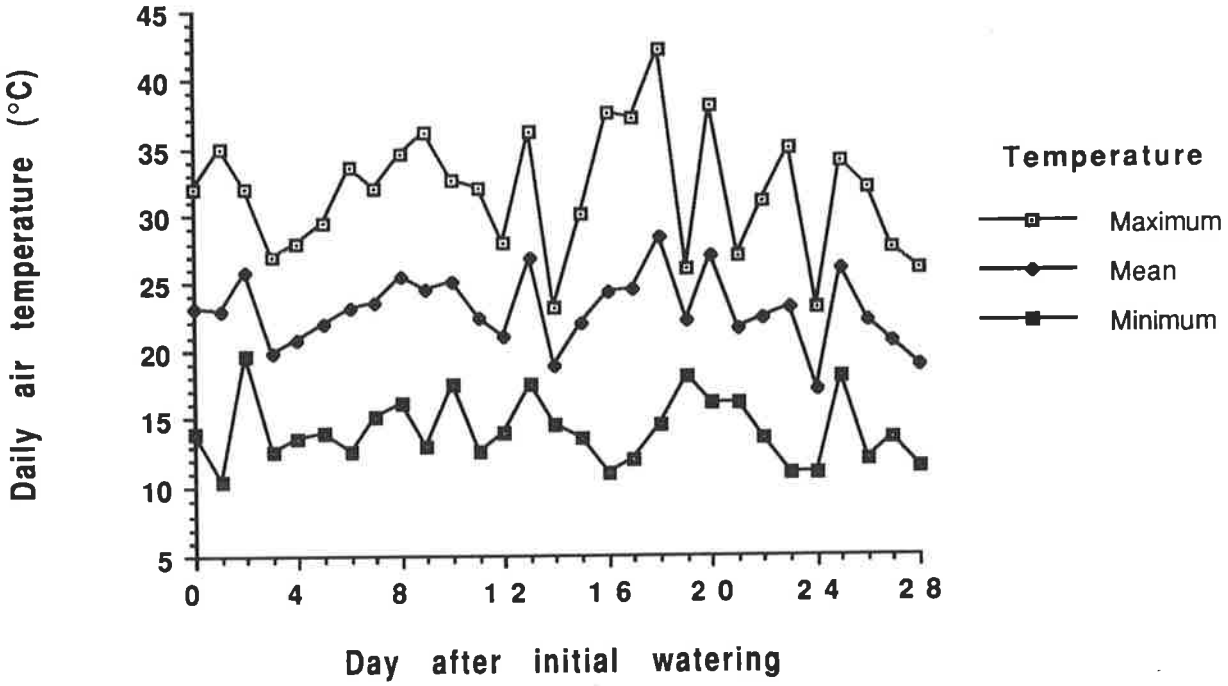


Figure 4.1 The daily minimum, mean and maximum air temperatures recorded in the glasshouse during the experimental period.

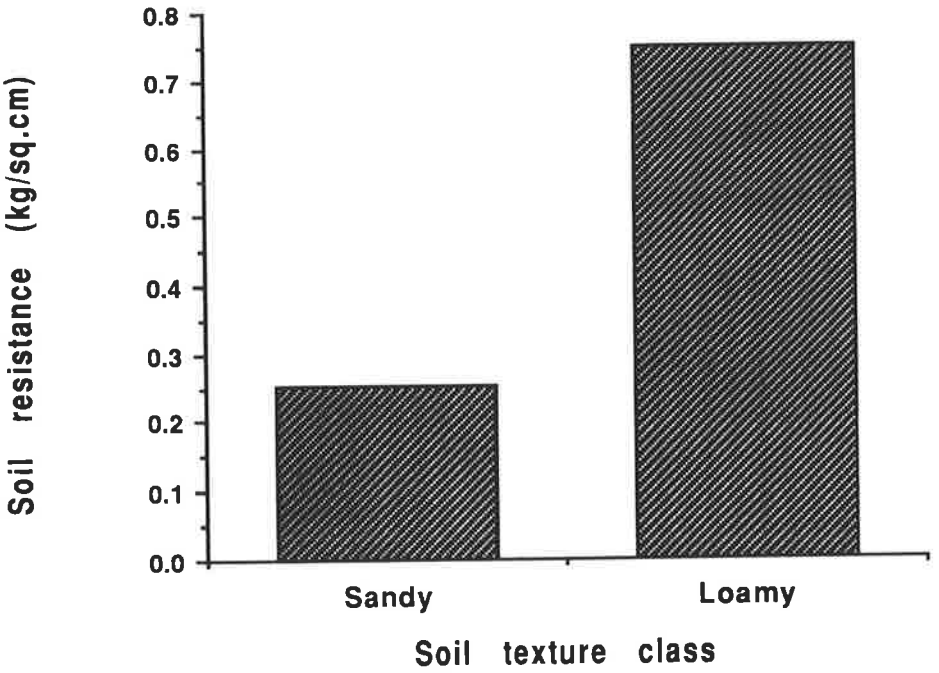


Figure 4.2 The resistance of soil estimated on sandy- and loamy-textured soils used in the study.

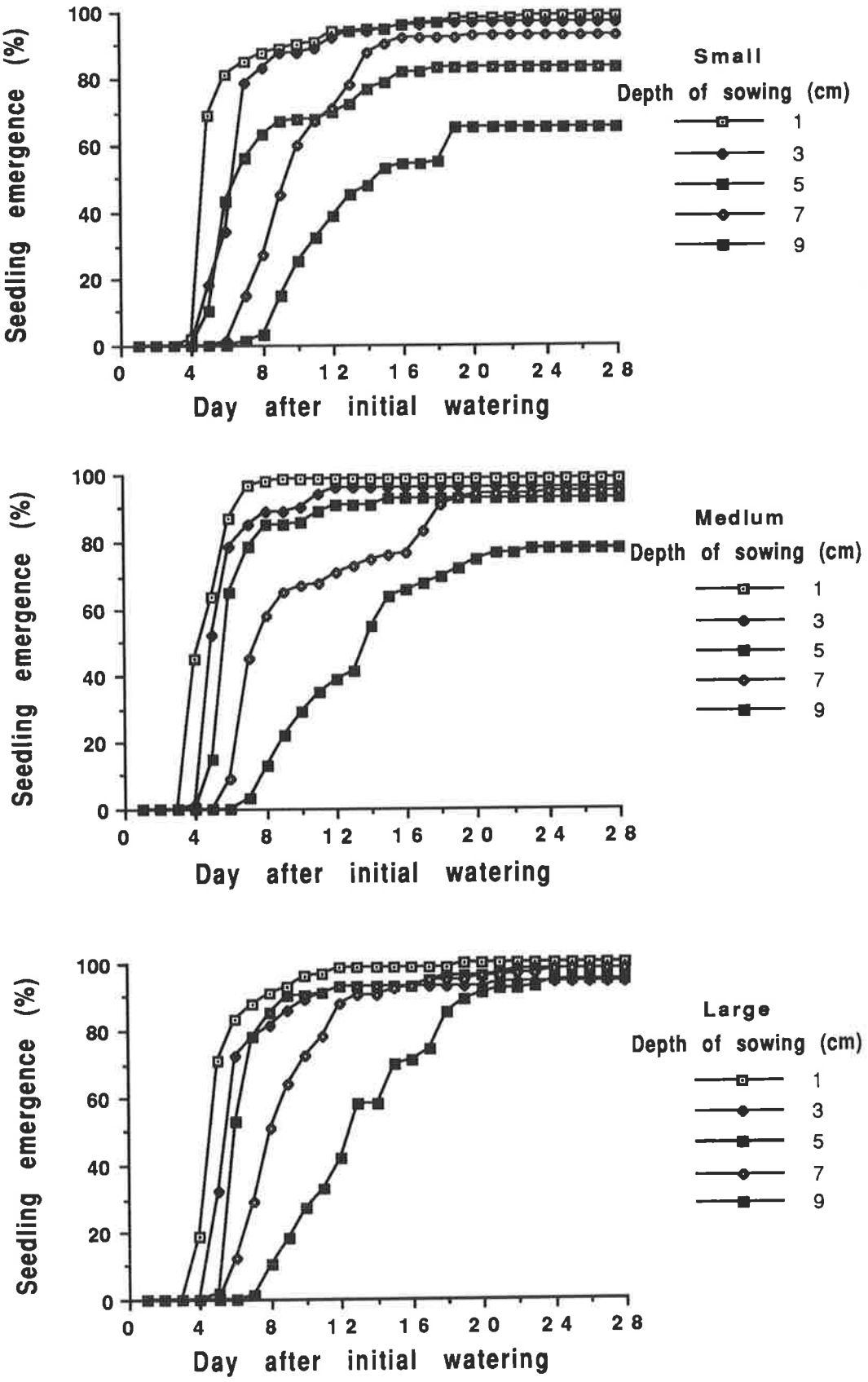


Figure 4.3 The trend in emergence of Sava snail medic seedlings sown in a sandy soil with small, medium and large seed-size categories.

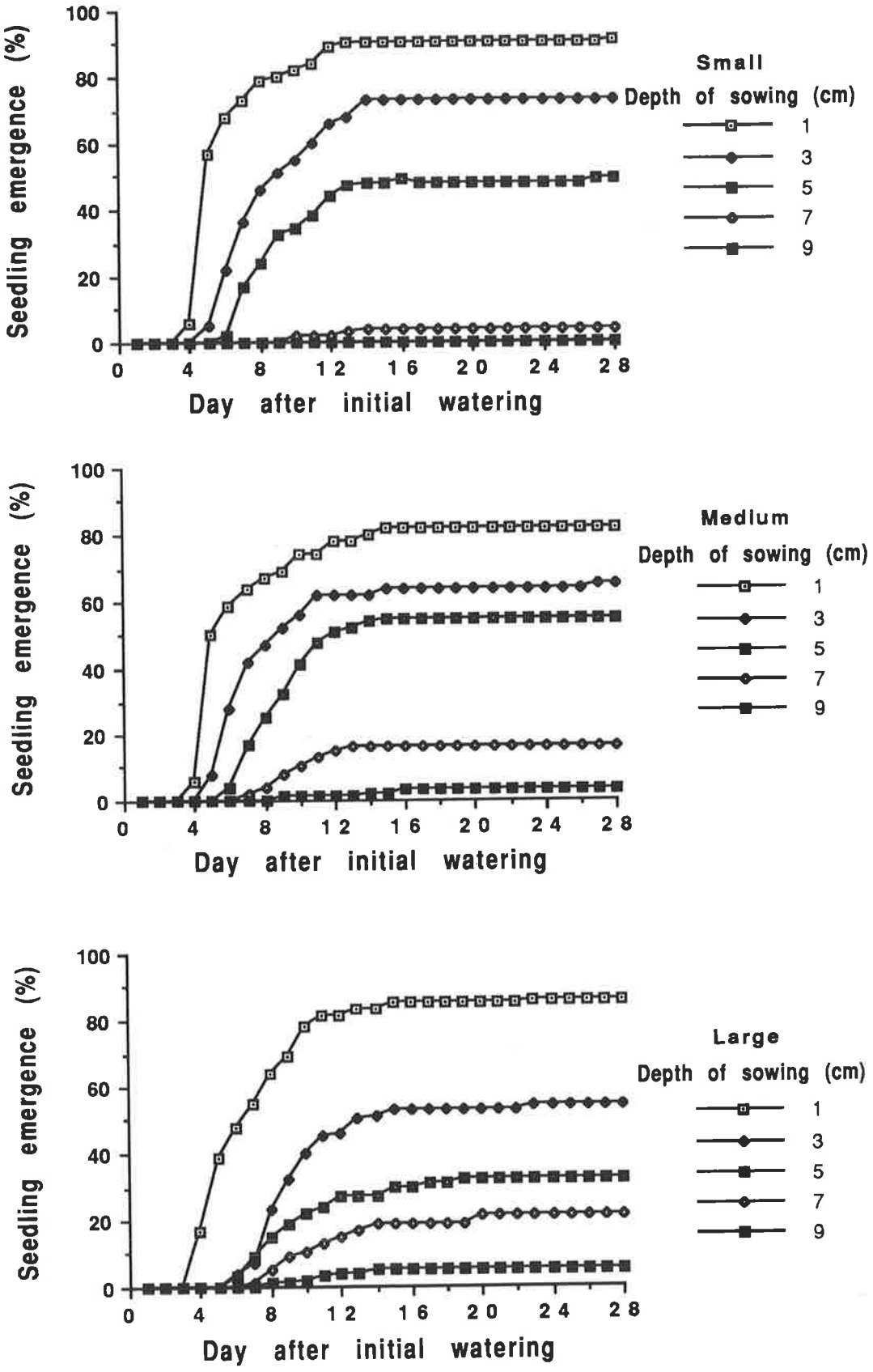


Figure 4.4 The trend in emergence of Sava snail medic seedlings sown in a loamy soil with small, medium and large seed-size categories.

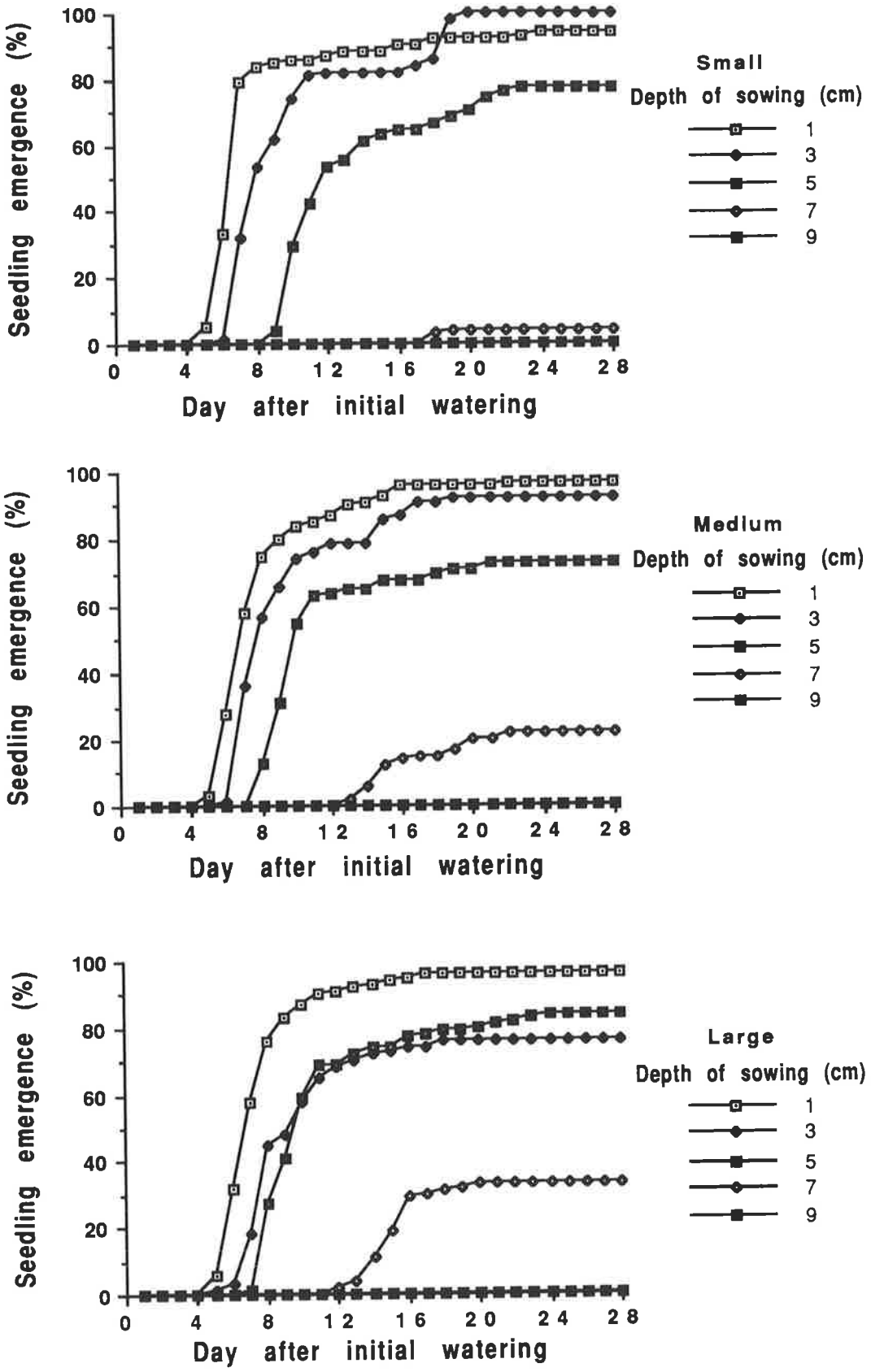


Figure 4.5 The trend in emergence of Trikkala subterranean clover seedlings sown in a sandy soil with small, medium and large seed-size categories.

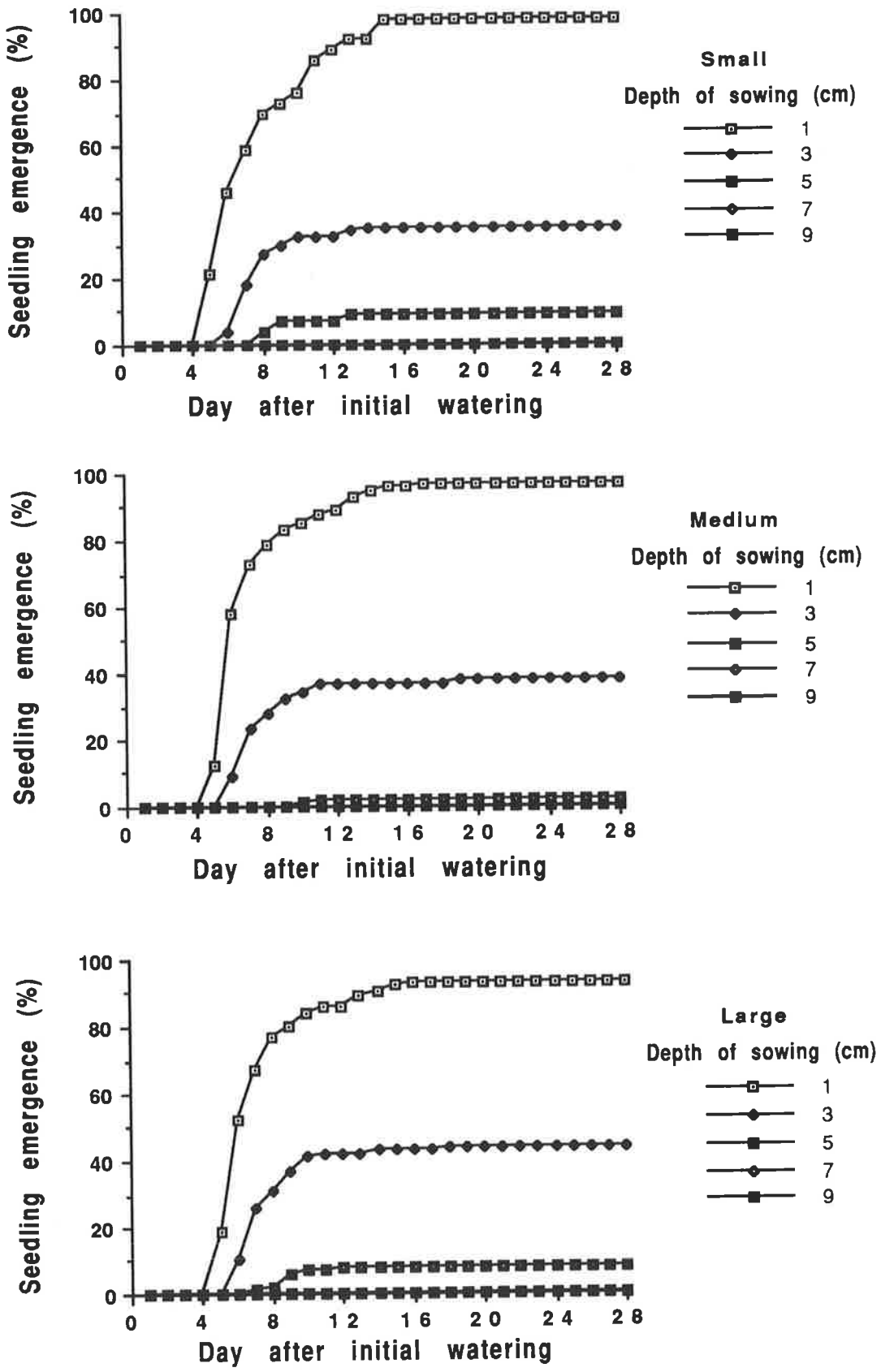


Figure 4.6 The trend in emergence of Trikkala subterranean clover seedlings sown in a loamy soil with small, medium and large seed-size categories.

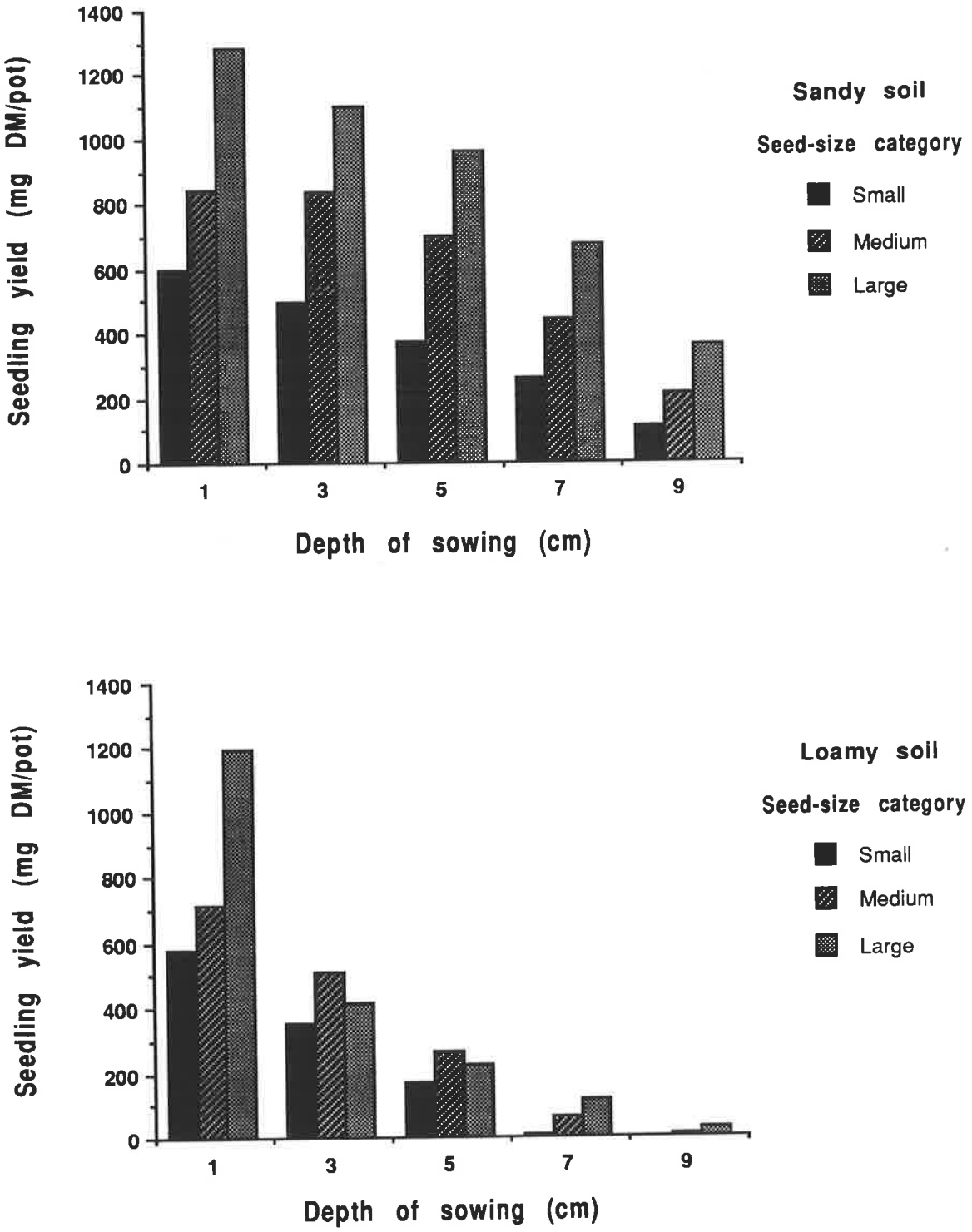


Figure 4.7 The yield of Sava snail medic seedlings emerged from a sandy and a loamy soil.

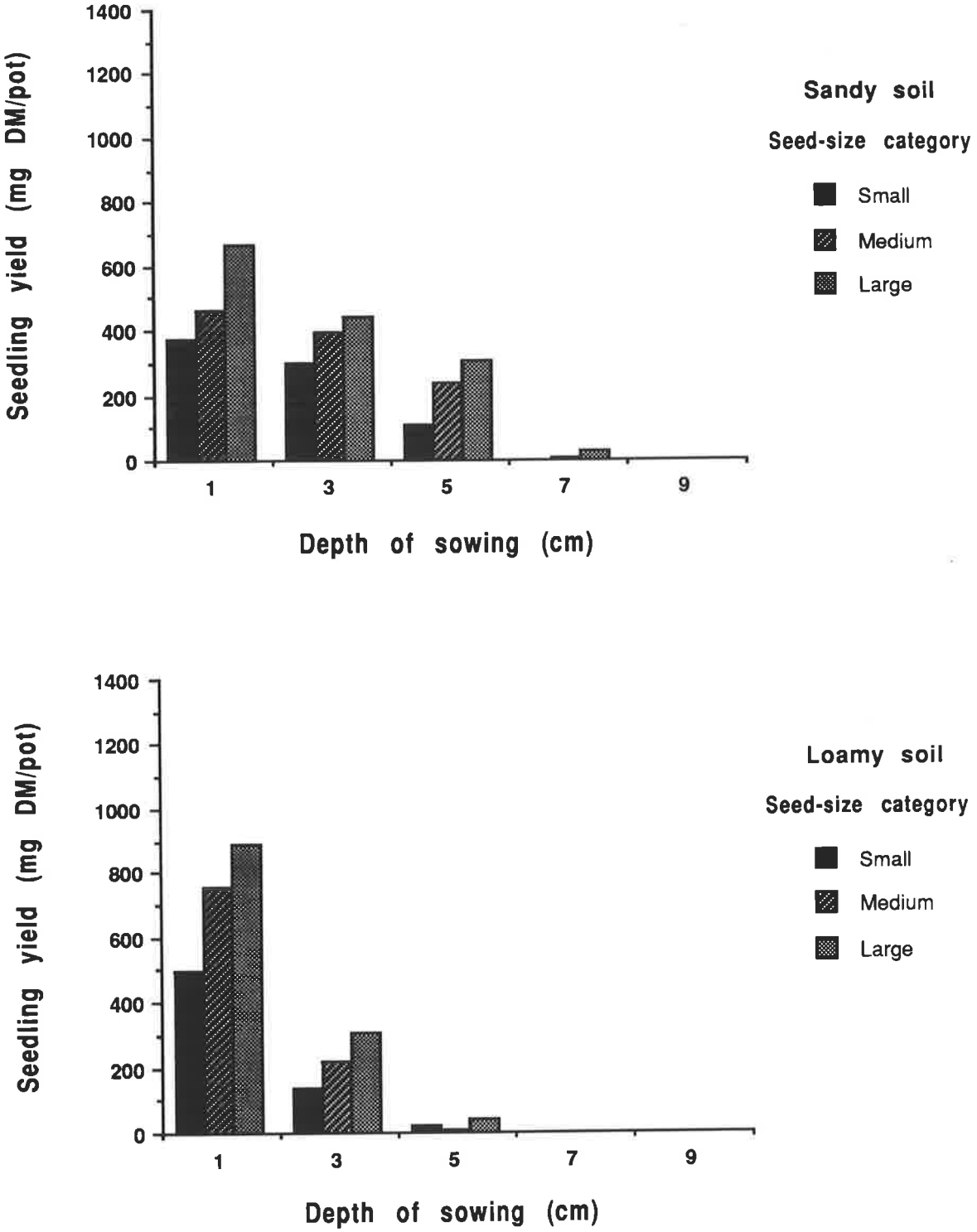


Figure 4.8 The yield of Trikkala subterranean clover seedlings emerged from a sandy and a loamy soil.

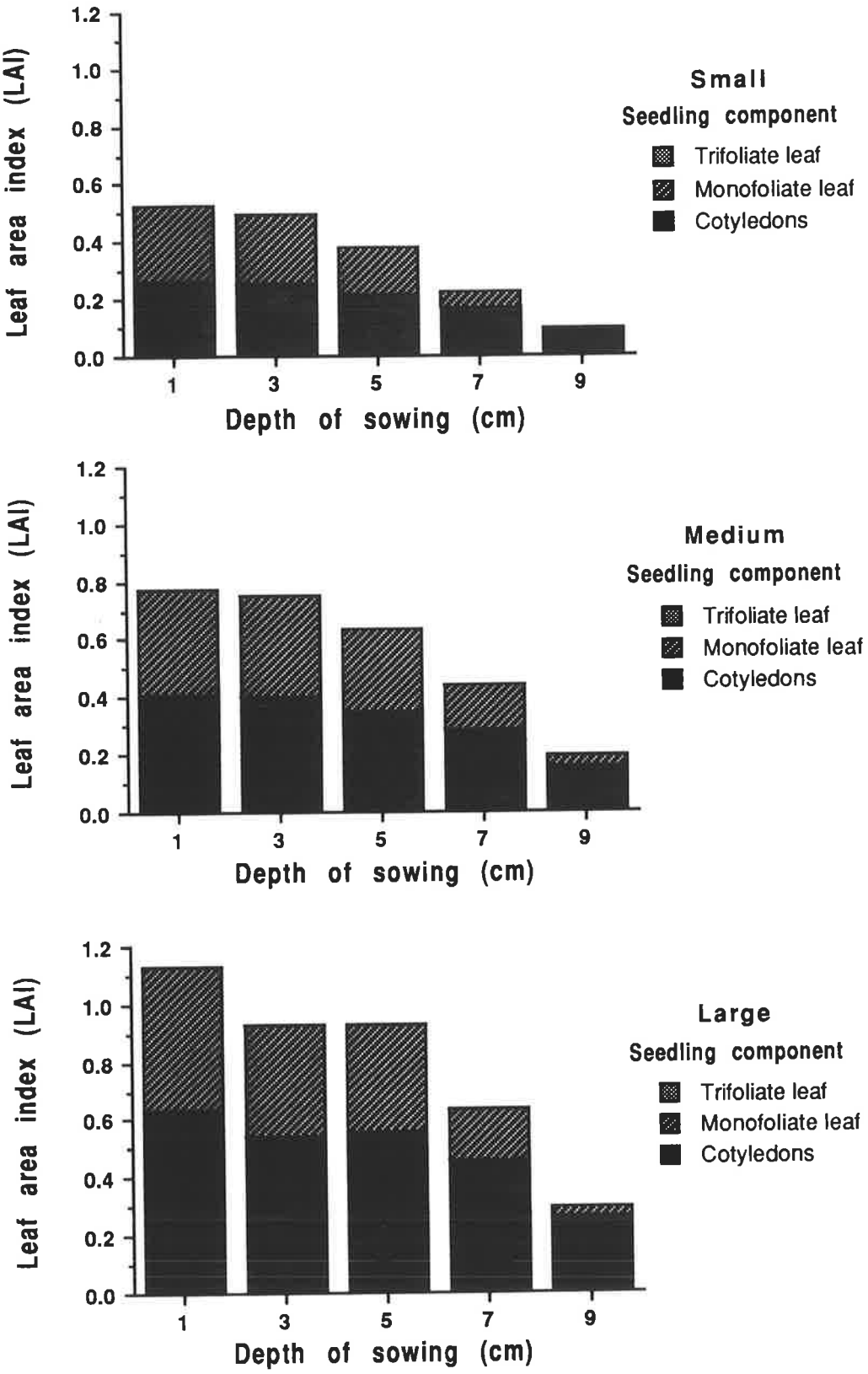


Figure 4.9 The leaf area index of Sava snail medic seedlings sown in a sandy soil with small, medium and large seed-size categories.

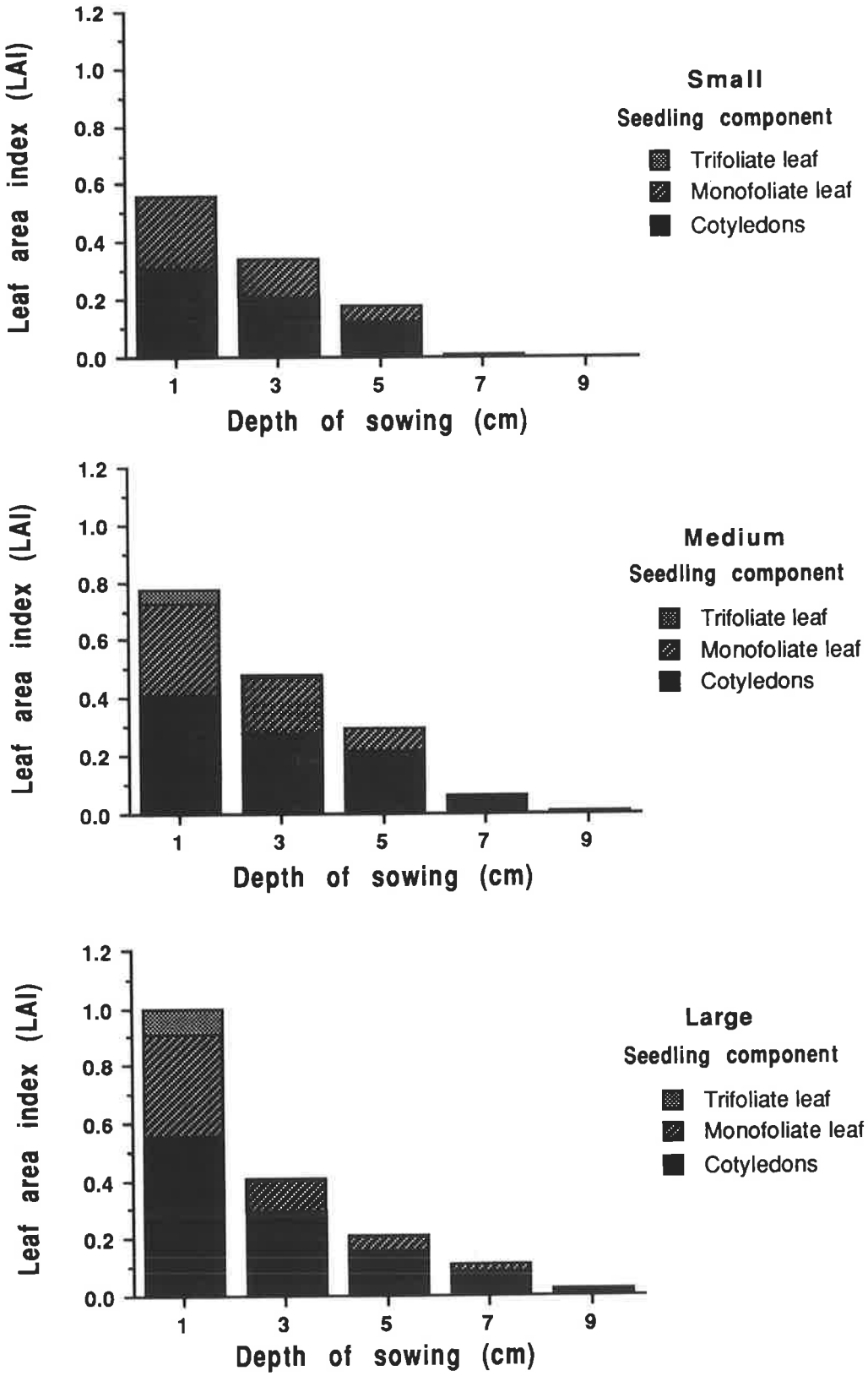


Figure 4.10 The leaf area index of Sava snail medic seedlings sown in a loamy soil with small, medium and large seed-size categories.

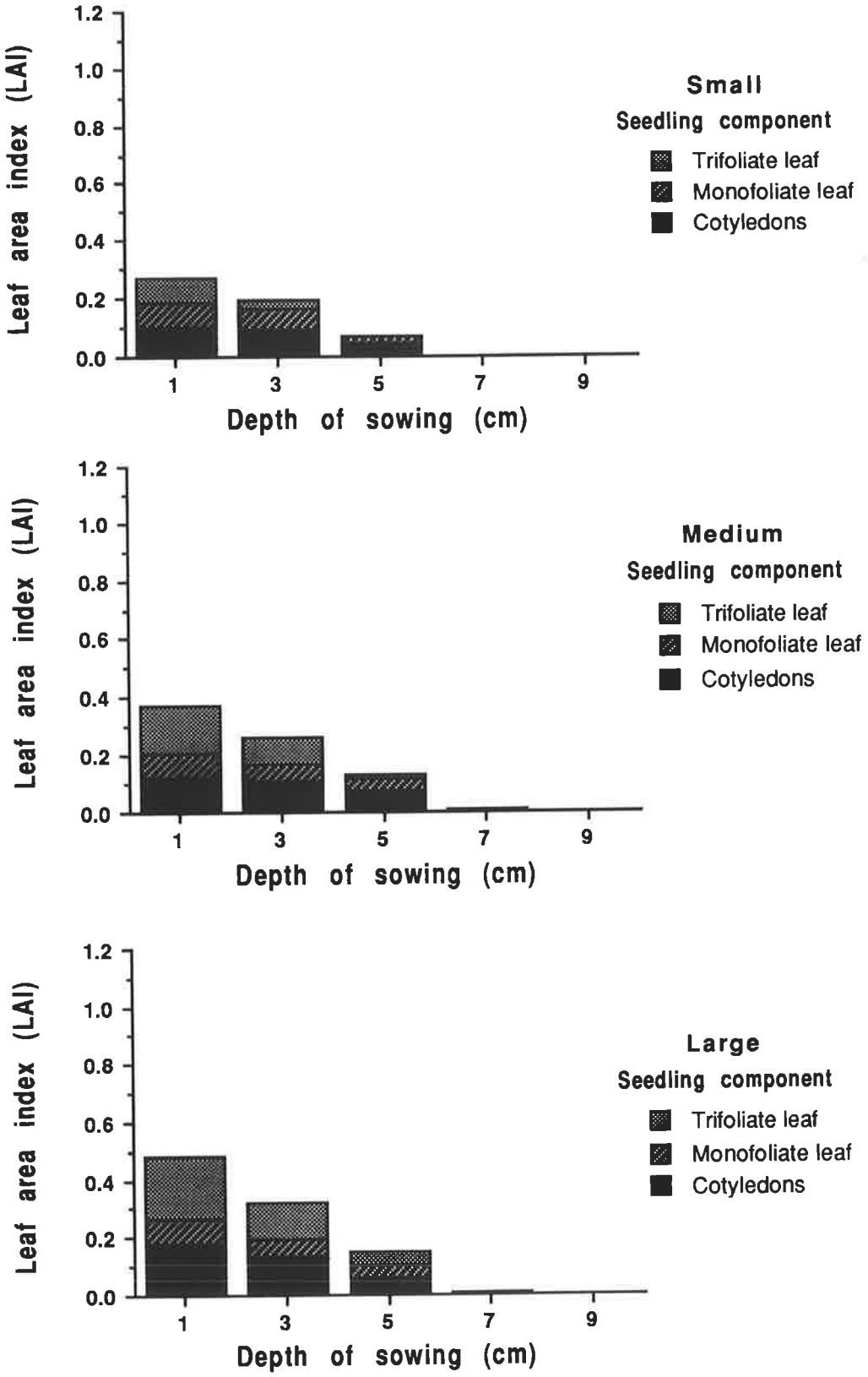


Figure 4.11 The leaf area index of Trikkala subterranean clover seedlings sown in a sandy soil with small, medium and large seed-size categories.

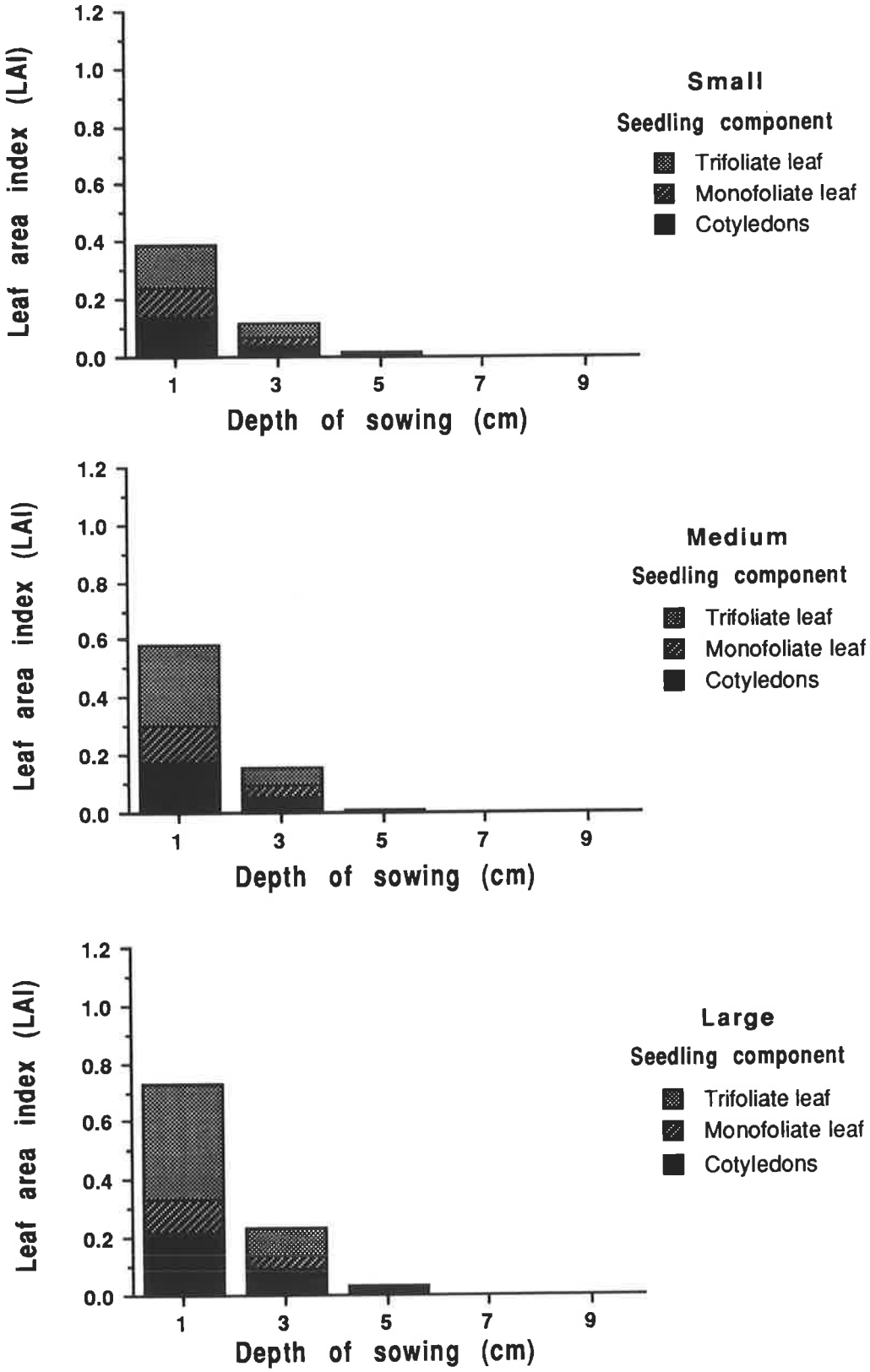


Figure 4.12 The leaf area index of Trikkala subterranean clover seedlings sown in a loamy soil with small, medium and large seed-size categories.

Plate 4.1 Close-up view of emergence and early growth of Sava snail medic seedlings in a sandy soil (upper) and in a loamy soil (lower) at harvest time (14 days after initial watering).

	Seed size
Front to rear:	Small (S), Medium (M), Large (L)
	Depth of sowing
Left to right:	1 cm (1), 3 cm (3), 5 cm (5), 7 cm (7), 9 cm (9)

Medicago scutellata cv. Sava

Sand



Medicago scutellata cv. Sava

Loam

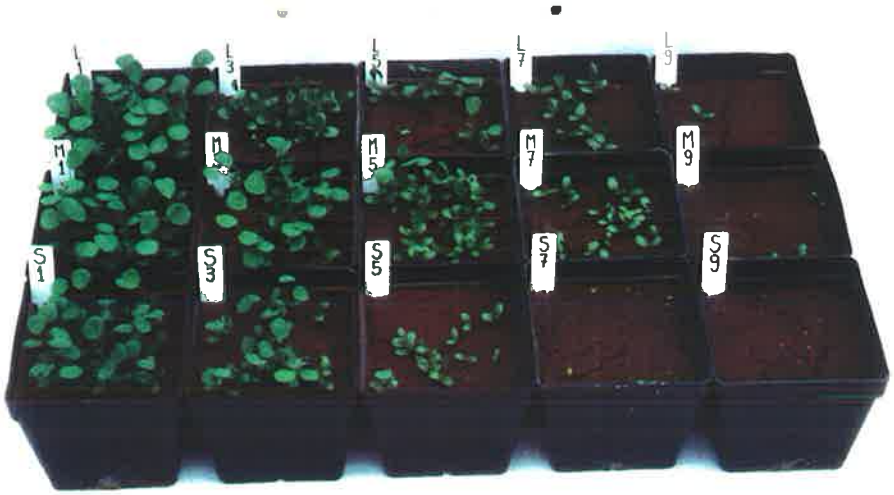


Plate 4.2 Close-up view of emergence and early growth of Trikkala subterranean clover seedlings in a sandy soil (upper) and in a loamy soil (lower) at harvest time (14 days after initial watering).

	Seed size
Front to rear:	Small (S), Medium (M), Large (L)
	Depth of sowing
Left to right:	1 cm (1), 3 cm (3), 5 cm (5), 7 cm (7), 9 cm (9)

Trifolium subterraneum ssp.
yanninicum cv. Trikkala
Sand



Trifolium subterraneum ssp.
yanninicum cv. Trikkala
Loam



4.3 Glasshouse Experiment 2: The effects of seed size, depth of sowing and cereal straw residues on the emergence and early growth of medic seedlings

4.3.1 Introduction

Previous studies revealed that physical impedance by cereal straw residues *per se* had minor influence on seedling emergence, but it was also recognized that more stressful conditions are required to show the impact of cereal straw residues on the emergence and seedling vigour of medic seedlings (Quigley 1988). For this reason, the ability of cultivar Sava pods containing contrasting seed sizes to emerge from different depths of sowing and levels of straw was investigated.

4.3.2 Materials and methods

A population of pods produced during the 1987 growing season at Brinkworth about 167 km north of Adelaide, South Australia was used. The collected pods were cleaned of impurities and sieved through a pair of 30 cm diameter, 9.5 mm and 12.5 mm round-holed steel laboratory sieves to obtain the desired small, medium and large pod-size categories (Table 4.5).

Sub-samples of 100 pods per pod-size category were selected and the individual dimension of the pods determined with a calipers (Table 4.5). Each sub-sample of 100 pods was divided in four lots of 25 pods, weighed, dissected by hand, and the number of seeds per pod and the individual weight of the seed estimated. A positive relationship between pod size and seed size became apparent (Table 4.6). After that, all the seed involved per pod-size category was bulked and sieved through a set of 20 cm diameter square-holed brass laboratory sieves ranging between 1.40 mm and 3.35 mm in aperture, to estimate the distribution of the seed by size and weight (Table 4.7). Finally, the seed was tested for germinability under the same conditions described in 3.3 Laboratory Experiment 1 (Table 4.8).

Two hundred and forty, freely drained black polypropylene pots 23 cm in height and 25 cm in diameter were filled with 5 cm of steam-sterilized wood chips and a hard-setting red-brown earth soil collected from the top soil (0-15 cm) at Waite Agricultural Research Institute. The soil material was processed in the same conditions shown before (4.2 Glasshouse Experiment 1), except that it was sterilized by use of a forced-draught dehydrator, programmed to operate at 105°C for a period of 48 h, in order to kill the embryos of any seed present, reduce the risk of fungal attack and to better conserve soil structure.

Pods were sown at four depths (2.5, 5.0, 7.5 and 10.0 cm). Twenty five pods were sown in each pot, using a 21 cm in diameter wooden template marker (Plate 4.3). The distance between pods was about 3 cm on the line of the circumference and 4 cm between lines. In order to avoid the emergence of seedlings between the soil and the interior wall of the pot, the external line of pods was located three centimetres from the interior side of the pot. The pods were covered with soil to the final level and slightly compacted. Unweathered wheat straw (87.2 %, DM basis) equivalent to 0, 2, 4, 6 and 8 ton DM/ha, was placed horizontaly in an uniform layer on the surface of the soil (Plate 4.4), giving an additional depth of 0.0 ± 0.0 , 1.5 ± 0.3 , 2.3 ± 0.4 , 3.1 ± 0.4 and 3.5 ± 0.4 cm ($\pm$ s.d.), respectively (Figure 4.13).

The pots were transferred to a glasshouse and the soil moistened with rain water to field capacity on 27 July, 1990. To prevent the pre-emergence attack of seedlings by soil fungi, the straw mulch, as well as the soil were treated with Ridomil® Granular fungicide (active constituent metalaxyl, 50 g/kg) applied at a rate of 0.5 g a.i. per m^2. After emergence, preventative treatments with Benlate® Granular fungicide, 2.5 g a.i. per m^2 were applied. During the experimental period, the soil was maintained at field capacity.

The emergence of seedlings, was defined as the complete appearance of cotyledons or monofoliate leaf above the soil surface or above the straw layer and was recorded daily

over 28 days after the initial watering (24 August). A single harvest was carried out on 17 August, 21 days after initial watering. The emerged seedlings were harvested, stored and processed by the same methods described before (4.2 Glasshouse Experiment 1).

The levels of readily-germinable seed recorded from the previous germination test (Table 4.8) differed between pod-size categories and the emergence that occurred under the glasshouse conditions actually exceeded those values. For these reasons, given the insignificant levels of dead seed, the emergence of seedlings was corrected for each pot by using the expected potential emergence, obtained by the difference between the total number of seeds per pot and the number of hard seed per pot obtained by hand dissection of the pods after the experiment was concluded. The average total number of seeds per pot was used because (according to a previous hand dissection of 40 lots of 25 pods of small, medium and large pod-size categories) it was less variable with coefficients of variation of 3.4%, 3.9% and 4.3% respectively.

Air temperature in the glasshouse was recorded during the experimental period by use a thermohydrograph located at plant level (Figure 4.14) and shielded from the direct sunlight. Also, one hundred readings of the resistance of soil (0.68 ± 0.17 kg/cm^2) to the emerging seedlings were made at field capacity by use of a soil penetrometer.

The experiment comprised factorial combinations (completely randomized block design) as follows: Pod size (3) x Depth of sowing (4) x Wheat straw concentration (5) x Blocks (4)= 240 pots. Analyses of variance were done using GENSTAT V on daily cumulative percentages of emergence calculated over the experimental period. Seedling vigour variables relating values of dry matter and leaf area were also considered in the analyses. Because the pod-size categories used in the study differed in expected potential emergence of seedlings, the analyses of variance of this variable were carried out considering individual values only, assuming that at that stage of plant development intraspecific competition was not occurring.

4.3.3 Results

Analyses of variance on daily cumulative increased percentages of germination obtained over the experimental period show a significant three-way interaction between straw level, pod size and depth of sowing on days 5, 6 and 7. Two-way interactions were mainly observed soon after the initial watering, where the combined effect of straw level and depth of sowing on the emergence of seedlings was more persistent over time. Despite these interactions, both factors were always significant through the experimental period, revealing that the influence of pod size appeared to be of least importance (Table 4.9).

The first appearance of seedlings started on day 5 at shallow sowing depths. Only seedlings from small and medium sized pods in the control treatment and seedlings from small pods in the 2 and 4 ton DM/ha of straw treatments were observed at that time. This suggests that seedlings produced by smaller seeds had higher initial rates of emergence. This effect persisted over time, but tended to be overcome by the combined effect of increasing depth of sowing and straw level. In these situations, seedlings from larger seeds had higher rates of emergence compared with seedlings from smaller ones (Figures 4.15-4.19).

Final emergence was most affected by depth of sowing, but this was exacerbated as the level of straw increased, especially at the greatest sowing depth, where the maximum negative combined effect was observed. The only situation where the level of straw used did not affect the final emergence of seedlings was at the most-shallow depth of sowing. However, the single exception occurred when medic seedlings from small pods sown at 2.5 cm depth were covered with an equivalent of 8 ton DM/ha of straw residues. Seed size appeared to affect the initial stages of emergence more than the later stages, but after 28 days seed size still had some influence. This was most apparent under the most extreme of the imposed conditions on emergence, where seedlings produced by larger seeds still showed higher final percentages of emergence than seedlings produced by smaller seeds. In contrast, under less restricted conditions of emergence (given by the

combined effects of lower levels of straw and shallower depth of sowing), a higher percentage emergence of seedlings came from small pods (Plate 4.5; Appendix 4.8).

The individual seedling dry weight was significantly affected by straw level, pod size and depth of sowing (Table 4.10). The size of the seed as well as the depth of sowing appeared to be the most important factors influencing the mean seedling weight. Despite this, the interaction of pod size and depth of sowing was not significant at harvest time. The weight of the seedlings decreased with deeper placement of pods in the soil and smaller seed size. To a lesser extent, increased levels of straw up to 4 ton DM/ha tended to increase the individual weight of seedlings, but higher levels of straw decreased their individual dry weight (Figures 4.20-4.21).

Analyses of variance carried out on the individual lamina area of cotyledons and monofoliate leaves, as well as on the mean lamina area comprising the whole seedling, showed a significant effect of the factors involved in the study. Only pod size, did not significantly affect the lamina area of the monofoliate leaf. Similarly, a two-way significant interaction between pod size and depth of sowing was observed in relation to the cotyledon area (Table 4.10), revealing that both factors play a role in determining the photosynthetic area of seedlings (Figures 4.22-4.23). At that stage of plant development, the cotyledons represent the seedling component with the highest proportion of dry matter (Appendices 4.9-4.13). Both the individual lamina area of cotyledons and monofoliate leaves were importantly determined by the size of the seed and became importantly affected by depth of sowing as well as by the concentration of wheat straw residues (Appendices 4.14-4.15).

4.3.4 Discussion

In the earlier study (4.2 Glasshouse Experiment 1) it was noted that seedlings produced by smaller seeds had higher initial rates of emergence than seedlings from larger seeds. This difference was confirmed in this study. Only the imposition of greater sowing depth and increased levels of straw, resulted in higher rates of seedling emergence from

larger pods rather than smaller ones. This response was most apparent when the soil surface was covered with 8 ton DM/ha of straw. This finding suggests that smaller seeds would germinate faster, given the better moisture relationship described previously (3.3-3.4 Laboratory Experiments 1-2). The force exerted into the soil by the emerging seedling is positively correlated with seed weight (Williams 1956; Jensen *et al.* 1972; Sinha and Ghildyal 1979; Longer *et al.* 1986) and the cross-section area of the seedling stem. The flexibility of stem tends to decrease and the axial force that can be exerted for given tissue pressure increases; however, greater lifting power tends to be offset by less flexibility (Arndt 1965).

Final emergence was more influenced by depth of sowing, but became more and more depressed by increased levels of straw. This may be due to the additional physical barrier imposed on elongation of the hypocotyl, reducing the potential seedling development by reducing photosynthesis (Robbins *et al.* 1942). Independent of the depth of sowing, the emergence of medic seedlings generated from small pods, was frequently found to be less affected than seedlings derived from larger pods, as a consequence of increased levels of straw.

Physical impedance produced by a cereal straw *per se* has a minor influence on seedling emergence, but more stressful conditions given by factors, such as depth of sowing, waterlogging, low temperatures, etc. are required in order to estimate the response of medic seedlings to cereal straw residues (Quigley 1988).

The same trend in individual seedling weight and lamina area of seedlings was obtained as a consequence of seed size and depth of sowing as in the previous study (4.2 Glasshouse Experiment 1). There was some positive response to the level of straw up to 4 ton DM/ha on those seedling variables observed possibly due to improved microenvironmental conditions being achieved. The presence of wheat stubble at the soil surface has been reported by Aston and Fisher (1986) to reduce the range of daily temperatures by decreasing the maximum temperature without having any effect on the minimum temperature. In this experiment the wheat straw was clean and fresh:

however, in the field there is frequently partial decomposition of straw residues which results in a dense matted layer which can retard breakdown of hard-seededness and completely suppress emergence of annual medics and subterranean clover seedlings.

Table 4.5 Characteristics of pod-size categories used to test the effects of seed size, depth of sowing and cereal straw residues on emergence and early vegetative growth of Sava snail medic seedlings.

Pod-size category	Sieve aperture (mm)	Height (mm)	Height ratio	Diameter (mm)	Diameter ratio	Diam./Height ratio
Small	< 9.5	0.8±0.101	1.00	0.9±0.077	1.00	1.21
Medium	9.5 - 12.5	1.0±0.080	1.29	1.1±0.059	1.17	1.10
Large	> 12.50	1.1±0.072	1.44	1.3±0.066	1.34	1.11

Table 4.6 Characteristics of pod and seed weights used to test the effects of seed size, depth of sowing and cereal straw residues on emergence and early vegetative growth of Sava snail medic seedlings.

Pod-size category	Pod weight (mg)	Pod weight ratio	Seeds per pod (#)	Seeds per pod ratio	Seed weight (mg)	Seed weight ratio
Small	149.4	1.00	4.12±0.23	1.00	12.56±0.36	1.00
Medium	250.7	1.68	4.68±0.09	1.14	16.87±0.48	1.34
Large	353.6	2.37	5.02±0.15	1.22	20.34±0.18	1.62

Table 4.7 Distribution of seed by size and weight in Sava snail medic pod-size categories.

Sieve aperture Min. - Max. (mm)	Pod-size category: Small (%)	Small (mg/seed)	Medium (%)	Medium (mg/seed)	Large (%)	Large (mg/seed)
1.41 - 1.70	5.91	5.65	0.44	7.40	-	-
1.71 - 2.00	34.28	10.11	11.26	11.20	1.57	12.21
2.01 - 2.36	48.23	14.11	52.93	15.53	32.09	17.49
2.37 - 2.80	11.58	18.16	35.14	19.48	65.55	21.49
2.81 - 3.35	-	-	0.23	24.60	0.79	24.65

Table 4.8 Germination test data on seeds in Sava snail medic pod-size categories.

Seed condition	Pod-size category: Small	Medium	Large
Germinated	35.3	21.7	21.4
Dead	0.0	0.0	0.0
Soft	35.3	21.7	21.4
Hard	64.7	78.3	78.6
Hard/Soft ratio	1.83	3.61	3.67
Hard seed weight, mg	12.20	16.63	19.99

Table 4.9 Summary of analyses of variance on daily cumulative increases in percentage emergence obtained during the experimental period.

Source of variation	Days after inital watering											
	5	6	7	8	9	10	11	12	13	14	15	16
	(Significance levels) †											
Straw	***	***	***	***	***	***	***	***	***	***	***	***
Pod size	*	***	***	***	***	***	***	***	***	***	***	*
Depth	***	***	***	***	***	***	***	***	***	***	***	***
Straw x Pod size	*	***	***	*	NS	NS	NS	NS	NS	NS	NS	NS
Straw x Depth	***	***	***	***	***	***	*	NS	NS	NS	NS	NS
Pod size x Depth	***	***	***	***	**	NS	NS	NS	NS	NS	NS	NS
Straw x Pod size x Depth	***	***	***	NS	NS	NS	NS	NS	NS	NS	NS	NS

Source of variation	Days after inital watering											
	17	18	19	20	21	22	23	24	25	26	27	28
	(Significance levels) †											
Straw	***	***	***	***	***	***	NS	***	***	***	***	***
Pod size	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Depth	***	***	***	***	***	***	***	***	***	***	***	***
Straw x Pod size	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Straw x Depth	NS	NS	*	*	*	*	NS	*	*	NS	NS	NS
Pod size x Depth	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Straw x Pod size x Depth	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Table 4.10 Summary of analyses of variance on individual seedling weight and leaf lamina areas of Sava snail medic seedlings.

Source of variation	Seedling weight	Individual leaf lamina area (cm^2)		
	(mg DM)	Cotyledon	Monofoliate	Seedling
	(Significance levels) †			
Straw	*	***	*	**
Pod size	***	***	NS	***
Depth	***	***	***	***
Straw x Pod size	NS	NS	NS	NS
Straw x Depth	NS	NS	NS	NS
Pod size x Depth	NS	**	NS	NS
Straw x Pod size x Depth	NS	NS	NS	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

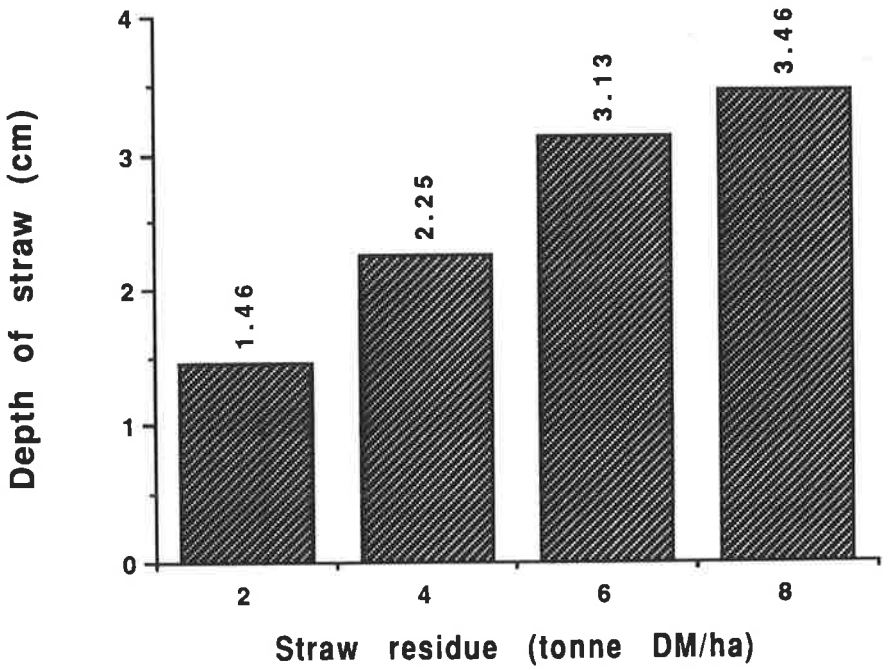


Figure 4.13 The depth of straw layers resulting from the amounts of material used for the different treatments.

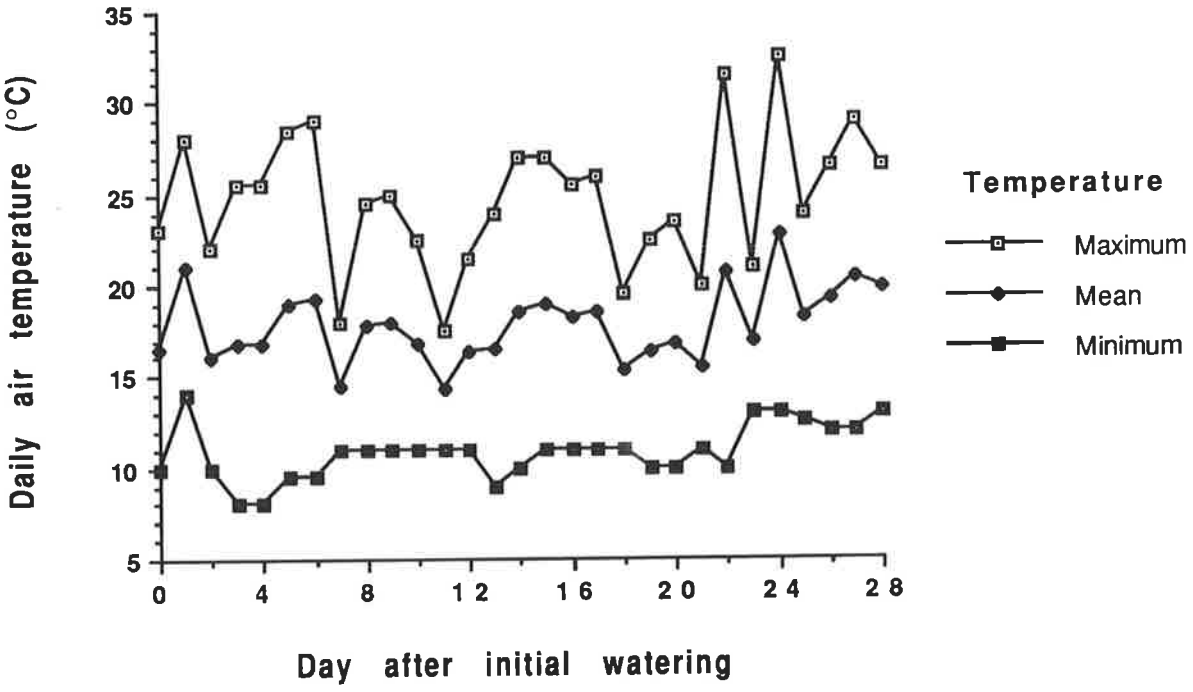


Figure 4.14 The daily minimum, mean and maximum air temperatures recorded in the glasshouse during the experimental period.

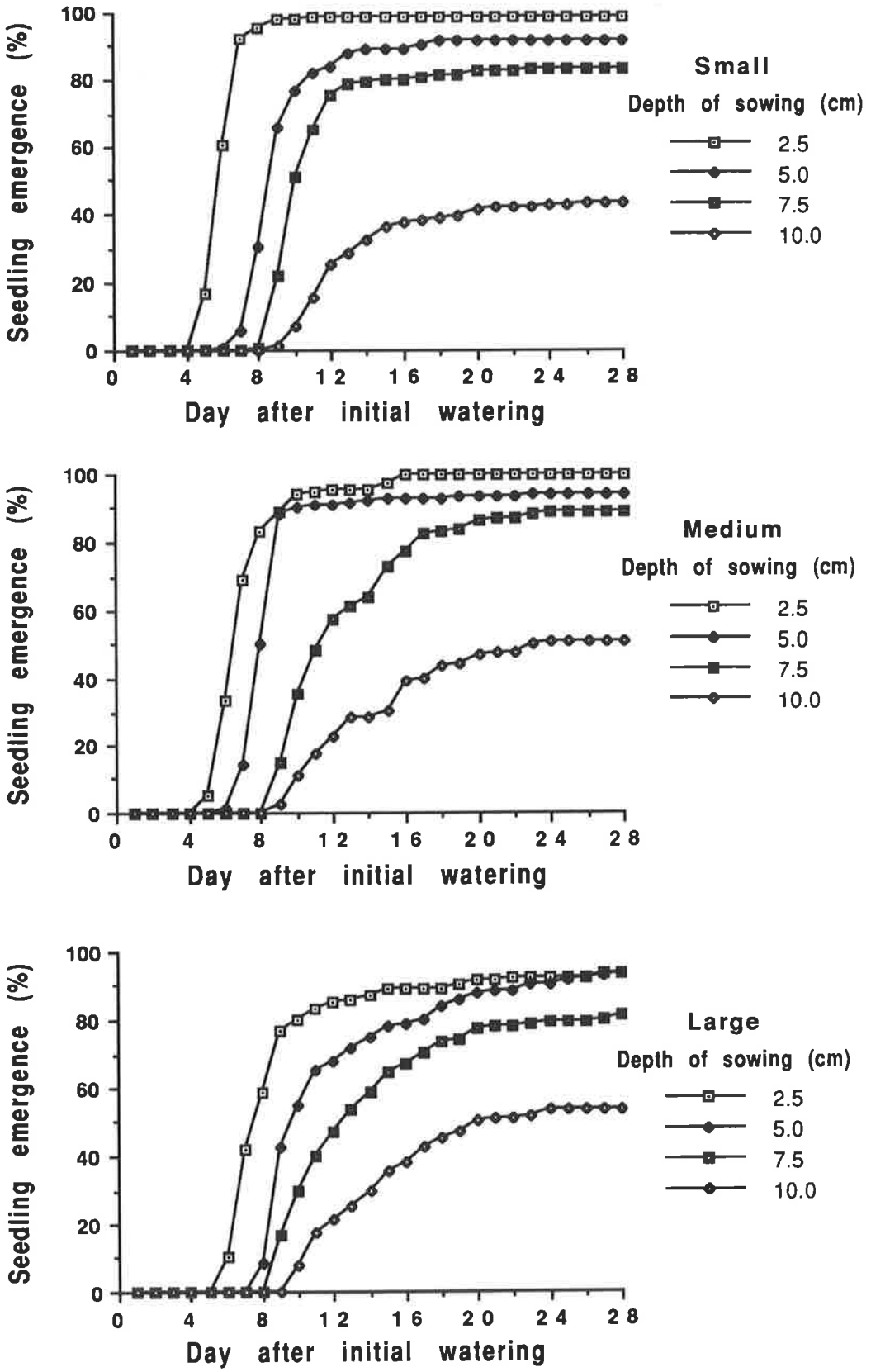


Figure 4.15 The trend in emergence of Sava snail medic seedlings from a soil without cover of wheat straw residues.

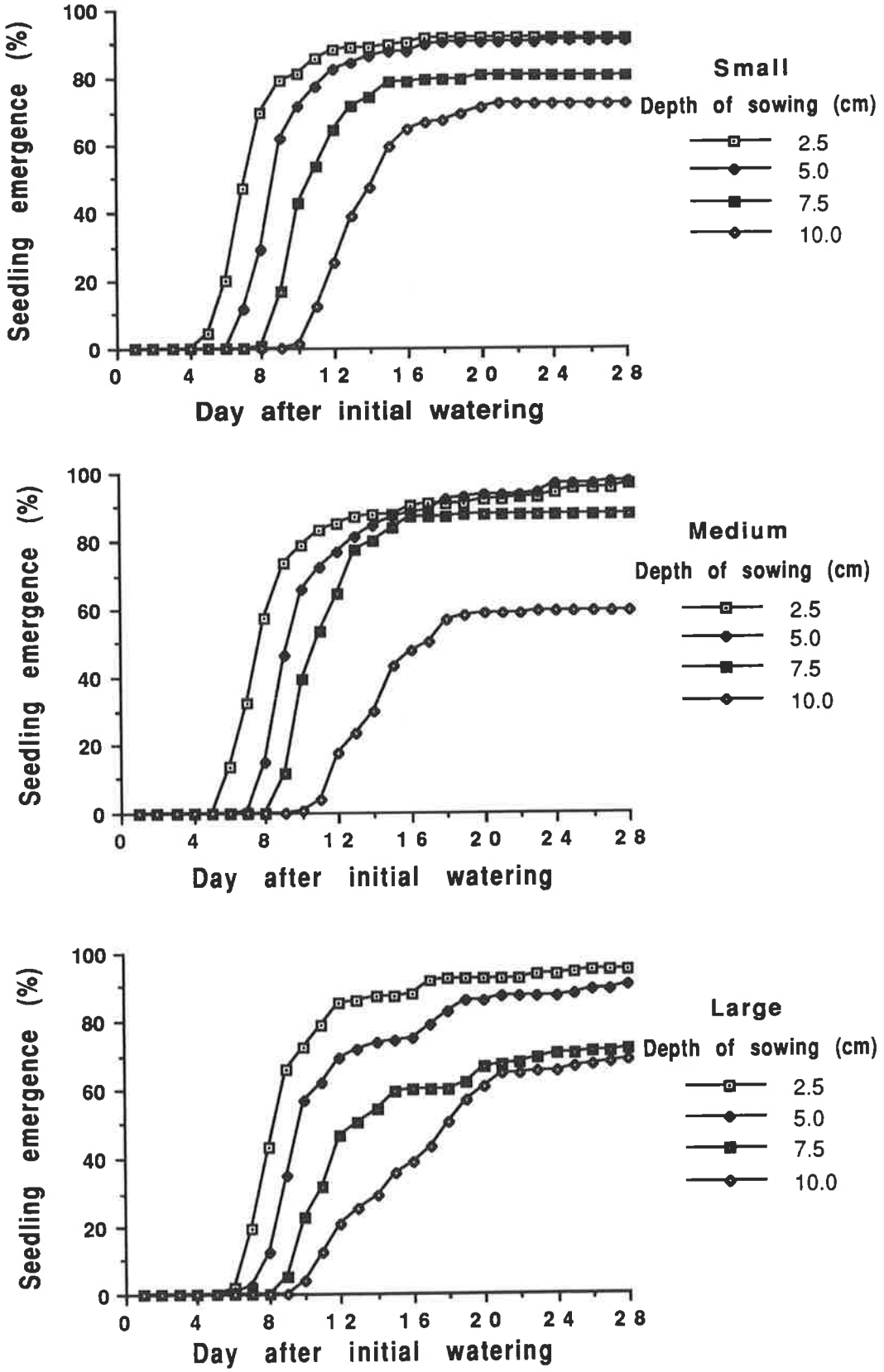


Figure 4.16 The trend in emergence of Sava snail medic seedlings from a soil covered with 2 ton DM/ha of wheat straw residues.

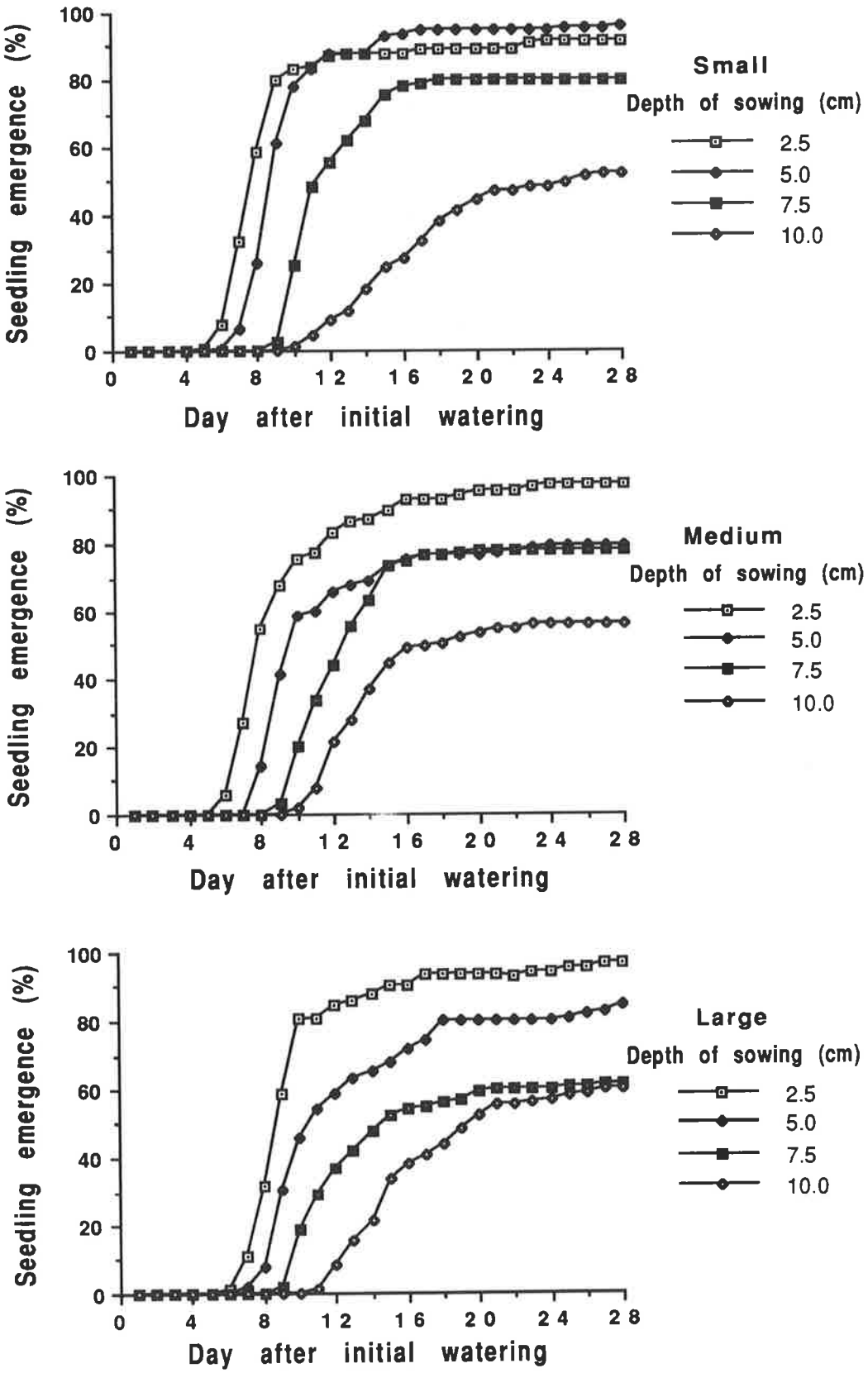


Figure 4.17 The trend in emergence of Sava snail medic seedlings from a soil covered with 4 ton DM/ha of wheat straw residues.

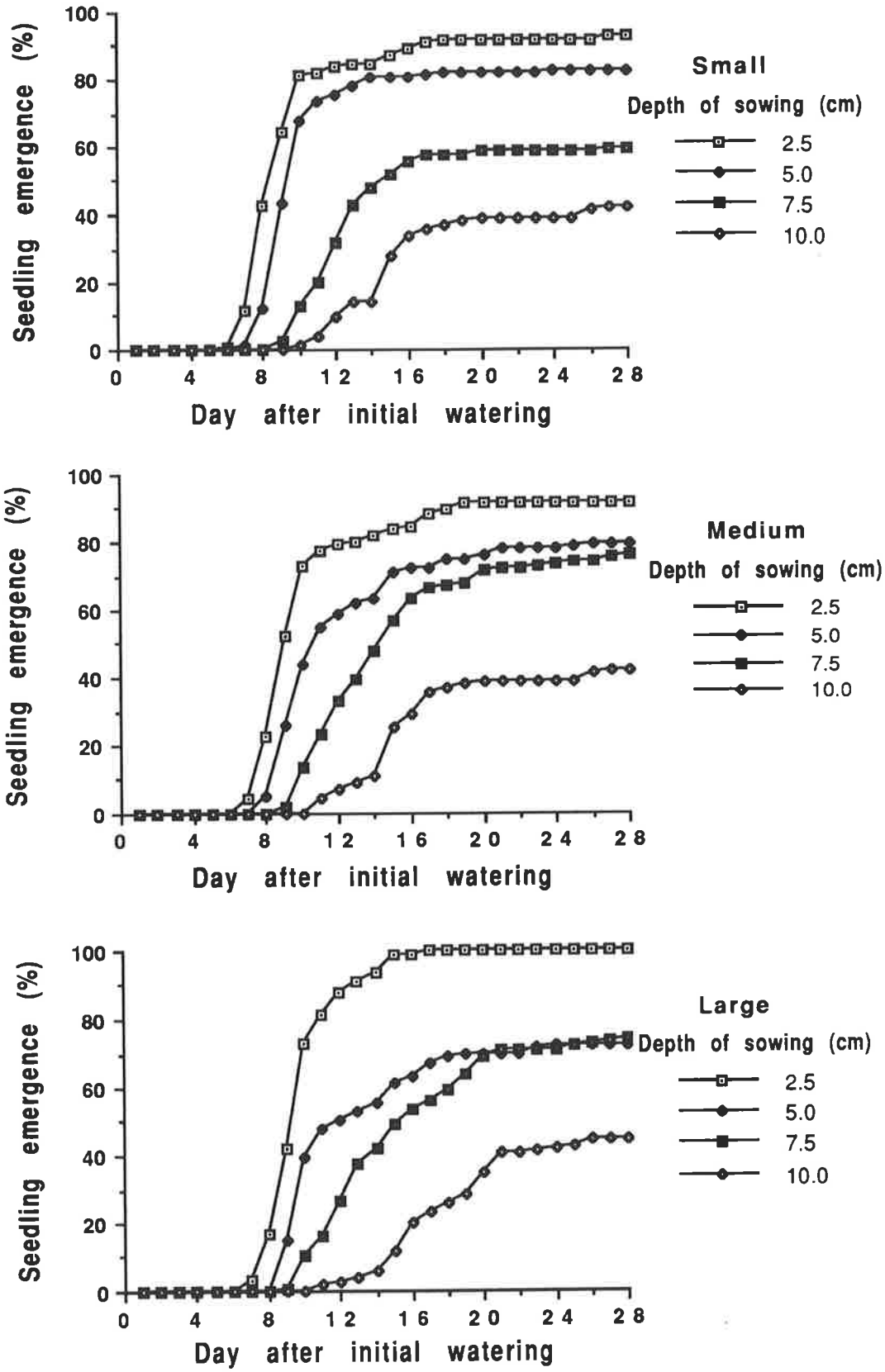


Figure 4.18 The trend in emergence of Sava snail medic seedlings from a soil covered with 6 ton DM/ha of wheat straw residues.

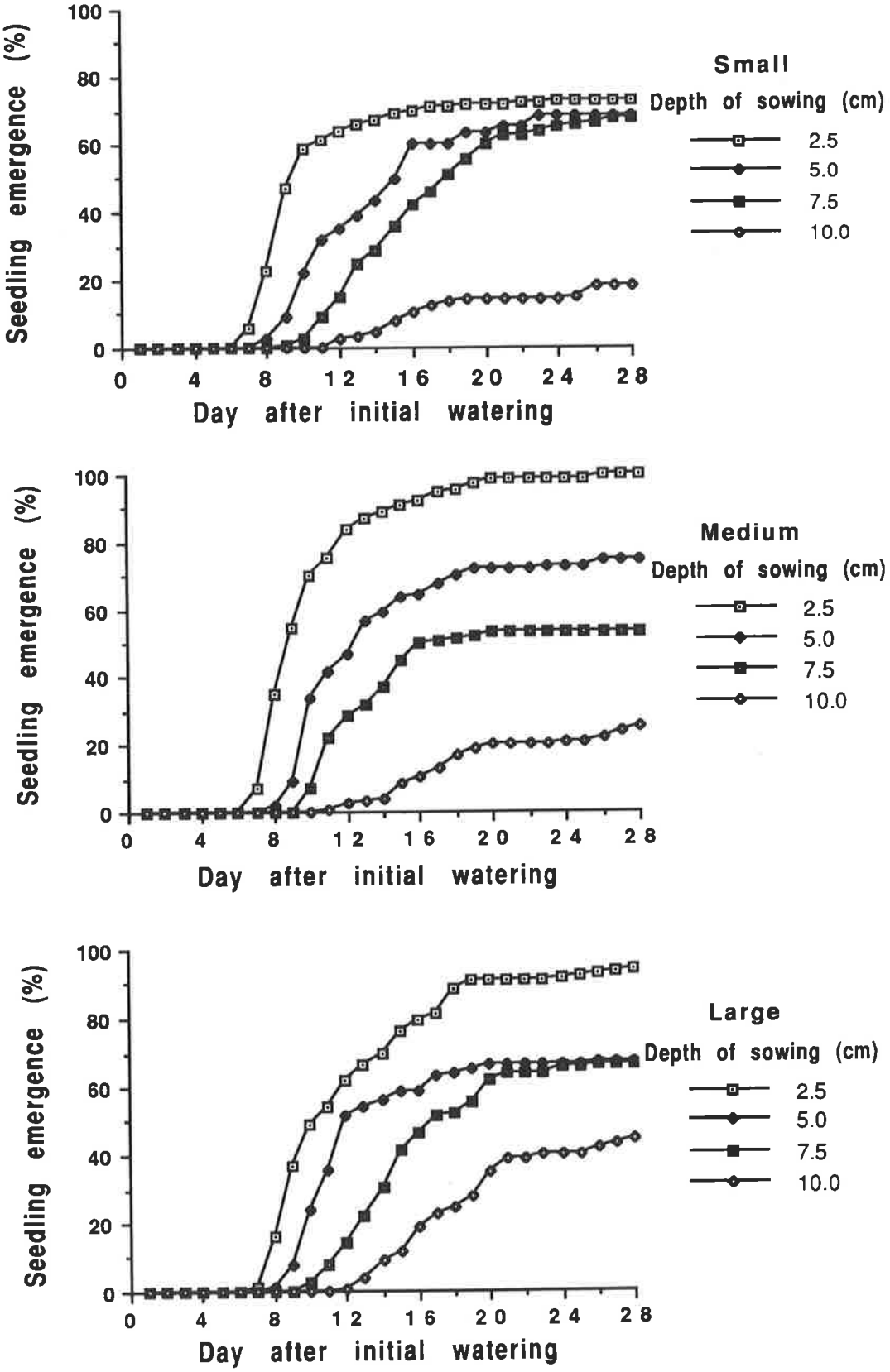


Figure 4.19 The trend in emergence of Sava snail medic seedlings from a soil covered with 8 ton DM/ha of wheat straw residues.

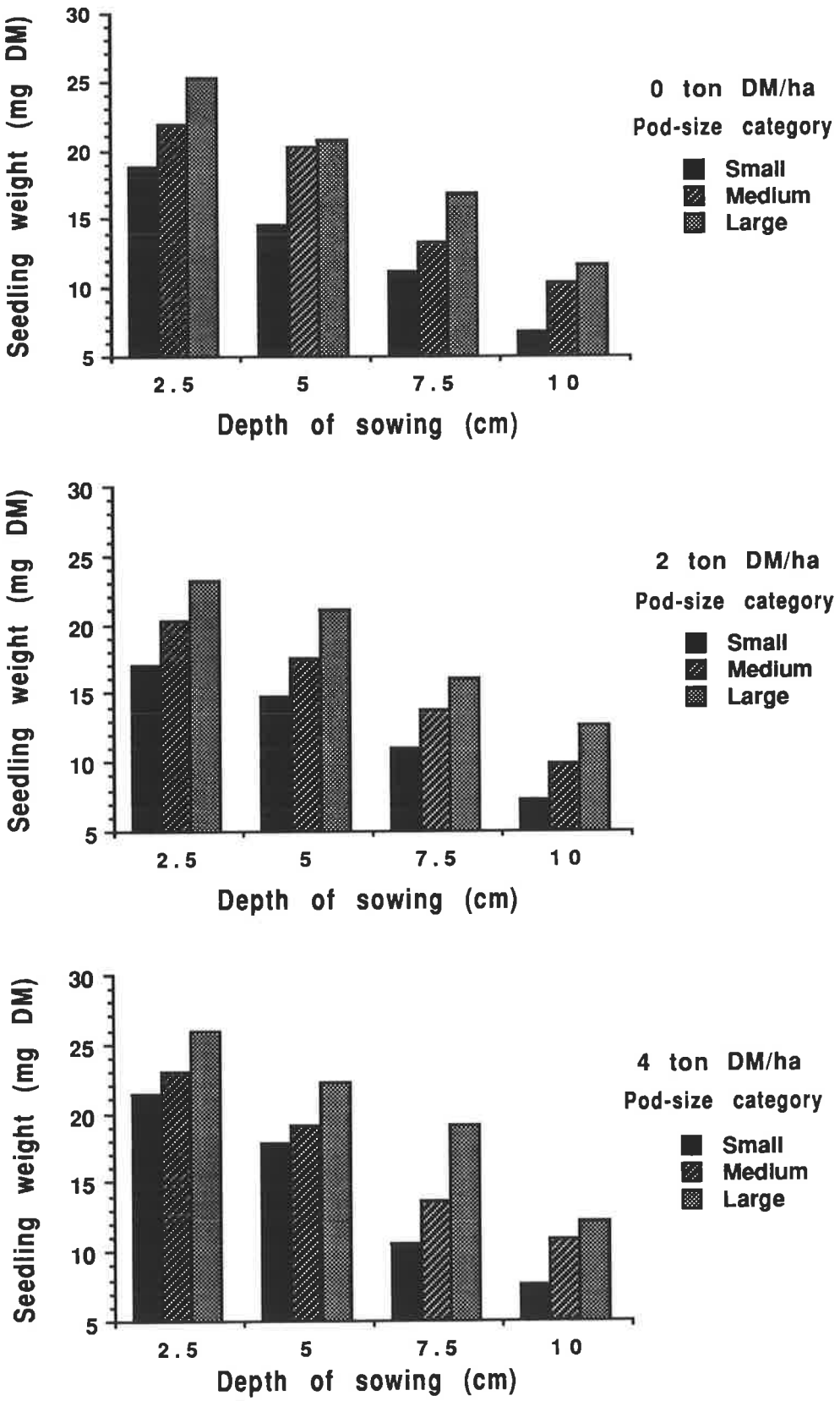


Figure 4.20 The individual weight of Sava snail medic seedlings emerged from a soil surface covered with 0, 2 and 4 ton DM/ha of wheat straw residues.

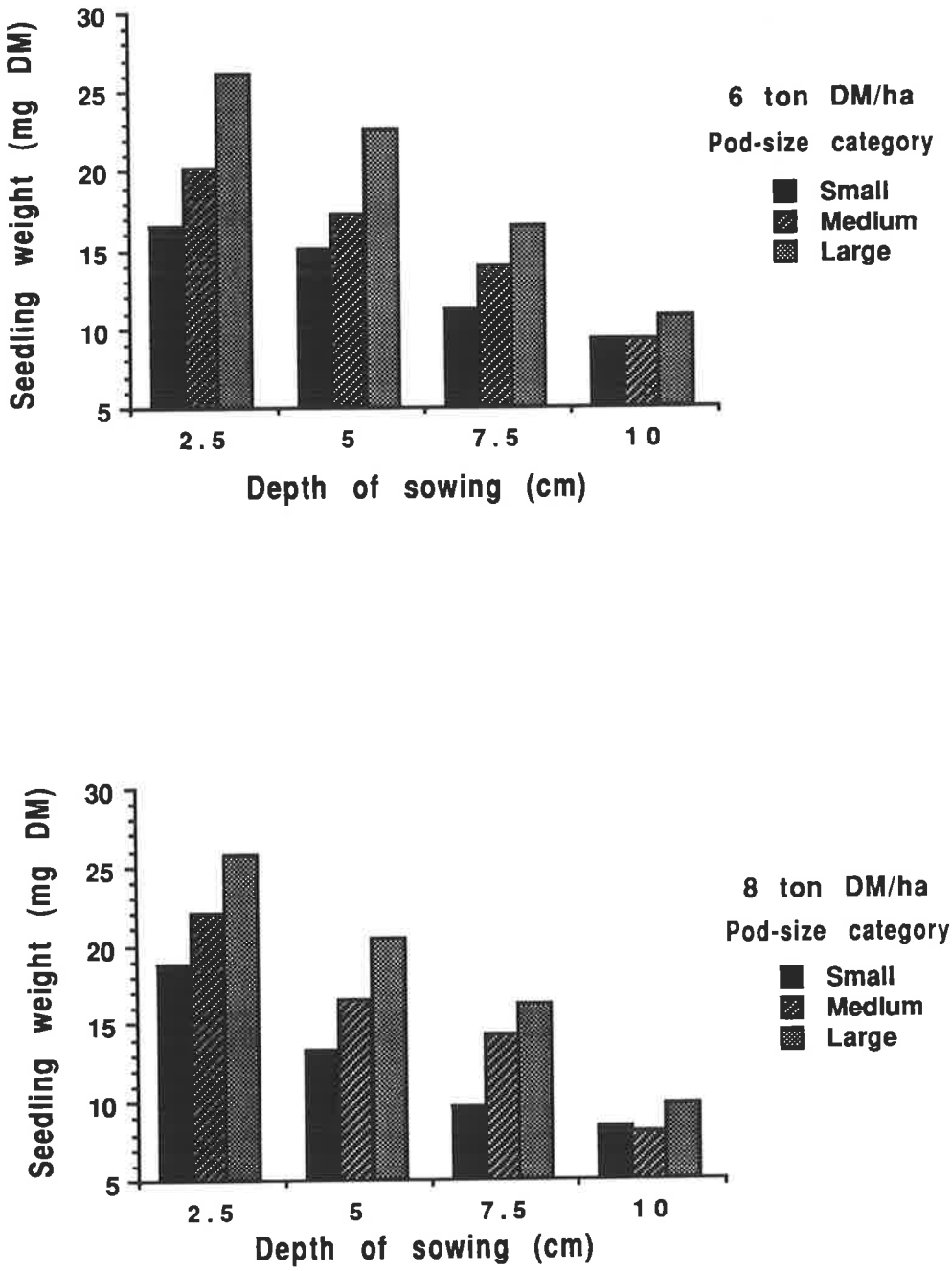


Figure 4.21 The individual weight of Sava snail medic seedlings emerged from a soil surface covered with 6 and 8 ton DM/ha of wheat straw residues.

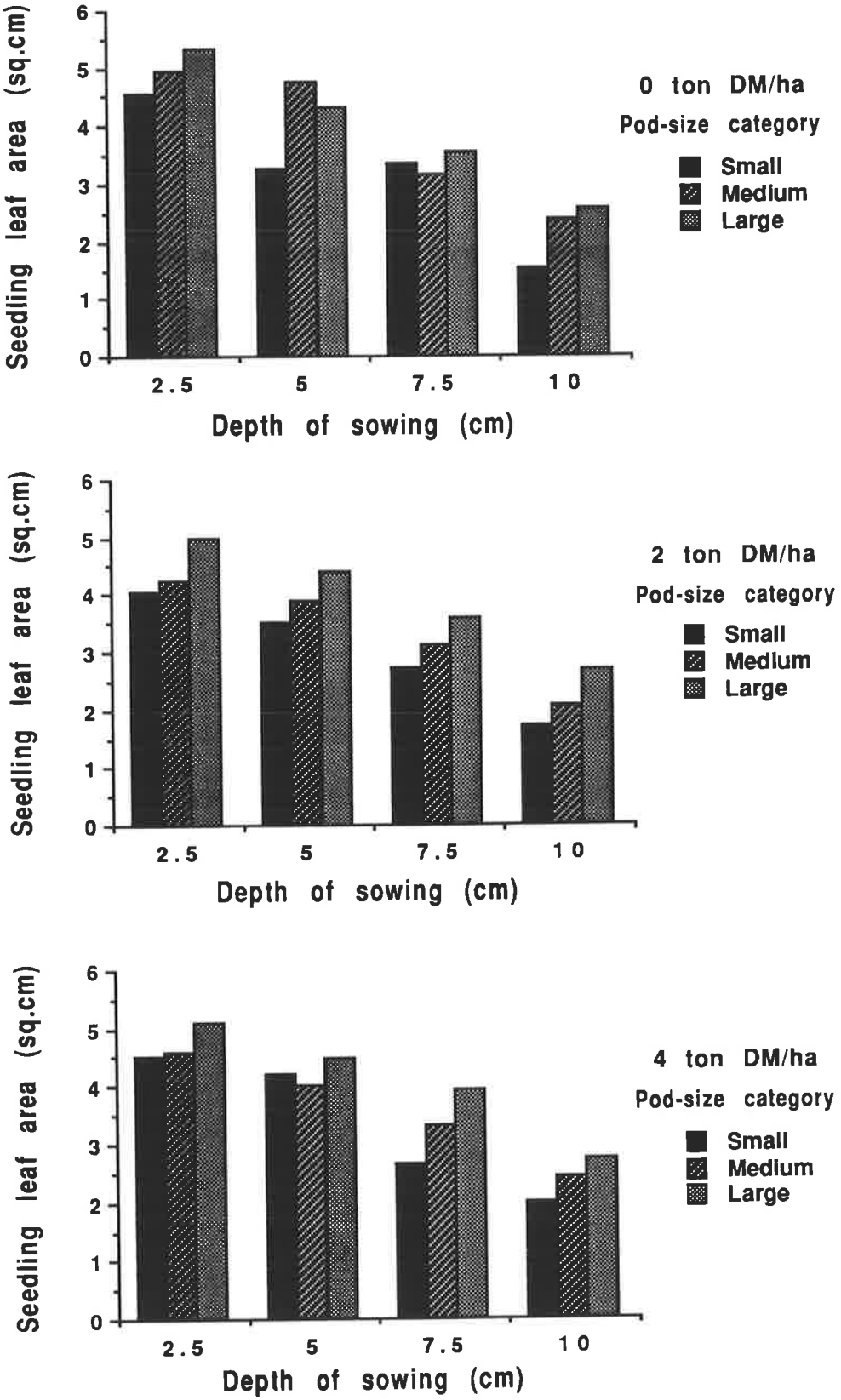
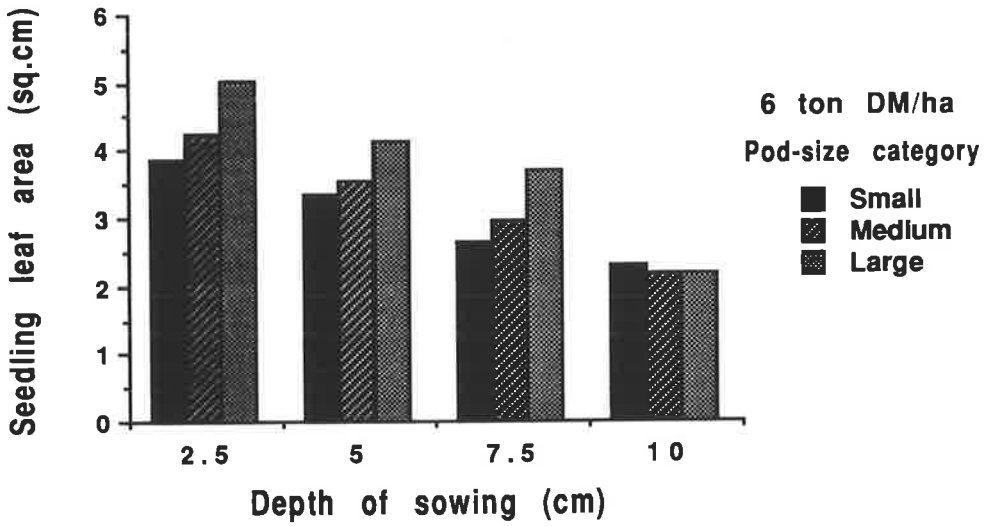


Figure 4.22 The leaf laminae area of Sava snail medic seedlings emerged from a soil surface covered with 0, 2 and 4 ton DM/ha of wheat straw residues.



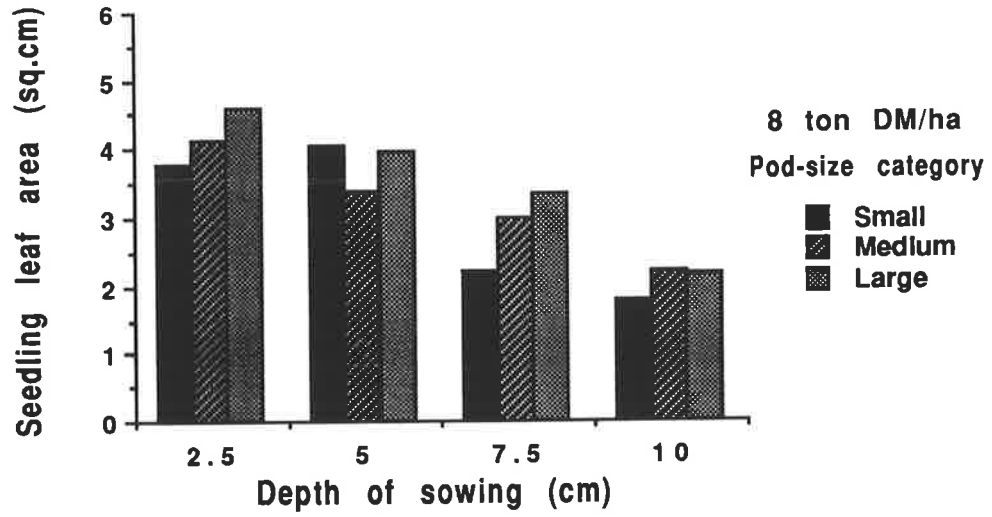
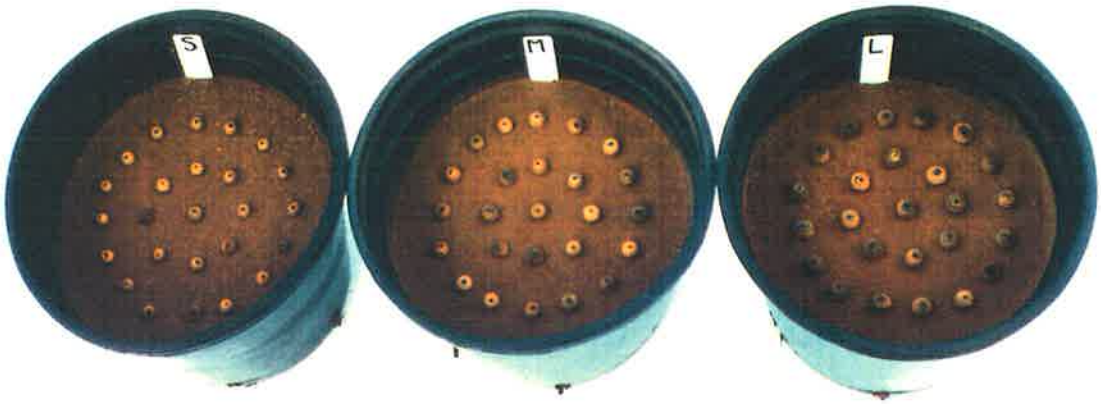


Figure 4.23 The leaf laminae area of Sava snail medic seedlings emerged from a soil surface covered with 6 and 8 ton DM/ha of wheat straw residues.

Plate 4.3 Close-up view of sowing pattern of Sava snail medic using small (S), medium (M) and large (L) pod-size categories.

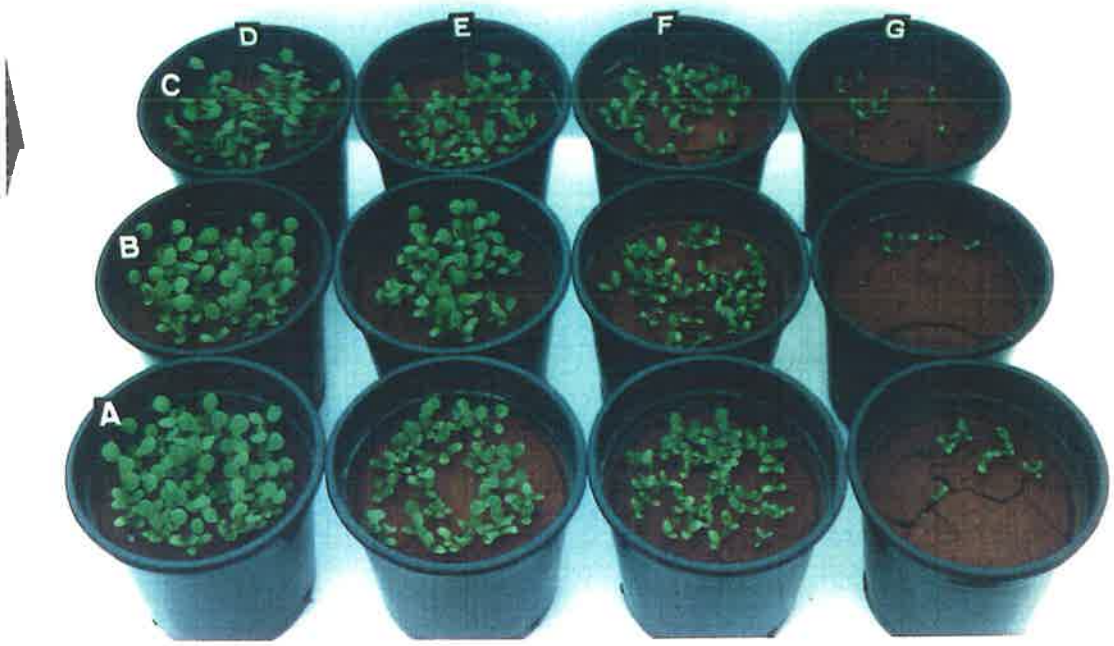
Plate 4.4 Close-up view of wheat straw residues (0, 2, 4, 6 and 8 tonne/DM/ha) used to test the emergence and early growth of Sava snail medic seedlings from different pod-size categories.


S
M
L


0
2
4
6
8

Plate 4.5 Close-up view of emergence and early growth of Sava snail medic seedlings from various pod-size categories sown at different soil depths with no straw (upper) and 8 ton DM/ha of wheat straw (lower) at harvest time (21 days after initial watering).

	Pod size
Front to rear:	Small (A), Medium (B), Large (C)
	Depth of sowing
Left to right:	2.5 cm (D), 5.0 cm (E), 7.5 cm (F), 10 cm (G)


D
E
F
G
C
B
A


D
E
F
G
C
B
A

4.4 Raised-bed Experiment 1: The effects of seed size and plant density on early growth of medic and subterranean clover seedlings

4.4.1 Materials and methods

To test the effects of seed size and plant density on early productivity of Sava snail medic and Trikkala subterranean clover, both cultivars were grown on raised-beds located outdoors at the Waite Agricultural Research Institute (WARI).

Treatments consisted of three selected seed-size categories (small, medium and large) obtained as before (3.2 Laboratory Test 1), and four plant densities (low, regular, high and very high) adjusted for readily-germinable (soft) seed percentages according to a previous germination test (Table 4.11 and Appendix 4.16) to give potential plant densities of 100, 500, 2500 and 12,500 seedlings per m^2 (Appendix 4.17). The treatments were sown in micro-plots of 400 cm^2 (20 x 20 cm) and arranged in a completely randomized block design with a factorial treatment structure and three replicates. An independent experiment was carried out with each species.

Four raised-beds were used, each being oriented lengthwise north-south. For each cultivar, six sub-plots or harvest areas with the corresponding combinations of three seed sizes and four plant densities, assigned at random, were set aside within each block. Because some shading effect was expected on the north side of the northern raised-beds, the sub-plots were not randomized within a block. Thus, the first harvest was done from the northern end of each block and the consecutive harvests were moved progressively southward. This procedure had proved entirely reliable in several previous experiments (e.g. de Koning 1984; Ababneh 1991). The total combination for each experiment comprised: Seed sizes (3) x Plant densities (4) x Harvests (6) x Blocks (3)= 216 micro-plots.

The permanent raised beds filled with sandy loam soil were scraped to remove any legume or weed seed before applying a complete mineral-mix fertilizer at a rate of 200 kg/ha. Then a layer of approximately 5 cm of aerated, steam-sterilized University of California potting mixture (sand 1 : peatmoss 1), also provided with a complete mineral-mix fertilizer, was spread. This layer was carefully levelled, and a galvanized mesh with 20 x 20 cm openings placed flat upon the surface of the soil. The 20 x 20 cm micro-plots in which the different treatments were sown were made by cutting some wire from the mesh prior to placement.

The seed of Sava and Trikkala were inoculated with the specific commercial Nodulaid® peat inoculum group A and C respectively. In the micro-plots to be sown with the low and regular seed densities, a 19 x 19 cm stamp provided with 25 evenly-spaced 0.5 cm diameter round-holes was used. To ensure the desired seedling densities (100 and 500 plants/m^2), 10 and 50 seeds respectively were sown 1 cm deep. At the high and very high densities, a layer of soil 1 cm deep was removed and 150 and 660 seeds were broadcast evenly by hand and then covered with the same soil previously removed (Appendix 4.16). After full emergence, the excess seedlings were removed, leaving the corresponding densities selected as treatment (Appendix 4.17). Galvanized fly-wire mesh sleeves were later placed around each micro-plot at high and very high densities to contain the swards within the microplots.

The Trikkala subterranean clover experiment was sown on 12 April, 1989 and the Sava snail medic experiment was sown on 13 April. To eliminate competition between contrasting density treatments, a 10 cm wide buffer zone, which entirely surrounded each treatment micro-plot was sown on 14 April by using some of the original commercial Sava snail medic and Trikkala subterranean clover (inoculated) seed at a rate designed to give a potential of 1500 established seedlings/m^2. After sowing, the experimental area was covered with a thin layer of about 0.5 cm deep of steam sterilized coarse sand, in order to improve the conditions of emergence. As required, irrigation

was used during the entire period of the study and weeds were removed continuously by hand.

The first harvest was made 16 days after sowing (seven days after full emergence) when the counts and corrections in plant density were made. The second harvest was carried out 14 days later with subsequent harvests at intervals of 7 days. At each harvest, the plants were cut at ground level with a scalpel, and all the collected material was enclosed in sealed plastic bags and stored at 4°C until the plant counts could start. The plants were washed and a sub-sample of 10 plants per treatment were dissected into cotyledons, monofoliate laminae, trifoliate laminae, petioles and stems. The areas of the leaf laminae were measured with an electronic planimeter. All harvested herbage (previously hand-separated in photosynthetic and dead plus senescent plant material), was oven-dried at 90°C over a period of 48 h and then weighed separately. The root fraction was not estimated.

Analyses of variance were made using the program GENSTAT V on selected plant variables, such as shoot yield (g DM/m^2), individual weight of plant (mg DM) and leaf laminae area (cm^2/plot). The analyses of variance for each cultivar were carried out firstly considering all harvests together and then individually by harvest. No further transformation of the data was required.

4.4.2 Results

Monthly climatic data for the Waite Meteorological Station, located about 200 m from the experimental site, is summarized for the experimental period in Appendix 4.18.

At the very high plant density, a progressive mortality of plants was observed over the experimental period (Table 4.12). Plants of Sava were highly susceptible to intraspecific competition and 44.6%, 45.0% and 55.7% of initial plant density disappeared in small, medium and large seed-size categories respectively. The presence of *Pythium* species, mainly associated with the highest plant density, may also have been responsible for some mortality. By contrast, plant populations of Trikkala were more persistent and

only 12.3%, 12.8% and 15.6% reductions in plant density due to intraspecific competition were estimated in each seed-size category, respectively. In both species, the size of seed was positively correlated with plant mortality.

Analyses of variance carried out on data for all harvests together showed a significant two-way interaction for both cultivars and all plant variables between harvest and plant density, and seed size and plant density. The combined effect of Harvest x Seed size was only significant for individual plant weight in cultivar Sava, and for shoot yield and individual plant weight in cultivar Trikkala. The single effect of factors involved in the study consistently revealed a major influence on plant variables examined (Table 4.13).

When the analyses of variance were made on each harvest individually, a continuous significant interaction between seed size and plant density for shoot yield persisted until 37 days after full emergence for Sava (Table 4.14), and throughout the experimental period for Trikkala (Table 4.15). For other variables, the interaction was not significant at most harvests. The single significant effect of seed size and plant density was continuously observed.

The changes in shoot dry matter over time produced by increasing sowing rates of small, medium and large seed sizes of Sava snail medic and Trikkala subterranean clover are shown in Figures 4.24-4.25 and Appendices 4.19-4.20, respectively. From the first harvest until the end of the experimental period, the combined effect of seed size and plant density were important factors in determining the level of dry matter produced by the corresponding pure swards of Sava and Trikkala species (Plates 4.6-4.7).

At the first harvest, the shoot dry matter of Sava produced by the small and medium seed-size category compared to the shoot yield produced by the large seed were 59.4% and 60.0%; 46.0% and 68.2%; 62.7% and 74.2%, and 48.6% and 67.9% at low, regular, high and very-high plant densities, respectively. At the last harvest, similar proportional values were observed at low, regular and high plant densities. Due to the mortality of plants at the very-high plant density, a reduction in the differences of dry matter in the large seed size compared with the others was observed. The shoot dry

matter estimated on the first harvest at the low, regular and high plant densities compared to the very high one, yielded 1.7%, 7.3% and 25.0%; 1.5%, 7.7% and 28.7%, and 1.4%, 7.7% and 26.3% for small, medium and large seed sizes, respectively. At the end of the period, the low, regular and high plant densities showed increased proportional values of dry matter equivalent to 9.0%, 32.8% and 75.8%; 8.0%, 25.5% and 58.7%, and 10.9%, 30.8% and 86.5% for small, medium and large seed sizes, respectively.

In Trikkala subterranean clover, the shoot dry matter yields from small and medium seed sizes compared to the shoot yields from the large seeds at the first harvest, were 65.0% and 80.0%; 65.9% and 86.8%; 56.1% and 82.9%, and 63.8% and 81.9% at low, regular, high and very-high plant densities, respectively. When the changes in the proportion of shoot dry matter were estimated at the end of the experimental period, smaller increases obtained from the medium seed size compared with small seed size revealed the progressive decline in rate of growth over time. Simultaneously, there was an increase in the rates of senescence and plant mortality, first in plants from large seeds at higher plant densities and finally in plants from small seeds at lower plant densities. Comparisons of shoot yield values obtained at the first harvest between the low, regular and high plant densities, and values estimated at the very-high plant density, showed proportional percentages of 1.3%, 6.0% and 23.6%; 1.2%, 6.2% and 27.2%, and 1.3%, 5.8% and 26.9% for small, medium and large seed-size categories respectively. However, at the end of the experimental period, the low, regular and high plant densities had increased proportional values with respect to the very-high plant density of 8.1%, 25.5% and 69.0%; 9.6%, 26.0% and 70.5%, and 8.5%, 25.3% and 68.7% for small, medium and large seed sizes, respectively.

Given that there was a positive trend in plant mortality at very high plant densities through the experimental period, it was also necessary to examine the mean individual weights of plants. Changes in the individual weight of plants over the experimental period for contrasting seed sizes sown at different rates are shown in Figure 4.26 for Sava and in Figure 4.27 for Trikkala.

Very consistent effects of seed size and plant density were observed, especially for cultivar Trikkala, where lower rates of plant mortality through intraspecific competition at the highest plant density were achieved. Heavy plants were associated with lower densities and large seed size.

At the same time, the allocation of dry matter at plant level was influenced by time and by the factors involved, especially plant density (e.g. Appendices 4.21-4.22 for Sava and Appendices 4.23-4.24 for Trikkala). Heavier plants obtained at lower densities showed a more prostrate habit of growth with stronger stems that permitted greater leaf areas. This leaf load was principally trifoliate leaves which by 30 days after sowing (23 days after full emergence) became the most important active photosynthetic part of the plant. At that time, most of the cotyledons and monofoliate leaf laminae had senesced, especially under higher plant densities growing from large seeds. Increased plant densities tend to reduce the leaf area, enhancing the allocation of dry matter to petioles, causing a more erect growth habit. Increased amounts of senescent and dead material were observed and represented in the corresponding Figures (Appendices 4.21-4.24) by the difference between the 100% limit and the total percentage value allocated to each component over time.

Figures 4.28-4.29 and Appendices 4.25-4.26 show that leaf area index (LAI) in Sava and Trikkala pasture swards was influenced by the factors involved in the study. For both cultivars, LAI increased during the entire period of growth at low and regular plant densities and was related to the trend in shoot dry matter yields shown in Figures 4.24-4.25. At higher densities and especially at the very high one, LAI tended to increase up to a certain level of dry matter, and then decreased with further increases in shoot dry matter. A maximum LAI, independent of pasture species and seed size, was reached at the highest plant density 30 days after sowing (23 days after full emergence), when the dry matter, consisting largely of cotyledons and monofoliate leaves, was mostly replaced by the trifoliate leaves. A further decrease in LAI was also due to a reduction in plant number and increased rates of senescence and mortality of trifoliate leaves, the

most important foliar component after that time (e.g. Appendices 4.21-4.24). The LAI of Sava at the two higher plant densities produced by small seeds, as well as the highest plant density produced by medium-sized seeds, is the result of an increased allocation of dry matter to trifoliate leaves which occurred as a consequence of intraspecific competition and the presence of foliar diseases.

4.4.3 Discussion

These experiments demonstrated that early growth in Sava snail medic and Trikkala subterranean clover was influenced by seed size and plant density. According to Black (1959), the period of early growth of a pasture can be defined as the time until increasing inter-plant competition begins to reduce the rate of growth of individual plants.

The results of these studies are in agreement with Black (1956, 1957 a), who demonstrated a direct relationship between seed weight, and dry weight and leaf area in the early growth stages of subterranean clover. More recent work on forage legumes by Hur and Nelson (1985) verified this finding and revealed that larger seed also produced accelerated cotyledon expansion that was closely associated with seedling growth. This allows an earlier onset of leaf production and earlier leaf development. Both are important factors determining the initial growth of seedlings.

During the earlier harvests, greater increments in shoot yield were observed as a consequence of seed size. Over time, these differences diminished, suggesting that progressive declines in plant growth occur first in plants produced by large seeds and then in small ones. There is ample evidence that the relative differences between seed weight in herbage legumes influence plant yields during initial growth, but later these differences may diminish. A gradual onset of plant competition is expected to occur first amongst plants which are more developed, indicating that at more advanced stages of growth, differences should be eliminated (Black 1959). Taylor (1972) stated that smaller seeds of subterranean clover ultimately produced relatively bigger plants than

larger ones, because higher relative growth rates occurred. Similarly, Lawson (1969) showed that growth rate is not independent of seed size, even in spaced plants.

The same situation was observed when increased plant densities were present. According to Donald (1951), plant density and yield have a clearly-defined relationship. At sowing time, a linear relationship between seed density and weight of embryos is present, and plant competition first starts to operate at the highest density and subsequently appears with advanced stages of growth at lower densities. At the end of the season, lower densities tend to approach the yield achieved by higher densities. Thus, the weight per plant is inversely proportional to plant density.

These aspects are of great importance in forage production, because large seed size (Black 1957 b) and a dense plant population (Davidson and Donald 1958) influence the early values of leaf area index (LAI) and affect the trend of dry matter production (Donald and Black 1958).

It has been demonstrated in previous experiments carried out on subterranean clover, that density of sowing influences the rate of growth by affecting light distribution to the leaves in the canopy (Davidson and Donald 1958; Black 1960b; Black and Wilkinson 1963).

In the present studies, the time taken to attain a full canopy was 30 days after sowing for the highest plant density. According to Silsbury and Fukai (1977), this stage represents an important part of the growth of the pasture sward, and the length of this phase is strongly influenced by plant density. The maximum LAI values obtained by Sava and Trikkala species were seed-size-dependant and similar to the maximum canopies of LAI 9 reported by Black (1963) used to define the optimum relationship between pasture growth rate and LAI by young plant populations growing at high densities.

After the ceiling LAI was reached, inter-plant competition increased the rate of leaf senescence and plant mortality, especially in cultivar Sava where the formation of new

leaves in the remaining plants tended to produce a new rise in LAI. These phenomena have been reported by Black (1960 a) as changes in plant population.

When plants were growing at high densities and independent of the size of the seed, important changes in the allocation of dry matter were observed. Especially important were the increases in the proportion of leaf at high, rather than at low, densities caused by a progressive allocation of dry matter in petioles rather than in lamina. However, unlike the situation described by Cocks (1974), the proportion of leaf in both cultivars increased with time, principally for Trikkala subterranean clover growing at higher densities where the allocation of dry matter in stems declined with time. Results obtained by Prioul and Silsbury (1982) on individual growth characteristics of subterranean clover plants showed that the presence of longer petioles, smaller leaf area and less dry weight for individual leaf laminae and less branching was characteristic of plants grown at high density. This confirms the results obtained by Stern (1965) who estimated the effect of plant density on the performance of individuals plants in subterranean clover swards.

Table 4.11 Characteristics of seed-size categories of Sava snail medic and Trikkala subterranean clover used in the raised-bed experiment.

Seed-size category	Sieve aperture (mm) Min.	Max.	Individual seed weight: As stored (mg)	ratio	Oven-dried (mg)	ratio	Moisture content (%)	Germinability (%)
	SAVA							
Small	1.71	2.00	10.27	1.00	9.74	1.00	5.16	76
Medium	2.01	2.36	14.66	1.43	13.95	1.43	4.84	79
Large	2.37	2.80	22.30	2.17	21.27	2.18	4.62	79
	TRIKKALA							
Small	1.71	2.00	6.45	1.00	6.13	1.00	4.96	83
Medium	2.01	2.36	9.70	1.50	9.23	1.51	4.85	82
Large	2.37	2.80	13.10	2.03	12.46	2.03	4.89	82

Table 4.12 Changes in plant density of Sava snail medic and Trikkala subterranean clover at the very high sowing rate in the raised-bed experiment.

Harvest (#)	Days after full emergence	Pasture species: Sava Small	Sava Medium	Sava Large	Trikkala Small	Trikkala Medium	Trikkala Large
		(plants/m^2)					
1	9	12467	12500	12500	12442	12442	12417
2	23	11767	11908	11858	12192	11992	12150
3	30	11575	11783	11733	12408	12392	12442
4	37	9767	10833	9758	11683	11883	11942
5	44	10500	7600	7575	11633	11308	11742
6	51	6925	6875	5533	10958	10900	10550
Mean		10500	10250	9826	11886	11820	11874
Mortality (%)		44.6	45.0	55.7	12.3	12.8	15.6

Table 4.13 Summary of analyses of variance on total shoot yield, individual plant weight and total leaf area values for Sava snail medic and Trikkala subterranean clover for all harvests.

Plant variable	Harvest (H)	Seed size (S)	Plant density (D)	HxS	HxD	SxD	HxSxD
			(Significance levels) †				
	SAVA						
Shoot yield (g DM/pot)	***	***	***	NS	***	***	NS
Plant weight (mg DM)	***	***	***	***	***	**	NS
Leaf area (cm^2/pot)	***	***	***	NS	***	***	NS
	TRIKKALA						
Shoot yield (g DM/pot)	***	***	***	***	***	***	NS
Plant weight (mg DM)	***	***	***	***	***	***	NS
Leaf area (cm^2/pot)	***	***	***	NS	***	**	NS

† NS = Not significant; ** = P<0.01; *** = P<0.001

Table 4.14 Summary of analyses of variance on plant variables for Sava snail medic at each harvest.

	Harvest time (days after full emergence)					
Plant variable	**9**	**23**	**30**	**37**	**44**	**51**
Shoot yield (g DM/pot)			(Significance levels) †			
Seed size	***	***	***	***	***	*
Plant density	***	***	***	***	***	***
Seed size x Plant density	***	***	***	*	NS	NS
Plant weight (mg DM)						
Seed size	***	***	*	***	***	***
Plant density	***	***	***	***	***	***
Seed size x Plant density	**	NS	NS	NS	NS	*
Leaf area (cm^2/pot)						
Seed size	***	NS	*	***	*	*
Plant density	***	***	***	***	***	***
Seed size x Plant density	***	NS	NS	***	NS	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Table 4.15 Summary of analyses of variance on plant variables for Trikkala subterranean clover at each harvest.

	Harvest time (days after full emergence)					
Plant variable	**9**	**23**	**30**	**37**	**44**	**51**
Shoot yield (g DM/pot)			(Significance levels) †			
Seed size	***	***	***	***	***	***
Plant density	***	***	***	***	***	***
Seed size x Plant density	***	***	***	***	*	***
Plant weight (mg DM)						
Seed size	***	***	***	***	***	*
Plant density	***	***	***	***	***	***
Seed size x Plant density	NS	NS	NS	***	**	NS
Leaf area (cm^2/pot)						
Seed size	***	***	***	*	*	***
Plant density	***	***	***	***	***	***
Seed size x Plant density	***	**	NS	NS	NS	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

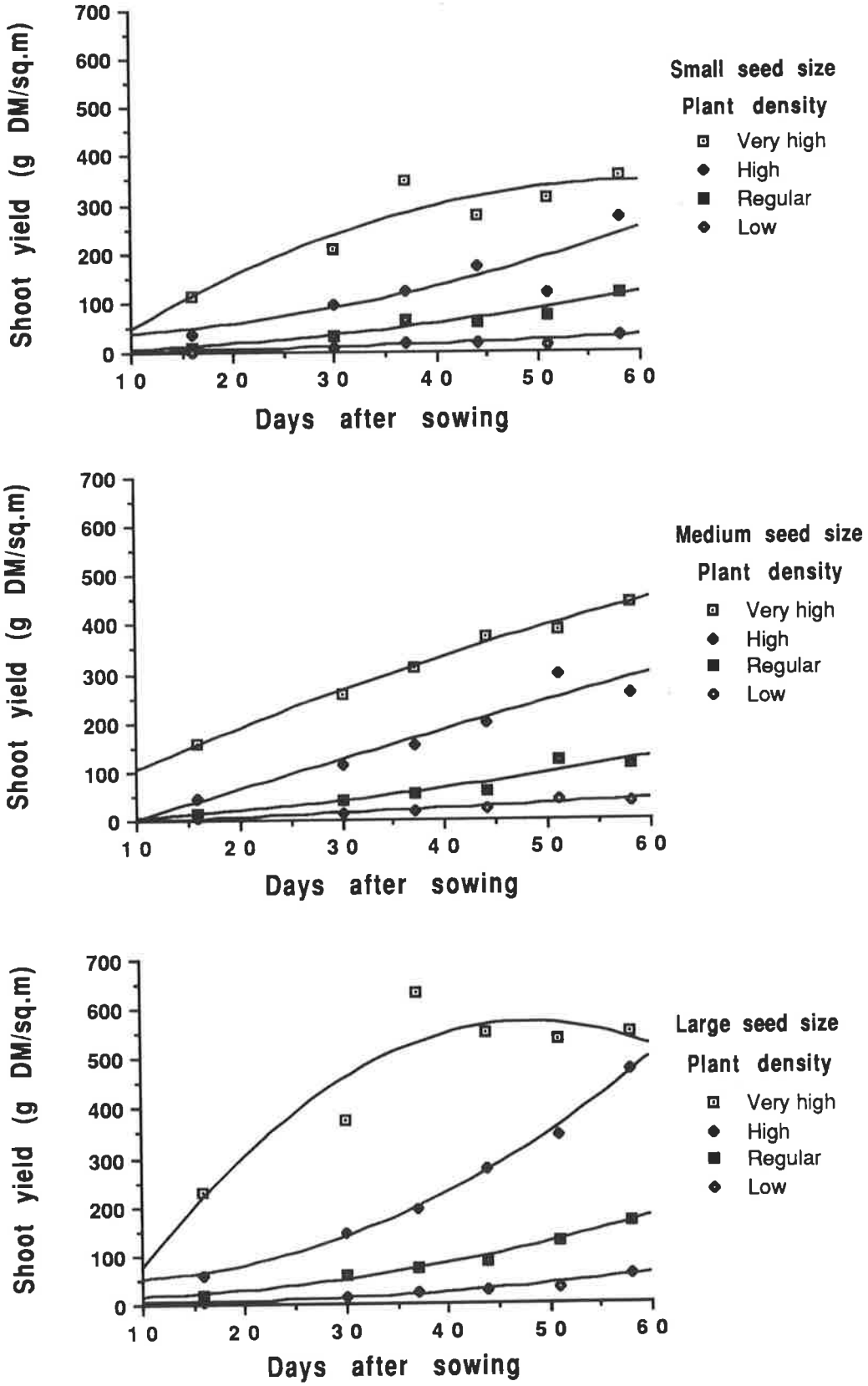


Figure 4.24 The trend in shoot yield produced by various sowing rates of small, medium and large seed of Sava snail medic.

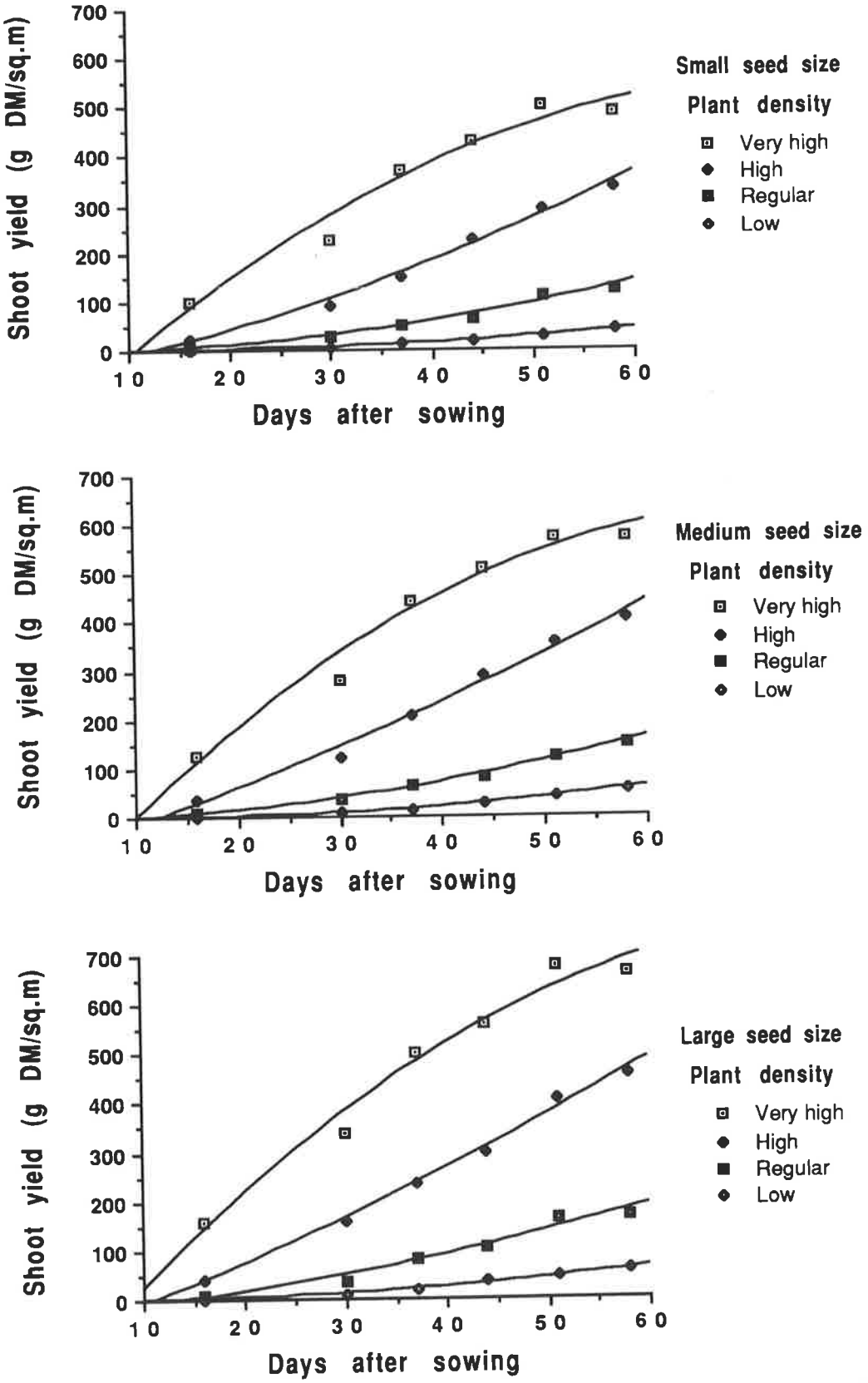


Figure 4.25 The trend in shoot yield produced by various sowing rates of small, medium and large seed of Trikkala subterranean clover.

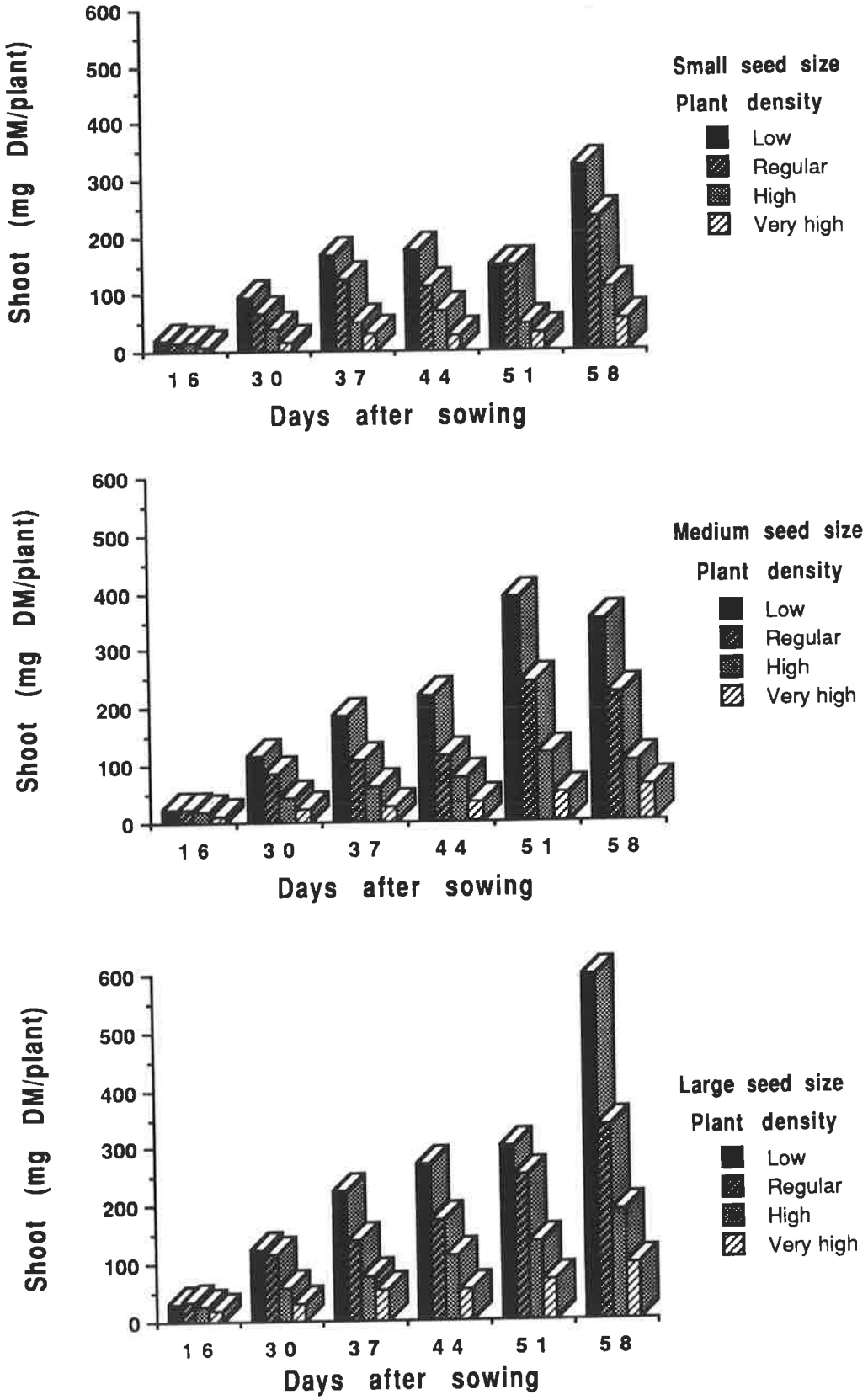


Figure 4.26 The trend in plant weight resulting from sowing various rates of small, medium and large seed of Sava snail medic.

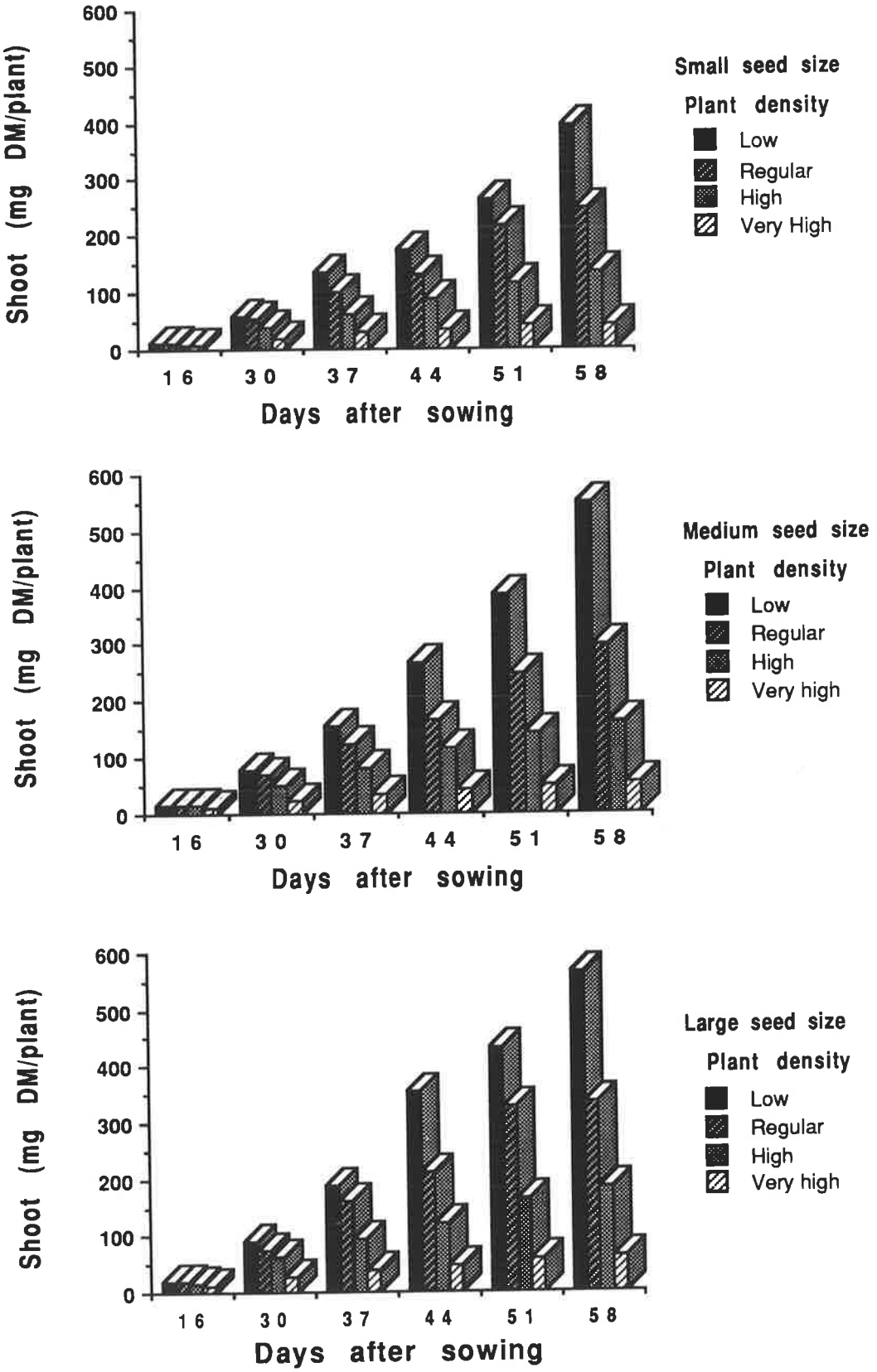


Figure 4.27 The trend in plant weight resulting from sowing various rates of small, medium and large seed of Trikkala subterranean clover.

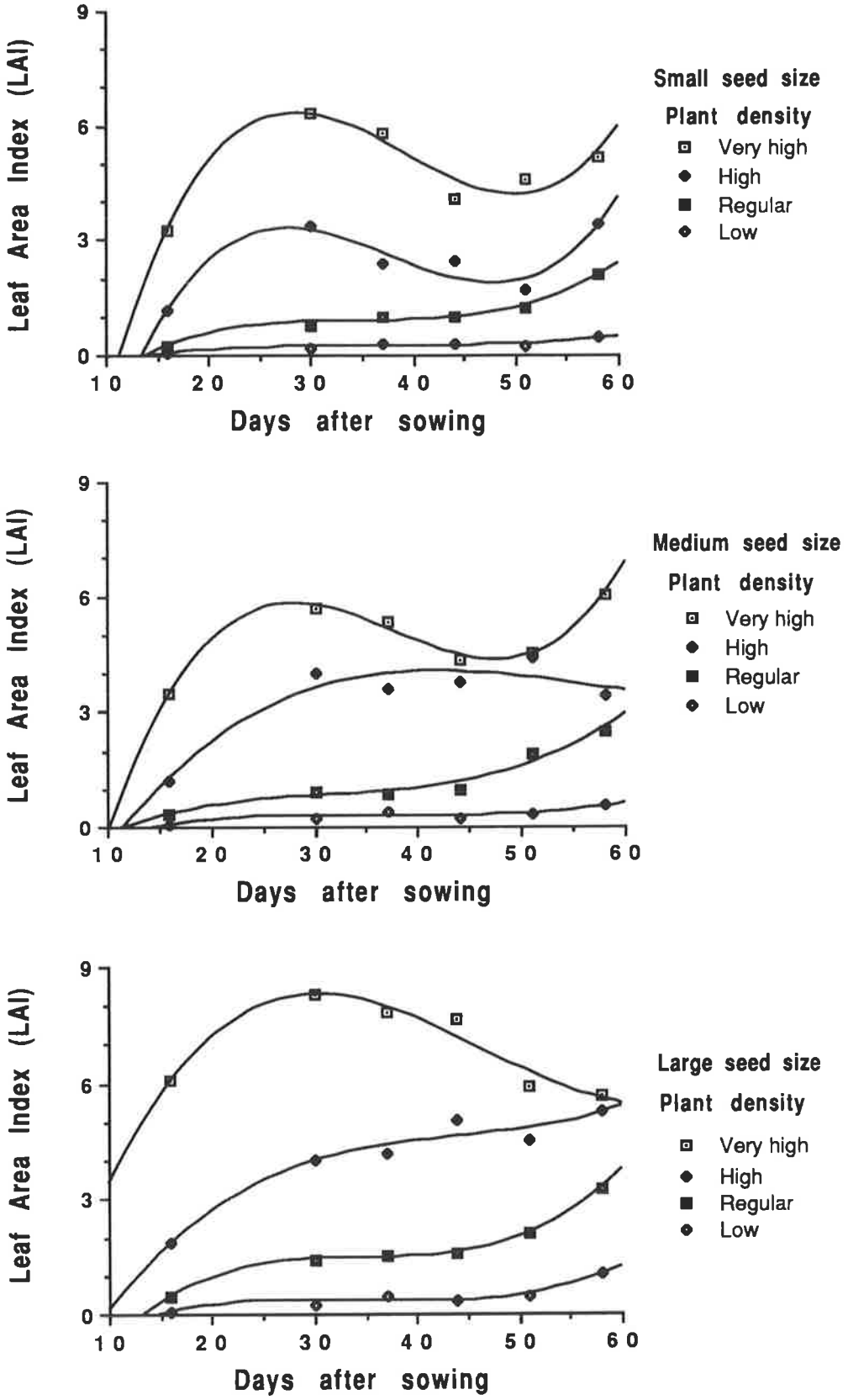


Figure 4.28 The impact of seed size and sowing rate on Leaf Area Index of Sava snail medic.

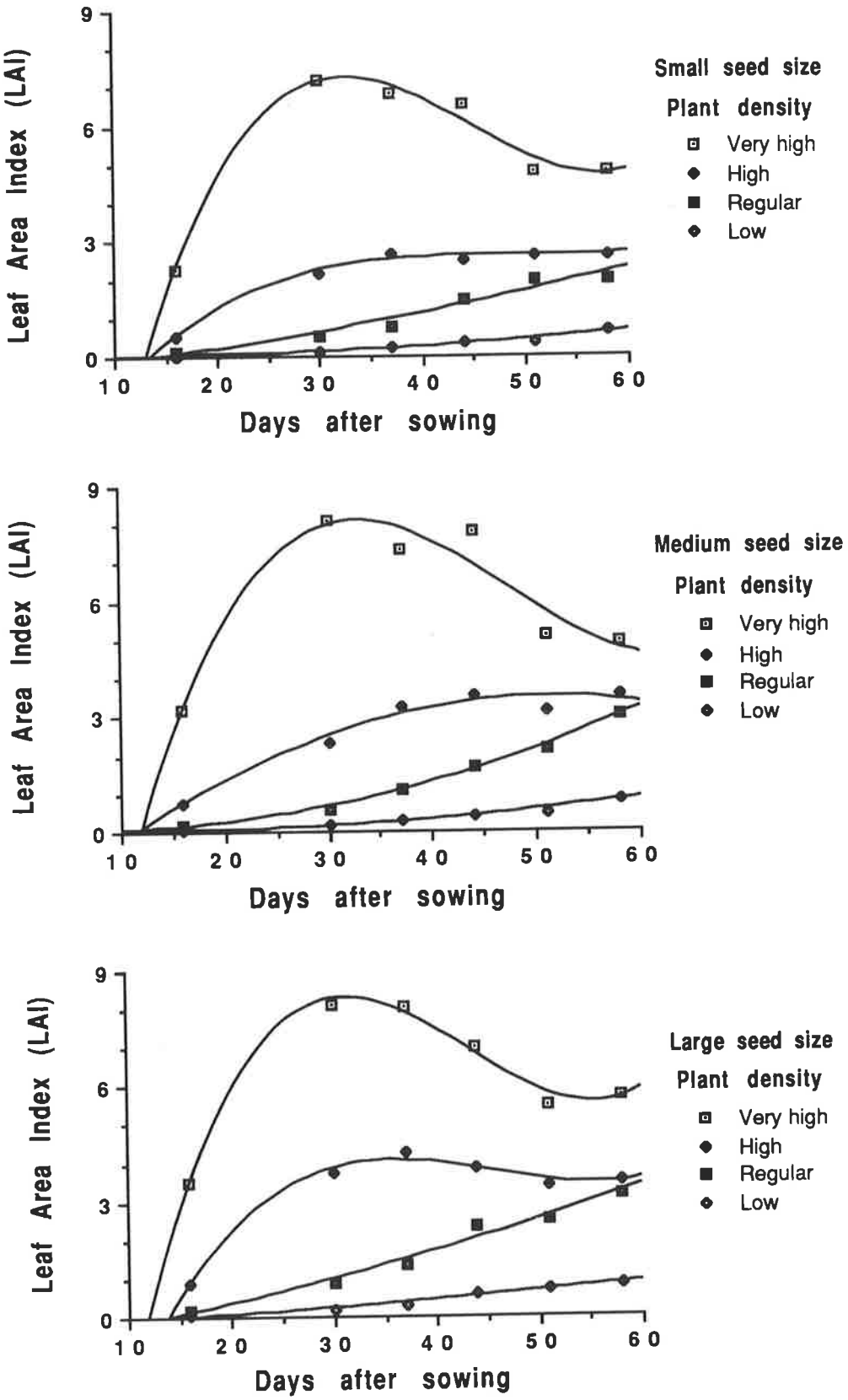


Figure 4.29 The impact of seed size and sowing rate on Leaf Area Index of Trikkala subterranean clover.

Plate 4.6 Close-up view of early forage production from three Sava snail medic (S) seed-size categories (A=small, B=medium and C=large) grown at four densities (1=100 plants/m^2, 2=500 plants/m^2, 3=2,500 plants/m^2 and 4=12,500 plants/m^2) on days 9 (H1=upper), 30 (H3=middle) and 51 (H6=lower) after full emergence.



Plate 4.7 Close-up view of early forage production from three Trikkala subterranean clover (T) seed-size categories (A=small, B=medium and C=large) grown at four densities (1=100 plants/m^2, 2=500 plants/m^2, 3=2,500 plants/m^2 and 4=12,500 plants/m^2) on days 9 (H1=upper), 30 (H3=middle) and 51 (H6=lower) after full emergence.



4.5 Conclusions from Glasshouse and Raised-bed Experiments

Two main conclusions may be derived from the Glasshouse Experiment 1. Firstly, there is an important influence of seed size, depth of sowing and soil type in delaying emergence and affecting the potential of emergence. These responses, especially the rate of emergence, are of critical importance under natural conditions because of the influence on the rate of seedling establishment and subsequent early forage production. Some evidence suggests that seedlings emerging from small seeds gave higher initial rates of emergence than seedlings from larger seeds. Secondly, the experiments have demonstrated the critical effects of delaying emergence on seedling vigour, affecting the rate at which the original seed material can be employed to generate further plant material. Trikkala seedlings were almost always more affected than seedlings of cultivar Sava. However, under some circumstances, the large size of cotyledons could be an additional constraint for seedling emergence. When moisture is limiting in the surface soil, the optimum depth of seed placement according to the soil type did not represent the best strategy to ensure a better performance of emerging seedlings.

When wheat straw was used in the Glasshouse Experiment 2 to test the emergence and seedling vigour of plants produced from Sava pods containing contrasting seed sizes, a similar finding in relation to the effects of seed size and depth of sowing as the previous study was observed. The physical impedance of cereal straw *per se* had minor influence on seedling emergence compared to depth of sowing, but at greater concentrations straw was an additional constraint which was reflected in lower rates of emergence. However, in terms of seedling vigour, a positive influence of straw on seedling development after emergence was noted up to 4 ton DM/ha. This suggests that when major impediments such as allelopathic and pathogenic factors are not present (Quigley 1988), the cereal residues may play an important protective role, probably by moderating the environmental conditions for seedling growth.

Differences in the potential of forage production obtained in the Raised-bed Experiment 1 ascribed to the size of seed, as well as to plant density, have considerable implications in pasture production and management, especially under a Mediterranean-type climate where there are serious feed deficiencies during the autumn-early winter period. The results of these experiments reveal that only at higher plant densities, and mainly with larger seed sizes, is it possible to ensure early feed supply with a reasonable level of pasture availability (1200-1600 kg DM/ha), above which there is no further advantage to sheep growth (Willoughby 1959; McFarlane 1965; Arnold and Dudzinski 1967). This is of great importance, because one of the main problems in achieving a high level of early forage production at the farm level is related to low leaf areas, which are a consequence of low plant densities resulting from inadequate seed reserves, poor regeneration and inadequate grazing practices. According to Carter and Day (1970) and Smith *et al.* (1972), a maximum forage utilization under continuous grazing occurred when the availability of forage was about 1000 kg DM/ha. However, this amount may result in low LAI (between 1 and 3) and forage yields of 20-70% of the potential (Cocks 1974).

THIRD SECTION

5 FIELD EXPERIMENT 1

AND

FIELD SURVEY 1

5 SEED SIZE AND OTHER FACTORS INFLUENCING THE SURVIVAL OF ANNUAL LEGUME SEEDS UNDER GRAZING

5.1 Introduction

In the Mediterranean-type climate of southern Australia, there is a marked seasonal fluctuation in the amount and nutritive value of forage available to grazing animals (Puckridge and French 1983; Purser *et al.* 1987).

The persistence of annual self-regenerating pasture legumes in such environments is ensured mainly by the ability of a species to produce adequate viable seeds, to conserve them through hard-seededness, and by adaptation to soil conditions (Gramshaw *et al.* 1989; Reed *et al.* 1989). Several factors including diseases, insect pests, fertilizer practices, cropping intensity, grazing management and fodder conservation practices have been identified by many workers (Carter *et al.* 1982; Gillespie 1983; Carter and Cochrane 1985; Carter and Lake 1985; Dear and Loveland 1985; Carter 1987) as contributing to the recent deterioration in the quality of annual legume pastures in southern Australia. Furthermore, it has been suggested that an inadequate level of seed in the soil is frequently the most important factor influencing the commercial success obtained with this type of resource.

Carter (1981) and de Koning and Carter (1989) found that seed reserves in pastures based on annual medic and subterranean clover on hard-setting soils were significantly depleted by sheep grazing the dry pasture residues in summer and autumn. Sheep initially select the heavier pods and burrs containing the most seeds and the larger seeds. Under heavy grazing pressure, small seeded clovers such as cluster clover have been reported to increase (Carter 1966; Reed and Cocks 1982; Carter and Lake 1985).

Jones and Carter (1989) proposed that a greater use of legume mixtures, combining several cultivars and/or species, is a reasonable alternative to mono-specific swards. A variety of species minimises the adverse effects of environmental factors and management on the persistence of a pasture. This strategy represents a combination of diverse and more efficient seed and plant traits, in which the size of the seed should have important ecological implications for the survival of the seed, the establishment of the pasture sward, the production of forage and animal performance.

The following studies examined the effects of grazing by sheep on the seed and seedling dynamics in annual legume pastures consisting of species with contrasting seed sizes. The first field study investigates the survival of seeds of subterranean clover *(Trifolium subterraneum)*, balansa clover *(Trifolium balansae)* and cluster clover *(Trifolium glomeratum)* when sheep graze dry pasture residues over the summer-autumn period on a hard-setting red-brown earth at the Waite Institute. The selectivity of harvesting of seed by ants is also considered. The second field study is concerned with the impact of two contrasting long-term management strategies on the incidence of cluster clover, in a subterranean clover-perennial ryegrass pasture established on a sandy-loam lateritic podzolic soil at Parawa.

5.2 Field Experiment 1: The survival of seeds of subterranean clover, balansa clover and cluster clover when sheep graze dry pasture residues

5.2.1 Materials and methods

5.2.1.1 Location and previous history of the paddock

The site selected for the grazing study was part of paddock W5, located on a hard-setting, red-brown earth at the Waite Agricultural Research Institute (WARI), South Australia (34° 58' S. Lat. and 138° 38' E. Long., Altitude 122.5 m). In May 1984 the paddock was sown with a mixture of subterranean clover cultivars Clare (early mid-season flowering) and Daliak (early flowering) in equal proportion by weight at 20

kg/ha. In the following years, until 1988, the paddock was used in a pasture-cereal crop rotation. In April 1989, it was sown with 7.6 kg/ha of balansa clover seed and a mixed sward of subterranean clover and balansa clover species became established. The paddock was grazed for one month during winter at a stocking density of about 96 sheep/ha and seed was allowed to set. Total rainfall during 1989 was 576 mm (Appendix 5.1).

5.2.1.2 Pre-experimental period

In order to select ten Merino wether (castrated male) sheep to use during the experimental period, twelve adult (3.5 years old) full-mouth animals were taken from the sheep flock and weighed to the nearest 0.1 kg (Appendix 5.2). Sheep were placed in separate digestibility pens in the Animal House (WARI) for 7 days (24 January, 1990 to 31 January, 1990) in order to avoid a further contamination of the animal's digestive tract by related clover seeds, and to accustom them to the use of faecal-collection harnesses.

The sheep were fed daily each morning at 0900 h with a standard diet of 600 g of prepared sheep pellets (Appendix 5.3) with 89.4 ± 0.21% (± s.d.) DM, 150 g of chaffed oaten hay with 88.8 ± 0.89% DM and 50 g of chaffed lucerne hay with 86.4 ± 0.87% DM per head. A progressive substitution of chaffed oaten hay by pellet was done during the pen feeding stage. Water was consumed by sheep *ad libitum*.

In order to check for contamination by related seed species, sheep faeces were examined before the completion of the pre-experimental period. A sub-sample of 100 g of fresh faeces per head was obtained using collection harnesses. The faeces was macerated under running tap water and washed through 1.00 mm and 0.50 mm square-holed 30 cm diameter brass laboratory test sieves. None of the sub-samples of sheep faeces was contaminated with annual clover seeds. At the end of the period, ten selected wethers were drenched, weighed (Appendix 5.2) and delivered to the grazing site.

5.2.1.3 Experimental period

Faeces collection, seed recovery and emergence test:

The ten Merino wether sheep, each fitted with a faecal-collection harness, were introduced to the grazing area on 31 January, 1990 at 0900h (Plate 5.1). The dry pasture residues in a 0.20 ha site were grazed continuously for 70 days at a stocking density of 50 D.S.E./ha. At the end of the experimental period, the sheep were weighed and returned to the original farm flock (Appendix 5.2).

The sheep were penned daily at 0900 h in order to collect the faeces and replace the bags. Two polyethylene bags 335 x 205 mm x 100 mm were used daily for each sheep. The daily sample of fresh faeces obtained from each animal was weighed, well mixed and a sub-sample of 20 g was taken to determine the moisture content of the material by oven-drying at 80°C for 48 h period. A sub-sample of 100 g was kept to measure the daily recovery of seeds. An additional 100 g sub-sample was taken every seventh day during the grazing period to estimate seedling emergence from sheep faeces, exposed for different periods of time under field conditions. The remaining faeces were dried at 40°C for 48h in a forced-draught dehydrator and then stored at room temperature for eventual later analysis.

The sub-samples used to estimate the presence of seeds in faeces were thoroughly macerated under tap water using a scrubber within 1.00 mm and 0.50 mm square-holed 30 cm diameter brass laboratory test sieves. These sieves retain the washed out seeds of subterranean clover, balansa clover and other small-seeded, desirable volunteer clover species such as cluster clover, respectively.

The material collected by each sieve was transferred to Petri dishes, which had a pad of three Whatman N°1 filter papers at the bottom and one at the top, and moistened with about 10 ml of 0.16% w/v solution of fungicide Thiram. The dishes were placed in an incubator in the dark at 19.0 ± 0.5 °C (± s.d.) for ten days with moisture maintained in order to favour the germination and emergence of seedlings. Dishes were maintained at

room temperature for a further 4 days. Recording of the emerged normal seedlings was performed according to the suggestions of Bekendam and Grob (1979).

The remaining faecal material was dried at 40°C for 48h. Seed was separated from the undigested material by using an exhaust cupboard provided with a fan and scrubber unit. The number of seeds was counted and weighed to the nearest 0.1 mg. For the small-seeded clovers such as balansa clover and cluster clover, a sub-sample of 50% by weight of the dried material was taken to recover the seed when the whole sample was difficult to process. The daily, cumulative and total recovery of undigested seeds as normal seed and hard seed (considered also those seeds that imbibed water and remain in that condition through the entire period of testing) were calculated by correcting the number of seeds obtained in the sub-samples by the original sample value of fresh faeces by sheep. On some occasions not all the faeces were dropped inside the collection bags. A further collection of faeces from the soil surface was made, and the bulk sample thus obtained was processed independently and included in the corresponding calculations.

The sub-samples of 100 g of fresh faeces, which were taken every seventh day (10 sheep x 10 weeks = 100 sub-samples), were used to examine the emergence of seedlings from faeces. Sampling was started on day 8 of grazing (8 February) and finished on day 71 (after 70 days of grazing i.e. 12 April), to better synchronize the output of seed in faeces with the intake of seed by sheep every seventh day and corresponding with the frequency of sampling of dry pasture residues and their components. At each sampling, the faecal sub-samples were placed at ground level in the grazing area and protected from disturbance by the use of galvanized weldmesh exclosure cages of 90 cm height. Every week, the cages and the corresponding faecal sub-samples were moved to a new grazing area, to ensure that the faecal pellets were subjected in time to the real conditions imposed by grazing (Plate 5.2)

One week after the finish of the grazing period (19 April), the faeces were moved to a raised bed, top-filled with 5 cm potting mixture (sand 1 : peatmoss 1) and located

outdoors at the WARI. The faecal sub-samples were assigned at random and placed within 15.0 cm square white plastic rims and the faecal pellets partially covered with soil. The material was kept under natural conditions until the first important rainfall event occurred on 17 May (11.2 mm), and then watered as required.

Counts of emerged seedlings were made on 31 May and 14 June. A final harvest of the seedlings was made on 28 June, four weeks after the initial count. The seedlings were cut at ground or pellet level with a scalpel and counted. At each count, the species were grouped in subterranean clover cultivars, balansa clover and cluster clover species.

The remaining sub-sample material was collected, macerated under tap water as before, and placed in a uniform layer of about 1.5 cm depth in a square white plastic container (15.0 x 15.0 x 9.5 cm). The containers were incubated in the dark at 19.0 ± 0.5 °C (± s.d.) over a 14-day period. The material was re-moistened as required and the final emergence of seedlings recorded. The resulting residual faecal material was dried at 40°C for 48 h and processed as before, and the recovered seed cleaned of impurities, counted and weighed. The total normal emerged seedlings, as well as the hard, undigested seeds were adjusted for the corresponding original sample values.

Evaluation of dry herbage residues :

In order to evaluate the decline of dry herbage residues under grazing, two parallel transect lines oriented east-west were established. Ten permanent wooden pegs were placed at 5 m intervals along each line (Diagram 5.1). Each peg represented the centre of a circle 8 m in diameter in which quadrat samples were taken. The initial harvest of twenty samples was taken on 24 January, 1990 by use of a 28.5 cm diameter (637.9 cm^2) galvanized steel cylinder centred at the northern position on the circumference of the corresponding circle. Subsequent weekly samplings during the grazing period were carried out at 45° intervals following an anti-clockwise pattern. Because at that interval only eight samplings were allowed, the following ones were taken at successive intervals of 22.5° (Diagram 5.1).

The dry pasture residues were cut at the soil surface level with a knife and the remaining material, mainly soil particles and balansa clover and cluster clover seeds, was collected with a brush and a small dust pan (Plate 5.3). The 'clean' dry pasture residue samples and the corresponding 'dirty' swept samples were kept separately in paper and plastic bags, respectively. The dry pasture component was cleaned of impurities, hand separated in the subterranean clover-cluster clover stems and leaves, subterranean clover burrs, balansa clover heads, cluster clover stems and leaves, cluster clover heads and a bulk sample of other undesirable volunteer species and dried in a forced-draught dehydrator at 40°C for 24 h. The material was weighed and the burrs and heads counted. The seeds were obtained by rubbing the burrs and heads over an enclosed rubber surface and were then cleaned, counted and weighed. A sub-sample of 100 burrs of subterranean clover were retained at each harvest for later germination tests as well as additional material for use in future *in sacco* digestibility analyses.

At the first harvest, before grazing commenced, and at the final one, carried out one day before the animals left the area, circular samples were also taken of the surface 0-2.5 cm soil to estimate the proportion and characteristics of buried burrs of subterranean clover and their enclosed seeds. This material was processed as described above.

Germination tests were conducted for each species at each time of harvest. Seeds from buried and unburied burrs of subterranean clover, and seeds of balansa clover and cluster clover obtained from the heads and/or collected from the soil surface were used. The seed from 25 burrs of subterranean clover obtained by hand dissection and 100 seeds of small-seeded clovers were used in each germination test, which was conducted using the method previously reported for seed recovered from faeces. Each test was replicated four times and germination was recorded over a period of 14 days.

At each time of sampling, the percentage bare ground was estimated by using a point method of pasture analysis (Levy and Madden 1933). Four replicate analyses of 200 points each, were made on two perpendicular 5m long transect lines oriented (N-S and E-W) which were located between the two parallel transect lines used to evaluate the dry

pasture residues (Diagram 5.1). All the analyses and replicates in time were undertaken at the same points that were previously used.

Rumen degradability of dry pasture residues:

Four Merino wether sheep with initial mean body-weight of 33.0 ± 0.77 kg (± s.e.), mean fat score of 3 (Jefferies and Nash 1989) (Appendix 5.4) and about 11 months old (full milk-tooth mouth), were fitted with a rumen fistula (of 30 mm of external diameter and 25 mm internal diameter) about 6 weeks before the start of the experiment (Plate 5.4). The sheep were kept in individual pens in the Animal House (WARI) and were fed twice each day (0900h and 1700h) in equal proportion by weight with a fixed daily standard diet of 600 g of pellets (Appendix 5.3) with 89.4 ± 0.17% (± s.d.) DM, 150 g of chaffed oaten hay with 87.0 ± 0.37% DM and 50 g of chaffed lucerne hay with 82.2 ± 0.43% DM per head. Water was consumed by sheep *ad libitum*. The mean daily intake of feed per sheep was about 708 g DM with a mean digestibility (DM) measured *in sacco* and *in vivo* of 70.5% and 62.2%, respectively (Table 6.5; Appendix 6.7). This was sufficient to maintain the sheep in good condition, slightly above the maintenance level.

The selected dried samples of the different plant components harvested from the grazing experiment were milled through a 1.0 mm sieve and two 1.00 ± 0.01 g sub-samples were used to determine the moisture content of the plant material. The corresponding samples were enclosed in aluminium drying tins, dried at 100°C for 24 h, cooled for 20 minutes in a desiccator provided with active particles of silica gel, and weighed. From the remaining material, sets of 1.00 g sub-samples were enclosed in powder paper sheets (11.3 x 8.8 cm) and kept in plastic hermetic phials for the *in sacco* digestibility analyses.

The dimensions of the nylon bags used in the study were about 18 cm long and 9 cm wide between the sewing lines (2.0 : 1.0 ratio). According to microscopic examination

there were 22,500 square holes/cm^2, a mean pore size of about 40 μm and a real aperture area of about 40%.

Up to six nylon bags can be placed in the rumen without interfering with the ruminal process of digestion (CSIRO 1990). Individual samples were placed at random in each bag, and each bag was assigned at random according to its position in the line. Each sample was duplicated within each ruminal site and replicated four times between the animals. Six bags each numbered and containing a known amount of dry matter, were tied 5 cm apart with a nylon fishing line (strength 110 N and 0.50 mm of diameter). After tying, an effective bag surface of about 80 cm^2 was obtained equivalent to approximately 12.5 mg of plant material (tops, stem, leaf, burrs, heads, chaff and seed) per cm^2 of bag area. The other end of the line was tied to an external ring fitted outside the fistula. The free length between the top of the fistula and the first tied bag was 25 cm, allowing the bags to move freely after entering into the ventral sac of the rumen. A steel bearing of 12.5 mm diameter and 8.4 g weight was placed in each bag to minimize floating.

The prepared line of bags was soaked in tap water for about one minute and introduced into the rumen before the morning feeding when the rumen was relatively empty. After a 48 h period of incubation the bags were removed from the rumen, washed by hand under running cold tap water until it became colourless and placed to dry in an oven at 60°C for 24 h. The material was crushed several times to aid drying. After 24 h, the undigested material was transferred to sheets of powder paper, enclosed in open plastic phials and dried to a constant weight. In order to express the disappearance of the dry matter as a proportion of organic matter, the resultant undigested material (DM) as well as an original duplicate sub-sample was ignited at 550°C over an 8 h period and the ash content determined.

After use the bags were turned inside out, rinsed and left in a soap solution for 24 h then placed under running cold tap water, washed and oven-dried at 60°C for 12 h.

Seedling regeneration :

During week nine (28 March), twenty sampling areas were selected within the grazed area. The positions were based on the same pattern described for the weekly harvesting of dry pasture residues (Diagram 5.1). A permanent steel infiltrometer ring (638 cm^2) was centrally placed (29 March) at sampling point No.10 at each of the 20 sampling sites (Diagram 5.1). The rings were protected against grazing sheep by using galvanized weldmesh cages (78 x 78 x 90 cm). The area within the rings was watered on 30 March with the equivalent of 75 mm of water to ensure the germination and emergence of the seedlings (Plate 5.5). Emerged seedlings of subterranean clover, balansa clover and cluster clover were recorded one week after the initial watering (6 April). A final harvest of the seedlings was made on 13 April by cutting the plants at ground level with a scalpel. The number of seedlings was counted and ten randomly-selected seedlings of each species were dissected into cotyledons and monofoliate laminae, petioles and stems. The lamina area was measured by using an electronic planimeter. The material was oven-dried at 80°C for 48 h and weighed separately.

After harvesting, the sampling areas were left to dry naturally and the burrs and/or seeds remaining at the ground level were collected by brushing them into a small dust pan. Samples were also taken of the 0-2.5 cm soil to estimate the presence of buried burrs and enclosed seeds of subterranean clover. This plant/soil material was dried at 40°C for 48 h, cleaned of impurities and the subterranean clover burrs counted and weighed. The processing of the seed was carried out by using the same procedures described previously.

Seed harvested by ants :

To estimate the loss of pasture seeds due to ants (Plate 5.6) a survey was conducted before the start of grazing (15 January). The number of ant nests and nest entries were counted in the grazed area. Twenty nests with a total of 80 entries were found. These were mainly visible in the south-eastern area of the grazing site where a higher proportion of bare soil was present. Thirty eight active entries were selected at random

(24 January) in accordance with the importance of the nest, and all the seed harvested by ants and left around each entry was collected, cleaned as before, separated in seed size categories by using square-holed 20 cm brass laboratory sieves differing in apperture size, counted and weighed. The day after the initial survey (16 January) and until the date that the sheep arrived on the grazing area (31 January), sixteen successive daily (0900h) collections of seed were made on two selected active entries at three different nests to estimate the rate at which the seed had been harvested by ants. The material obtained was processed under the same conditions described previously. Then at the eighth week (28 March) when a large proportion of the ground surface was bare, further counts of the active nest entries were made. At that time a total number of 311 entries were recorded in the entire grazing area (2000 m^2).

5.2.2 Results

5.2.2.1 Herbage availability and seed reserves in the pasture-soil complex

Dry pasture material available at the start of grazing was composed almost entirely of subterranean clover, balansa and some volunteer cluster clover: weeds had all disappeared by 49 days of grazing. At the start of the grazing period there was 5432 kg DM/ha, which declined to 97 kg DM/ha at the end of 10 weeks (Plate 5.7). At the completion of grazing the surface was about 95% bare soil. Subterranean clover-balansa clover stems and leaves were the principal components of the dry pasture residues during most of the period of grazing. Until day 56 more than the 50% of the dry matter readily available was composed of this material. After day 56, subterranean clover burrs became the major source of feed for grazing sheep (Figures 5.1-5.3 and Appendix 5.5).

Most of the heads of balansa clover, were selected by sheep very early during the grazing period. They initially represented a more-accessible and digestible material (57.7%, OM base) comparable with subterranean clover burrs (48.1%, OM base). Only when the heads of balansa clover were severely reduced after the second week of

grazing, did sheep start to graze the subterranean clover burrs more intensively. At the end of grazing, the subterranean clover burrs had almost disappeared including the buried ones. Cluster clover plant residues, which were very poorly represented in the mixture, disappeared rapidly during the early part of the grazing period (Table 5.1; Figures 5.4-5.6 and Appendices 5.6-5.9).

The subterranean clover burrs progressively declined in individual weight until 56 days of grazing. Later, the total availability of forage became very low and the grazing pressure was almost entirely concentrated on larger and heavier buried burrs that were embedded in the soil surface. This produced a rise in the weekly estimated weight of individual burrs. A similar trend was observed for the individual weight of the seed. The number of seeds per burr tended to decrease in time, revealing that the latest available burrs had enclosed few heavier seeds (Appendices 5.6-5.7).

Because most of the subterranean clover seeds are completely retained in the burrs and the proportion of the seed by weight in the burr does not change greatly, except at the end of the grazing period, the decline in the seed density over time followed the same trend observed with burrs. About 95% of the seed disappeared from the grazing area. This demonstrates the susceptibility to grazing of subterranean clover seed, especially when cultivars with inefficient burr-burying traits are grown on hard-setting soils (Figure 5.7 and Appendix 5.6).

In both cluster clover and especially balansa clover, there was a significant reduction in the weight of the heads, number of seeds per head and individual seed weight over time. Selective grazing of heavier heads containing higher numbers of seeds, natural ripening of the seeds, as well as trampling effects produced by grazing sheep could be the main factors involved. The last two effects can explain the increase over time in the amount of pure seed at soil level. However, a further increase in seed density of balansa clover is observed at the end of the grazing period in the absence of heads. The progressive disturbance of the soil surface by sheep seems to affect the original distribution of the harvested seed around the ant nest entries. This seed was therefore available for

sampling and its appearance may explain the later rise in seed density (Figures 5.8-5.9 and Appendices 5.8-5.9).

Germination tests carried out on subterranean clover seeds enclosed in buried and unburied burrs during the experimental period, showed increases in germination from 23.0% to 42.5% and 28.5% to 52.5% respectively. Germination of balansa clover seed collected from the surface increased from 6.8% to 32.8% and 1.0% to 18.0% in cluster clover. No changes in germination were observed when the seed was enclosed in heads in plant residues for the duration of the experimental period (Figure 5.10 and Appendices 5.10-5.12).

5.2.2.2 Digestibility of pasture components

Digestibility analyses on various plant materials using the nylon bag *in sacco* technique before grazing revealed differences in the digestibility of the annual pasture species and among plant components within a species. The whole pasture sample (subterranean clover-balansa clover), was 56.3% (OM basis) digestible. Stems of subterranean clover and balansa clover showed similar values, but the leaves of subterranean clover were 3.0% higher in digestibility (OM basis) than balansa clover. Differences between the burrs and heads are mainly produced by the higher digestibility of the chaff in small-seeded clovers, as well as the seed component that represents the part of the plant with the greatest nutritive value (Table 5.1).

This demonstrates the importance of seed in causing selectivity in grazing by sheep. This is especially so when more accessible seed is enclosed in comparatively more-digestible material, such as balansa clover heads during the early stage of grazing. At that stage the availability of forage was not a limiting factor and selectivity played an important role in the disappearance of the clover heads. Because the stems and leaves of the sown species and the burrs of subterranean clover cultivars were the main forage available throughout the experiment, digestibility analyses were done on these materials at different times during the grazing period. The highest value of digestibility (55.9%,

OM basis) in stems and leaves occurred before the start of the experiment. At this time, the proportion of leaves was at a maximum. Following 49 days of grazing the values ranged between 48.4% and 50.9% (OM basis), declining to a minimum value of 39.1% (OM basis) at the end of the grazing period (Table 5.2). A progressive change in the digestibility of the organic matter of subterranean clover burrs was also detected up to 49 days of grazing. Subsequently, increased values were observed and maintained until the end of the grazing period (Table 5.3). A higher proportion of seed by weight in burrs at that time could explain the increased values of digestibility obtained (Appendix 5.6).

5.2.2.3 Seed transmission through the digestive tract

The daily output of faeces obtained from the sheep flock was very low after one day of grazing (2071 g, DM basis), increasing later up to 5545 g DM after 25 days of grazing. Then a progressive decline was obtained until the end of the grazing period when the amount of faeces dropped by the sheep flock was 2854 g DM. A mean rate of about 433 g DM/sheep/day was calculated for the entire period (Figure 5.11 and Appendix 5.13). A similar trend was observed for the percentage of dry matter in faeces, except that at the end of the grazing period, especially the last ten days, an increase in the dry matter content of faeces occurred, probably as a consequence of greater output of inert soil particles ingested by sheep (Figure 5.12 and Appendix 5.13).

Initially the number of subterranean clover seeds voided by the sheep flock was very low. It increased during the first 30 days of grazing, but after that a sustained decline up to 56 days of grazing was observed. A relatively constant output occurred in the last two weeks (Figure 5.13 and Appendix 5.14). This pattern is a consequence of the changes in the selectivity of grazing by sheep and reflects the utilization of the seed enclosed in buried burrs at the end of the grazing period. This seed became the only forage material available to the sheep at the end of the grazing period. When the total daily output of seed in faeces was partitioned into seed that became a normal seedling or remained hard, the same curvilinear trend for each component was obtained. Mean

values of 35.8% of emerged seedlings and 64.2% of hard seed were calculated for the complete grazing period (Appendix 5.14).

Adjusted values of the daily mean individual weight of the undigested hard seed showed an almost constant upward trend over 56 days of grazing, reaching similar levels to those in the field. This was due to more heavy and hard seed enclosed in buried burrs becoming readily available for grazing during the last two-week period (Figure 5.14). Assuming that the total disappearance of the subterranean clover seed from the grazing area was a consequence of the utilization of the pasture residues by sheep, the total output of seed voided in faeces was estimated at 1.79% (emerged seedlings = 0.64 % and hard seed = 1.15%).

The initial preference for balansa clover heads by sheep was clearly reflected in the level of seed recovered during the early stages of the grazing period. Higher levels of balansa clover seed output were recorded at the end of the first week, after which seed output decreased markedly up to 28 days, remaining at a low level until the end of the grazing period (Figure 5.15 and Appendix 5.15). It may be that sheep could collect some seeds directly from the soil surface, especially near the entry to ant nests. However, the persistence in excretion of seed over time suggests a longer retention period for small hard seed in the digestive tract of the sheep. This could also be responsible for increased values of emerged seedlings from faeces obtained in the last part of the experiment. A mean value of 11.3% for emerged seedlings and 88.7% for hard seed was estimated during the grazing period (Appendix 5.15).

When the daily mean weight of the individual hard seed was plotted over time, a gradual decrease occurred up to 63 days of grazing, then a rise in the weight of the seed was observed. This suggests that the sheep were forced to collect seed from the ground surface and consequently more heavy undigested seed became available in the faeces (Figure 5.16 and Appendix 5.15). The recovery of balansa clover seed in faeces was estimated at 14.94% (emerged seedlings = 1.68% and hard seed = 13.26%) of the calculated intake of balansa clover seed.

The daily levels of cluster clover seed voided in faeces by the sheep flock followed a similar trend to that observed for balansa clover. An early depletion of the heads by grazing became effective after the sheep selected most of the balansa clover heads (Figures 5.5-5.6). The highest output of seed was recorded after one week of grazing: then the voided seeds declined until the end of the grazing period. The proportion of seeds that germinated or remained hard did not change significantly over the grazing period. A mean of 1.7% of recovered seed was soft and gave rise to emerged seedlings while 98.3% was hard seed. (Figure 5.17 and Appendix 5.16).

No increase in individual hard seed weight was detected in cluster clover at the end of the grazing period. A sustained decline with time in the individual weight of seeds voided in faeces was observed. Given that cluster clover seeds were not likely to have been taken by sheep from the soil surface, the decline may be due to cluster clover being retained for longer in the digestive tract of the sheep (Figure 5.18 and Appendix 5.16). When the total disappearance of cluster clover seed from the grazing area, as well as the total output of seed voided in faeces, was considered, a throughput of 75.59% of seed was calculated (emerged seedlings =1.28% and hard seed = 74.31%).

5.2.2.4 Seed content and seedling emergence from faecal material

The content of seed and emergence of seedlings from faeces sub-samples collected every seven days during the grazing period and kept under field conditions were tested under optimum conditions in the raised-beds. These were compared with the values obtained from daily faeces samples that were washed out in order to estimate the survival of seeds in the sheep pellets.

The trend in the content of seed in faeces observed for each annual legume species and recorded in the raised beds confirmed the trends obtained previously for the throughput of seed by the sheep flock during the grazing period. However, there was an important

decrease in seed survival between samples that were washed-out immediately at collection time and those kept under field conditions (Figure 5.19).

For subterranean clover, the amount of hard seed recovered from washed-out samples was similar to the total recovery of both hard and soft seed from the field-stored samples. The difference could be explained by mortality of seed in faeces due to desiccation and death of the original imbibed soft seed available on the day of sampling. However, values obtained during the first three weeks of grazing, suggest that some hard seeds softened inside the sheep pellet were also unable to survive the conditions imposed by the surrounding environment. In the same manner, the small-seeded clover species were affected, and a survival of 63.2% of subterranean clover, 71.7% of balansa clover and 80.5% of cluster clover seeds originally voided in faeces was able to persist until the emergence of seedlings was recorded (Figures 5.20-5.22).

For the subterranean clover seed stored in faeces, 35.8% emerged and 64.2% remained as hard seed. However, over time there is a decline in the number of seedlings emerging from faeces. This suggests a progressive decrease in the amount of soft seed. This could be explained by the shorter times of exposure of the seed in the sheep pellets and the environment reducing the rate of softening of hard seed (Table 5.4). The opposite trend was observed in the proportion of emerged seedlings from washed-out samples (Appendix 5.14).

For the small-seeded clovers, a mean proportion of emerged seedlings and hard seed of 15.8% and 84.2% for balansa clover and a 2.4% and 97.6% for cluster clover were recovered from the field-stored samples (Tables 5.5-5.6). There was no clear trend over time. This may be explained by the fact that seed from the small-seeded clovers was not permanently available and the output of seed voided in faeces represents a combination of sporadic intakes of seed from the ground surface and hard seed retained and softened at different rates in the digestive tract of the sheep.

Given the total disappearance of annual clover seeds from the grazing area and the recovery of seeds from the sub-samples of faeces kept under the grazing conditions, survival values of 1.14% (emerged seedlings = 0.41% and hard seed = 0.73%) for subterranean clover, 10.72% (emerged seedlings = 1.70% and hard seed = 9.02%) for balansa clover and 58.66% (emerged seedlings = 1.41% and hard seed = 57.25%) for cluster clover were estimated.

5.2.2.5 Regeneration of seedlings in the field

The potential regeneration of the annual legume species was measured 56 days after the start of grazing by watering small areas enclosed in infiltrometer rings. At this time there was 395 kg/ha of dry pasture (Appendix 5.5) and the estimated number of seeds of subterranean clover, balansa clover and cluster clover were 2737/m^2, 13,749/m^2 and 1164/m^2 respectively (Table 5.7). Following watering, 22.8% of the seeds of subterranean clover emerged as seedlings, somewhat less than the 36.5% expected based on laboratory germination tests of seed collected at the same time (Table 5.7 and Appendix 5.10). In contrast, 13.0% and 4.5% respectively of balansa clover and cluster clover emerged following watering, which was similar to the laboratory germination tests (Table 5.7 and Appendices 5.11-5.12).

Despite the differences in the density of the emerged seedlings of subterranean clover and balansa clover obtained at harvest time, similar amounts of dry matter were observed and subterranean clover had about twice the leaf laminae area index (LAI) of balansa clover (Table 5.7).

5.2.2.6 Seed harvested by ants

During the 1989 growing season, subterranean clover, balansa clover and cluster clover established in the experimental area had almost completed seed set by the end of October. However, some small and isolated patches of subterranean clover cv. Clare persisted and set seed until the second week of November. Therefore, the established

colonies of ants represented by the Genus *Iridomyrmex* and most probably species *Iridomyrmex chasei*, length 2-3 mm, (identified by Mr. Peter Davis, Western Australia Department of Agriculture) only started to harvest seed from November. At the first estimation of dry pasture residues (24 January, 1990) the processing of the seed collected from the 38 selected active nest entries indicated the obvious selection by ants of more numerous and accessible small seeds of balansa clover, especially those ripened from the heads and readily available as pure seed at the soil surface. In contrast, the less numerous and more strongly retained seeds in the heads of cluster clover and seeds in the burrs of subterranean clover were taken to a lesser degree (Tables 5.8-5.9).

During the 85 days of harvesting, the ants took an average 25 seeds of subterranean clover, 22,301 seeds of balansa clover and 142 seeds of cluster clover at each nest entry. This represents less than one seed, about 262 seeds, and about 2 seeds of each species per nest entry per day respectively. Considering the total production of seed by each species as the sum of the seed recorded before the start of the grazing experiment (Appendices 5.6, 5.8 and 5.9), the seed harvested by ants (Table 5.9) during that period was 10.6% of the balansa clover seed produced and equivalent to 27.2 kg/ha. In contrast, less than one percent of the cluster clover seeds (0.7%) and subterranean clover seeds (0.03%) produced became part of the seed bank harvested by ants and initially stored around the nest entries.

Some ant nest entries were selected to record the daily harvesting of seed by ants during a period of 16 days. The total seed harvested was 49 seeds of subterranean clover, 11,252 seeds of balansa clover and 324 seeds of cluster clover per entry for the entire period (Figure 5.23). This represents a rate of about 3, 703 and 20 seeds/nest entry/day, respectively. An important increase in the rate of harvesting, as well as an increased selection of smaller seeds of balansa clover and cluster clover (Table 5.10) was observed compared to the values obtained during the previous 85 days of harvesting (Tables 5.8-5.9).

5.2.3 Discussion

In the Mediterranean-type climatic regions of southern Australia, seasonal limitations to animal production occur. The main constraints are the poor nutritive value of the pasture on offer in summer and the low availability of forage in autumn (Purser *et al.* 1987). In the present study, the changes in the proportion of plant components during the grazing period reflect the ability of sheep to graze selectively in order to improve the nutritive value of their diet compared with the average dry pasture residues on offer. The early selection of balansa clover and cluster clover heads, mainly located in the upper part of the pasture canopy and containing the seed with higher values of digestibility, was due to the acceptability and accessibility of the plant material (Curll and Jones 1989). However, the initial abundance of subterranean clover and balansa clover leaves of high digestibility, suggests that the sheep were also selecting a high proportion of these plant components in their diets (Arnold *et al.* 1966; Leigh and Holgate 1978).

Differences in acceptability normally arise because of different digestibility levels among plant species or plant parts, and also because of differences in taste, odour and surface characteristics of the forage plants. However, the previous experience of an animal to a particular feed and the degree of satiation can modify the selection behaviour (Arnold 1981; Black 1987). Kenney and Black (1984) indicate that the diet selected by sheep is mainly determined by how easily the plant material can be harvested and eaten, the chemical and physical characteristics of the forage, the amount available and the spatial distribution of the different plant components in the pasture (Black and Kenney 1984).

In this Waite Institute study the later selection by sheep of subterranean clover-balansa clover stems and leaves with lowered leaf/stem ratios and less digestible subterranean clover burrs (Wilson and Hindley 1968) can be attributed to a progressive reduction in the scope for selection due to reduced amounts of forage on offer to the grazing sheep (Hamilton *et al.* 1973; Langlands and Sanson 1976). This coincides with the onset of the decline in the digestibility and quality of the pasture residues on offer (Curll 1980).

The reduction in the weight of the heads, number of seeds per head and the individual weight of the seed through the grazing period in both small-seeded clovers, especially balansa clover, can be explained by the initial selective grazing of heavier heads containing a higher number of larger seeds. The natural ripening of the seed, its small size and the trampling effect produced by the grazing sheep allows seeds to avoid grazing by falling onto the soil surface. This contributes to the survival of the seed under grazing by remaining in the seed reserve on or in the soil. However, a loss of 56.6% of balansa clover seed and a 70.6% of cluster clover seed from the grazing area was estimated for the entire period of grazing.

With subterranean clover, 94.4% of the burrs were produced above ground level and most of the seed was retained in burrs through the grazing period. This is a similar trend to that observed by Carter (1981) and de Koning (1990) with grazed dry medic and subterranean clover residues. The sheep initially selected the heavier pods or burrs containing a higher number of heavier seeds. However, in the present study the sheep were forced to graze almost all the available forage. This caused previously-buried burrs to be dislodged and ingested by the end of the experimental period: these burrs had a higher individual weight. This suggests that the later-developing burrs above ground and readily accessible to grazing sheep contained fewer, lighter seeds, because of the trampling effect, compared with burrs still buried in the soil (Yates 1958).

As a consequence, 95.4% of the subterranean clover seed disappeared in the WARI grazing experiment. This supports the conclusion of de Koning (1990) who found that subterranean clover seed reserves were susceptible to grazing. This was especially relevant under the conditions of this study where a combination of an inefficient-burr-burying cultivar viz. Clare (Francis *et al.* 1972) and a good above seed producer, such as cultivar Daliak (Collins *et al.* 1976), set seed under ungrazed conditions on a hard-setting soil (Taylor and Rossiter 1969; Quinlivan and Francis 1971) followed by the imposition of heavy grazing during the summer-autumn period.

Increased seed softening during the 70 days of grazing with a consequent rise in germination of seeds was detected for all clover species. In this study, seeds of subterranean clover enclosed in buried burrs showed lower germinability than seeds contained in unburied burrs (Taylor 1984; Taylor and Ewing 1988). Despite the differences among subterranean clover cultivars in the pattern of seed softening (Bolland 1987a), cultivar Clare shows a clear advantage compared to cultivar Daliak (Gladstones and Collins 1984). Softening occurs gradually during the summer months under the influence of high and fluctuating temperatures at the soil surface (Quinlivan 1961; 1966). In the present study, the progressive removal of dry pasture residues improved the exposure of the subterranean clover burrs and small-seeded clover seeds mainly located at the soil surface to the diurnal variation of temperatures (Quinlivan 1965; Taylor *et al.* 1984) increasing the rate of breakdown of hard seeds.

Excretion of faeces from livestock can affect the legume content of a pasture by modifying the level of nutrients in the soil, by scorching plants, by influencing the behaviour of grazing, and by dispersing the seed in faeces (Curll and Jones 1989). The ability of a pasture legume seed to survive and become dispersed through the faeces of grazing livestock is a desirable feature (Carter and Lake 1985; Jones and Carter 1989).

The trends in seed recovery from faeces through the grazing period was obviously a consequence of the preference of selection by sheep. The persistent output of subterranean clover seeds until the end of the grazing period reflects the ability of sheep to dispose of buried burrs which have higher seed/chaff ratios. However, on the hard-setting red-brown earth of the experiment site most of the burrs are available to sheep because of only partial covering with soil or being located in cracks at the soil surface. A similar trend in the changes of the output of subterranean clover seeds were reported by de Koning (1990), working with a mixed pasture of subterranean clover cultivars grown on the same soil type. However her results differed in that a sustained decline in the amount of seed voided in faeces was observed until the end of the grazing period. This may be a consequence of a progressive change in the priorities of selection by

sheep given the higher and more diverse availability of dry pasture residues at that time. However, the proportion of germinated seed and hard seed found in faeces reported by de Koning were similar to those in this study.

It has been suggested that only hard seed can survive the process of chewing and the subsequent effects in the digestive tract of the sheep (Yamada and Kawaguchi 1971; Simao Neto and Jones 1987). The present study suggests that a natural softening and mortality of some hard seeds occurs after seed leaves the rumen site (6.1 Pen Experiment 1 and 6.2 Pen Experiment 2). The increase in the relative number of emerged seedlings compared to hard seed in faeces through the grazing period, also suggests that the different rates of softening may be related to different times of retention of small quantities of seed in the digestive tract (increased retention with decreasing digestibility of feed) or to a higher degree of susceptibility of hard seed to softening (through removal of the dry pasture insulation and longer exposure of the seed to diurnal fluctuations in temperature) imposed by grazing for 70 days.

In the present study, data on subterranean clover seed differs from that of de Koning (1990) who found that individual weight of the subterranean clover hard seed during the grazing period did not decline with time and tended to increase at the end of the period. The almost steady initial state could have been due to a narrow range of variation in the weight of the hard seed available for grazing at that period of time (Appendix 5.10). The later increase in weight of the hard seed could have resulted from heavier seeds being retained in previously-buried burrs, especially if the sheep were able to select burrs with higher seed/chaff ratios.

At the end of this present study, the differences between the weight of the hard seed on offer and the weight of the hard seed voided in the faeces suggest that small and hard seeds are the most suitable seed to survive the natural process occurring in the digestive tract of the sheep. Finally, only 1.79% (emerged seedlings = 0.64% and hard seed = 1.15%) of the subterranean clover seeds that were removed from the grazing area were able to survive grazing, corroborating the estimate of 1.5% made by de Koning (1990)

and confirming the extreme susceptibility of larger subterranean clover seed to ingestion utilization by sheep.

There was also a persistent output of small-seeded clover seed in faeces until the end of the grazing period. Unlike subterranean clover, all the seed of balansa clover and cluster clover were available on the surface of the soil after 42 days of grazing. Although the sheep were collecting some seeds from the ground, it is suggested that smaller (and harder) seeds were retained longer than subterranean clover seeds in the digestive tract of the sheep.

A recovery of 14.94% (emerged seedlings = 1.68% and hard seed = 13.26%) of balansa clover seeds and a 75.59% (emerged seedlings = 1.28% and hard seed = 74.31%) of cluster clover seeds was estimated. A higher value (38%) of survival is reported by Carter *et al.* (1988) for balansa clover seeds fed to penned sheep in combination with a standard diet. It is possible that the higher quality of the diet used in the penned sheep experiment and consequent faster passage of the seed compared to the diet selected by sheep under grazing influenced the recovery of seeds in faeces (Jones and Simao Neto 1987). Also, the almost exclusive selection of balansa clover heads by sheep during the initial part of the grazing period may have had a negative effect on the survival of the seed. A lower percentage (56.7%) of ingested cluster clover seed was found to pass in sheep faeces (Franklin and Powning 1942). The high seed survival obtained in the present study may be due to the contrasting seed sizes and levels of hard-seededness. This further confirmed that small, hard seed is better able to survive the passage through the digestive tract of the sheep (Playne 1969; Thomson *et al* 1987).

Of the seeds in the faeces stored in the field, 63.2% of subterranean clover, 71.7% of balansa clover and 80.5% of cluster clover originally voided were able to persist until the emergence of the seedlings in the raised-beds was recorded. The loss of subterranean clover seeds in storage was similar to the original number of normal emerged seedlings. This suggests that most of the mortality of seed in faeces was due to the desiccation of germinating seeds following excretion, and may also be the effect of

alternate wetting and drying as a consequence of erratic pulses of rainfall. The timing of initial imbibition of water in a seed is critical, because as more advanced metabolic processes are achieved the resistance of the embryo to dehydration decreases (Bewley and Black 1982). In contrast, softened seeds in faeces seem to be less affected as most of them germinated and seedlings emerged in the raised-beds following artificial watering. However, during normal summer grazing faecal pellets rapidly desiccate, preventing germination of soft seed before the occurrence of adequate falls of rain.

According to Suckling (1950), Lennartz (1956), Simao Neto (1985) and Simao Neto and Jones (1986), only those seeds that passed through the digestive tract of livestock in hard condition are able to survive the fermentation process in faeces. However, under the conditions of this WARI study, in which the sheep pellets rapidly dried after placement in the field, the processes of change and decomposition of faeces have been prevented and consequently delayed until the level of moisture in the pellets was improved, principally after transfer from the field to the raised-beds, and the commencement of artificial watering.

In the small-seeded clover species, the reduction in viable seeds exceeded the original number of normally emerged seedlings, indicating a difference with subterranean clover. Some softened seeds of the small-seeded species contained in pellets were not able to survive the conditions of storage. The combination of high temperatures and the presence of bacteria during the process of fermentation of faeces reduces the viability of seeds by damaging the embryo (Ozer 1979). Moreover, the activities of other organisms that are involved in the decomposition of faeces, such as insect larvae, lumbricid worms and coprophagous beetles (White 1960; Barley 1964; Gillard 1967) may also cause the disappearance of seed.

Despite the reduced potential emergence and establishment of seedlings from sheep pellets expected under field conditions (Suckling 1950; de Koning and Carter 1989; de Koning 1990) a recovery of 1.1% of subterranean clover, 9.0% of balansa clover and 58.7% of cluster clover seed was ultimately recorded.

The regeneration of a pasture based on annual legume species is frequently limited by the presence of inadequate levels of seed reserve induced by poor grazing practices during the dry stage of the pasture sward (Carter 1987). Despite the heavy stocking density imposed on the dry pasture residues, a density of 625, 1786 and 52 seedlings/m^2 of subterranean clover, balansa clover and cluster clover respectively, was recorded after 56 days of grazing. Based on information obtained for barrel medic (Carter 1983), subterranean clover (Collins *et al.* 1983; Taylor *et al.* 1984) and Verano stylo (Gardener 1982) an emergence of at least 500 seedlings/m^2, representing a 10% of emergence at the start of the growing season, is a reasonable minimum limit for success in the regeneration of an annual pasture legume (Jones and Carter 1989).

The adequate but contrasting level of regeneration observed between the sown pasture species as well as the higher and more relevant hard seed reserve occurring in balansa clover, as a consequence of dissimilar abilities to avoid and survive the impact of grazing by sheep, suggest that adequate utilization of dry pasture residues at the proper time to ensure exposure of the seed to the fluctuating temperatures in order to favour the breakdown of hard-seededness, would be an important strategy to ensure sufficient density of soft seed with a higher potential for regeneration. There must be a compromise between excess consumption of seed and having an adequate reserve of soft seed for satisfactory regeneration of a dense and productive pasture.

The most available and accessible seeds of balansa clover were collected by ants in preference to other annual legume pasture species and other annual grass seeds mainly located at the unsown edge of the grazing area at WARI. There is a positive relationship between the availability of seed and the preference shown for its collection (Hocking 1975). The acceptability of the seed as a food resource is not only because seeds can be stored in the nests for a long period, but also because of high nutritive value (Janzen 1971; Baker 1972). The ease of accessibility of seed at the surface of the soil and a matching between the size of the ant and the seed (Culver 1972; Briese 1974), seem to be the main factors influencing the selection of balansa clover seeds. Cluster clover

seeds were both small and less numerous while subterranean clover seeds were contained in burrs which were less-easily accessed and transported.

Despite the various selective forces involved in the selection of seeds by ants (Smith 1975), the basic food strategy of the harvester ants is to maximize the predation activities when the seasonal production of seed occurs. This leads to a rapid accumulation and storage of the seeds in the nests. The colony uses them as a source of food during the period of the year in which conditions for seed collection are poor and/or the seed is not available (Briese 1974). Consequently, a large number of small ants such as *Iridomyrmex chasei* (length 2-3 mm) collect many small, easily-carried seeds like balansa clover. This is probably the most efficient strategy for the ants to cope with the food requirements of the colony (Shoener 1969).

In the present study, about 10% of the balansa clover seed produced in the pasture mixture was taken by ants in a relatively short time of harvesting. Considering that the subterranean clover seed enclosed in burrs were protected from predation and also that they were less preferred as a free seed than other pasture seeds [especially lighter ones such as of annual grasses (Campbell 1966; McGowan 1969)], it is suggested that some attention should be given to this topic in the future, especially when the presence of grazing sheep follows overgrazing patterns that favour the increase of ant populations under pasture conditions (Hutchinson and King 1980).

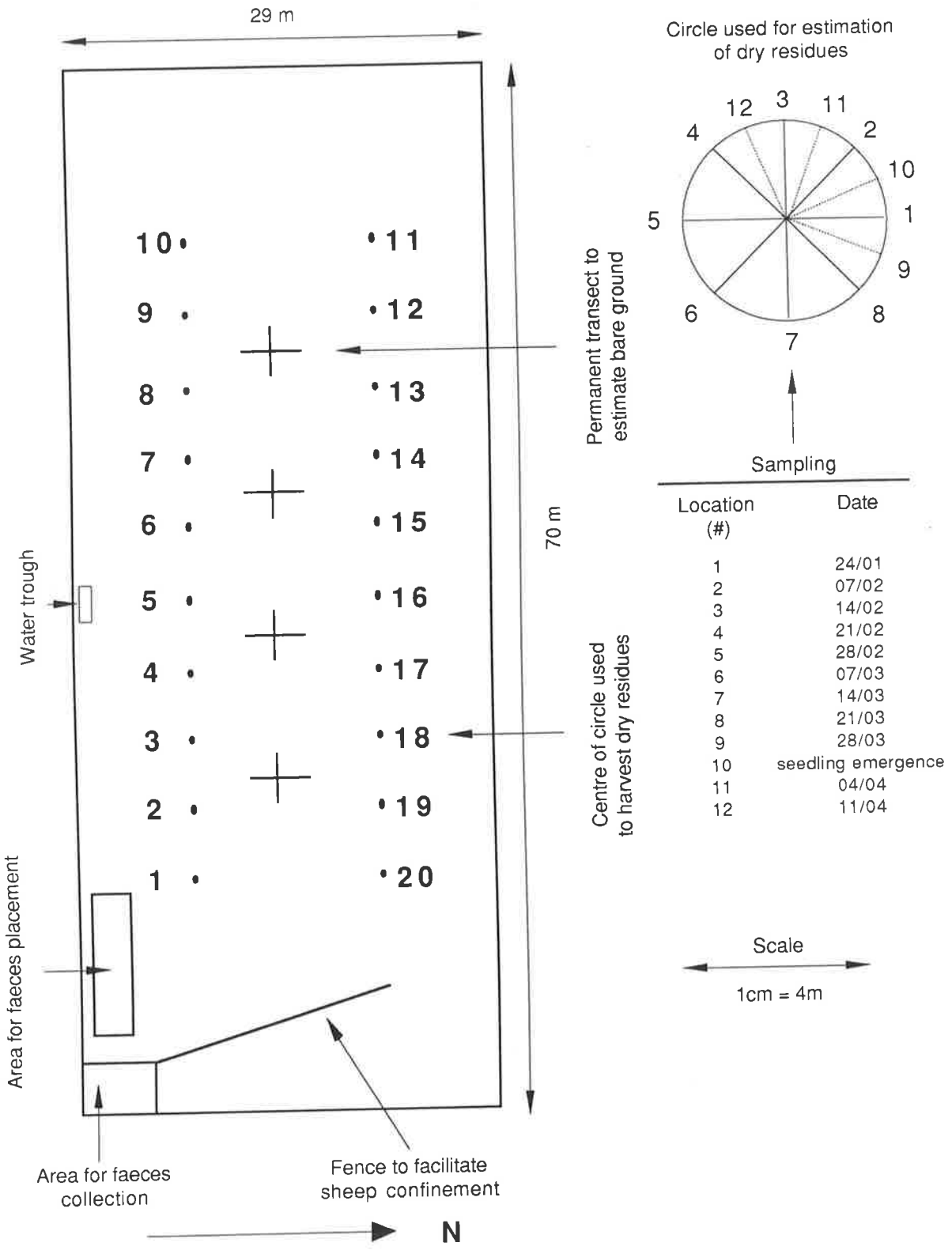


Diagram 5.1 Site used to test the survival of clover seeds under grazing by sheep at the Waite Agricultural Research Institute.

Table 5.1 Digestibility and ash content of subterranean clover, balansa clover and cluster clover plant components before starting the grazing experiment at Waite Institute.

Clover species	Plant component	Digestibility		Ash content	
		Dry matter	Organic matter	Whole sample input	Undigested output
		(%)		(%)	
Subterranean-balansa	Top	56.7	56.3	9.5	8.6
Subterranean	Stem	52.1	50.1	7.6	3.7
	Leaf	68.3	67.6	9.8	7.8
	Burr	49.3	48.1	6.6	4.3
	Chaff	6.9	5.7	4.2	3.0
	Seed	86.8	86.7	4.3	3.3
Balansa	Stem	52.8	50.9	7.4	3.7
	Leaf	65.3	64.6	9.0	7.1
	Head	57.8	57.7	4.6	4.5
	Chaff	35.3	34.1	5.8	4.1
	Seed	96.7	97.9	6.8	39.6
Cluster	Head	68.4	68.3	6.7	6.5
	Seed	99.2	99.4	3.6	24.8

Table 5.2 Digestibility and ash content of subterranean clover-balansa clover stems and leaves available to sheep at different times during the grazing experiment at Waite Institute.

Grazing period (days)	Digestibility		Ash content	
	Dry matter	Organic matter	Whole sample input	Undigested output
	(%)		(%)	
0	55.7	55.9	7.5	5.9
7	50.1	50.1	6.7	4.9
14	48.9	49.9	5.7	4.5
21	49.0	48.9	6.8	4.8
28	48.0	48.4	6.4	4.5
35	50.0	50.9	6.0	4.8
42	49.3	49.8	6.4	4.6
49	49.4	49.9	5.6	5.1
56	45.4	46.0	6.4	4.7
63	43.9	44.8	5.5	3.0
70	39.5	39.1	7.2	3.1

Table 5.3 Digestibility and ash content of subterranean clover burrs available to sheep at different times during the grazing experiment at Waite Institute.

Grazing period (days	Digestibility		Ash content	
	Dry matter	Organic matter	Whole sample input	Undigested output
	(%)		(%)	
0	48.1	48.0	7.4	5.8
7	48.1	47.1	7.6	5.8
14	47.6	46.3	5.8	3.5
21	49.1	47.8	6.6	4.1
28	44.8	43.1	7.2	4.3
35	46.4	44.2	7.4	3.2
42	47.1	45.7	6.7	4.2
49	45.7	44.5	6.0	3.9
56	50.7	49.4	6.5	3.7
63	50.6	48.0	7.1	3.3
70	50.0	49.2	6.8	3.5

Table 5.4 Seed-seedling dynamics of subterranean clover seeds voided in the faeces by the sheep flock at different times during the grazing experiment at Waite Institute.

Grazing period (days)†	Seed output (#) that became seedling at:					Hard seed output (#)	Total	Seed output		Hard seed weight (mg)
	Raised beds			Incubator	Sub-total	Sub-total		Seedling (%)	Hard (%)	
	31/05	14/06	28/06	12/07						
7	243	475	1029	105	1134	1638	2772	40.9	59.1	3.75
14	508	967	1836	328	2164	3046	5210	41.5	58.5	3.85
21	1050	1197	2711	366	3077	4079	7156	43.0	57.0	3.80
28	710	932	2139	352	2491	3745	6236	39.9	61.0	3.51
35	611	917	1614	344	1958	4296	6254	31.3	68.7	3.81
42	319	515	1282	240	1522	3040	4562	33.4	66.6	3.88
49	239	458	789	154	943	2310	3253	29.0	71.0	3.87
56	141	314	598	66	664	1544	2208	30.1	69.9	3.93
63	200	324	634	186	820	2149	2969	27.6	72.4	4.26
70	220	279	496	140	636	1835	2471	25.7	74.3	4.87
Total	-	-	-	-	15409	27682	43091	-	-	-
(%)	-	-	-	-	-	-	-	35.8	64.2	-

† Faecal seed collections made one day later, i.e. from Day 8 to Day 71.

Table 5.5 Seed-seedling dynamics of balansa clover seeds voided in the faeces by the sheep flock at different times during the grazing experiment at Waite Institute.

Grazing period (days)†	Seed output (#) that became seedling at:					Hard seed output (#)	Total	Seed output		Hard seed weight (mg)
	Raised beds			Incubator	Sub-total	Sub-total		Seedling (%)	Hard (%)	
	31/05	14/06	28/06	12/07						
7	8660	11946	12467	1677	14144	135092	149236	9.5	90.5	0.742
14	11032	21828	27677	1011	28688	112621	141309	20.3	79.7	0.738
21	2518	7379	11614	834	12448	58937	71385	17.4	82.6	0.743
28	826	2301	2787	477	4210	20503	24713	17.0	83.0	0.711
35	926	2356	3733	260	3047	12975	16022	19.0	81.0	0.696
42	347	911	1064	300	1364	7881	9245	17.3	82.7	0.679
49	192	841	865	212	1077	5708	6785	15.9	84.1	0.709
56	148	483	816	136	952	2314	3266	29.1	70.5	0.678
63	129	525	993	370	1363	3250	4613	29.5	70.5	0.682
70	397	1056	1619	247	1866	9831	11697	16.0	84.0	0.671
Total	-	-	-	-	69159	369112	438271	-	-	-
(%)	-	-	-	-	-	-	-	15.8	84.2	-

† Faecal seed collections made one day later, i.e. from Day 8 to Day 71.

Table 5.6 Seed-seedling dynamics of cluster clover seeds voided in the faeces by the sheep flock at different times during the grazing experiment at Waite Institute.

Grazing period (days)†	Seed output (#) that became seedling at:					Hard seed output (#)	Total	Seed output		Hard seed weight (mg)
	Raised beds			Incubator	Sub-total	Sub-total		Seedling (%)	Hard (%)	
	31/05	14/06	28/06	12/07						
7	1251	2139	2391	207	2598	115489	118087	2.2	97.8	0.433
14	930	1723	1870	165	2035	79368	81403	2.5	97.5	0.427
21	636	915	1006	108	1114	43427	44541	2.0	98.0	0.430
28	321	776	861	92	953	35725	36678	2.6	97.4	0.420
35	256	443	543	52	595	23204	23799	2.5	97.5	0.410
42	213	300	317	30	347	14727	15074	2.3	97.7	0.382
49	125	212	270	15	285	13959	14244	2.0	98.0	0.403
56	92	126	216	15	231	11323	11554	2.0	98.0	0.399
63	146	285	388	30	418	13054	13472	3.1	96.9	0.395
70	62	132	183	18	201	6488	6689	3.0	97.0	0.387
Total	-	-	-	-	8777	356764	365541	-	-	-
(%)	-	-	-	-	-	-	-	2.4	97.6	-

† Faecal seed collections made one day later, i.e. from Day 8 to Day 71.

Table 5.7 Artificial regeneration of the annual clovers under field conditions after 56 days of grazing.

Variable	Clover species						
	Subterranean					Balansa	Cluster
	Unburied	%	Buried	%	Total		
BURR							
Density (#/m^2)	1020	76.9	307	23.1	1327	-	-
Weight (mg)	16.2	-	27.1	-	18.8	-	-
HARD SEED/BURR							
Number (#)	1.58	-	1.64	-	1.59	-	-
Weight (%)	47.3	-	38.4	-	44.3	-	-
HARD SEED							
Density (#/m^2)	1607	76.1	505	23.9	2112	11962	1112
Weight (mg)	4.87	-	6.34	-	5.22	0.752	0.418
Reserve (g/m^2)	7.83	71.0	3.20	29.0	11.03	9.000	0.465
SEEDLING							
Density (#/m^2)	-	-	-	-	625	1787	52
Emergence (%)	-	-	-	-	22.84	13.00	4.47
Dry matter (g/m^2)	-	-	-	-	3.80	3.48	0.06
Leaf Area Index (LAI)	-	-	-	-	0.095	0.047	trace

Table 5.8 Seed size distribution by number of seeds of subterranean clover, balansa clover and cluster clover harvested by ants during the 85-day period before the start of the grazing experiment at Waite Institute.

Sieve aperture (mm) Max. - Min.	Clover species								
	Subterranean			Balansa			Cluster		
	(%)	Seed (mg)	weight ratio	(%)	Seed (mg)	weight ratio	(%)	Seed (mg)	weight ratio
2.36 - 2.01	7.3	8.72	5.70	-	-	-	-	-	-
2.00 - 1.71	43.7	5.32	3.48	-	-	-	-	-	-
1.70 - 1.41	30.9	3.17	2.08	-	-	-	-	-	-
1.40 - 1.19	17.9	2.04	1.33	-	-	-	-	-	-
1.18 - 1.01	0.2	1.53	1.00	16.8	0.933	2.97	-	-	-
1.00 - 0.711	-	-	-	82.7	0.755	2.41	90.1	0.413	1.38
0.710 - 0.501	-	-	-	0.5	0.314	1.00	9.1	0.300	1.00
Mean	-	4.40	-	-	0.781	-	-	0.333	-

Table 5.9 Seeds of subterranean clover, balansa clover and cluster clover harvested by ants before the start of the grazing experiment at Waite Institute.

Clover species	Statistic (n = 38)				
	Sum	Mean	s.d.	C.V. (%)	s.e.
Subterranean					
Seed (# / entry)	930	25	38.9	158.8	6.3
Seed (g / entry)	4.06	0.108	0.190	177.6	0.03
Seed weight (mg)	-	4.32	-	-	-
Balansa					
Seed (# / entry)	847442	22301	16820	75.4	2729
Seed (g / entry)	664	17.48	13.19	75.5	2.14
Seed weight (mg)	-	0.784	-	-	-
Cluster					
Seed (# / entry)	5378	142	336.2	236.8	54.5
Seed (g / entry)	1.662	0.0437	0.1071	245.1	0.0174
Seed weight (mg)	-	0.308	-	-	-

Table 5.10 Seed size distribution by number of seeds of subterranean clover, balansa clover and cluster clover harvested by ants during the 16-day period before the start of the grazing experiment at Waite Institute.

Sieve aperture (mm) Max. - Min.	Clover species								
	Subterranean			Balansa			Cluster		
	(%)	Seed (mg)	weight ratio	(%)	Seed (mg)	weight ratio	(%)	Seed (mg)	weight ratio
2.36 - 2.01	17.4	7.52	4.42	-	-	-	-	-	-
2.00 - 1.71	47.5	5.04	2.96	-	-	-	-	-	-
1.70 - 1.41	28.7	3.13	1.84	-	-	-	-	-	-
1.40 - 1.19	6.4	1.70	1.00	-	-	-	-	-	-
1.18 - 1.01	-	-	-	12.9	0.916	1.27	-	-	-
1.00 - 0.711	-	-	-	87.1	0.719	1.00	81.2	0.421	1.35
0.710 - 0.501	-	-	-	-	-		18.8	0.313	1.00
Mean	-	4.57	-	-	0.745	-	-	0.403	-

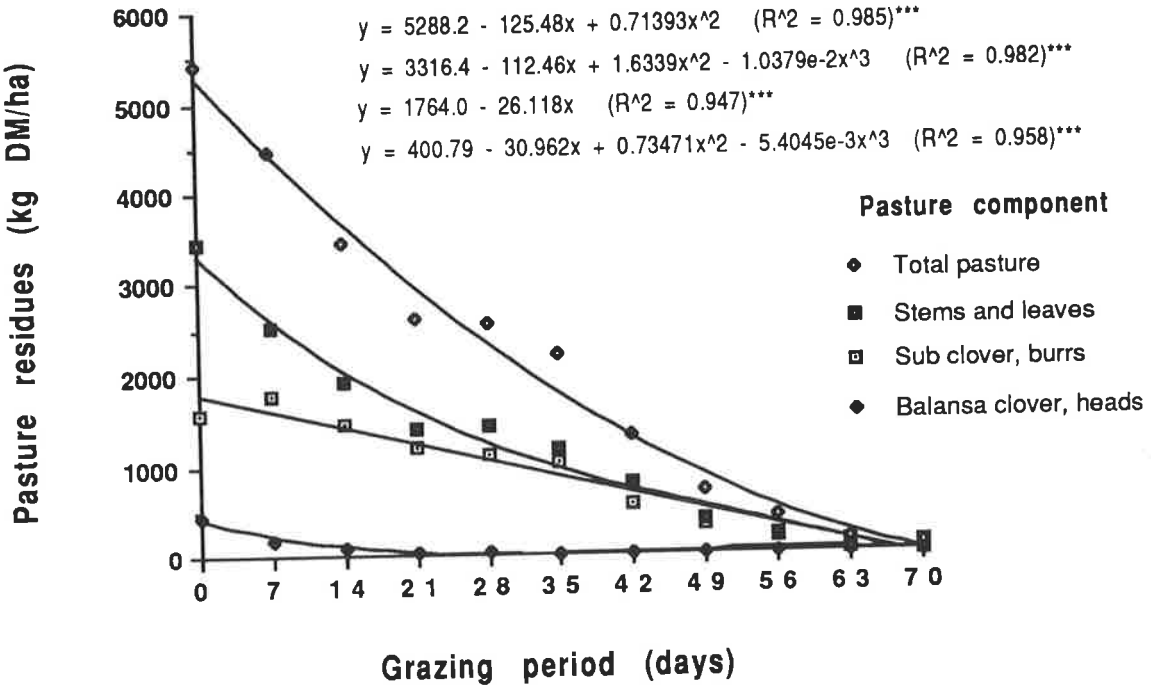


Figure 5.1 The trend of dry pasture residues during the grazing period.

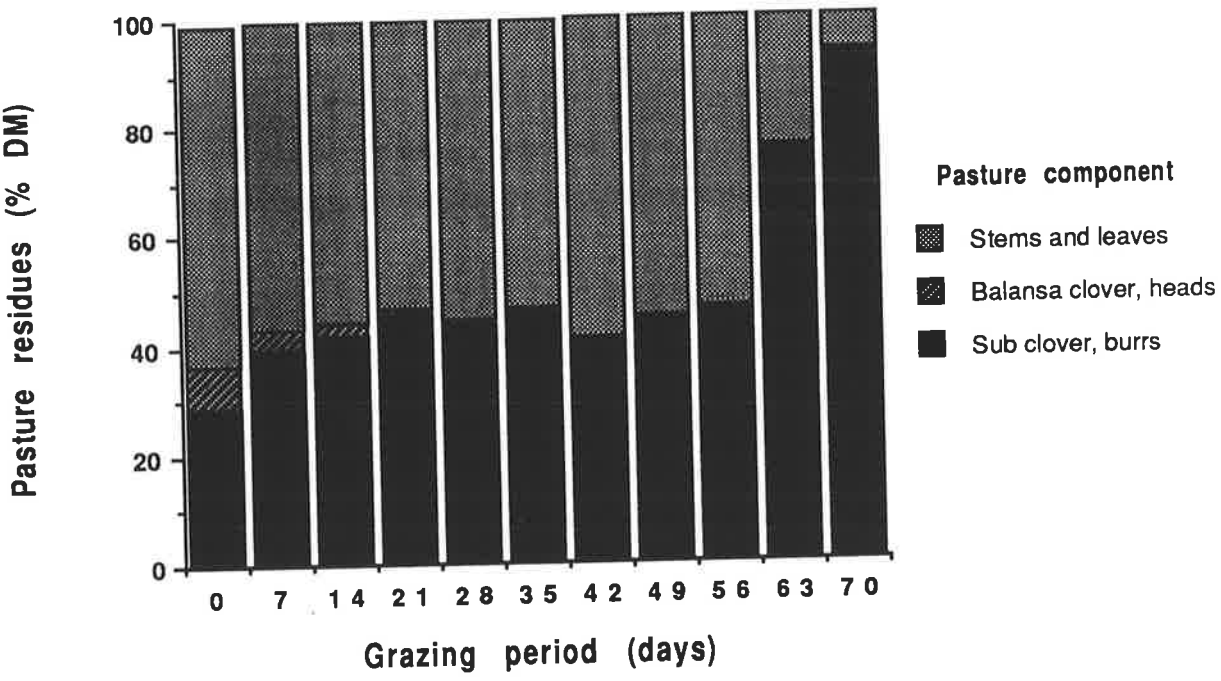


Figure 5.2 The changes in the availability of dry pasture components during the grazing period.

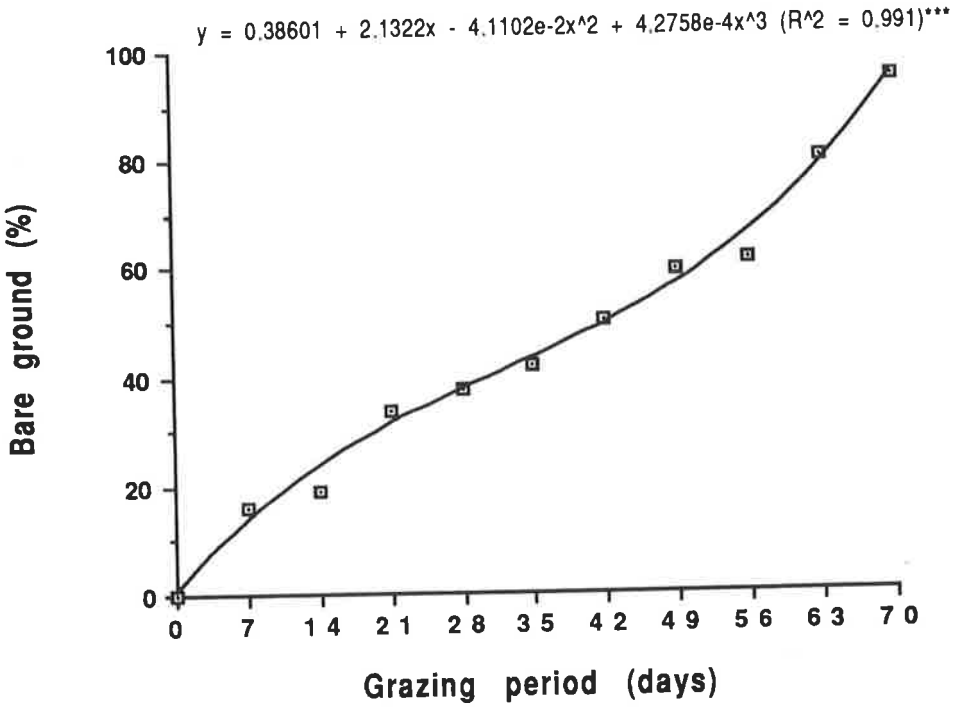


Figure 5.3 The increases of bare ground during the grazing period.

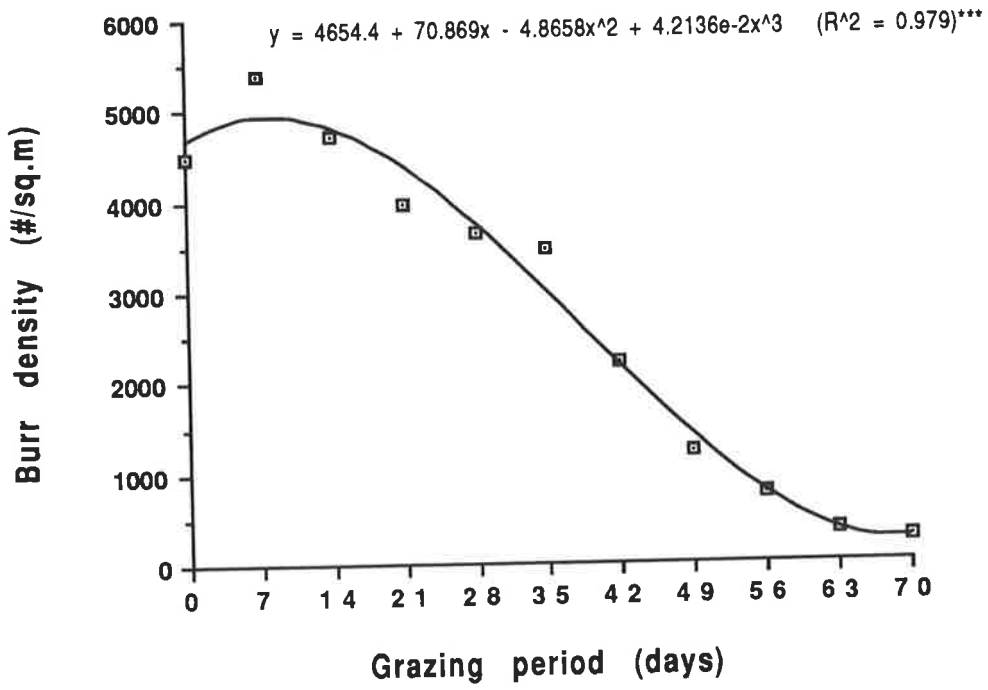


Figure 5.4 The decline of subterranean clover burrs during the grazing period.

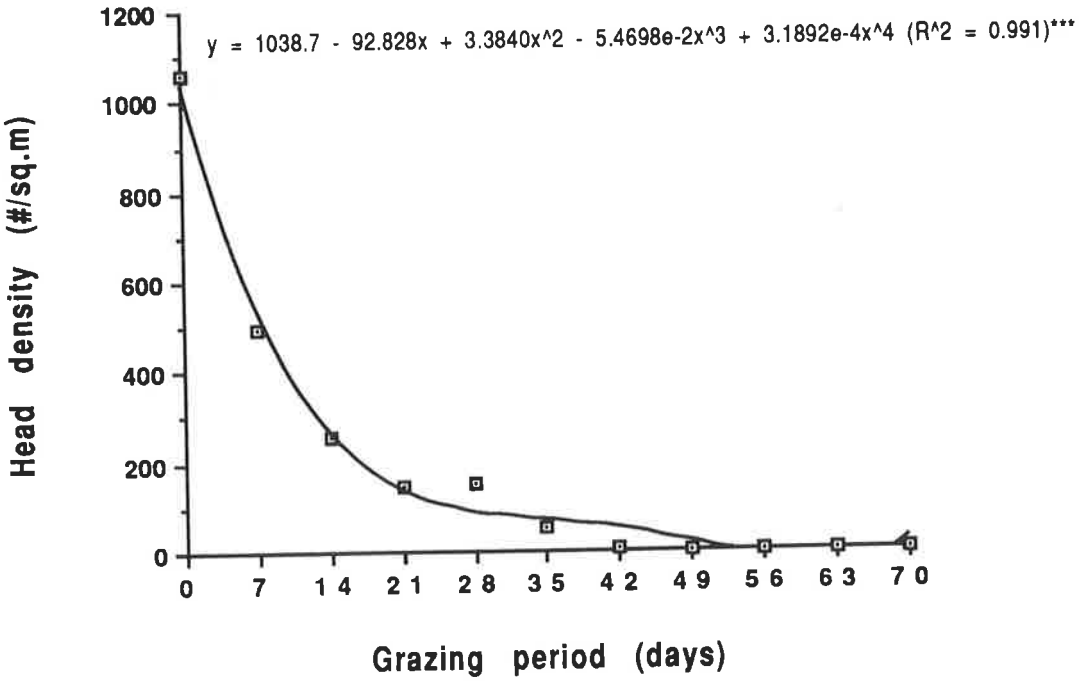


Figure 5.5 The decline of balansa clover heads during the grazing period.

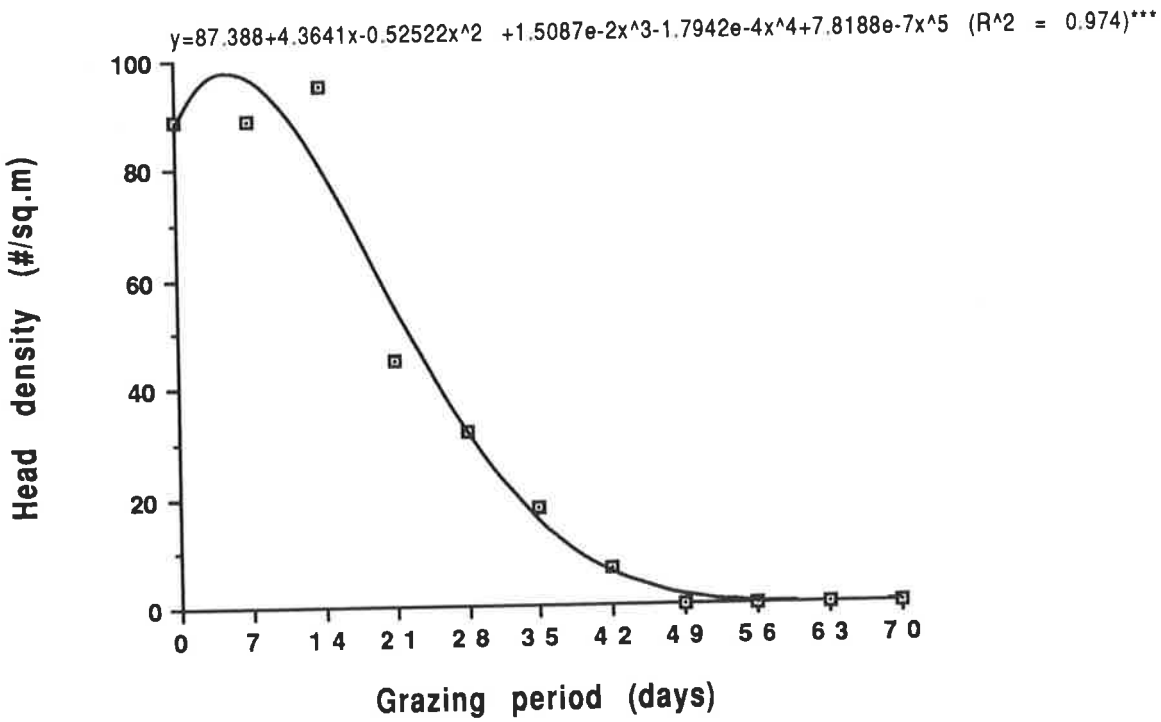


Figure 5.6 The decline of cluster clover heads during the grazing period.

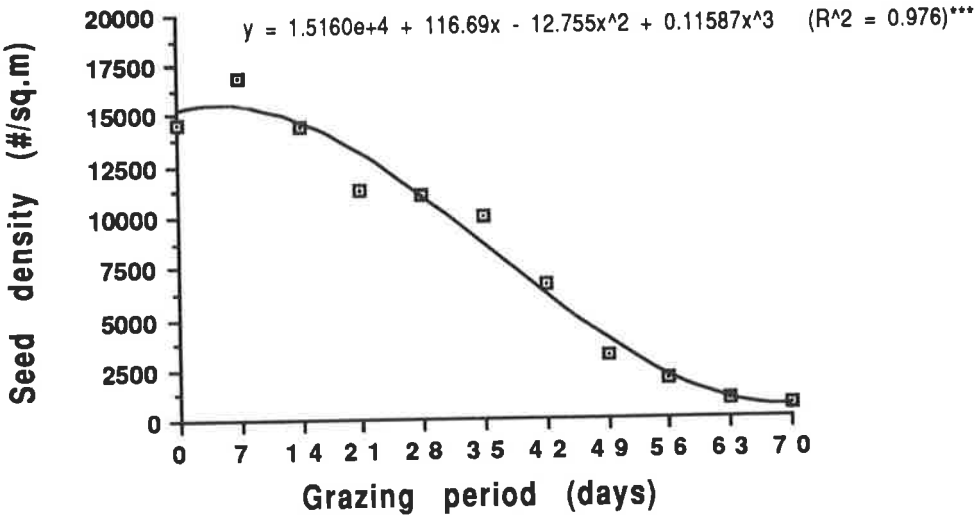


Figure 5.7 The trend of subterranean clover seeds during the grazing period.

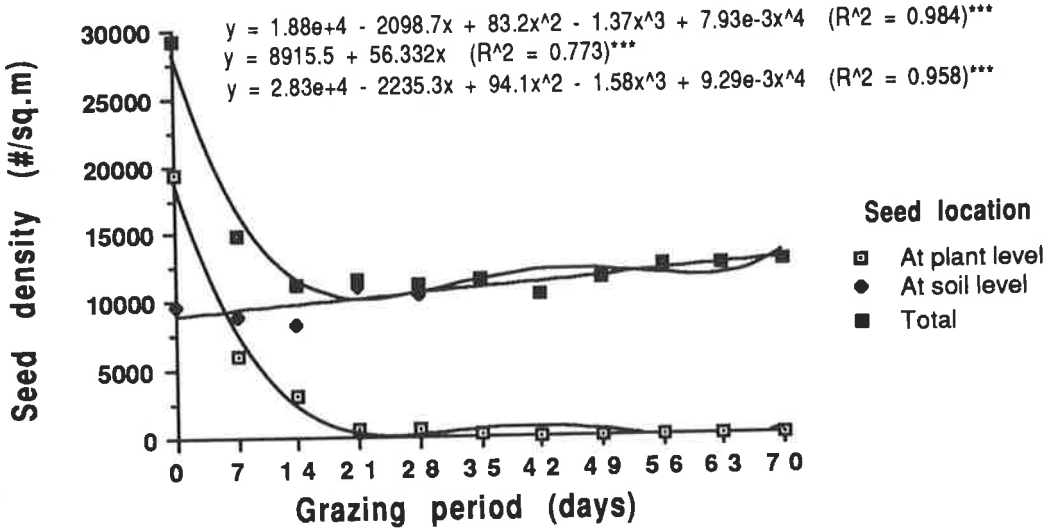


Figure 5.8 The trend of balansa clover seeds during the grazing period.

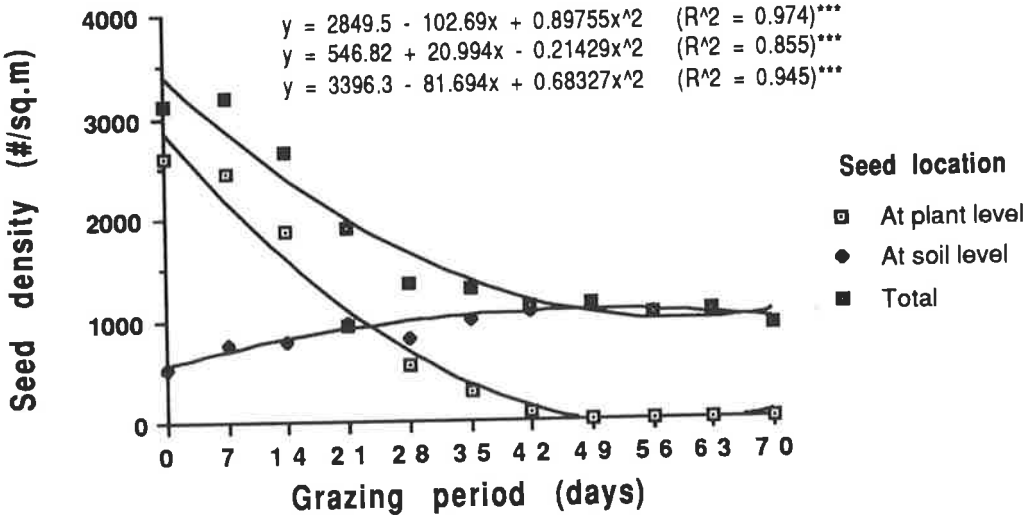


Figure 5.9 The trend of cluster clover seeds during the grazing period.

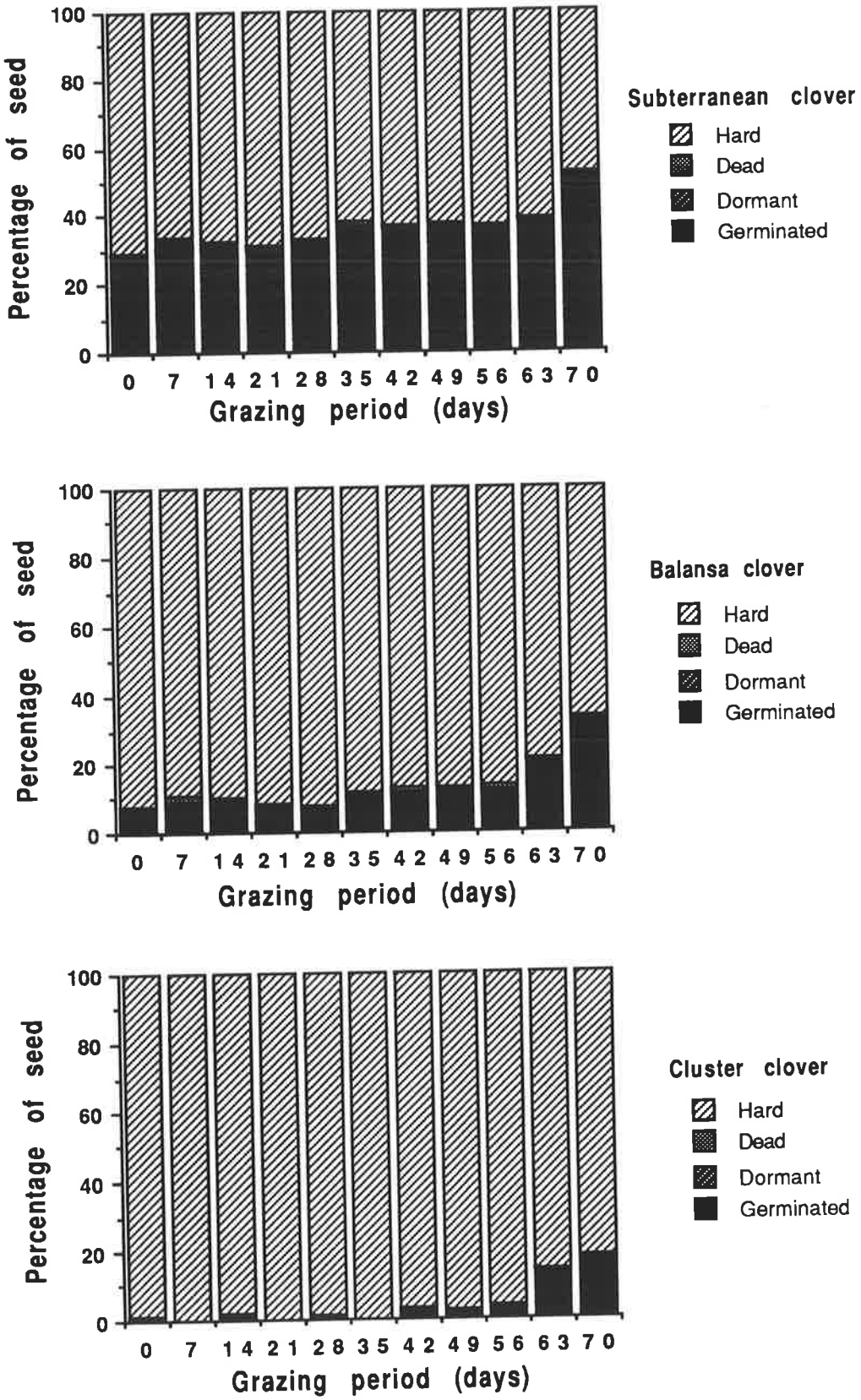


Figure 5.10 The changes in the germinability of subterranean clover, balansa clover and cluster clover seeds during the grazing period.

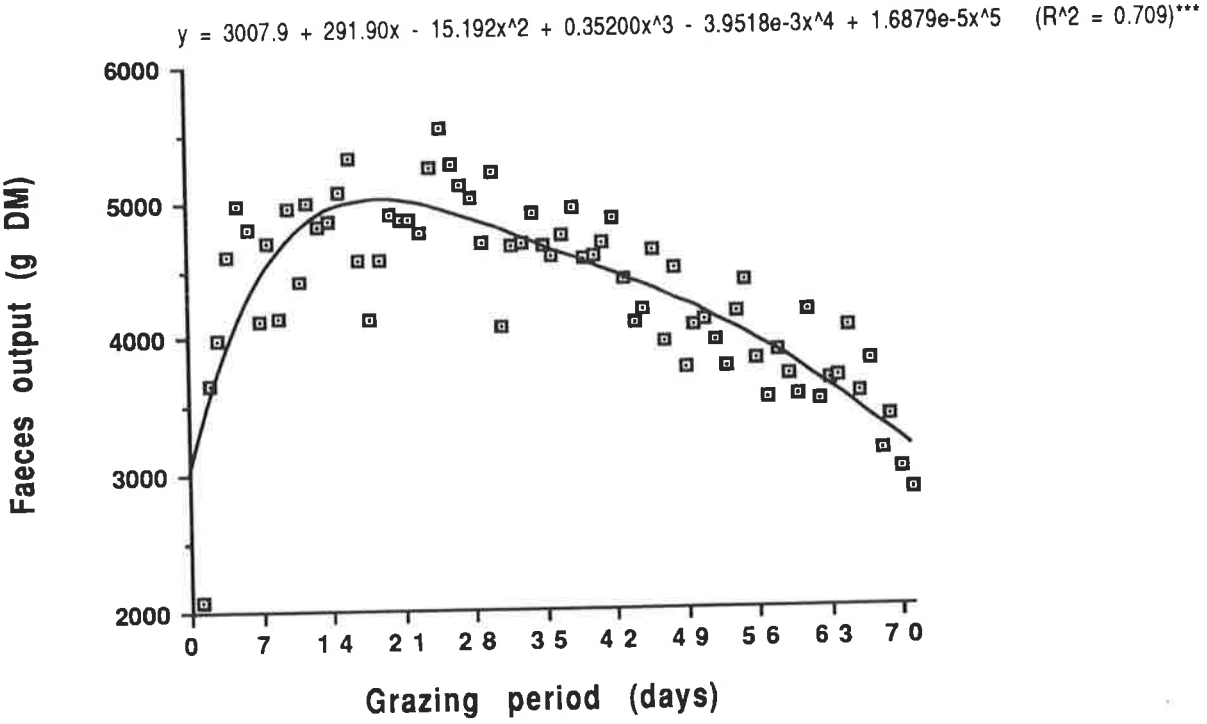


Figure 5.11 The trend of the flock faeces output during the grazing period.

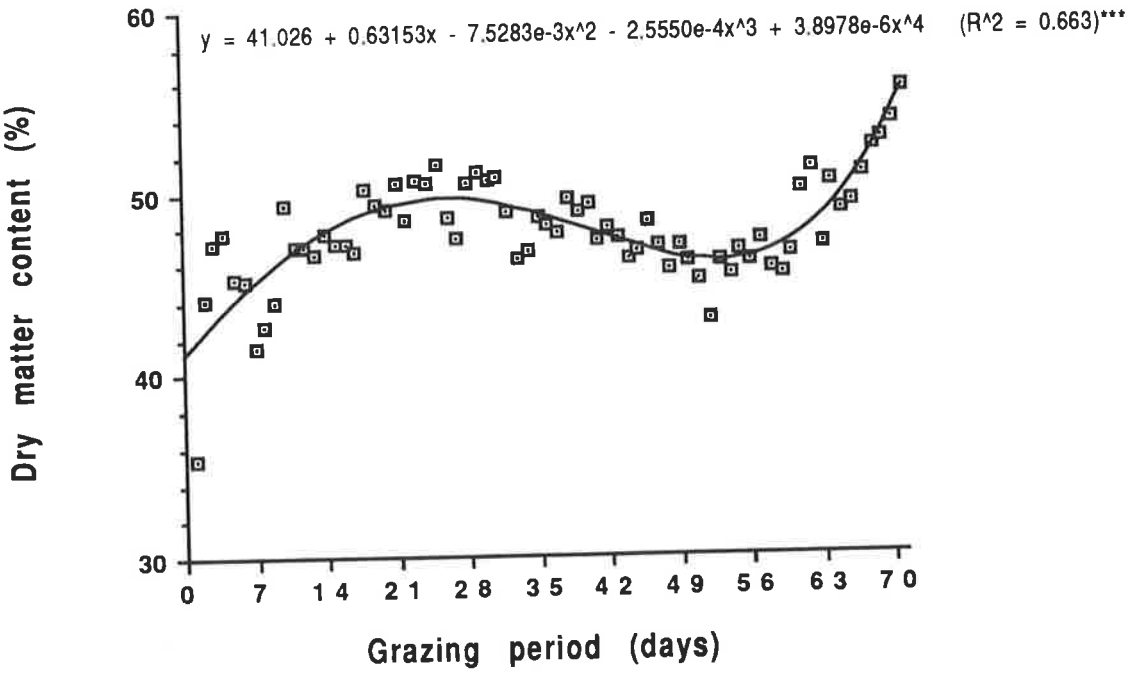


Figure 5.12 The trend of the dry matter content of faeces during the grazing period.

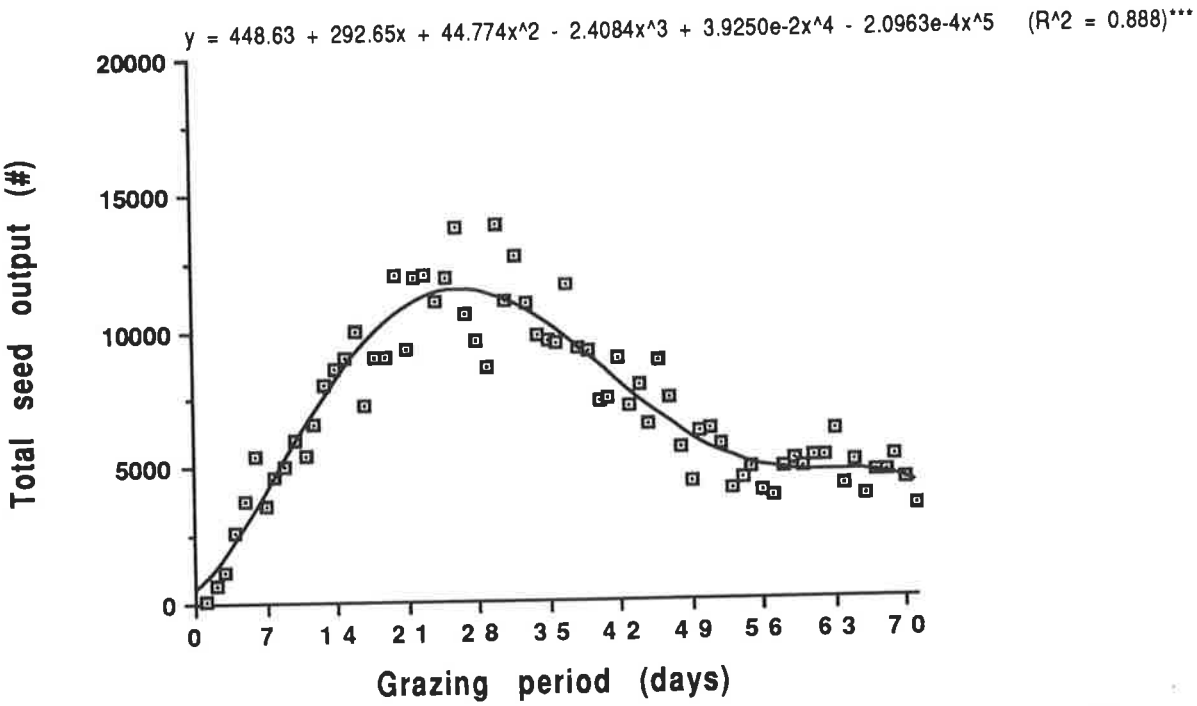


Figure 5.13 The changes in the output of subterranean clover seeds during the grazing period.

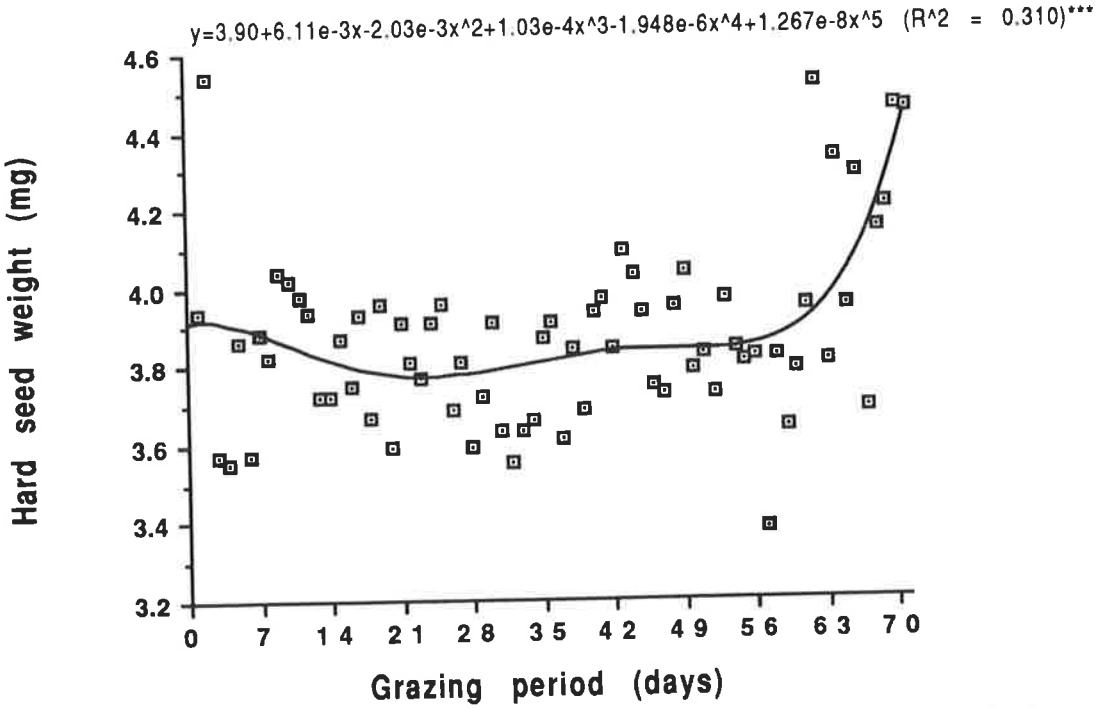


Figure 5.14 The changes in the seed weight of subterranean clover hard seeds voided in the faeces by the flock during the grazing period.

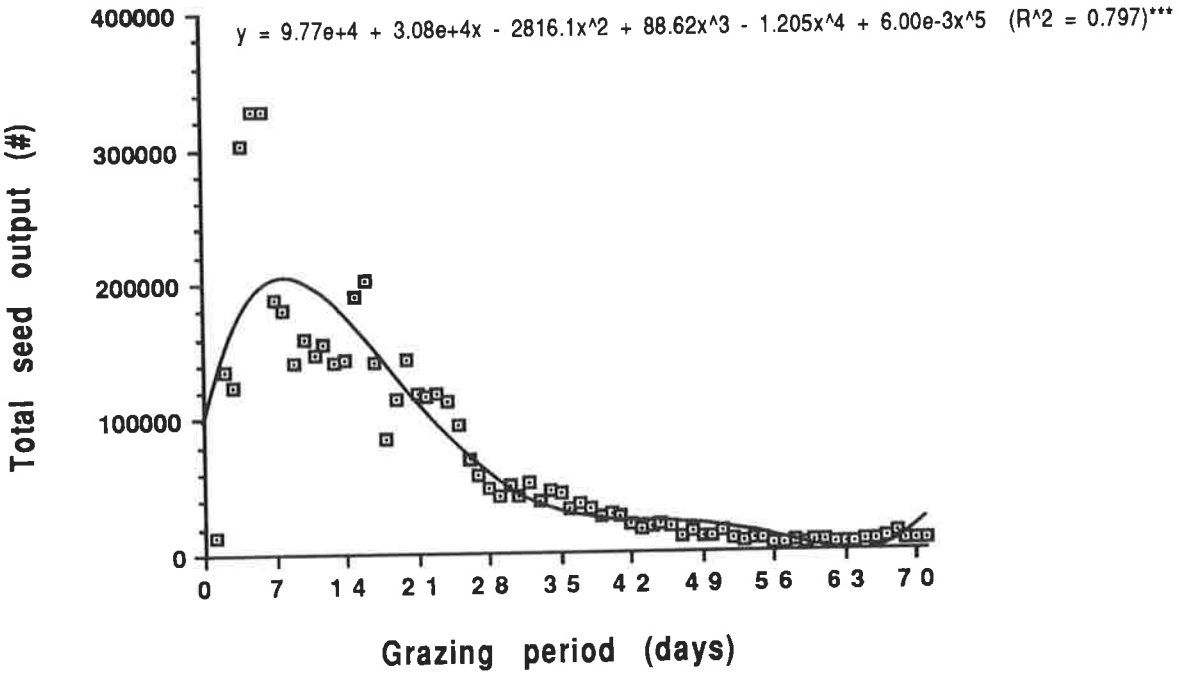


Figure 5.15 The changes in the output of balansa clover seeds during the grazing period.

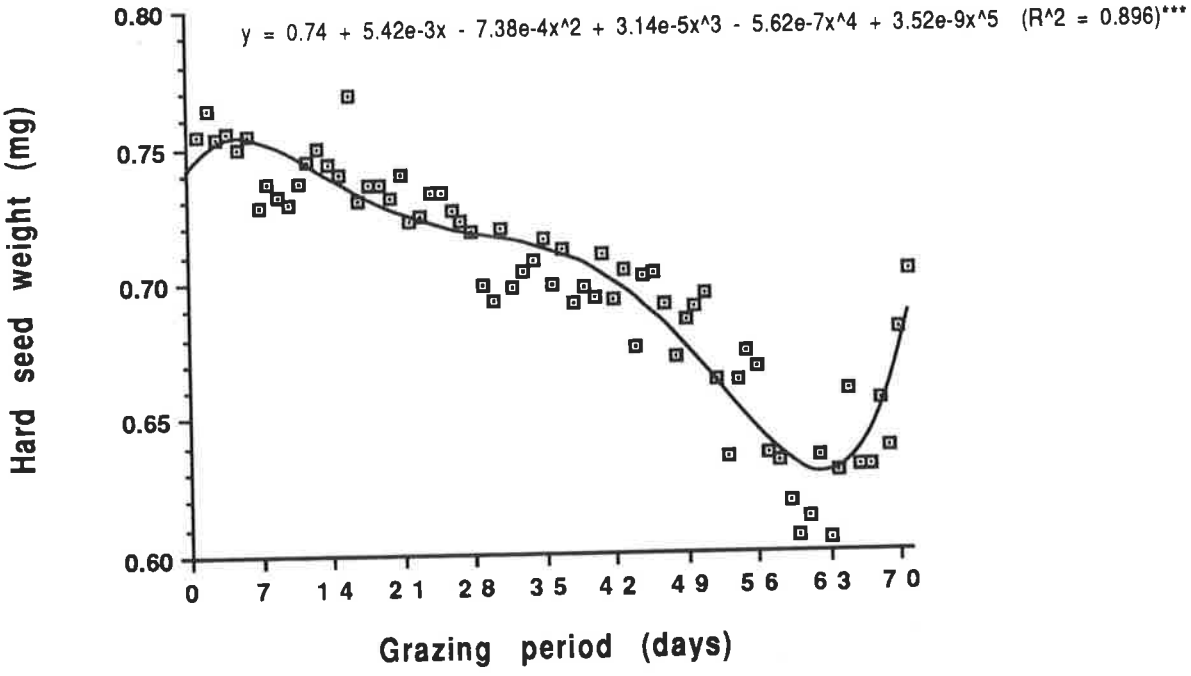


Figure 5.16 The changes in the seed weight of balansa clover hard seeds voided in the faeces by the flock during the grazing period.

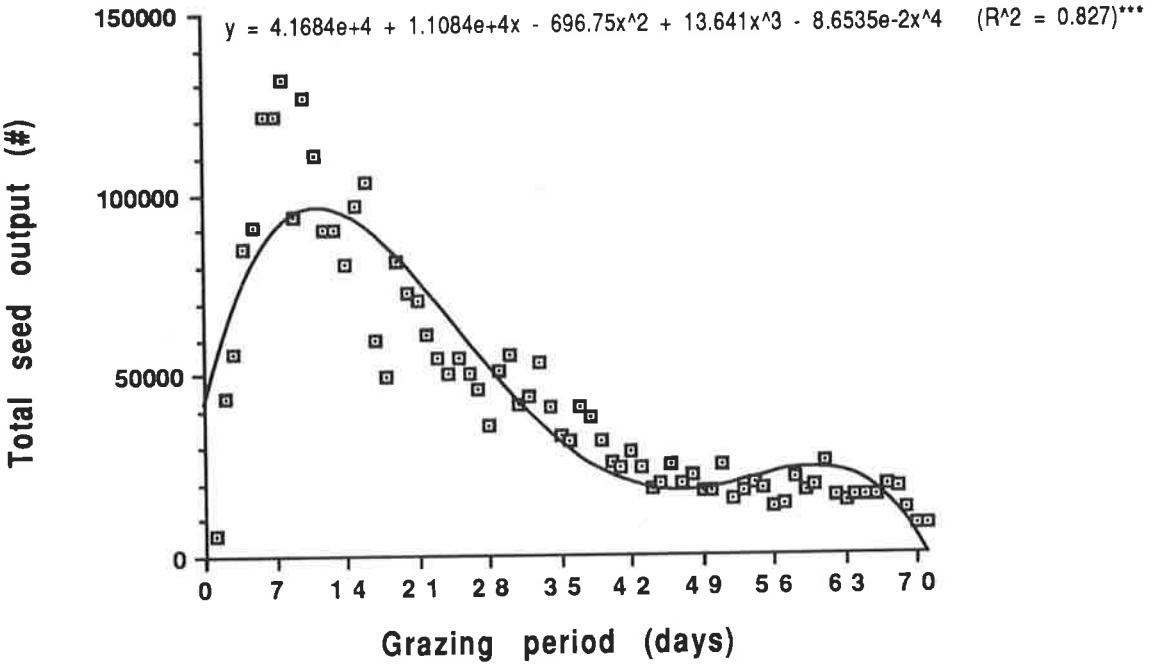


Figure 5.17 The changes in the output of cluster clover seeds during the grazing period.

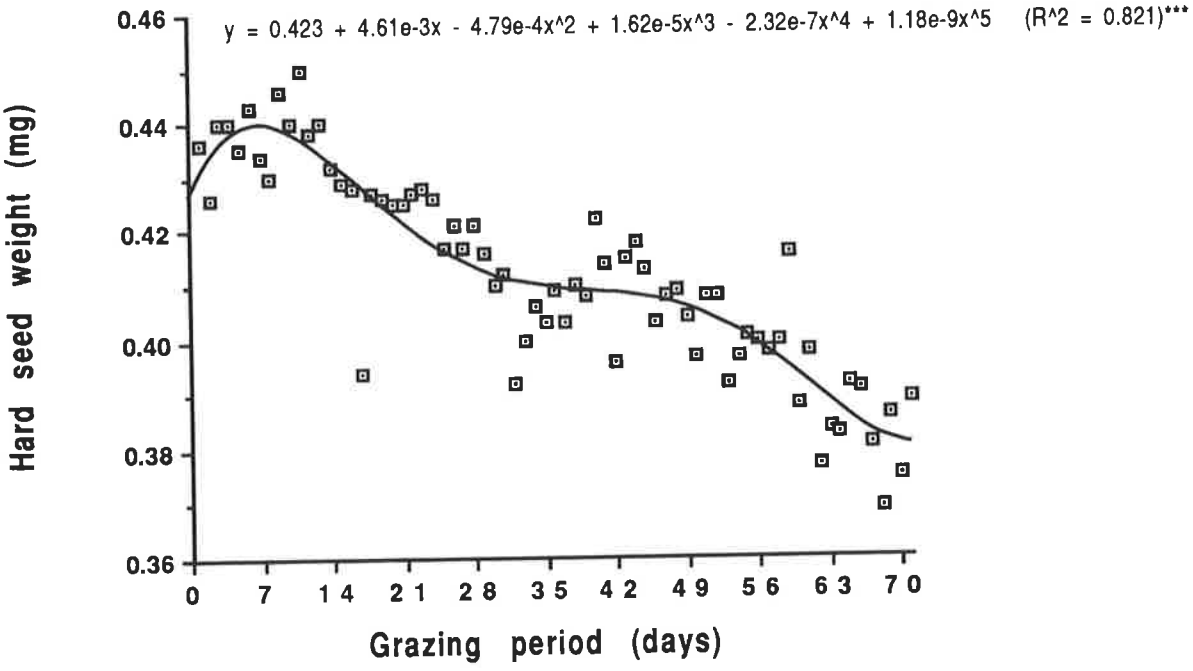


Figure 5.18 The changes in the seed weight of cluster clover hard seeds voided in the faeces by the flock during the grazing period.

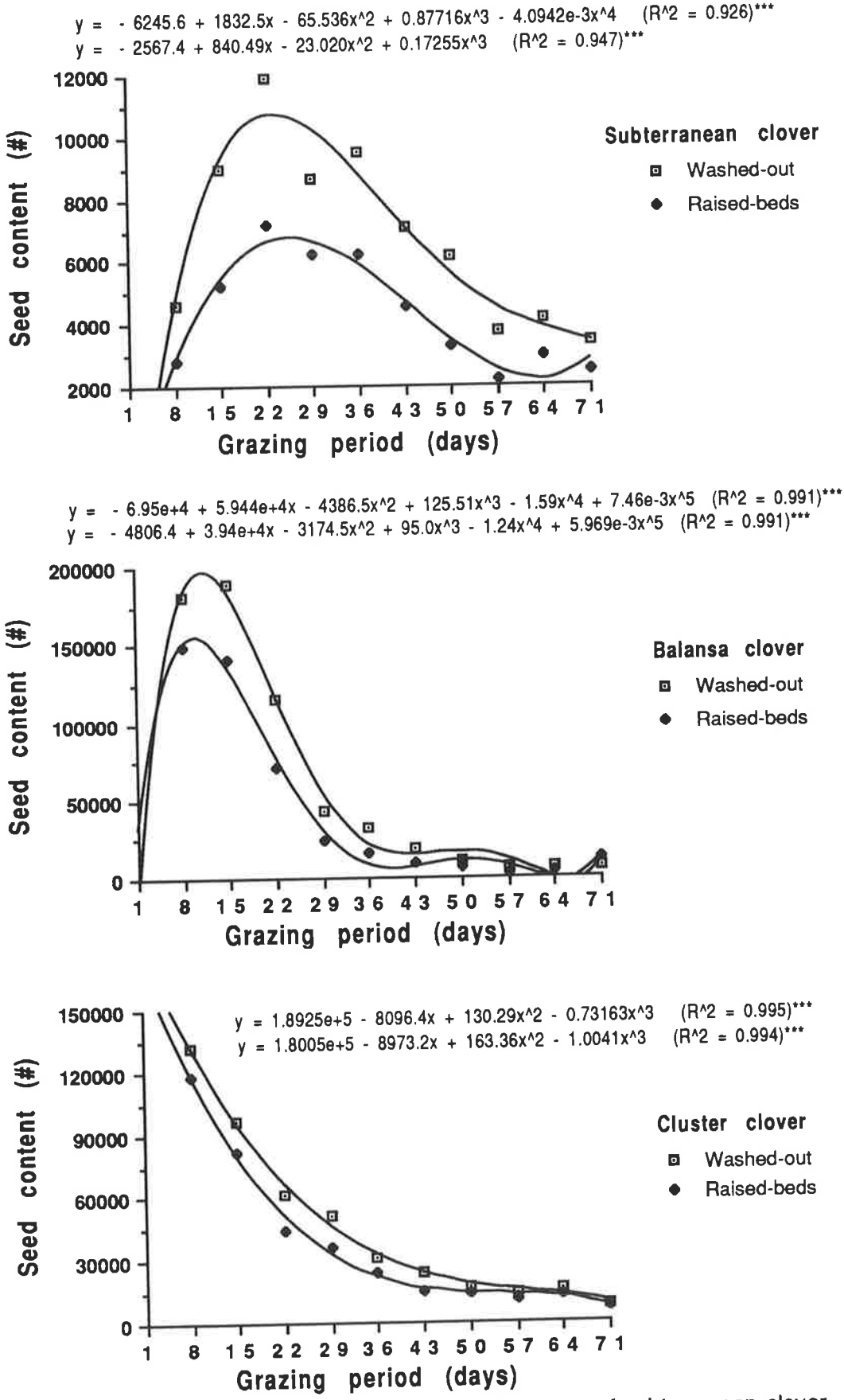


Figure 5.19 Differences in the trend of total seed content of subterranean clover, balansa clover and cluster clover voided in the faeces by the flock and recorded in samples washed-out and in samples tested in raised-beds.

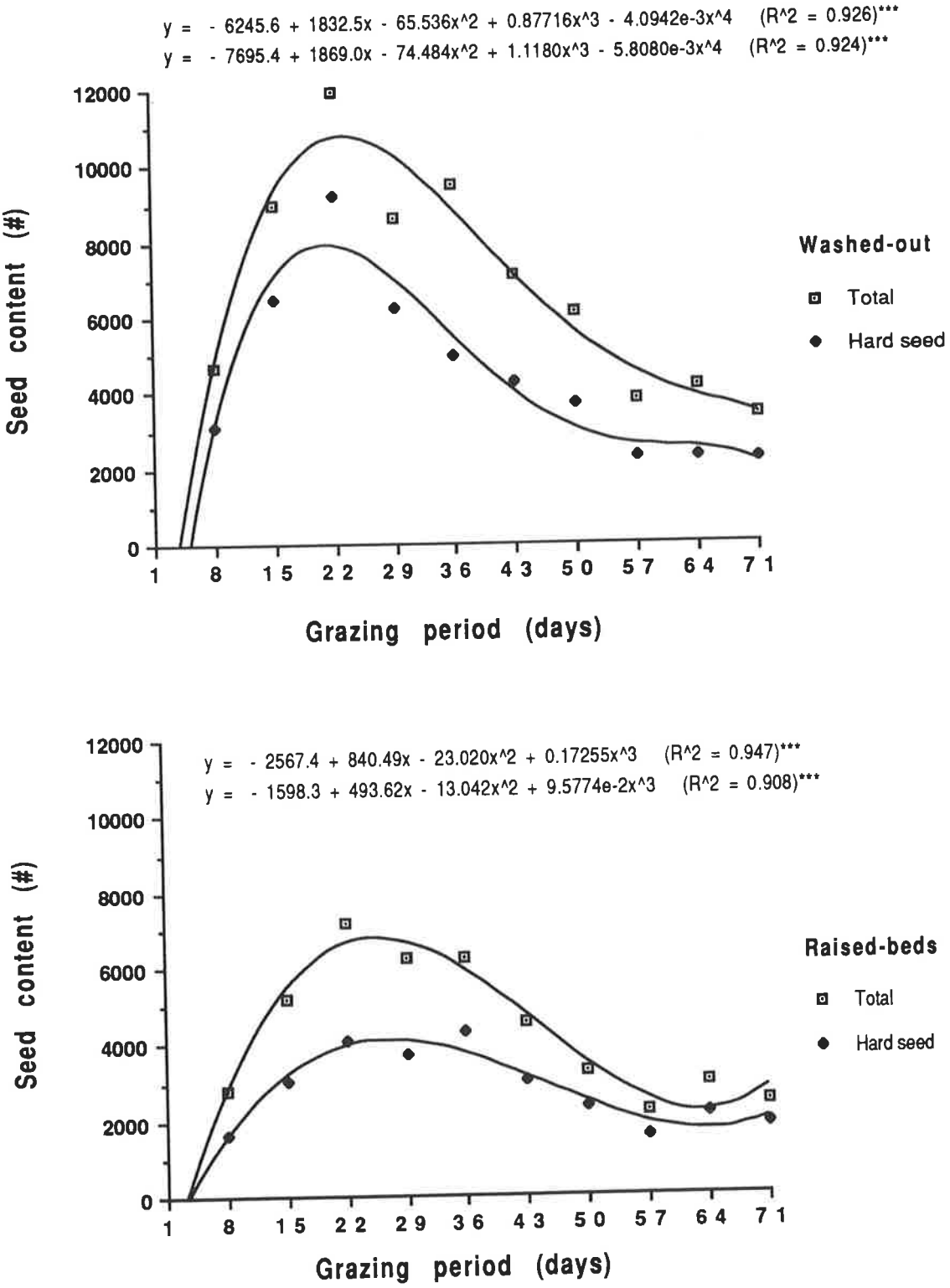


Figure 5.20 Differences in the trend of total and hard seed of subterranean clover voided in faeces by the sheep flock and recorded in samples washed-out and in samples tested at raised-beds.

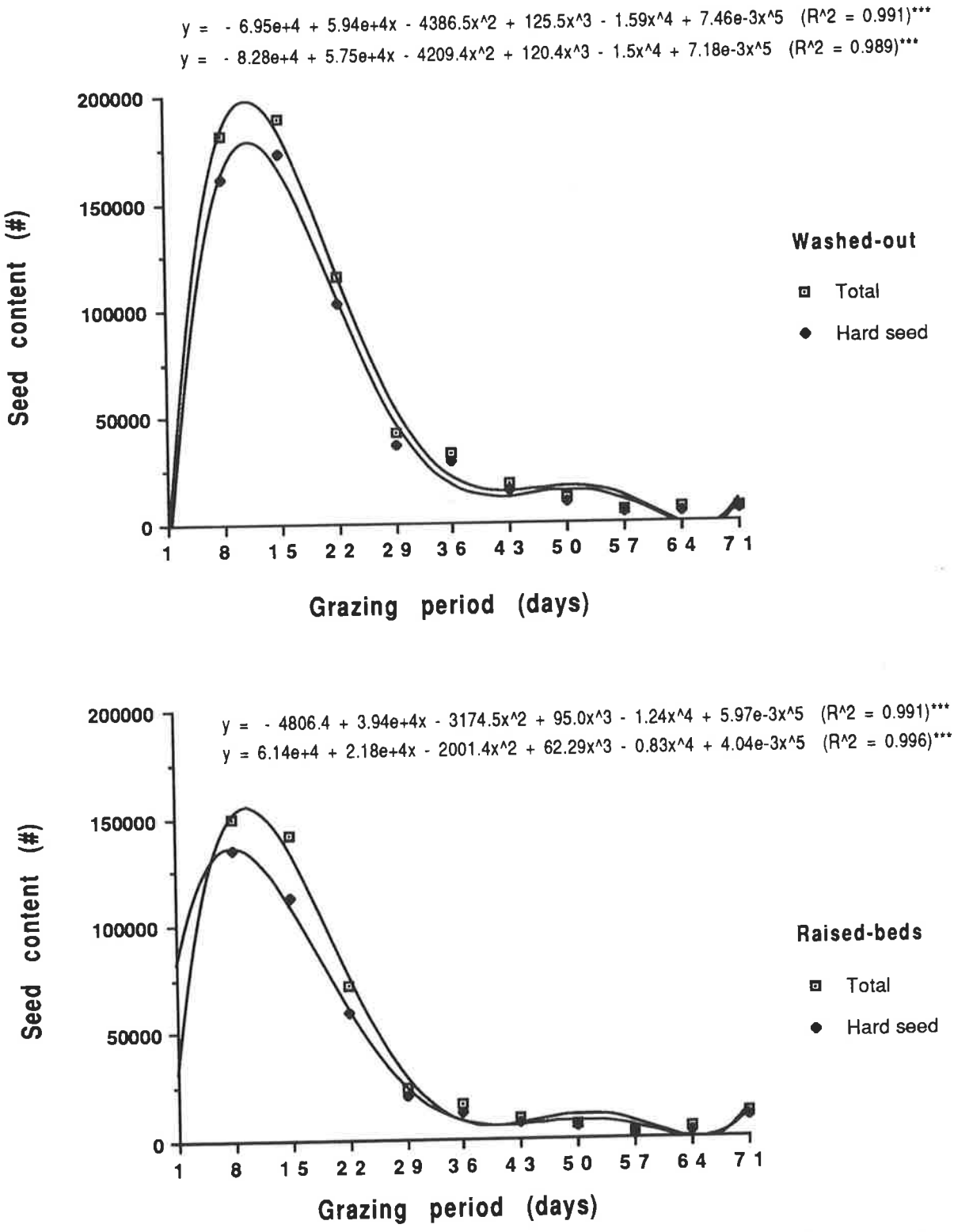


Figure 5.21 Differences in the trend of total and hard seed of balansa clover voided in faeces by the sheep flock and recorded in samples washed-out and in samples tested in raised-beds.

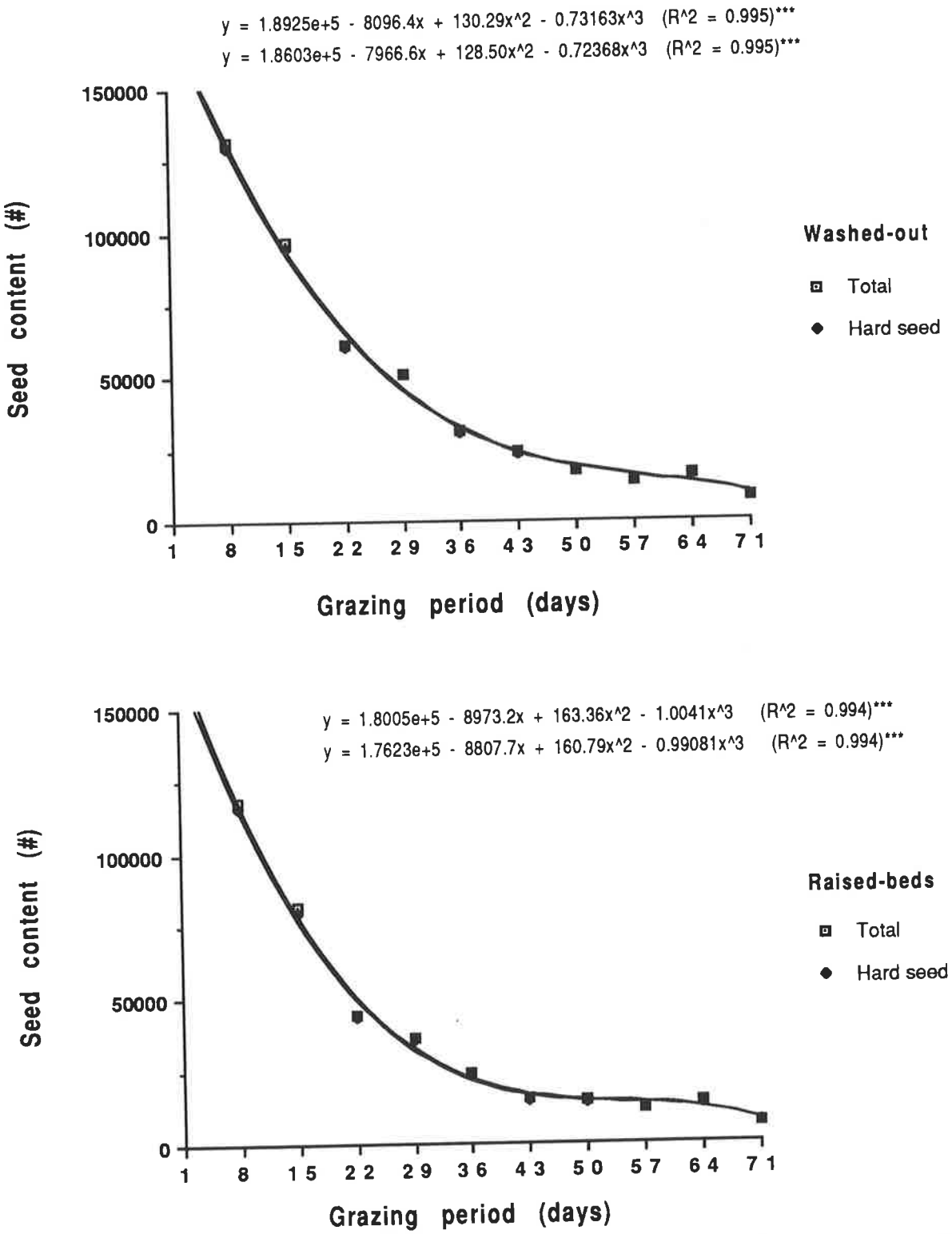


Figure 5.22 Differences in the trend of total and hard seed of cluster clover voided in faeces by the sheep flock and recorded in samples washed-out and in samples tested in raised-beds.

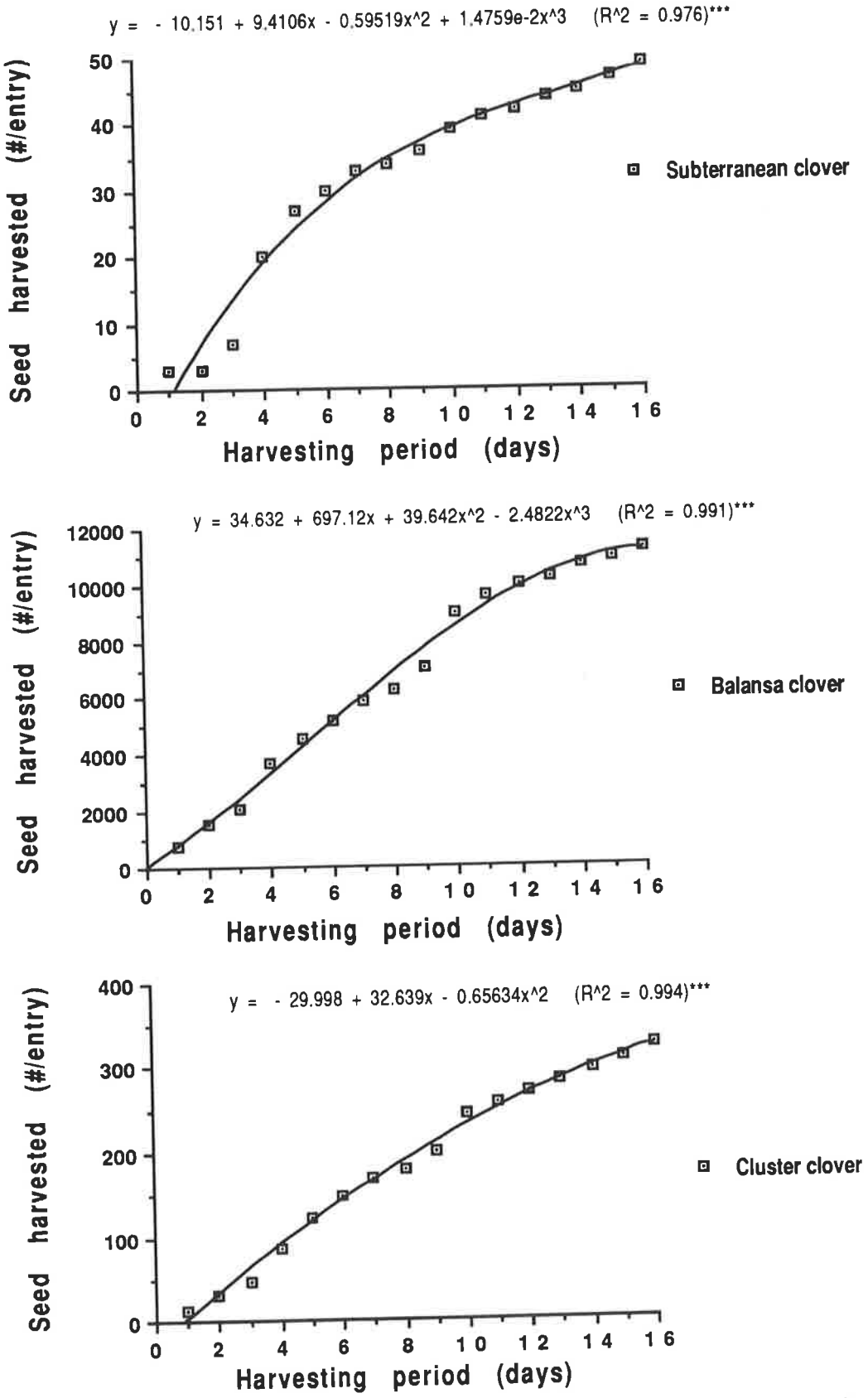


Figure 5.23 Cumulative values of subterranean clover, balansa clover and cluster clover seeds harvested by ants.

Plate 5.1 General view of the sheep flock on the dry pasture residues in the field grazing study.

Plate 5.2 Close-up view of sub-samples of sheep faeces placed weekly under field grazing conditions in a sheep exclosure cage.





Plate 5.3 Close-up view of a sampling site after harvesting the dry pasture residues.

Plate 5.4 Close-up view of a sheep fitted with a rumen fistula.



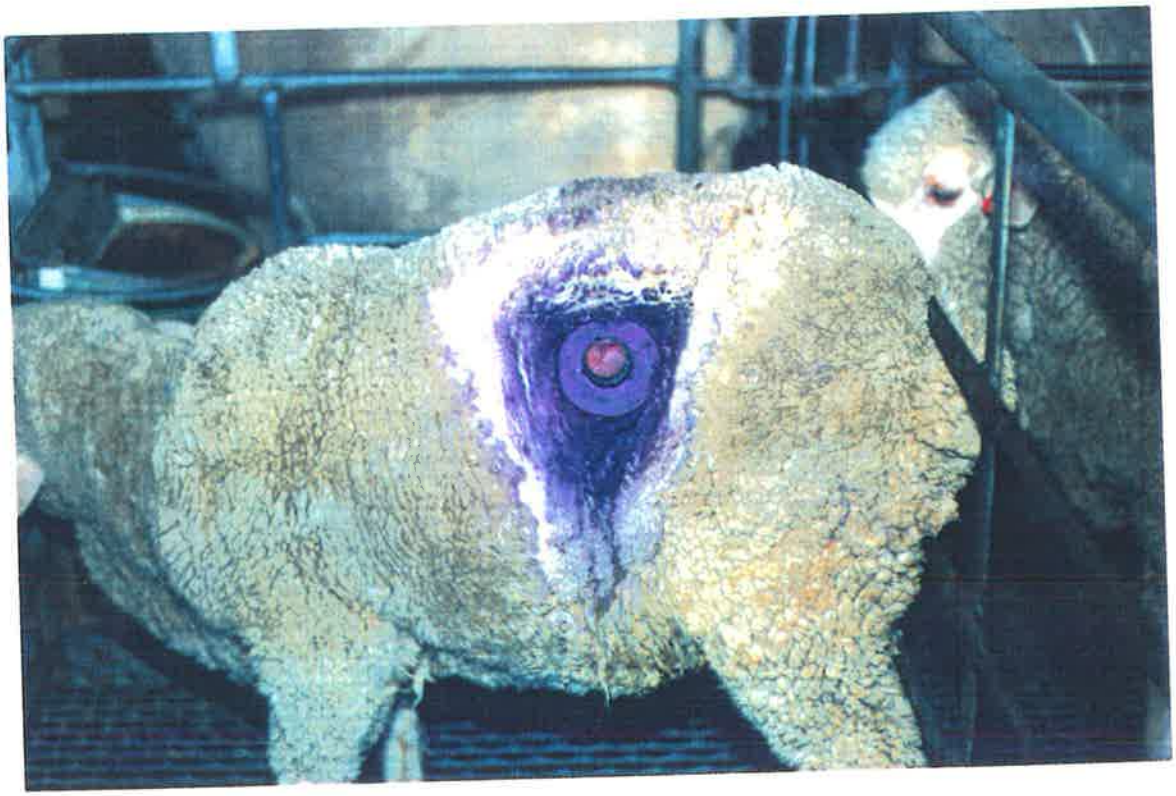


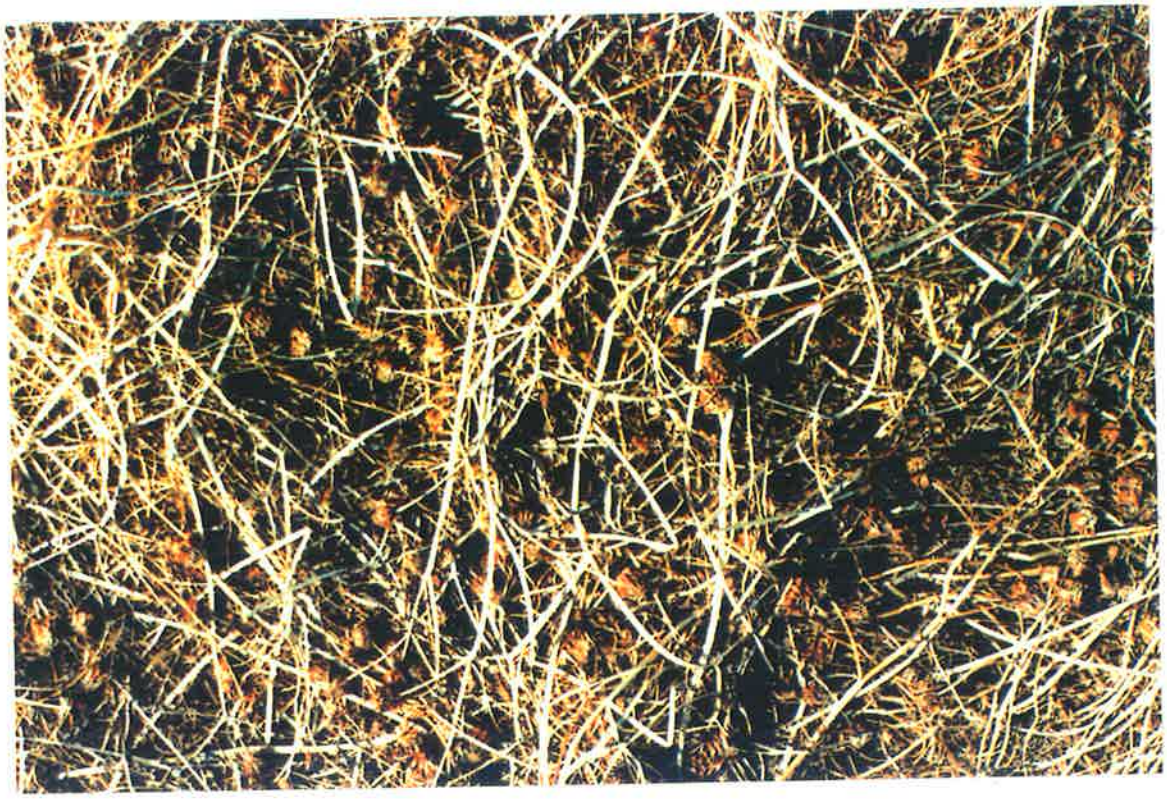
Plate 5.5 Close-up view of the ring-watering system used to test the regeneration of annual clover species in the field.

Plate 5.6 Close-up view of an active ant nest entry surrounded by clover seeds collected by ants.





Plate 5.7 Close-up view of dry pasture residues at the start of the grazing period on 31 January, 1990 (upper) and at the end of the grazing period on 11 April, 1990 (lower).





5.3 Field survey 1: The significance of cluster clover in a subterranean clover-perennial ryegrass pasture under two contrasting management systems.

5.3.1 Materials and methods

5.3.1.1 Location and previous history of the paddocks

The grazing survey was carried out on the grazing property 'Maylands' located at Parawa on Fleurieu Peninsula, c. 100 km south of Adelaide, South Australia. The property consists of 600 ha located in an area of about 900 mm of rainfall (Appendix 5.17). The soil is mainly lateritic podzol with sandy loam surface. The farm is stocked with 2700 crossbred ewes (Border Leicester x Merino) joined with Poll Dorset rams each July, and 100 Simmental and Simmental cross cows used for prime lamb and vealer production respectively (Mayfield 1990).

In order to examine and ascertain the ecological significance of small- seeded naturalized cluster clover (Woodward and Morley 1974) two paddocks (subsequently abbreviated and referred to as Paddock I and Paddock II) containing a subterranean clover-perennial ryegrass pasture mixture established on sandy loam textured soils with contrasting management histories were selected.

Paddock I of 5 ha has been heavily set stocked permanently during the last fifteen years with about 20 crossbred ewes/ha and their lambs. In 1980, just after the opening rains, the area was sprayed with a Spray-seed knockdown herbicide and sown with a mixture of late mid-season maturity subterranean clover cultivars Karridale and Mount Barker (var. *subterraneum*) and cultivar Larisa (var. *yanninicum*), and perennial ryegrass (cv. Victorian). Then in 1987, the paddock was oversown again with a mixture of subterranean clover (cvv. Karridale and Larisa) and Victorian perennial ryegrass (Plate 5.8).

Paddock II comprises an area of about 6.5 ha that had been cut for hay over the last twelve years (1976-1989) and stocked with about 13.8 crossbred ewes/ha plus lambs.

During the early 80's, subterranean clover cv. Mount Barker and the early-maturity cv. Yarloop (var. *yanninicum*) were the dominant legume components of the pasture. In 1984, the early-maturity cv. Trikkala (var. *yanninicum*) was sown and finally between 1986-1988, a mixed pasture of subterranean clover (cvv. Karridale and Larisa) and Victorian perennial ryegrass was established (Plate 5.8). During the period of the study (14 September 1990 - 16 May 1991) the paddocks were grazed permanently by sheep and stocked according to the rates indicated.

5.3.1.2 Pattern of sampling

A transect was established to estimate the trend of the herbage components, the seed reserves in the soil-plant-animal faeces complex and the further regeneration of the pasture sward in both paddocks. The transect was 100 m long with 20 permanent wood pegs spaced 5 m apart in the same direction (North-South) on the main slope of both paddocks. Each peg represented the centre point of a sampling area (2 x 2 m) used to identify the proper place of sampling of each variable involved at each time of evaluation (Diagram 5.2).

5.3.1.3 Evaluation of plant components in the soil-plant-faeces complex

The first evaluation was done on 14 September, 1990 before the legume species commenced flowering (Plate 5.9). The objectives were to estimate the reserves of hard seed in the soil and to measure some sward characteristics, such as plant density, herbage availability and botanical composition.

Forty cores (two per sampling area, taken 40 cm apart), 10 cm in diameter were taken from the topsoil (0-2.5 cm) of each paddock at each sampling area. Using the techniques of Hutchinson (1967), Carter *et al.* (1977), Jones and Bunch (1977) and Forcella (1984), the soil cores were washed with a high-pressure jet of water through 1.00 mm and 0.50 mm square-holed 30 cm diameter brass laboratory test sieves to remove most

of the soil particles and to retain the subterranean clover and cluster clover seeds, respectively. The different plant species present in the samples were identified, separated by hand, counted and the dry matter of the tops determined by oven-drying at 80°C for a period of 48 h. The remaining material was compressed by hand, transferred to square plastic containers (15.0 x 15.0 x 9.5 cm) and dried in a forced-draught dehydrator at 40°C for 12 h.

The dried material was then inmersed in perchloroethylene (specific gravity of 1.623 at 20°C) to separate by flotation the seed from the inert material and re-dried at 30°C for about 2 h. Organic debris was then aspirated by the use of a cupboard provided with a fan. Finally, the pure seed was obtained by hand separation from the remaining organic particles of similar size. The corresponding seeds of subterranean clover and cluster clover were counted, weighed and tested for germinability and hard-seededness by using four replicate lots of 100 seeds under the same conditions as described before (5.2 Field Experiment 1).

The Levy Point Quadrat (Levy and Madden 1933) was used to assess botanical composition and percentage bare ground. A frame with ten points (5 cm apart) was used to sample the 20 sampling areas along a specific sampling line and repeated in time in the same location (Diagram 5.2). In the following evaluations the factors previously considered in the study were estimated by the same methods described, but in some specified instances, depending on the pasture or the objectives of the study, some variables were or were not considered and some explanation of the adjustment in the sampling of the variables is required.

On 5 November, 1990 before the subterranean clover cultivars and cluster clover had set seed, a permanent quadrat cage (78 x 78 x 90 cm) was placed at each sampling area (Diagram 5.2), to determine the effect of ungrazed conditions in comparison to grazed ones on seed production, seed survival during the summer-autumn period and seedling regeneration.

When the seed of both clover species was mature (Plate 5.9), another evaluation was made on 9 January, 1991. At that time, the seed reserves were estimated under ungrazed and grazed conditions, at soil level, as well as at plant level. Because the use of a small 10 cm diameter core sample was inappropiate for the ungrazed situation, a 28.5 cm diameter steel cylinder was used for sampling. All the plant material within the ring was cut at the soil surface level with a knife and then two soil cores were taken inside the cylinder to estimate the reserve of seed in the soil.

Sheep faeces from the corresponding areas was collected to determine the amount of faeces, the seed reserve in faeces and the further emergence of seedlings from faeces. The sampling transect (Diagram 5.2) was divided into three segments, according to the position on the slope as high (sampling areas, 1-5), intermediate (sampling areas, 8-12) and low (sampling areas, 16-20). The sheep droppings were collected from ground level in the projection (East-West) of the sampling areas at each position on the slope by meaning 20 m at each side and then taking 10 random samples of faeces surrounded by a long, narrow rectangular frame (400 x 50 cm), that reduces the variance between samples, especially when the faeces tend to be concentrated (Hilder 1966). A sub-sample of 20 g of pellets was used to determine the moisture content of the faeces by oven-drying at 80°C for 48 h. An additional sub-sample of droppings, equivalent to 50 g as collected was kept under room temperature and then placed on a raised-bed (WARI) on 27 March, 1991 (3 positions in the slope x 10 sub-samples x 2 paddocks = 60). the samples were treated in the same manner as previously described (5.2 Field Experiment 1). A single count of all emerged seedling species from the droppings was carried out four weeks later on 24 April, 1991.

On 18 March, 1991, before the opening of the normal rainfall season, a further evaluation of the previous variables was carried out only for grazed conditions (Plate 5.10). The corresponding sampling areas to be used in the following evaluations after the initiation of rains, were cleaned of sheep faeces in order to minimize the influence of seedlings provided by droppings. A second collection of sheep faeces was carried out

and treated as before. Finally, on 16 May, 1991, and after 48.5 mm of rainfall was recorded (Appendix 5.17), a complete evaluation was made (Plate 5.10). The main objective was to examine the re-establishment of the pasture sward species under ungrazed and grazed conditions.

5.3.2 Results

5.3.2.1 Rainfall patterns

The monthly rainfall recorded throughout the field survey (1990-1991) is given in Appendix 5.17. During 1990, rainfall of 880 mm was slightly below average, which 56.4% falling between June and August. The regeneration of the annual species as well as the regrowth of perennial grasses in the pasture sward started principally during April when the first rainfall became effective. Further good rains during spring ensured flowering and seed set of the different cultivars of subterranean clover as well as cluster clover. In 1991 and until the last evaluation was completed (16 May), 48.5 mm of rain was recorded and the regeneration of the pasture species started late in March.

5.3.2.2 Evaluation of plant components

Before the legume species started flowering (September, 1990), the paddocks differed in the dominance of the species involved in the corresponding pasture swards (Tables 5.11-5.12). In Paddock I, higher densities of winter grass, subterranean clover, cluster clover and capeweed were present (further information about the different species, varieties and cultivars recorded during the study is given in Appendix 5.18). In Paddock II, subterranean clover, winter grass, storksbill and chickweed were the most frequent species recorded. Herbage availabilities of 1486 kg DM/ha in Paddock I and 1170 kg DM/ha in Paddock II were estimated in September. Although vitually no bare soil was recorded in either paddock, only 33.5% of the available dry matter in Paddock I was produced by the sown species compared with 66.0% in Paddock II. In both cases, subterranean clover cultivars contributed more to dry matter than perennial ryegrass.

Cluster clover had significantly ($P<0.001$) greater plant density and dry matter availability in Paddock I. Subterranean clover plant density and dry matter availability was similar in both paddocks: however, there was a highly significant ($P<0.001$) difference in the hard seed reserve in the top-soil. Paddock II had more than twice the number of subterranean clover seeds of Paddock I, also a more even distribution of var. *subterraneum* and *yanninicum*. The cluster clover seed reserve and the individual seed weight was significantly greater in Paddock I. The higher plant density of cluster clover found in Paddock I was related to the higher hard-seed reserves of these small seeds located in the top-soil.

In November, 1990, pasture availability in Paddock I was still higher (2686 kg DM/ha) than in Paddock II (2213 kg DM/ha). There were improved contributions of forage provided by the sown species, equivalent to 45.7% and 81.0% of the dry matter, respectively. As before, subterranean clover cultivars made a greater contribution to forage production than perennial ryegrass in both paddocks (Table 5.13).

When the clover species finished seed set early in summer (January, 1991), a complete evaluation of the plant components at soil-plant level was made. The availability of dry residues was similar with a non-significant difference between paddocks under ungrazed and grazed conditions respectively. However, these differed significatly ($P<0.001$) within paddocks where important differences in the level of bare ground were recorded at each situation. Similar relative contributions in forage provided by the sown species were observed under the ungrazed (63.0%) and grazed (54.7%) conditions in Paddock I, and under ungrazed (91.0%) and grazed (92.1%) conditions in Paddock II. Unlike the previous evaluations, perennial ryegrass made a higher contribution to dry matter than subterranean clover under all conditions (Tables 5.14-5.15).

In Paddock I, the exclusion of grazing sheep during the last part of the spring growing season, when the seed of subterranean clover cultivars was maturing, produced a significant decline of 1316 seeds/m^2 in the seed reserve compared with the grazed area.

Only 56.5% of the seed, mainly cultivar Larisa, was buried in the top-soil when grazing was absent compared with 86.1% under grazing. Cluster clover seed which is principally trampled into the soil by sheep, also showed a decline of 1958 seeds/m^2 as a consequence of the prevention of grazing (Table 5.16).

In Paddock II, similar seed production by subterranean clover was obtained under ungrazed and grazed conditions. However, compared with Paddock I, there were higher relative levels of buried seed of the variety yanninicum cultivars Larisa, Trikkala and Yarloop, equivalent to 64.0% (ungrazed) and 93.5% (grazed). In contrast, the total seed reserve of cluster clover, in the top-soil, was depressed by the imposed grazing and a reduction of 969 seeds/m^2 was observed (Table 5.17).

At that stage of the year, comparisons between paddocks indicated highly significant differences ($P<0.001$) in the seed reserves of subterranean clover and cluster clover under ungrazed and grazed conditions respectively. This confirmed the trend observed in hard seed reserves, estimated during the previous growing season (September, 1990).

At the end of summer, before the first effective autumn rains, the grazing areas had similar amounts of available dry residues and a little green perennial ryegrass totalling 261kg DM/ha in Paddock I and 276 kg DM/ha in Paddock II. Bare ground was estimated to be 56.5% and 59.5% respectively. The contributions to total dry matter by the sown species was 60.4% in Paddock I, of which each pasture species provided the same quantity of dry matter and 93.9% in Paddock II, of which most of the forage (80.8%) was produced by ryegrass. This was similar to the previous evaluation. Amongst the undesirable volunteers, barley grass became a more dominant species under grazed conditions than in the ungrazed area. Barley grass residues were very persistent through the summer-autumn period, representing about 32% of the dry matter available in Paddock II by the end of the summer (Table 5.18).

Consistent with earlier evaluations, highly significant ($P<0.001$) differences between the seed reserves of clover species in both paddocks were observed. The slightly lower amounts of seed reserves of subterranean clover estimated in both grazing areas, compared with the previous evaluation, suggest that, despite the efficient burial capacity of subterranean clover cultivars in that soil, the trampling of unburied burrs by sheep could affect positively the survival of the seed. In contrast, there was a decrease in the density of cluster clover seeds equivalent to 5418 seeds/m^2 in Paddock I and 360 seeds/m^2 in Paddock II. This suggests selection by sheep of the more accessible heads of cluster clover compared with the well-buried subterranean clover burrs. Predation of seeds by the numerous colonies of seed-harvesting ants (higher population in Paddock I) would be responsible for the disappearance of the seed from both paddocks (Table 5.19).

About 50 days after the opening of the rainfall season, a re-evaluation of the paddocks was carried out in May 1991 in order to estimate the regeneration of the pasture swards. In Paddock I, the dominant plant species were winter grass, subterranean clover, cluster clover and barley grass under ungrazed conditions, and winter grass, cluster clover, subterranean clover and chickweed under grazed conditions. Similar amounts of available herbage were recorded, even the differences in ground cover under ungrazed and grazed conditions. However, 55.8% of the available dry matter under ungrazed conditions was provided by the sown species compared with 27.3% under grazed conditions. In both cases, perennial ryegrass made the highest contribution to forage (Tables 5.20-5.21).

In Paddock II, the dominant species were winter grass, subterranean clover, storksbill and perennial ryegrass under ungrazed conditions, and winter grass, subterranean clover, storksbill and chickweed under grazed conditions. Available herbage and ground cover were higher under ungrazed conditions, in which 66.8% of the dry matter was contributed by the sown species compared to 32.8% estimated under grazed conditions. Perennial ryegrass was the main contributor in both situations (Tables 5.22-5.23).

There was better suppression of winter grass in both paddocks when the area was excluded from grazing sheep. It is possible that there was both interspecific competition from the sown species during the critical period of seed production the previous spring and the actual period of regeneration of the pasture. The vigorous regrowth of the perennial ryegrass, would be responsible for the low contribution of winter grass to the total dry matter on offer in the excluded area. Conversely, continuous heavy grazing appears to favour the dominance of winter grass in the pasture sward. During the regeneration of the pasture the selection of grazing by sheep was concentrated on the sown species, particularly ryegrass. The less available and accessible small seedlings of cluster clover grew mostly in conjunction with winter grass or around the edges of turfy ryegrass plants. Here, they are expected to be less affected by grazing but have been influenced by plant competition.

The final seed reserve of clover species that was represented by the seed bank estimated in January, 1991 under ungrazed conditions, and under grazed conditions in March, 1991 showed contrasting levels of seedling density and remaining hard seed reserve in the top-soil observed within paddocks (Table 5.24). Only 7.5% of subterranean clover and 32.2% of cluster clover original seed reserves were depleted under the excluded conditions: however, under grazing 47.1% and 57.8% of the reserves were depleted, respectively. The decline in cluster clover reserves under ungrazed conditions, suggests that plant competition and predation by ants may have occurred in Paddock I.

In Paddock II, original seed reserves of the 9.0 % of subterranean clover and 24.1% of cluster clover were depleted under the ungrazed conditions. Under grazing, 28.8% and 27.4% were depleted, respectively. Higher levels of seedling emergence expected under grazed conditions, mainly as a consequence of increasing levels of soft seeds achieved by the clover seeds compared to the ungrazed situation, revealed the susceptibility of clover seedlings (especially subterranean clover) to grazing at this stage of plant development (Table 5.25).

Significant differences ($P<0.001$) between paddocks under ungrazed and grazed conditions were observed for seedling density, hard seed density and individual weight of the hard seed of cluster clover. There was a significant difference ($P<0.001$) also in the subterranean clover seedling density and hard seed density between paddocks under grazed conditions. However, only hard seed density was significantly greater ($P<0.001$) under ungrazed conditions.

5.3.2.3 Seed reserve in faeces

During the summer (January, 1991) and autumn (March, 1991), three collections of faeces were made at the high, intermediate and lower portions of the main slopes in each paddock. In summer, there was 666 kg DM/ha of faeces in Paddock I and 401 kg DM/ha of faeces in Paddock II (Appendices 5.19-5.20). In autumn there were 941 kg DM/ha and 596 kg DM/ha of faeces respectively (Appendices 5.21-5.22).

Cluster clover (25 seedlings/m^2) and winter grass (25 seedlings/m^2) in Paddock I, and winter grass (11 seedlings/m^2) and chickweed (5 seedlings/m^2) in Paddock II, were the most numerous seedling species regenerated from the faeces collected in summer (Appendix 5.23). In faeces collected in autumn, cluster clover and winter grass were the best represented species with 101 seedlings/m^2 and 55 seedlings/m^2 in Paddock I, and 32 seedlings/m^2 and 30 seedlings/m^2 in Paddock II, respectively (Appendix 5.24).

The densities of clover seeds voided and distributed in faeces by the grazing sheep are listed in the Tables 5.26 and 5.27. The presence of more seeds of cluster clover compared with subterranean clover was obtained in the corresponding evaluations of both paddocks. Higher levels of clover seeds were voided in faeces between evaluations, a period of high grazing pressure. The high level of cluster clover seeds recorded in the summer evaluation, suggests that sheep (given the lower accessibility of subterranean clover burrs) started to select the heads of cluster clover at an early stage favouring the distribution of this seed.

5.3.3 Discussion

Given that the environmental factors involved in the development of the corresponding pasture swards were similar, the long-term management of the paddocks is the most relevant factor influencing the population dynamics of the sown species and companion volunteers. According to Carter (1990), the grazing pressure applied in a closed system, is more influential in determining the botanical composition of a pasture than the initial potential of the mixture of sown species.

In the heavy set stocking rate used in Paddock I, a decrease of perennial grasses (Baker 1957; Reed 1974), a progressive dominance of clovers (Cameron and Cannon 1970) and an increase in the participation of annual grasses in the pasture (Sharrow *et al.* 1981) is expected. In contrast, the lighter stocking rate used in Paddock II, in combination with fodder conservation practices, avoids the defoliation of the sward during the period of higher rates of plant growth. This allows the accumulation of forage to be harvested for hay. A dominance of erect-growing grasses over the more prostrate clovers would therefore be expected (Brougham 1960).

The evaluation of the pasture swards prior to the flowering of legumes (September, 1990), indicated important differences between the paddocks. In Paddock II, despite the better grass/clover ratio for the sown species compared to Paddock I, similar plant densities of subterranean clover were found. This suggests a lower rate of seed softening in the paddock normally kept for fodder conservation. The permanent and dense pasture cover present during the spring and early part of the summer seasons restricts the impact of natural variations of temperature on clover burrs (Quinlivan 1965; Taylor *et al.* 1984) and explains the comparative higher levels of hard seed present, in cultivars Karridale and Mount Barker (var. *subterraneum*). These cultivars have lower levels of hard-seededness in comparison to the var. *yanninicum* cultivars (Collins *et al.* 1984; Oram 1990).

Contrasting differences in plant density and hard seed reserves of cluster clover were observed. The heavier set stocked grazing area (Paddock I) was dominated by the small-seeded clover. This confirmed that stocking rate strongly affected the dominance of one species or another in the pasture. Work done by Carter (1968b), Cameron and Cannon (1970), Carter and Lake (1985) and de Koning (1990) demonstrated the increase of volunteer clovers such as cluster clover in heavily-stocked grazing areas. This is due to a decline in the sown species such as subterranean clover, and the ability of small seed to survive the passage through the digestive tract of sheep. Similar observations were made for winter grass and capeweed, which increased in density under the grazing conditions imposed in Paddock I. (Carter 1968b; Cameron and Cannon 1970; Reed 1974; Carter and Lake 1985). Important losses of winter grass seeds occur from the surface seed population, due to the activity of invertebrates which bury the seed (Mortimer 1976). Despite this, winter grass has the ability to colonize bare ground caused by poaching and urine scorch and become successfully established, whilst the vigour of ryegrass is reduced by frequent defoliation (Wells 1974).

The pasture species composition changes from legume dominance in the growing season to a grass dominance in the later summer-autumn period. This suggests that despite the natural losses of forage, there is an important selective defoliation of clover species. However, after the clover species set seed (January, 1991), there was an increase in the density of subterranean clover and cluster clover seeds, especially in Paddock I and under grazing conditions. The deferment of grazing at that time, negatively influenced the production of seed, enhancing the dominance of ryegrass, which, being a perennial grass, is able to compete more efficiently than clover species. Prevention of grazing affected the ability of subterranean clover cultivars to bury their seed by preventing the proper changes in growth habit in response to defoliation. This is important given that the sandy loam texture, as well as the higher levels of organic matter in the top soil, provides an ideal medium for the burial of seed enclosed in burrs out of the reach of grazing animals. Frequent close grazing increases the proportion of petioles that became

prostrate, assisting the survival of some legume species such as subterranean clover (Broom and Arnold 1986; Curll and Jones 1989).

The more accessible and digestible heads of cluster clover (5.2 Field Experiment 1) were able to persist well under grazed conditions in the heavily set-stocked grazing area. The natural shattering of the seed, as well as the trampling effects provided by the grazing sheep cause the burial of seed favouring its regeneration. This is also important for subterranean clover and others volunteer species.

The effects of grazing and the greater impact of environmental factors on the seed located in more-denuded soils, favored the early softening of seed. This improved regeneration of the less-numerous seeds of cultivars Karridale and Mount Barker (var. *subterraneum*) in Paddock I, and to some extent, of cluster clover seeds. This process reached its greatest extent under grazed conditions before the opening rains (March, 1991). At this stage most of the seed was buried in the soil and presented higher levels of germination than the scarce, unburied ones.

For subterranean clover, the higher amounts of seed produced, resulted in negligible change in the seed reserves during the summer-autumn period. This contrasts with the results obtained on hard-setting, red-brown earths by de Koning (1990) and results reported in the previous study (5.2 Field Experiment 1), also on a hard-setting, red brown earth soil. There are several reasons for the contradictory results of these experiments: increases in seed coat permeability as a consequence of management practices that allowed the proper insulation of the seeds (Taylor *et al.* 1984), efficient burr burial at Parawa but not on the red-brown earths, and the presence of cultivars with very low to moderate degree of hard-seededness (Collins *et al.* 1984; Oram 1990). The satisfactory levels of seed reserves in both paddocks, especially the lightly-stocked one at the time of the opening rains (Carter and Cochrane 1985), reflects how significant is the survival of subterranean clover seeds under the specific circumstances of the study.

A different situation was observed for cluster clover, especially in Paddock I. Seed density was significantly higher at the end of the seed-set period (January, 1991) in comparison to the hard seed bank recorded previously during September, 1990. Despite this, a large amount of seed disappeared from the grazing area. Some losses could be expected because grazing sheep were still selecting some remaining heads from the soil surface. It is probable also, that the populations of ants observed in that paddock during the summer-autumn period were contributing to the disappearance of the seed.

As reported (5.1 Field Experiment 1) with overgrazing conditions, colonies of invertebrates, like ants, tend to increase in pastures (Hutchinson and King 1980). Small, more-available and accessible seeds located at the soil surface can be easily taken to the corresponding nests. Earthworms, abundant in this type of soil with high levels of organic matter, are also able to move seeds and bury them deeply in the ground (Chippindale and Milton 1932). Earthworms have also been reported as important agents in casting buried seeds to the surface, producing favourable microsites for germination (McRill 1974). A wide variation in survival of seeds recovered from casts was reported by McRill and Sagar (1973), indicating that small-seeded clovers seem to be more resistant to the digestion processes than larger-seeded ones.

The deferment of grazing on pasture swards up to 50 days after an effective rainfall (May, 1991) allowed a better establishment of subterranean clover seedlings in both paddocks and gave higher plant densities, greater availabilities of dry matter and much higher contributions of clover to the pasture swards. However, large amounts of ryegrass were always present, due to the higher rates of growth of the perennial species at that stage of the growing season. Small seedlings of cluster clover, normally growing in conjuction with winter grass at the edge of perennial ryegrass plants, showed similar plant densities under ungrazed and grazed conditions. Higher levels of hard seed reserves of cluster clover remained in the soil due to lower rates of softening occurring during the previous dry period. The suppression of winter grass under ungrazed

conditions seems to be more related to competition effects during the period of seed production rather than effects occurring during the seedling establishment. According to Brown (1976) deferred grazing did not prevent the invasion of winter grass at higher stocking rates. This suggests that seed set in winter and early spring, while the soil surface is soft, is easily trampled into the soil before the surface hardens. However, given the characteristics of the soil in the present study, a further trampling effect during the dry period is expected.

There was evidence from this Parawa study that subterranean clover cultivars have been affected more severely under set stocking, than by the regular fodder conservation practices adopted in Paddock II. Fodder conservation may lead to lower seed production because of increased plant competition, loss of seed through harvesting and less seed burial because of a more erect growth habit. However, reserves of seed were set in both paddocks which were strategically conserved through the summer-autumn period, ensuring a high potential regeneration of the pastures, due to a large proportion of the seed becoming seed-coat-permeable. Seedling mortality is high during the early stages of growth, especially under grazed conditions, when plants and animals are in a critical stage. Defoliation and treading cause a severe reduction in plant density, with subterranean clover more affected than annual grass (Carter and Sivalingam 1977; Jones and Bunch 1987). Competition for light, nutrients (Cook and Ratcliff 1985) and moisture as well as damage caused by pest and diseases may also be important constraints (Jones and Bunch 1987).

Despite the disappearance of cluster clover seeds from Paddock I (presumably harvested by ants, during the dry season) and a low rate of breakdown of hard-seededness, the small seedlings seem to persist better during the critical period of establishment than subterranean clover seedlings. This may partially explain the dominance of cluster clover in more-heavily-stocked situations. Another component in the success and persistence of cluster clover in the pasture, is the ability of the small seed to better survive the passage through the digestive tract of sheep which favours its dissemination,

while subterranean clover seeds have much higher mortality. A density of 823 seeds/m^2 in Paddock I was estimated compared with 233 seeds/m^2 in Paddock II. This supports the finding obtained by Carter and Lake (1985) and confirms the results obtained in the previous study (5.2 Field Experiment 1), where about 76% of the seed ingested by sheep was recovered in the voided faeces.

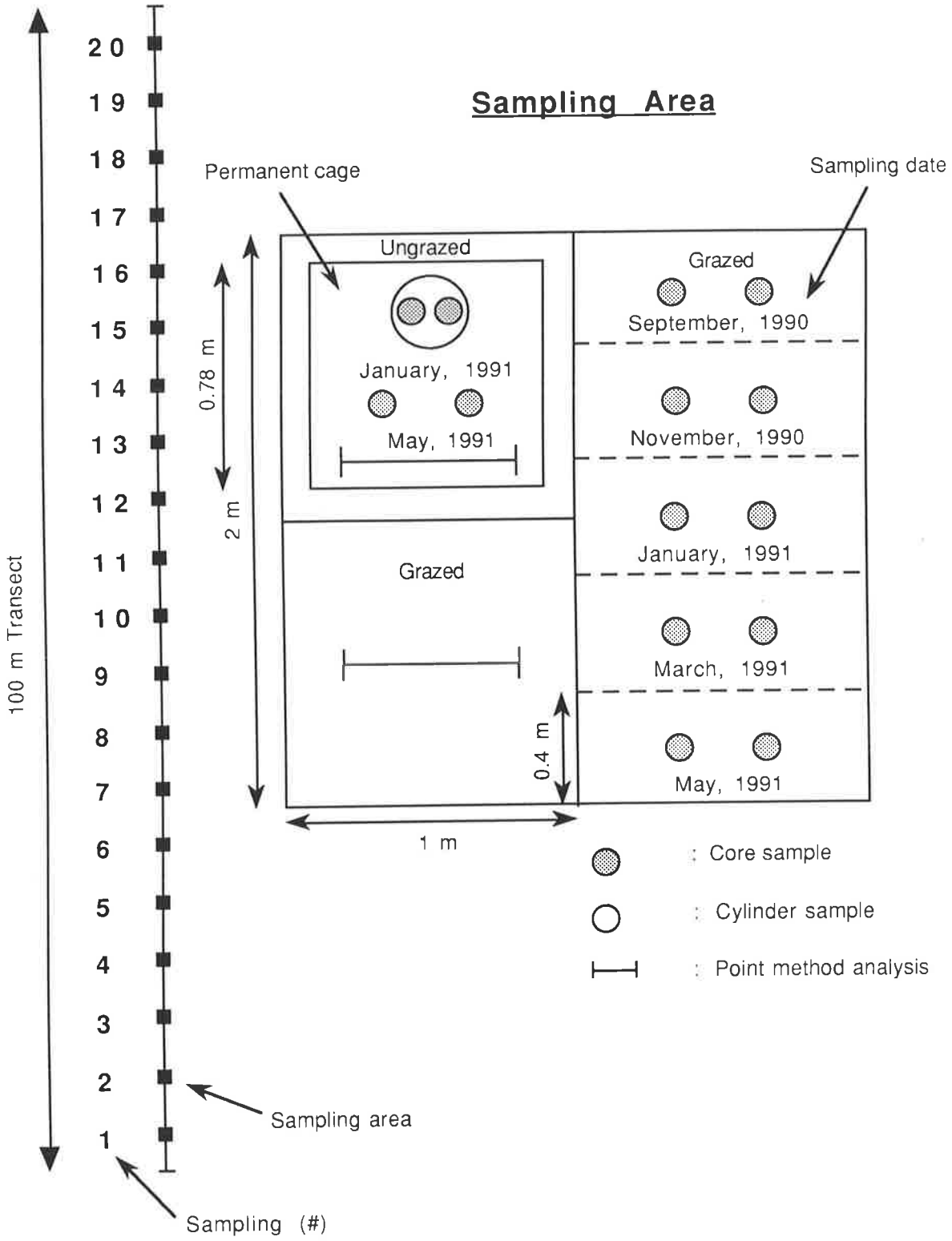


Diagram 5.2 Pattern of sampling used to estimate the pasture components in the soil-plant complex at Parawa.

Table 5.11 Plant density, herbage availability, botanical composition and seed reserve in a subterranean clover-perennial ryegrass pasture at Parawa (Paddock I; September, 1990).

Species	Plant density (#/m^2)	Herbage availability (gDM/m^2)†	Botanical composition (%, DM)††	(%)†††	Seed reserve (#/m^2)	(kg/ha)	(mg/seed)
A. SOWN							
Subterranean clover	991	34.0	22.9	23.2	3489	214.3	6.14
var. subterraneum	287	7.4	5.0	-	258	13.0	5.04
var. yanninicum	704	26.6	17.9	-	3231	201.3	6.23
Perennial ryegrass	76	15.8	10.6	13.4	-	-	-
B. VOLUNTEER DESIRABLES							
Cluster clover	341	3.0	2.0	1.2	7901	23.7	0.300
C. VOLUNTEER UNDESIRABLES							
Winter grass	1563	28.8	19.4	31.9	-	-	-
Barley grass	60	3.7	2.5	0.9	-	-	-
Storksbill	10	0.3	0.2	0.0	-	-	-
Capeweed	293	60.8	40.9	27.9	-	-	-
Chickweed	137	2.2	1.5	1.5	-	-	-
Total	**3471**	**148.6**	**100.0**	**100.0**	**11390**	**238.0**	-
D. BARE GROUND (%)	-	-	-	0.0	-	-	-

† Legume seed reserve is not included
†† Hand-separation method
††† Levy Point Quadrat method

Table 5.12 Plant density, herbage availability, botanical composition and seed reserve in a subterranean clover-perennial ryegrass pasture at Parawa (Paddock II; September, 1990).

Species	Plant density (#/m^2)	Herbage availability (gDM/m^2)†	Botanical composition (%, DM)††	(%)†††	Seed reserve (#/m^2)	(g/ha)	(mg/seed)
A. SOWN							
Subterranean clover	1089	43.9	37.5	25.5	8248	497.0	6.03
var. subterraneum	825	28.8	24.6	-	3839	171.0	4.46
var. yanninicum	264	15.1	12.9	-	4409	326.0	7.39
Perennial ryegrass	169	33.3	28.5	28.9	-	-	-
B. VOLUNTEER DESIRABLES							
Cluster clover	54	0.3	0.2	0.2	1251	4.1	0.331
C. VOLUNTEER UNDESIRABLES							
Winter grass	681	19.2	16.4	28.0	-	-	-
Barley grass	6	0.1	0.1	0.0	-	-	-
Storksbill	372	16.1	13.8	13.3	-	-	-
Capeweed	10	0.9	0.8	0.5	-	-	-
Chickweed	325	3.3	2.7	3.6	-	-	-
Total	**2706**	**117.0**	**100.0**	**100.0**	**9499**	**501.1**	-
D. BARE GROUND (%)	-	-	-	**0.5**	-	-	-

† Legume seed reserve is not included
†† Hand-separation method
††† Levy Point Quadrat method

Table 5.13 Herbage availability and botanical composition in a subterranean clover-perennial ryegrass pasture at Parawa (Paddocks I and II; November, 1990).

Species	Paddock I			Paddock II		
	Herbage availability (gDM/m^2)†	Botanical composition (%, DM)††	(%)†††	Herbage availability (gDM/m^2)†	Botanical composition (%, DM)††	(%)†††
A. SOWN						
Subterranean clover	98.8	36.8	40.6	103.1	46.6	34.4
Perennial ryegrass	23.9	8.9	16.4	76.1	34.4	44.0
B. VOLUNTEER DESIRABLES						
Cluster clover	7.8	2.9	0.6	0.7	0.3	0.6
C. VOLUNTEER UNDESIRABLES						
Winter grass	7.3	2.7	5.0	2.7	1.2	2.9
Barley grass	8.9	3.3	3.2	0.0	0.0	0.0
Soft brome	1.3	0.5	0.6	0.0	0.0	0.0
Storksbill	0.8	0.3	0.0	19.5	8.8	7.5
Capeweed	104.5	38.9	25.9	9.1	4.1	2.0
Chickweed	15.3	5.7	7.7	10.1	4.6	8.6
Total	**268.6**	**100.0**	**100.0**	**221.3**	**100.0**	**100.0**
D. BARE GROUND (%)	-	-	0.0	-	-	0.0

† Buried legume seed reserve is not included
†† Hand-separation method
††† Levy Point Quadrat method

Table 5.14 Herbage availability and botanical composition of ungrazed and grazed subterranean clover-perennial ryegrass pasture at Parawa (Paddock I; January, 1991).

Species	Ungrazed		Grazed	
	Herbage availability (gDM/m^2)†	Botanical composition (%)††	Herbage availability (gDM/m^2)	Botanical composition (%)
A. SOWN				
Subterranean clover	-	16.7	-	17.5
Perennial ryegrass	-	46.3	-	37.2
B. VOLUNTEER DESIRABLES				
Cluster clover	-	2.0	-	3.3
Cocksfoot	-	0.6	-	0.0
C. VOLUNTEER UNDESIRABLES				
Winter grass	-	0.3	-	4.9
Barley grass	-	18.6	-	35.0
Soft brome	-	2.8	-	0.0
Silver grass	-	1.1	-	0.0
Storksbill	-	0.0	-	0.0
Capeweed	-	9.6	-	2.1
Chickweed	-	2.0	-	0.0
Sorrel	-	0.0	-	0.0
Total	**213.2**	**100.0**	**63.4**	**100.0**
D. BARE GROUND (%)	-	8.5	-	36.5

† Legume seed reserve is not included

†† Levy Point Quadrat method

Table 5.15 Herbage availability and botanical composition of ungrazed and grazed subterranean clover-perennial ryegrass pasture at Parawa (Paddock II; January, 1991).

Species	Ungrazed		Grazed	
	Herbage availability (gDM/m^2)†	Botanical composition (%)††	Herbage availability (gDM/m^2)	Botanical composition (%)
A. SOWN				
Subterranean clover	-	15.5	-	23.4
Perennial ryegrass	-	75.5	-	68.7
B. VOLUNTEER DESIRABLES				
Cluster clover	-	0.0	-	0.0
Cocksfoot	-	0.0	-	0.0
C. VOLUNTEER UNDESIRABLES				
Winter grass	-	2.4	-	7.5
Barley grass	-	0.0	-	0.0
Soft brome	-	0.0	-	0.0
Silver grass	-	0.0	-	0.0
Storksbill	-	0.0	-	0.0
Capeweed	-	1.2	-	0.0
Chickweed	-	5.0	-	0.4
Sorrel	-	0.4	-	0.0
Total	**189.7**	**100.0**	**55.4**	**100.0**
D. BARE GROUND (%)	-	7.5	-	31.0

† Legume seed reserve is not included
†† Levy Point Quadrat method

Table 5.16 Total seed reserve of ungrazed and grazed subterranean clover-perennial ryegrass pasture at Parawa (Paddock I; January, 1991).

Species	Seed reserve								
	Above and/or at ground level			Below ground level			Total		
	(#/m^2)	(kg/ha)	(mg/seed)	(#/m^2)	(kg/ha)	(mg/seed)	(#/m^2)	(kg/ha)	(mg/seed)
Ungrazed									
Subterranean clover	3324	187.5	5.63	4320	283.8	6.57	7644	471.1	6.31
var. subterraneum	1093	57.5	5.26	563	31.7	5.63	1656	89.2	5.45
var. yanninicum	2231	130.0	5.82	3757	252.1	6.69	5988	381.9	6.51
Cluster clover	3348	10.0	0.299	12100	37.4	0.309	15448	47.4	0.308
Grazed									
Subterranean clover	1244	68.3	5.52	7716	465.0	6.03	8960	533.3	5.96
var. subterraneanum	213	10.4	4.85	754	42.4	5.62	967	53.3	5.45
var. yanninicum	1031	57.9	5.62	6962	422.6	6.07	7993	480.0	6.01
Cluster clover	-	-	-	17406	47.7	0.274	17406	47.7	0.274

Table 5.17 Total seed reserve of ungrazed and grazed subterranean clover-perennial ryegrass pasture at Parawa (Paddock II; January, 1991).

Species	Seed reserve								
	Above and/or at ground level			Below ground level			Total		
	(#/m^2)	(kg/ha)	(mg/seed)	(#/m^2)	(kg/ha)	(mg/seed)	(#/m^2)	(kg/ha)	(mg/seed)
Ungrazed									
Subterranean clover	5099	237.2	4.65	9053	587.1	6.49	14152	824.3	6.08
var. subterraneum	4264	184.8	4.33	4224	213.7	5.06	8488	398.5	4.82
var. yanninicum	835	52.4	6.29	4829	373.4	7.73	5664	425.8	7.62
Cluster Clover	709	2.4	0.334	1311	4.0	0.308	2020	6.4	0.314
Grazed									
Subterranean clover	914	44.5	4.88	13198	803.9	6.09	14112	848.4	6.01
var. subterraneum	535	29.0	4.45	5969	279.5	4.68	6504	308.5	4.66
var. yanninicum	379	15.5	5.48	7229	524.4	7.25	7608	539.9	7.17
Cluster clover	-	-	-	1343	3.7	0.275	1343	3.7	0.275

Table 5.18 Herbage availability and botanical composition under grazed conditions in a subterranean clover-perennial ryegrass pasture at Parawa (Paddocks I and II; March , 1991).

Species	Paddock I		Paddock II	
	Herbage availability (gDM/m^2)†	Botanical composition (%)††	Herbage availability (gDM/m^2)	Botanical composition (%)
A. SOWN				
Subterranean clover	-	30.2	-	103.1
Perennial ryegrass	-	30.2	-	80.8
B. VOLUNTEER DESIRABLES				
Cluster clover	-	0.0	-	0.0
C. VOLUNTEER UNDESIRABLES				
Winter grass	-	4.2	-	5.1
Barley grass	-	32.3	-	1.0
Storksbill	-	0.0	-	0.0
Capeweed	-	3.1	-	0.0
Chickweed	-	0.0	-	0.0
Total	**26.1**	**100.0**	**27.6**	**100.0**
D. BARE GROUND (%)	-	56.5	-	59.5

† Legume seed reserve is not included
†† Levy Point Quadrat method

Table 5.19 **Total seed reserve under grazed conditions in a subterranean clover-perennial ryegrass pasture at Parawa (Paddocks I and II; March, 1991).**

Species	Seed reserve								
	Above and/or at ground level			Below ground level			Total		
	(#/m^2)	(kg/ha)	(mg/seed)	(#/m^2)	(kg/ha)	(mg/seed)	(#/m^2)	(kg/ha)	(mg/seed)
Paddock I									
Subterranean clover	82	4.7	5.69	8868	534.3	6.03	8950	539.0	6.02
var. subterraneum	51	2.9	5.61	992	51.5	5.20	1043	54.4	5.22
var. yanninicum	31	1.8	5.83	7876	482.8	6.13	7907	484.6	6.13
Cluster clover	-	-	-	11988	37.6	0.314	11988	37.6	0.314
Paddock II									
Subterranean clover	193	7.9	4.09	13683	801.2	5.86	13876	809.1	5.83
var. subterraneum	193	7.9	4.09	7029	322.6	4.59	7222	330.5	4.58
var. yanninicum	0	0	-	6654	478.6	7.19	6654	478.6	7.19
Cluster clover	-	-	-	983	3.3	0.337	983	3.3	0.337

Table 5.20 Seedling density, herbage availability, botanical composition and seed reserve under ungrazed conditions in a subterranean clover-perennial ryegrass pasture at Parawa (Paddock I; May, 1991).

Species	Seedling density (#/m^2)	Herbage availability (gDM/m^2)†	Botanical composition (%, DM)††	(%)†††	Seed reserve (#/m^2)	(kg/ha)	(mg/seed)
A. SOWN							
Subterranean clover	1091	7.6	14.9	10.9	5983	355.2	5.94
var. subterraneum	-	-	-	-	912	49.7	5.46
var. yanninicum	-	-	-	-	5071	305.5	6.02
Perennial ryegrass	102	21.0	40.9	49.6	-	-	-
B. VOLUNTEER DESIRABLES							
Cluster clover	952	0.7	1.4	3.5	9519	28.0	0.294
Cocksfoot	3	2.0	3.8	6.2	-	-	-
C. VOLUNTEER UNDESIRABLES							
Winter grass	2606	7.4	14.5	11.7	-	-	-
Barley grass	904	9.7	18.9	13.9	-	-	-
Soft brome	99	0.3	0.6	-			
Storksbill	3	0.2	0.4	0.2	-	-	-
Capeweed	170	2.3	4.5	3.5	-	-	-
Chickweed	54	0.1	0.1	0.5	-	-	-
Sorrel	0	0.0	0.0	0.0	-	-	-
Total	**5984**	**51.3**	**100.0**	**100.0**	**15502**	**383.2**	-
D. BARE GROUND (%)	-	-	-	5.5	-	-	-

† Legume seed reserve is not included
†† Hand-separation method
††† Levy Point Quadrat method

Table 5.21 Seedling density, herbage availability, botanical composition and seed reserve under grazed conditions in a subterranean clover-perennial ryegrass pasture at Parawa (Paddock I; May, 1991).

Species	Seedling density (#/m^2)	Herbage availability (gDM/m^2)†	Botanical composition (%, DM)††	(%)†††	Seed reserve (#/m^2)	(kg/ha)	(mg/seed)
A. SOWN							
Subterranean clover	538	3.7	7.4	8.6	4193	258.8	6.17
var. subterraneum	-	-	-	-	215	11.6	5.38
var. yanninicum	-	-	-	-	3978	247.2	6.21
Perennial ryegrass	68	9.8	19.9	13.1	-	-	-
B. VOLUNTEER DESIRABLES							
Cluster clover	989	1.4	2.8	9.3	4068	11.9	0.293
Cocksfoot	0	0.0	0.0	0.0	-	-	-
C. VOLUNTEER UNDESIRABLES							
Winter grass	12295	31.8	64.5	63.4	-	-	-
Barley grass	167	1.2	2.5	0.4	-	-	-
Soft brome	0	0.0	0.0	0.0			
Storksbill	9	0.2	0.3	0.0	-	-	-
Capeweed	111	1.0	2.0	3.7	-	-	-
Chickweed	306	0.3	0.6	1.5	-	-	-
Sorrel	0	0.0	0.0	0.0	-	-	-
Total	**14483**	**49.4**	**100.0**	**100.0**	**8261**	**270.7**	-
D. BARE GROUND (%)	-	-	-	17.5	-		

† Legume seed reserve is not included
†† Hand-separation method
††† Levy Point Quadrat method

Table 5.22 Seedling density, herbage availability, botanical composition and seed reserve under ungrazed conditions in a subterranean clover-perennial ryegrass pasture at Parawa (Paddock II; May, 1991).

Species	Seedling density (#/m^2)	Herbage availability (gDM/m^2)†	Botanical composition (%, DM)††	(%)†††	Seed reserve (#/m^2)	(kg/ha)	(mg/seed)
A. SOWN							
Subterranean clover	1102	19.2	26.7	28.2	11776	572.6	4.86
var. subterraneum	-	-	-	-	8702	355.9	4.09
var. yanninicum	-	-	-	-	3074	216.7	7.05
Perennial ryegrass	113	28.9	40.1	51.2	-	-	-
B. VOLUNTEER DESIRABLES							
Cluster clover	88	0.2	0.3	0.0	1446	5.0	0.348
Cocksfoot	0	0.0	0.0	0.0	-	-	-
C. VOLUNTEER UNDESIRABLES							
Winter grass	5479	18.0	25.0	11.1	-	-	-
Barley grass	0	0.0	0.0	0.0	-	-	-
Soft brome	0	0.0	0.0	0.0	-	-	-
Storksbill	190	1.4	2.0	5.5	-	-	-
Capeweed	6	0.1	0.1	0.2	-	-	-
Chickweed	68	0.1	0.1	1.4	-	-	-
Sorrel	51	4.1	5.7	2.4	-	-	-
Total	**7698**	**72.0**	**100.0**	**100.0**	**13222**	**577.6**	-
D. BARE GROUND (%)	-	-	-	5.0	-	-	-

† Legume seed reserve is not included
†† Hand-separation method
††† Levy Point Quadrat method

Table 5.23 Seedling density, herbage availability, botanical composition and seed reserve under grazed conditions in a subterranean clover-perennial ryegrass pasture at Parawa (Paddock II; May, 1991).

Species	Seedling density (#/m^2)	Herbage availability (gDM/m^2)†	Botanical composition (%, DM)††	(%)†††	Seed reserve (#/m^2)	(kg/ha)	(mg/seed)
A. SOWN							
Subterranean clover	1031	5.3	9.8	13.3	8848	552.4	6.24
var. subterraneum	-	-	-	-	3666	173.3	4.73
var. yanninicum	-	-	-	-	5182	379.1	7.32
Perennial ryegrass	85	12.4	23.0	19.5	-	-	-
B. VOLUNTEER DESIRABLES							
Cluster clover	116	0.1	0.1	1.2	598	2.1	0.355
Cocksfoot	0	0.0	0.0	0.0	-	-	-
C. VOLUNTEER UNDESIRABLES							
Winter grass	14686	34.8	64.6	56.0	-	-	-
Barley grass	0	0.0	0.0	1.5	-	-	-
Soft brome	0	0.0	0.0	0.0			
Storksbill	235	1.1	2.1	6.2	-	-	-
Capeweed	11	0.0	0.0	0.8	-	-	-
Chickweed	227	0.1	0.1	1.5	-	-	-
Sorrel	3	0.1	0.3	0.0	-	-	-
Total	**16394**	**53.9**	**100.0**	**100.0**	**9446**	**554.5**	-
D. BARE GROUND (%)	-	-	-	4.5	-	-	-

† Legume seed reserve is not included
†† Hand-separation method
††† Levy Point Quadrat method

Table 5.24 Changes in the reserve of legume seeds in the soil-plant complex of a subterranean clover-perennial ryegrass pasture at Parawa.

Seed and seedling reserve (#/m^2)	Paddock I		Paddock II	
	Subterranean clover	Cluster clover	Subterranean clover	Cluster clover
		Ungrazed		
January 1991				
Total seed density	7644	15448	14152	2020
May 1991				
Seedling density	1091	952	1102	88
Hard seed density	5983	9519	11776	1446
Total reserve density	7074	10471	12878	1534
		Grazed		
September 1990				
Hard seed density	3489	7901	8248	1251
January 1991				
Total seed density	8960	17406	14112	1343
March 1991				
Total seed density	8950	11988	13876	983
May 1991				
Seedling density	538	989	1031	116
Hard seed density	4193	4068	8848	598
Total reserve density	4731	5057	9879	714

Table 5.25 Cumulative germination of clover seeds tested at various times during the study.

Condition	Paddock I			Paddock II		
	Subterranean clover subt.†	Subterranean clover yann.††	Cluster clover	Subterranean clover subt.	Subterranean clover yann.	Cluster clover
	Germination (%)					
UNGRAZED						
January 1991						
Unburied	29.8	9.5	4.5	7.2	19.5	3.0
Buried	22.0	15.8	6.8	12.8	7.2	3.5
GRAZED						
September 1990						
Buried	21.3	22.3	0.8	35.6	59.2	0.0
January 1991						
Unburied	55.8	10.8	-	12.8	8.5	-
Buried	32.5	14.5	9.8	23.2	24.1	7.0
March 1991						
Unburied	77.8	10.0	-	21.0	12.5	-
Buried	59.5	44.5	58.0	47.8	46.3	31.0

† Variety subterraneum
†† Variety yanninicum

Table 5.26 **Total undigested seed voided in faeces, seedling emergence and hard seed weight of subterranean clover and cluster clover seeds present in faeces during early summer in both paddocks at Parawa.**

Variable	Species							
	Paddock I				Paddock II			
	Subterranean clover			Cluster clover	Subterranean clover			Cluster clover
	subt.†	yann.††	Total		subt.	yann.	Total	
Seed voided (#/m^2)	1	2	3	376	3	5	8	50
Hard Seed	1	1	2	351	1	5	6	47
Seedling emerged	0	1	1	25	2	0	2	3
Emergence (%)	0.0	50.0	33.3	6.6	66.7	0.0	25.0	6.0
Hard seed (g/m^2)	0.0049	0.0039	0.0088	0.0957	0.0183	0.0020	0.0203	0.0142
Hard seed (mg)	4.97	3.96	4.40	0.272	3.39	5.92	3.52	0.301

† Variety subterraneum
†† Variety yanninicum

Table 5.27 **Total undigested seed voided in faeces, seedling emergence and hard seed weight of subterranean clover and cluster clover seeds present in faeces during early autumn in both paddocks at Parawa.**

Variable	Species							
	Paddock I				Paddock II			
	Subterranean clover			Cluster clover	Subterranean clover			Cluster clover
	subt.†	yann.††	Total		subt.	yann.	Total	
Seed voided (#/m2)	3	10	13	823	38	5	43	233
Hard Seed	2	8	10	722	29	3	32	201
Seedling emerged	1	2	3	101	9	2	11	32
Emergence (%)	33.3	20.0	23.1	12.3	23.7	40.0	25.6	13.7
Hard seed (g/m^2)	0.0082	0.0344	0.0426	0.1991	0.0953	0.0169	0.1122	0.0557
Hard seed (mg)	3.83	4.67	4.40	0.276	3.34	4.88	3.51	0.278

† Variety subterraneum
†† Variety yanninicum

Plate 5.8 General view of Paddock I, heavily set stocked (upper) and Paddock II, normally saved to make hay (lower) at Parawa (September, 1990).



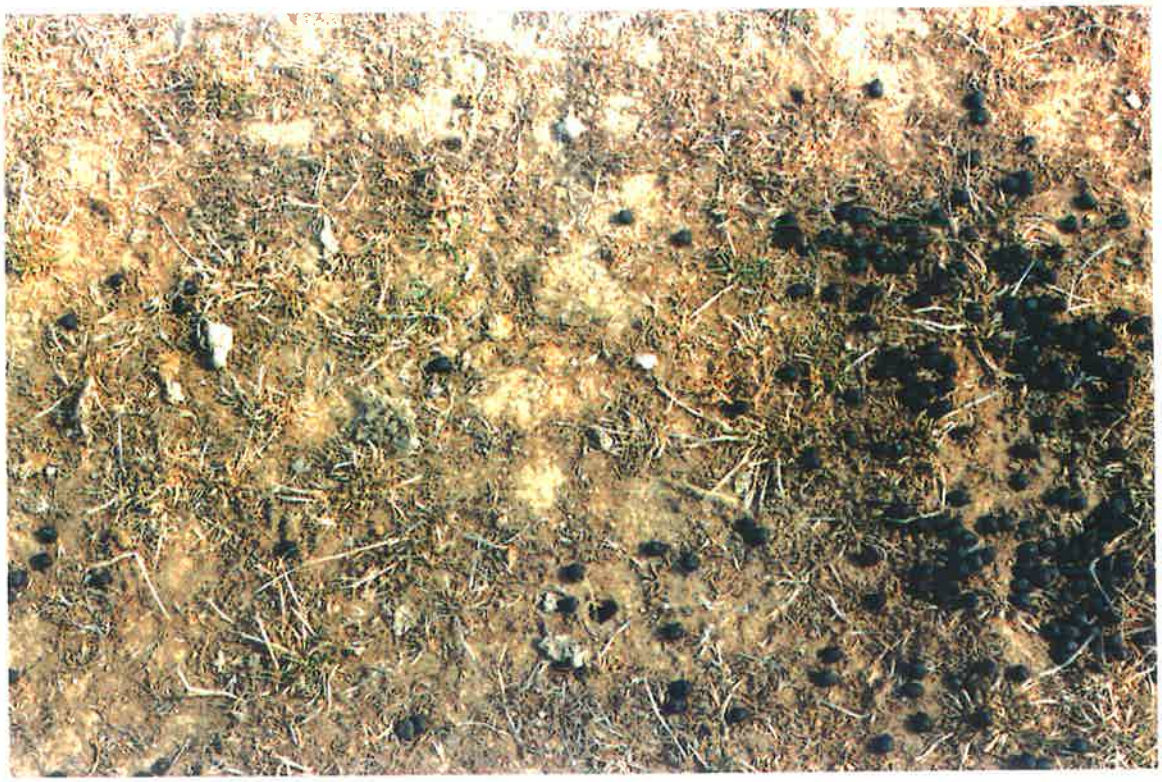


Plate 5.9 Close-up view of subterranean clover-perennial ryegrass pasture in Paddock I grazed by sheep in September, 1990 (upper) and in January, 1991 (lower).





Plate 5.10 Close-up view of subterranean clover-perennial ryegrass pasture in Paddock I grazed by sheep in March, 1991 (upper) and in May, 1991 (lower).





5.4 Conclusions from grazing studies

The results obtained from these grazing studies have shown that the acceptability and accessibility of annual pasture legume seeds to sheep plays an important role in the loss of seed from the pasture when sheep graze dry pasture residues.

On hard-setting red-brown earths at the Waite Agricultural Research Institute (WARI), the smaller seeds of balansa clover and cluster clover were more suited to avoid the impact of grazing by sheep than the larger subterranean clover seeds. In contrast, on the lighter-textured lateritic podzol at Parawa, the efficient burr burial capacity of subterranean clover cultivars, ensured an adequate level of seed reserve required for the regeneration of the pasture sward.

Smaller seeds were less affected by passage through the digestive tract of sheep. This is important in the dissemination of small-seeded clovers, especially cluster clover, as well as undesirable weed species such as winter grass, chickweed and capeweed.

FOURTH SECTION

6 PEN EXPERIMENTS 1-4

6 SEED SIZE AND SOME FACTORS INFLUENCING THE SURVIVAL OF ANNUAL PASTURE LEGUME SEEDS FED IN WHOLE PODS OR AS CLEAN SEED EXPOSED IN THE RUMEN

6.1 Introduction

In order to achieve a better understanding of the role of seed size on seed survival of annual pasture legumes, complementary pen experiments were carried out at the Waite Agricultural Research Institute Animal House (WARI).

To separate the effects of the initial mastication and rumination from the effects of digestion on seed survival, the following studies were principally aimed at examining the influence of digestion in the rumen by using the nylon bag *in sacco* technique. The species tested were subterranean clover, balansa clover and cluster clover seeds which were harvested from a previous study, in which sheep grazed dry pasture residues during the summer-autumn period (5.2 Field Experiment 1). Similarly, the survival of barrel medic (*Medicago truncatula* cv. Paraggio) and snail medic (*Medicago scutellata* cv. Sava) seeds was examined. Finally, the survival of barrel medic seeds of cultivar Paraggio, fed as the whole pod to sheep with contrasting seed sizes and levels of hard-seededness, and then subjected to the complete digestion process was examined.

6.2 Pen Experiment 1: The effects of seed size and time of harvesting on survival of seeds of subterranean clover, balansa clover and cluster clover exposed in the sheep rumen

6.2.1 Materials and methods

Two hundred selected seeds of subterranean clover, balansa clover and cluster clover with mean individual weights of 6.22 ± 0.43 mg (± s.d.), 0.757± 0.023 mg and 0.428 ± 0.010 mg respectively, collected at different times during the grazing period (0, 2, 4, 6, 8 and 10 weeks) of 5.2 Field Experiment 1 and tested for hard-seededness (Table 6.1) were enclosed in nylon bags and exposed in the rumen of four fistulated Merino

wethers for 48 h. After treatment, the seeds were washed, counted and tested for germination as before. Then to simulate the conditions in the abomasum, 25 undigested hard seeds were located in glass tubes and 50 ml of a acid pepsin solution (1000 ml of reverse osmosis water + 0.5 g of pepsin, 1 : 2500 + 8.9 ml of concentrated HCl, Normality about 11.5) per tube was added. The closed tubes were incubated for 24 h at 38 °C. The percentages of viable seed before and after digestion were obtained by adding to the readily germinable seed, the seed that had the potential for germination after scarification of the hard seed. The characteristics of the animals, the diet and the nylon bag *in sacco* technique were the same as used to test the degradability of dry pasture residues in the rumen (5.2 Field Experiment 1).

The experiment was arranged in a completely randomized block design with factorial treatments: Species (3) x Times of harvesting (6) x Replicate sheep (4) and analyses of variance were carried out on calculated percentages of germination and viability of seeds respectively.

6.2.2 Results

The characteristics and germination of clover species seeds harvested at different times during the grazing period, and tested before and after placement in the rumen are shown in Table 6.1. The exposure of seeds in the rumen decreased the germination and viability of the pasture species seeds tested. This was a consequence of complete mortality of readily-germinable (soft) seeds able to germinate prior to treatment and some changes in hard-seededness caused by digestion. There was practically no variation between the level of dead seed in the hard seed category before and after the digestion. There were no further changes in germination and viability of the seed observed after some hard seeds were placed under simulated conditions of the abomasum, where germinating seeds coming from the rumen are not expected to persist.

A significant interaction between species and time of harvesting was obtained for readily-germinable seed ($P<0.001$) and total viable seed ($P<0.005$). This indicates that the level of hard-seededness, associated principally with the small-seeded clovers, is important for the survival of the seed. Despite the further damage to the seed that may occur later during the passage through the intestine and even after defaecation, only hard seed ingested by sheep is able to persist, and only if it avoids the initial mastication and the chewing process which are part of digestion.

Table 6.1 Characteristics of clover seeds harvested fortnightly during the grazing experiment and tested before and after digestion in the rumen of sheep.

Week of Harvesting	Characteristics of seed							
	Before digestion				After digestion			
	Germinated	Hard	Dead	Viable germinable	Germinated	Hard	Dead	Viable germinable
	Subterranean clover							
0	28.0	70.0	2.0	98.0	4.1	65.4	30.5	69.5
2	32.5	66.0	1.5	98.5	1.1	67.4	31.5	68.5
4	33.5	64.5	2.0	98.0	0.5	65.4	34.1	65.9
6	37.5	60.0	2.5	97.5	0.4	59.3	41.1	58.9
8	38.5	60.5	1.0	99.0	1.3	57.1	41.6	58.4
10	51.0	46.5	0.5	97.5	0.9	51.2	48.8	52.1
	Balansa clover							
0	7.0	91.0	2.0	98.0	1.0	90.5	8.5	91.5
2	9.5	89.0	1.5	98.5	0.5	89.5	10.0	90.0
4	8.5	89.5	2.0	98.0	1.0	88.5	10.5	89.5
6	13.5	84.0	2.5	97.5	1.0	85.5	13.5	86.5
8	15.5	80.5	4.0	96.0	0.5	79.0	20.5	79.5
10	33.8	65.2	1.0	99.0	1.3	64.9	33.8	66.2
	Cluster clover							
0	2.5	95.5	2.0	98.0	0.0	93.1	6.9	93.1
2	3.5	94.0	2.5	97.5	0.0	93.4	6.6	93.4
4	3.5	94.5	2.0	98.0	0.0	93.4	6.6	93.4
6	4.5	94.0	1.5	98.5	0.1	94.4	5.6	94.5
8	7.5	90.0	2.5	97.5	0.1	90.3	9.6	90.4
10	19.0	81.0	4.0	96.0	0.3	76.3	23.4	76.6

6.3 Pen Experiment 2: The effects of seed size and time of incubation on survival of subterranean clover, balansa clover and cluster clover seeds exposed in the sheep rumen

6.3.1 Materials and methods

The same materials and methods as reported in the previous study were used (6.2 Pen Experiment 1). Lots of two hundred selected seeds per treatment of subterranean clover, balansa clover and cluster clover pasture species collected before the start of the grazing period (0 week) were incubated in the rumen of sheep for periods of 12, 24, 48, 72 and 96 h. The experimental design was a completely randomized block with factorial treatment: Species (3) x Periods of incubation (5) x Sheep (4). The analysis of variance was carried out on the percent germination and the viability of seeds.

6.3.2 Results

When the clover seeds were exposed in the rumen for different periods of time, only a significant effect for clover species was obtained (readily-germinable, $P<0.005$ and viable seed, $P<0.001$) indicating that the time of exposure of the seeds in the rumen did not cause significant changes in the level of hard-seededness of undigested seeds. Only soft seeds were completely digested (Table 6.2), confirming the resistance of the hard seeds of the pasture species tested to digestion in the rumen. Under the simulated conditions of the abomasum, there were no further changes in germination or viability of the seeds.

Table 6.2 Characteristics of clover seeds harvested prior to starting grazing experiment and tested after digestion in the rumen of sheep.

Period of exposure in rumen (h)	Characteristics of seed			
	Germinated	Hard germinable	Dead	Viable
	Subterranean clover			
0	28.0	70.0	2.0	98.0
12	1.4	69.9	28.7	71.3
24	1.4	69.6	29.0	71.0
48	0.8	69.8	29.4	70.6
72	0.9	69.2	29.9	70.1
96	0.6	71.7	27.7	72.3
	Balansa clover			
0	7.0	91.0	2.0	98.0
12	1.1	90.2	8.7	91.3
24	1.0	90.0	9.0	91.0
48	0.7	90.7	8.6	91.4
72	0.3	91.7	8.0	92.0
96	0.4	91.2	8.4	91.6
	Cluster Clover			
0	2.5	95.5	2.0	98.0
12	0.3	95.2	4.5	95.5
24	0.1	94.8	5.1	94.9
48	0.3	95.7	4.0	96.0
72	0.1	94.7	5.2	94.8
96	0.0	95.1	4.9	95.1

6.4 Pen Experiment 3: The effects of seed size and level of hard-seededness on survival of annual medic seeds exposed in the sheep rumen

6.4.1 Materials and methods

This study considered the effects of the rumen using the nylon bag *in sacco* technique (6.2-6.3 Pen Experiments 1-2) on the survival of small and large seeds of Paraggio and Sava medic cultivars selected for 100% soft and 100% hard seeds. The contrasting seed sizes were obtained by dissecting small and large pods of the selected cultivars (Table 6.3). The discrimination between soft and hard seed was made by placing the seeds in Petri dishes provided with wetted pads of filter paper. After 2 to 4 hours, swollen seeds and remaining hard seeds of each seed size of both cultivars were picked out and dried in a forced-draught dehydrator at 30 ^{o}C for a period of 12 h. Then, two hundred seeds per treatment: Cultivars (2) x Seed sizes (2) x Levels of hard-seededness (2) x Sheep replicates (4) and corresponding to a completely randomized block design with factorial treatments, were incubated in the rumen of sheep for a period of 48 h.

6.4.2 Results

All the seeds from the group selected for 100% small and large soft seeds for each cultivar, were almost completely digested and only small fragments of mainly cotyledons and seed coats were recovered from the nylon bags, showing a high susceptibility to digestion in the rumen. Contrasting results were obtained for hard seed, where the changes of hard-seededness caused by ruminal digestion reached only 3.5 % and 4.7% in cultivar Paraggio and 1.5 % and 3.7% in cultivar Sava for small and large seed respectively. No further variations were observed when some hard seeds were concurrently immersed in an acid pepsin solution for 24 h in order to simulate the conditions of the abomasum. Despite the non-significant values obtained between cultivars, significant differences ($P<0.005$) in percentage of viable seed between small and large seed were obtained. The experiment indicates the resistance of hard seed of

medic cultivars and the susceptibility of soft seed to digestion in the rumen. This suggests, as mentioned before, that much of the damage to the hard seed ingested by sheep occurred during the chewing process. It is during the chewing process that the size of the hard seed becomes an important factor in seed survival.

Table 6.3 **Characteristics of Paraggio and Sava medic pods and corresponding enclosed seeds used to test the survival of seed exposed in the rumen of sheep.**

	Medic			
	Paraggio		Sava	
Variable	Small	Large	Small	Large
Pod height (cm)	0.424	0.592	0.784	1.128
Pod diameter (cm)	0.492	0.592	0.912	1.244
Pod weight (mg)	40.6	78.2	158.2	347.2
Seeds/pod (#)	5.76	7.38	4.24	5.08
Seed weight (mg)	2.37	3.31	13.14	20.25
Seed/pod weight (%)	33.6	31.2	34.10	31.6

6.5 Pen Experiment 4: The effects of seed size and level of hard-seededness on survival of annual medic seeds in whole pods fed to sheep

6.5.1 Materials and methods

The objective of this experiment was to test the effects of seed size and level of hard-seededness on survival of *Medicago truncatula* cv. Paraggio seeds in whole pods fed to sheep. The experimental material was a population of pods produced in the 1987 growing season and it was collected in December at Arthurton, about 150 km north-west of Adelaide, South Australia.

The pods were cleaned of impurities and classified into small (< 5.00 mm) and large (> 5.00 mm) categories by using a 50 cm diameter square-holed 5 mm aperture sieve. To obtain the contrasting levels of hard-seededness, two sub-samples of 3 kg of pods were selected from each size category. One lot of both small pods and large pods was enclosed in a 4 x 2 mm rectangular-holed mesh plastic bag and placed in an alternating-temperature incubator located at Northfield Research Laboratories, S.A. Department of Agriculture for a period of 16 weeks (2 October, 1989 to 22 January, 1990). The incubator was programmed to operate in alternate cycles of 12 h duration at 15°C and 50°C respectively. The cycles of temperature were recorded by the use of a thermohydrograph (Figure 6.1). The pods were then removed from the incubator and placed outdoors on raised-beds (WARI) for a period of 14 weeks (23 January, 1990 to 1 May, 1990). The pods were enclosed and spread between two layers of a green plastic net of the same characteristics used to contain the pods in the alternating-temperature incubator, and protected against occasional falls of rain (Plate 6.1). The daily minimum and maximum soil surface temperatures were recorded during the entire period by using two maximum-temperature and two minimum-maximum temperature mercury bulb thermometers (Figure 6.2). The remaining small and large pods, were kept in paper bags and stored at a temperature of 12.7 ± 0.42°C (± s.d.) during the entire period (30 weeks). Later, all the pod samples referred to as small-untreated,

small-treated, large-untreated and large-treated were stored (12.7 ± 0.42°C) until they were used to feed the sheep.

To assess the extent of seed-softening in small and large pods under both storage conditions, germination tests were carried out using 25 pods per treatment replicated four times. The corresponding pods were placed in Petri dishes provided with three Whatman N°1 filter paper at the bottom and one at the top and moistened with 20 ml of reverse osmosis purified water (R.O.). To improve the moisture relationship between the pods and the surrounding media a 0.5 cm deep layer of non-absorbent cotton wool was placed over the pods and left for only the first 48 h of duration of the test. The Petri dishes were placed in an incubator at 19.0 ± 0.50°C (± s.d.) and re-moistened as required with a solution of fungicide Thiram (1.6 g/1000 ml of R.O. water). Emerged seedlings were recorded daily over a 14-day period. The pods were then dissected by hand and the remaining swollen (but not germinated) seeds, the dead seeds and the hard seeds were counted (Appendices 6.1-6.3). The resulting hard seed, was also weighed and those from the last germination test were sieved through a set of 20 cm diameter square-holed brass laboratory sieves ranging between 0.50 mm and 3.35 mm in aperture. The seeds obtained at each aperture size were counted, weighed and their percentage contribution calculated (Figures 6.3-6.4).

Finally, to estimate the characteristics of pods and seeds involved in the pen feeding study, sub-samples of one hundred randomly selected pods per size category and level of hard-seededness were sampled at week 30. The individual dimensions of pods were measured by use of a caliper graduated at intervals of 1.00 mm, weighed at the storage temperature, dissected by hand and the resulting seeds sieved and weighed as before. To estimate the moisture content of the seed, these were oven-dried at 80°C for a period of 48 h. Additional sub-samples of 100 pods were used to determine the moisture content of whole pods (Table 6.4).

Twenty mature, 3.5 year-old Merino wethers with mean body-weight (± s.d.) of 49.4 ± 4.4 kg (Appendix 6.4) plus two spares were penned individually in the Animal House

(WARI) on 1 May, 1990. During the following ten-day, pre-experimental period, in order to avoid a further contamination of the digestive tract of the sheep flock with related seeds and to accustom them to faecal-collection harnesses the animals were fed with 800 g/head/day of a standard diet containing 600 g of sheep pellets with 88.9 ± 0.44% (± s.d.) DM, 150 g of oaten hay with 87.9 ± 1.20% DM and 50 g of lucerne hay with 84.5 ± 2.03% DM (Appendix 6.5). Water was provided *ad libitum*.

To test for the possible presence of related medic seeds in the digestive tract of sheep, a sub-sample of 100 g of fresh faeces per sheep obtained the day before the start of the experiment (10 May, 1990) was processed. The sub-samples were macerated under running tap water within a set of 30 cm diameter square-holed brass laboratory sieves ranging between 1.00 mm and 0.5 mm in apperture. None of the sub-samples was found to contain related medic seeds.

At the start of the experimental period (11 May, 1990) four homogeneous groups of five sheep, were selected according to body-weight (Appendix 6.4) and each sheep within a group was assigned at random to a specific pen. Sheep numbered 1 to 5 were fed with small-untreated pods, 6 to 10 with large-untreated pods, 11 to 15 with small-treated pods and 16 to 20 with large-treated pods (Plate 6.2). All the sheep were fitted with faecal-collection harnesses and given two hundred grams of pods in addition to the standard diet at 0900 h. By midday, all pods were consumed by the sheep, except animal No. 6 that partially rejected the consumption of pods. After mixing the remaining pods with a small quantity of molasses, these pods were completely consumed during the day. The recovery of seed from faeces was recorded daily during 14 days after feeding with pods.

On 25 May, 1990 a second cycle of two weeks was started to ensure ten sheep replicates for each treatment using a cross-over design. At that time, the sheep numbered 1 to 5 were fed with large-untreated pods, 6 to 10 with small-untreated pods, 11 to 15 with large-treated pods and 16 to 20 with small-treated pods.

The same materials and methods (5.2 Field Experiment 1), were applied to estimate the output of seed voided in the sheep faeces. In addition, after completion of a cycle all the remaining hard seed was sieved as before and then scarified using sand paper to estimate the proportion of viable seed. The experiment was a completely randomized block design with factorial treatments (2 seed sizes x 2 levels of hard-seededness x 10 sheep replicates). The throughput of the seed as viable, germinable (able to produce a normal and an abnormal seedling) and hard was analysed as the percent of seed fed to sheep given the different number of seeds per unit pod weight.

Simultaneously, daily 100 g sub-samples of the dietary components (Appendix 6.5), 20 g of fresh faeces (Appendix 6.6) and rejected feed (Appendix 6.7), were taken to estimate the dry matter content by oven-drying at 80 °C for 48 h. From this, the *in vivo* apparent digestibility of the diet received by the animals for the two cycles (28 days) was calculated (Appendix 6.7) according to the following formula (Pryor and Gartner 1973).

$$\text{Apparent digestibility (DM)} = 100 \times \frac{\text{(Wgt of feed DM consumed - Wgt of faeces DM voided)}}{\text{(Wgt of feed DM consumed)}}$$

By using rumen-fistulated sheep as described before (5.2 Field Experiment 1), the digestibility of whole pods and the components of the diet were estimated, using the nylon bag *in sacco* technique.

6.5.2 Results

During the process of softening of seed by using an alternating-temperature incubator for a period of 16 weeks (Figure 6.5; Appendix 6.1) germination tests showed small variations in the level of hard-seededness. The hard/soft seed ratio only decreased from 4.4 to 2.1 in seed enclosed in small pods and 3.4 to 2.6 in seed enclosed in large pods. However, when the pods were removed from the incubator and placed on raised beds for a period of 14 weeks (Figure 6.5; Appendix 6.2), a greater range of temperatures was recorded (Figures 6.1-6.2) and higher rates of softening were achieved, especially

between weeks 16 and 22. At the end of the second period (30 weeks), the hard/soft seed ratio reached values up to 0.32 and 0.49 for exposed small and large pods, respectively. In contrast, seeds in pods kept at a relatively constant temperature for a period of 30 weeks showed only small variations in the level of hard-seededness (Appendix 6.3).

The distribution of seed by size in pods calculated prior to feeding (Figures 6.3-6.4) was similar between untreated and treated pods in the same pod-size category, but differed between the pod-size categories used in the study. When the seeds in pods were tested for germination (Figure 6.6), a decrease in the average size of seeds was obtained, especially in treated pods, suggesting that the process of softening occurred first in larger seeds and then progressed toward the smaller ones (Figures 6.7-6.8).

According to the daily dry matter output of faeces voided by sheep (Figures 6.9-6.10; Appendix 6.6), the daily dry matter content of the diet on offer (Figure 6.11; Appendix 6.5) and the proportion of feed rejected by the animals (Appendix 6.7), a mean daily intake of 658 g DM/head and *in vivo* digestibility of 61.9% were calculated (Figure 6.12; Appendix 6.7). Additional tests carried out by using rumen-fistulated sheep, showed higher values of *in sacco* digestibility for the diet on offer (70.5%, DM basis) and differences between whole small and large pods (Table 6.5). Perhaps the difference was due to a lower proportion of seed by weight in large pods (Table 6.4).

In all the situations, the highest recovery of viable seed was recorded on day 3 and almost finished one week after feeding with pods. However, it was importantly influenced by the variations between animals, especially when larger seeds enclosed in large pods were used (Appendix 6.8). Voided seeds that were enclosed in small pods, especially untreated ones, were more persistent in time, which suggests that smaller seeds could be retained longer than larger ones in the digestive tract of sheep (Figures 6.13-6.14). When the distribution by size of hard seed voided in faeces was considered, more large seeds were recovered from the untreated pods than those which had been treated (Table 6.6; Figures 6.15-6.16).

Analysis of variance carried out on results from the pen-feeding experiment, showed significant effects of the level of hard-seededness and seed size for voided viable seed ($P<0.001$ and $P<0.005$), readily-germinable seed that became a normal seedling ($P<0.001$ and $P<0.005$), and hard seed ($P<0.001$ and $P<0.001$), respectively. When the viable seed output was related to the total hard seed input (Table 6.6) in order to normalize the differences in the original level of hard-seededness between treatments, only the effect of seed size was significant ($P<0.001$).

The experiment indicates that the survival of Paraggio seed ingested in whole pods by sheep, is dependent on its resistance to digestion given by the level of hard-seededness and the size of the seed. It seems that larger seed enclosed in large pods is more damaged by the initial mastication and rumination processes than smaller seed enclosed in small pods .

Table 6.4 Characteristics of Paraggio medic pods and seeds used in the sheep-feeding experiment.

Variable	Pod size: SMALL Sum	Mean	s.d.	C.V. (%)	LARGE Sum	Mean	s.d	C.V. (%)
UNTREATED PODS								
Pod height (cm)	42.4	0.424	0.065	15.3	59.2	0.592	0.063	10.6
Pod diameter (cm)	49.2	0.492	0.039	8.0	59.2	0.592	0.27	4.6
Pod weight (mg)	4060	40.6	9.4	23.2	7820	78.2	18.1	23.1
Pod moisture content (%)	-	5.0	-	-	-	5.2	-	-
Seeds/pod (#)	576	5.76	1.41	24.5	738	7.38	0.97	13.1
Seed weight (mg)	237	2.37	0.64	27.0	331	3.31	0.73	22.1
Seed moisture content (%)	-	1.97	-	-	-	2.03	-	-
Seeds/pods (#/g)	-	141.8	-	-	-	94.4	-	-
Seed/pod weight (%)	-	33.6	-	-	-	31.2	-	-
TREATED PODS								
Pod height (cm)	42.5	0.425	0.065	15.3	59.5	0.595	0.065	10.9
Pod diameter (cm)	49.0	0.490	0.032	6.5	59.7	0.597	0.030	5.0
Pod weight (mg)	4055	40.6	10.5	25.9	7800	78.0	19.2	24.6
Pod moisture content (%)	-	5.0	-	-	-	5.3	-	-
Seeds/pod (#)	570	5.70	1.32	23.2	729	7.29	1.00	13.7
Seed weight (mg)	236	2.36	0.67	28.4	335	3.35	0.76	22.7
Seed moisture content (%)	-	2.05	-	-	-	2.10	-	-
Seeds/pods (#/g)	-	140.4	-	-	-	93.5	-	-
Seed/pod weight (%)	-	33.1	-	-	-	31.3	-	-

Table 6.5 Digestibility and ash content of Paraggio medic pods and standard diet components used in the sheep-feeding experiment.

Pods	Digestibility: Dry matter (%)	Organic matter (%)	Ash content: Whole sample input (%)	Undigested output (%)
Small - untreated	41.6	42.5	4.6	6.1
Small - treated	40.7	41.5	5.0	6.3
Large - untreated	36.8	37.5	4.0	5.1
Large - treated	37.9	40.1	3.9	5.3
Diet components				
Pellet	71.2	70.5	7.5	5.3
Oaten hay	64.6	62.2	10.6	7.2
Lucerne hay	76.2	75.3	9.4	6.2
Diet	70.5	69.5	8.1	5.6

Table 6.6 The survival of Paraggio medic seeds fed in whole pods to sheep.

Variable	Podsize-hardseededness			
	Small-untreated	Small-treated	Large-untreated	Large-treated
Total seed input (#/sheep)	28360	28080	18880	18700
Total soft seed input (#/sheep)	7515	21257	5060	12566
Total hard seed input (#/sheep)	20845	6823	13820	6134
TOTAL SEED OUTPUT (#/sheep)	1888	582	800	207
Viable seed output (%) that became or remain as:	98.9	98.5	98.6	99.5
Normal seedling	18.9	21.6	17.0	28.0
Abnormal seedling	0.2	1.3	0.3	0.5
Hard seed	79.8	75.6	81.3	71.0
Non viable seed output (%) from hard seed	1.1	1.5	1.4	0.5
Viable hard seed (%)	98.8	98.0	98.4	98.1
Hard seed weight (mg)	2.23	1.90	3.09	2.62
Viable seed output/total seed input (%) that became or remains as:	6.58	2.04	4.18	1.10
Seedling	1.27	0.47	0.74	0.31
Hard seed	5.31	1.57	3.44	0.79
Viable seed output/total hard seed input (%)	8.96	8.40	5.71	3.36

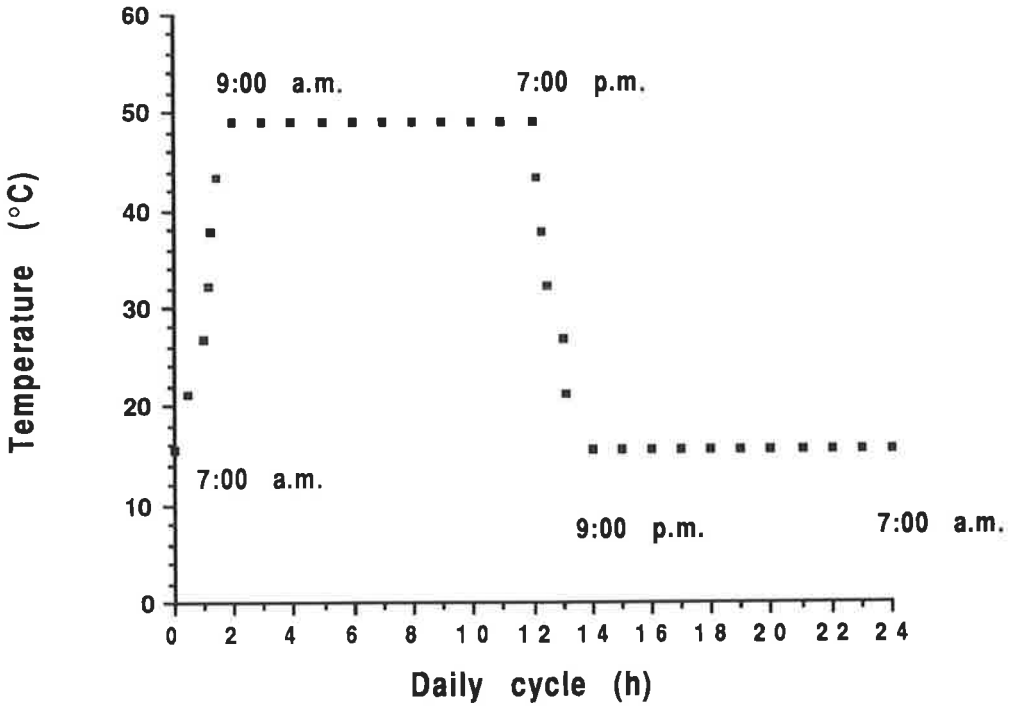


Figure 6.1 The daily alternating cycle of temperatures used to soften the Paraggio medic seeds in the incubator.

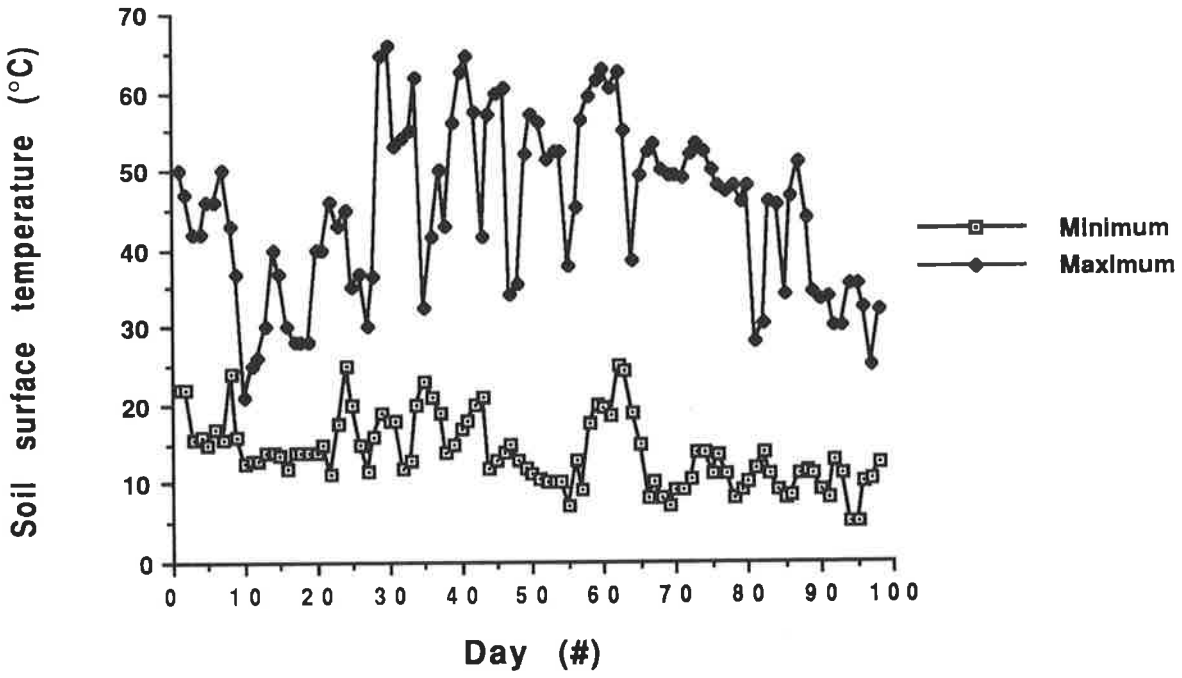


Figure 6.2 The minimum and maximum soil surface temperatures recorded during the period of softening of Paraggio medic seeds on the raised beds.

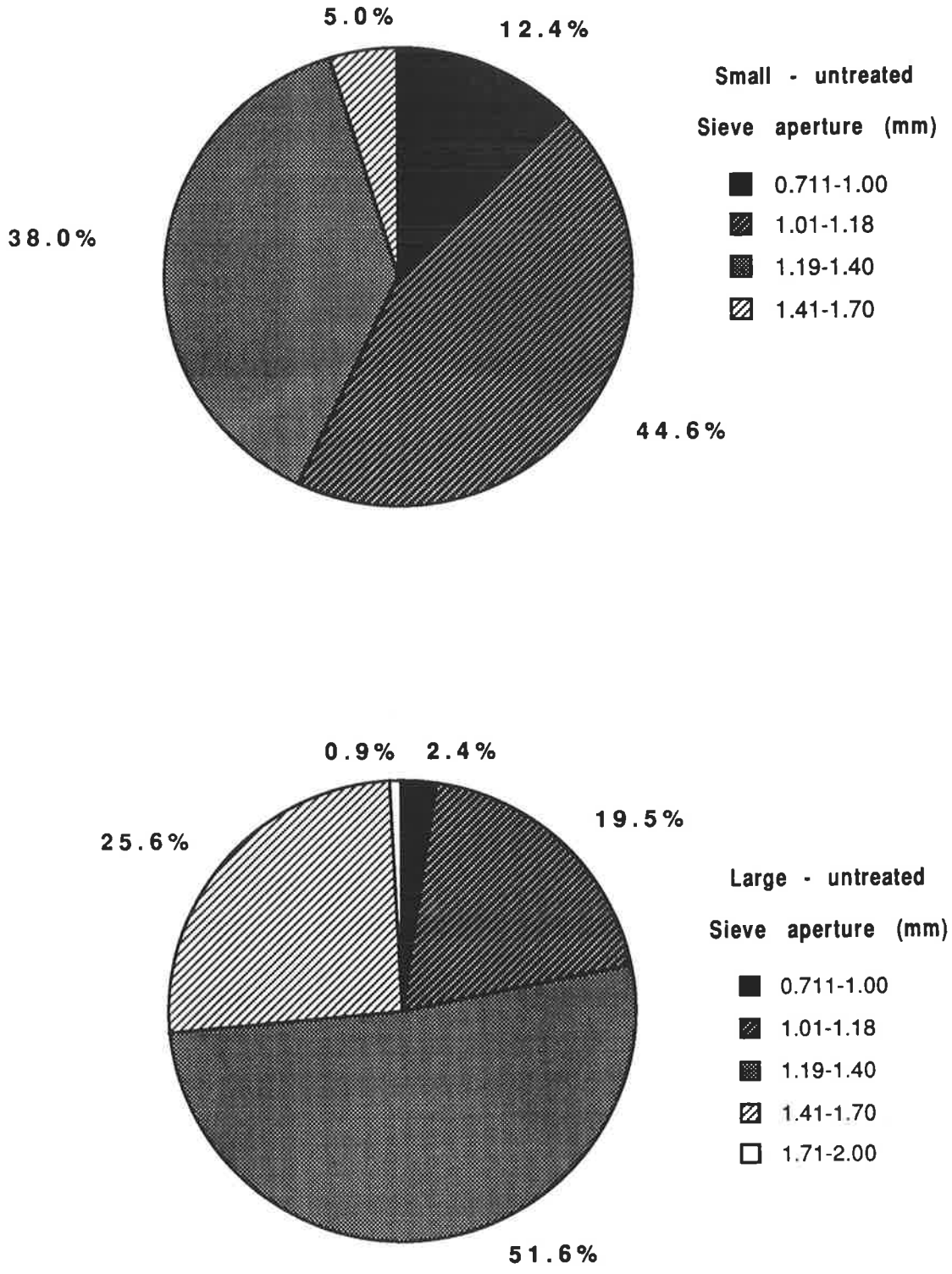


Figure 6.3 The distribution of seed by size in Paraggio medic pods comprising the small-untreated and the large-untreated populations.

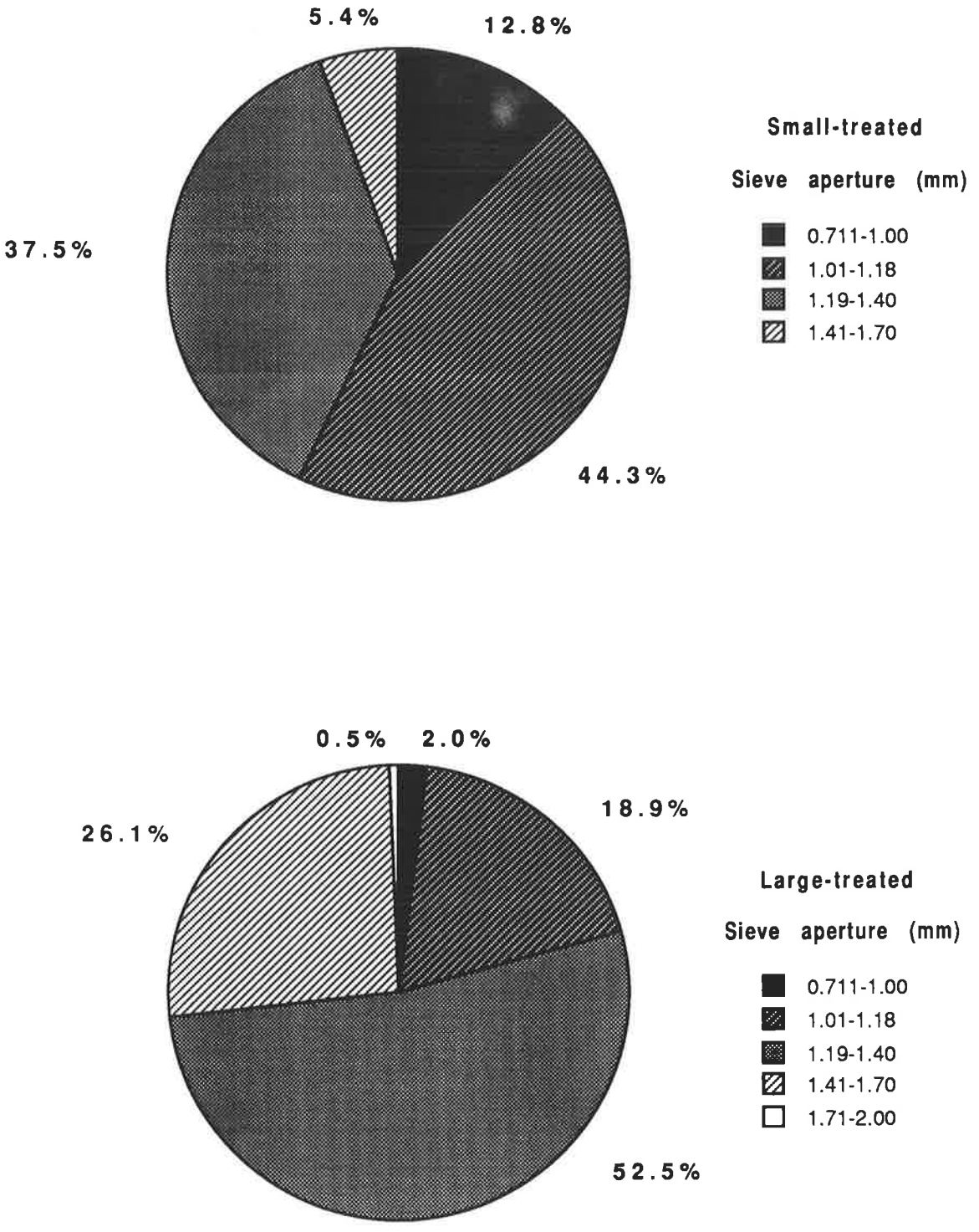


Figure 6.4 The distribution of seed by size in Paraggio medic pods comprising the small-treated and the large-treated populations.

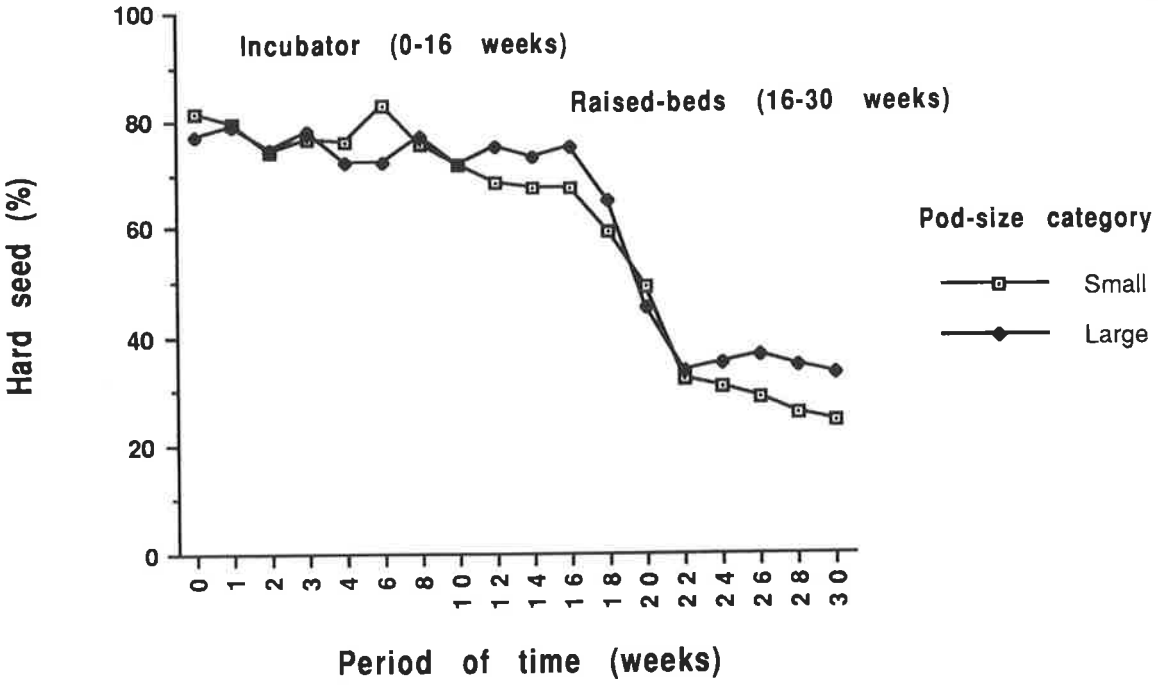


Figure 6.5 The decline in the level of hard-seededness of Paraggio medic seeds enclosed in pods used in the penned-sheep feeding experiment.

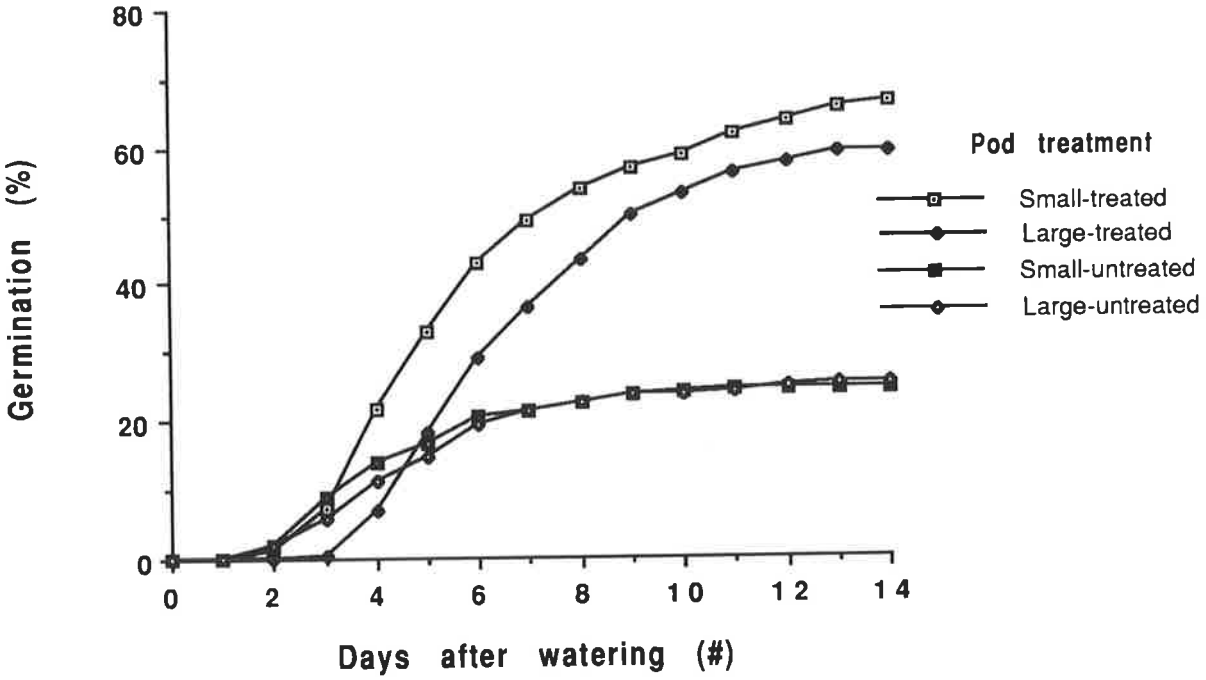


Figure 6.6 The mean cumulative germination of Paraggio medic seeds enclosed in pods used in the penned-sheep feeding experiment.

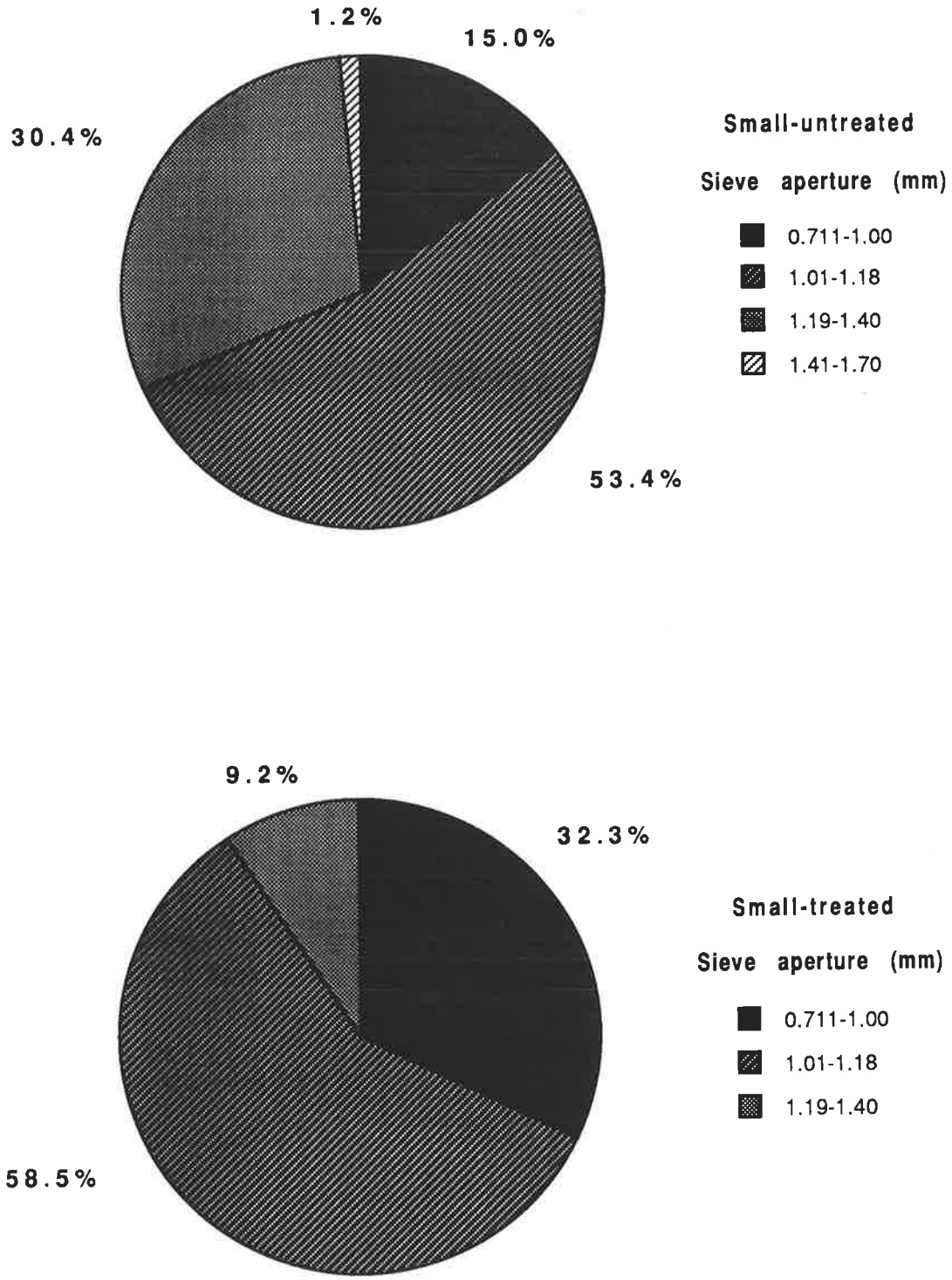


Figure 6.7 The distribution of hard seed by size in Paraggio medic pods after germination of seeds enclosed in small-untreated and small-treated pods.

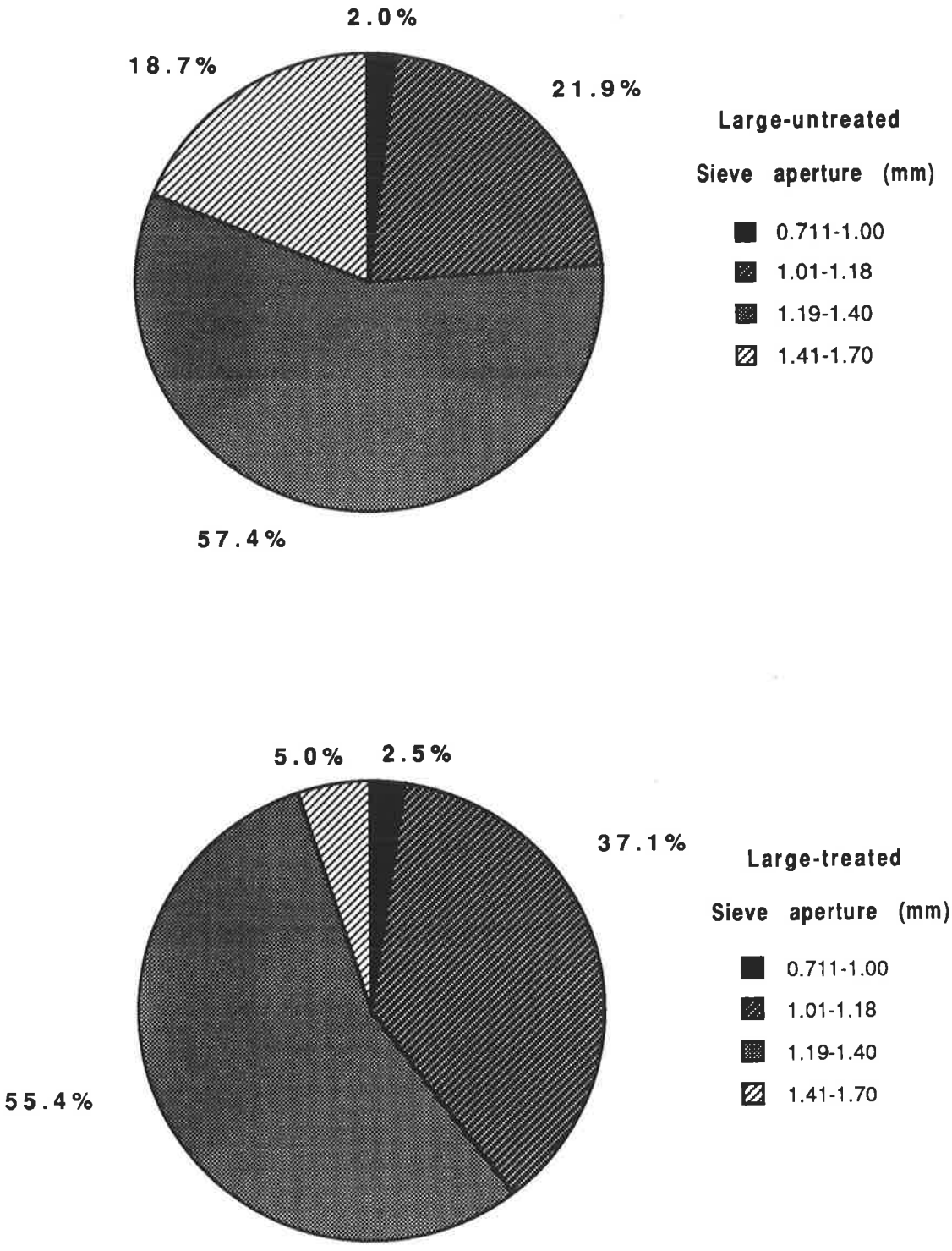


Figure 6.8 The distribution of hard seed by size in Paraggio medic pods after germination of seeds enclosed in large-untreated and large-treated pods.

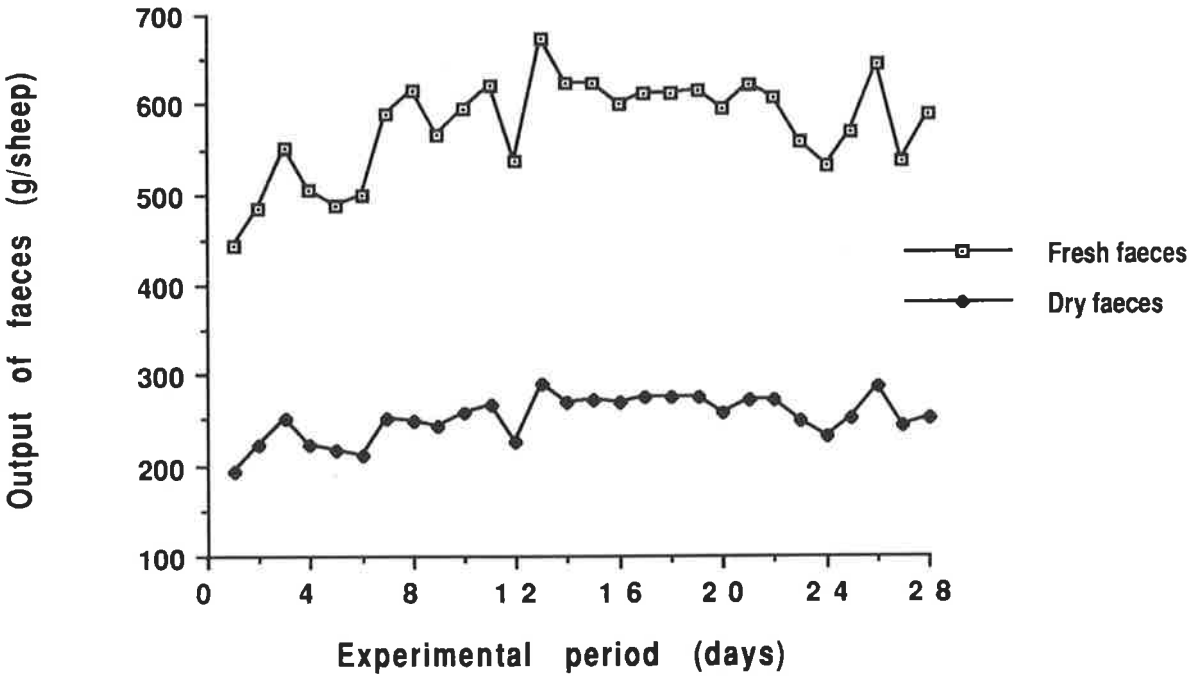


Figure 6.9 The mean daily output of faeces voided by sheep during the experimental period.

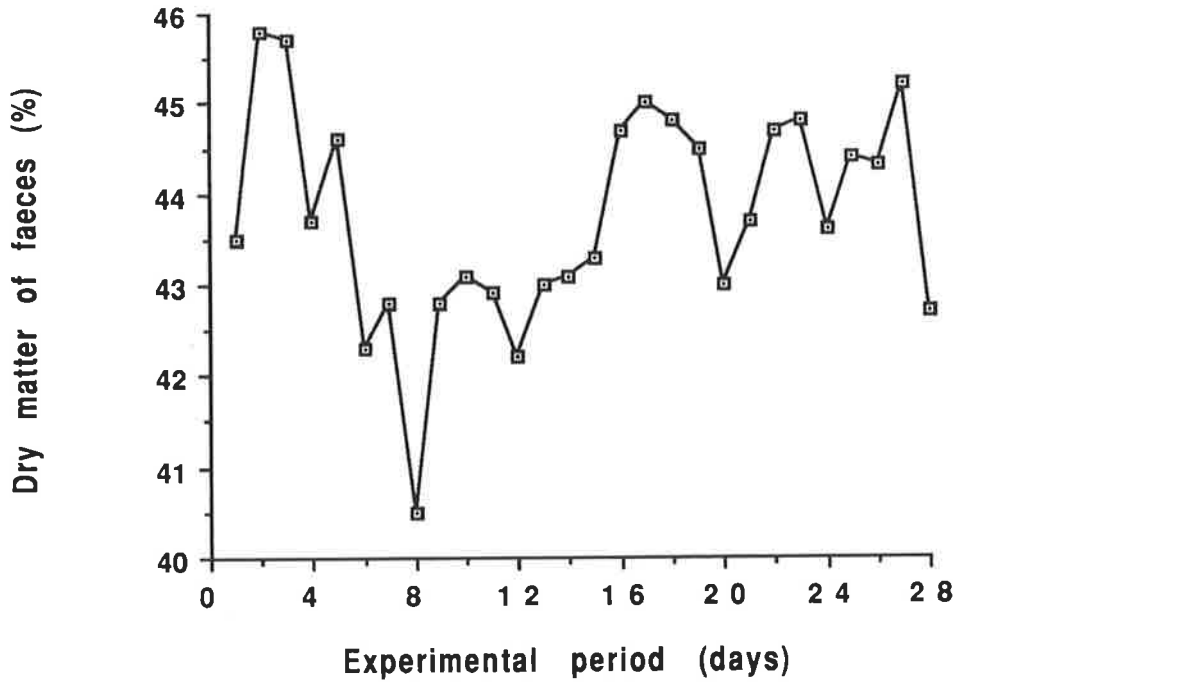


Figure 6.10 The mean daily content of dry matter of faeces voided by sheep during the experimental period.

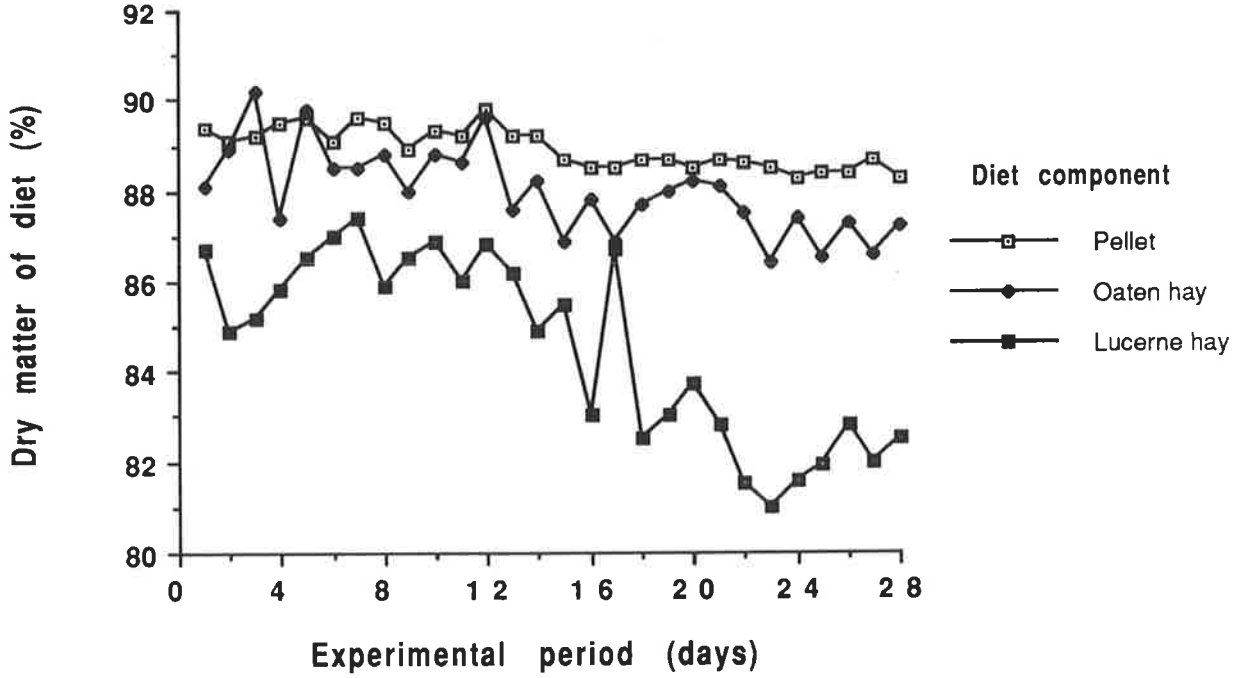


Figure 6.11 The daily content of dry matter of the diet components used in the experimental period.

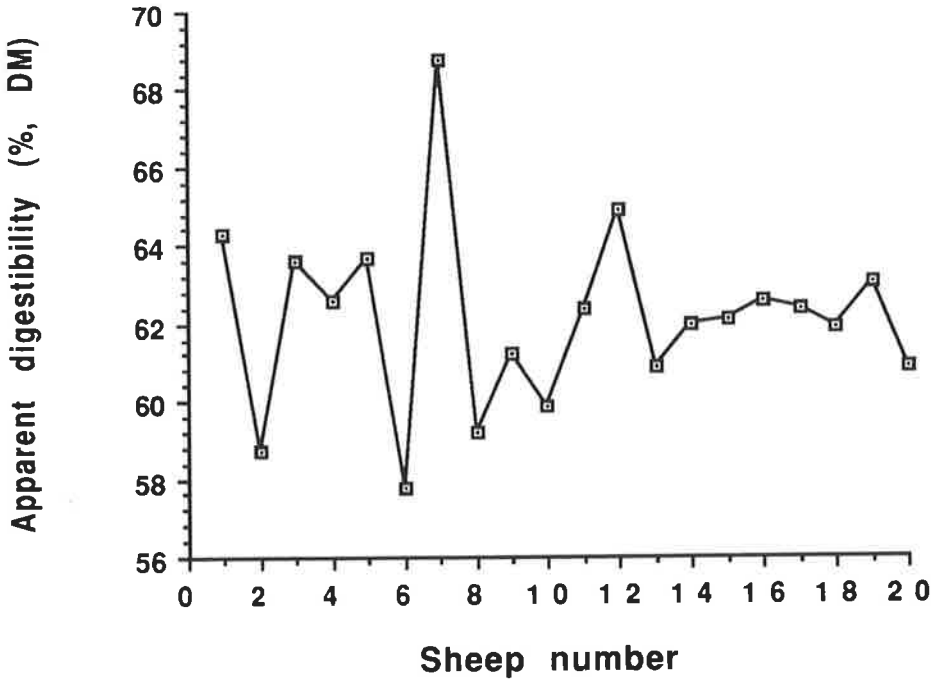


Figure 6.12 The changes in the apparent digestibility of the diet consumed by the sheep flock during the experimental period.

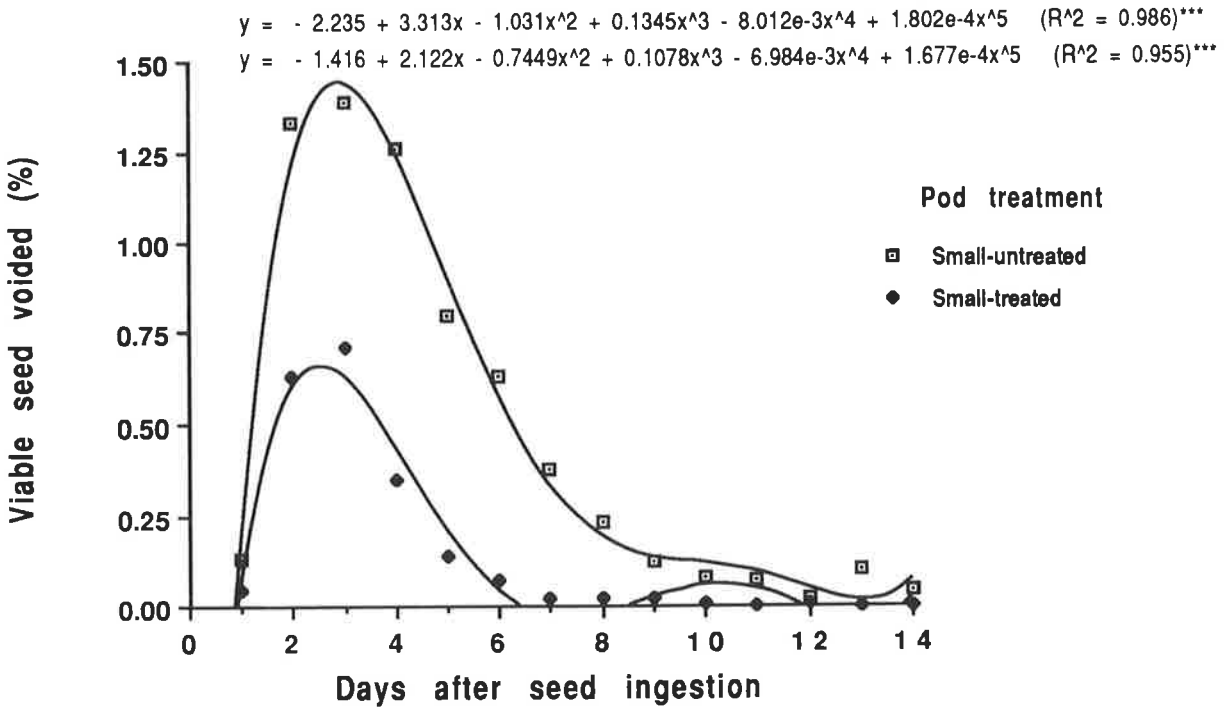


Figure 6.13 The daily output of viable seed voided in faeces by sheep fed with small-untreated and small-treated pods.

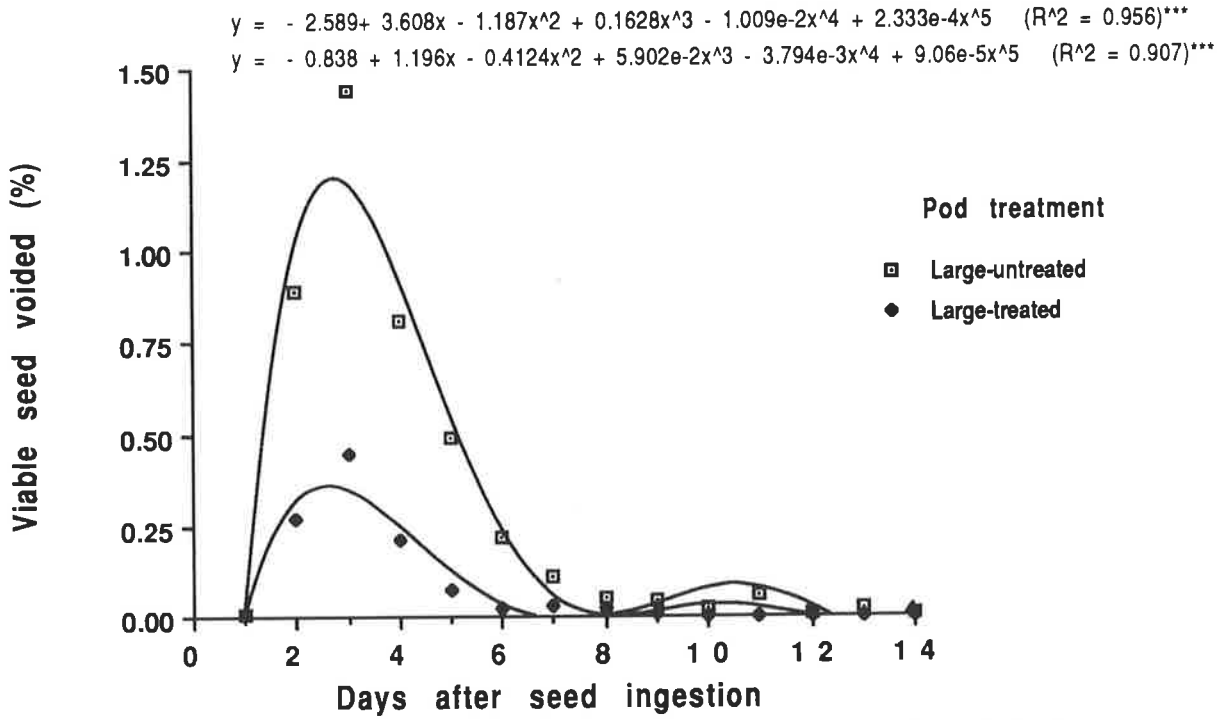


Figure 6.14 The daily output of viable seed voided in faeces by sheep fed with large-untreated and large-treated pods.

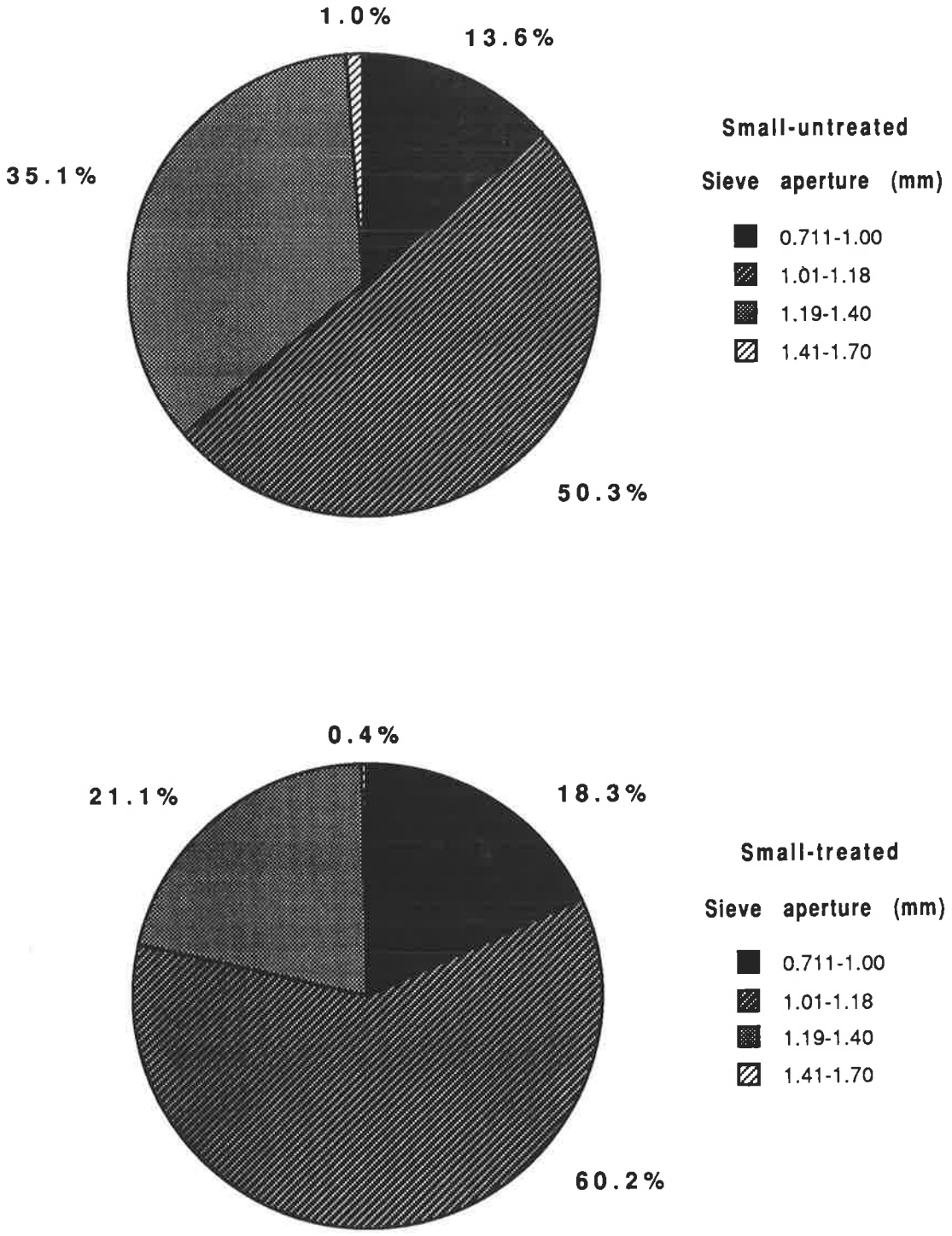


Figure 6.15 The distribution of hard seed by size voided in faeces by sheep fed with small-untreated and small-treated pods.

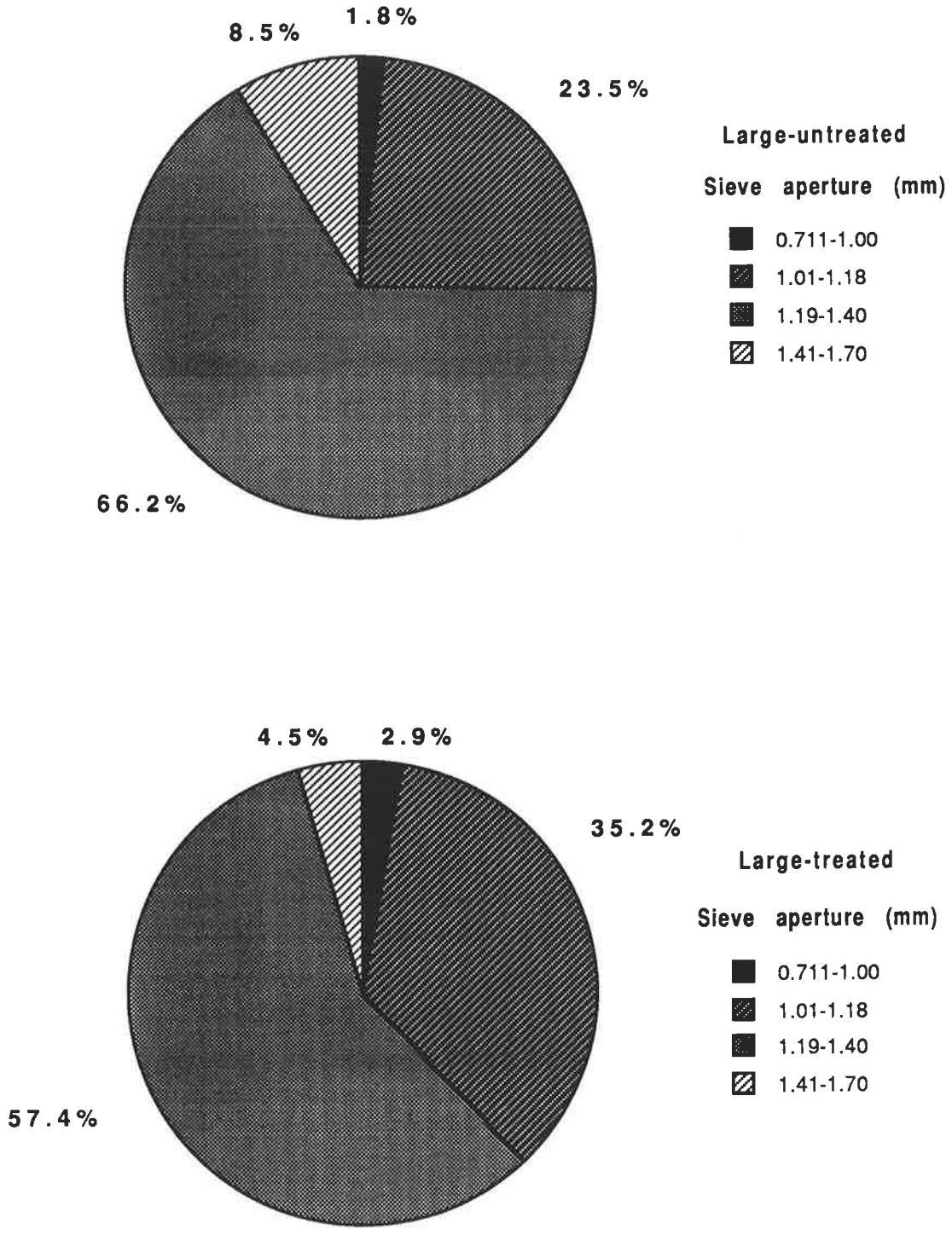
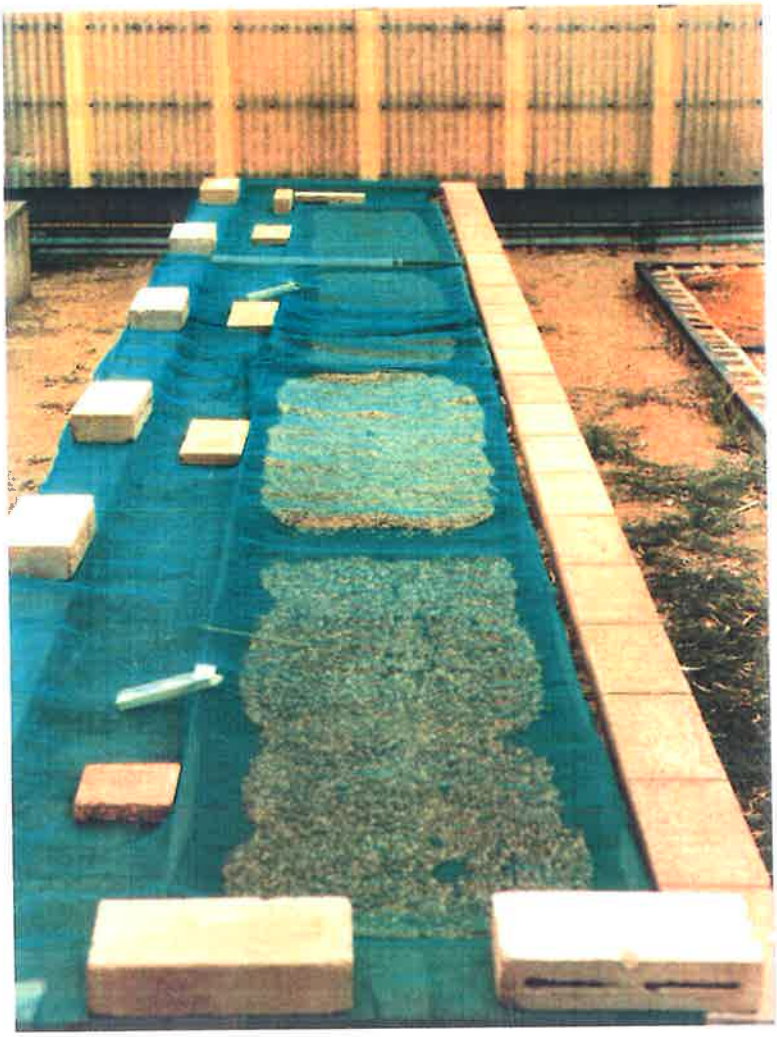


Figure 6.16 The distribution of hard seed by size voided in faeces by sheep fed with large-untreated and large-treated pods.

6.1 General view of the placement of Paraggio barrel medic pods on an exposed raised bed to allow the diurnal fluctuation in temperature to soften the seed over a period of 14 weeks (23 January, 1990 to 1 May, 1990).

6.2 General view of the sheep with faecal-collection harnesses used in the pen-feeding experiment (1st cycle).

Rear to front Sheep fed with pods	Left to right Sheep number
Small-untreated	1-5
Large-untreated	6-10
Small-treated	11-15
Large-treated	16-20




50
49
47
62
61
60

6.6 Discussion and conclusions of digestion and feeding experiments

The pen studies were conducted to examine the effects of ruminal digestion on survival of annual pasture seeds of clover and medic species. They clearly show that only hard seed, mainly associated with smaller-seeded species, is able to survive the digestion process in the rumen. Retention times as low as 12 hours were enough to cause severe and irreversible damage to the seed. The same response was found by Gardener *et al.* (1983) and Simao Neto and Jones (1987), working with tropical legume species using the *in sacco* technique in rumen-fistulated steers. Similarly, the results of Yamada and Kawaguchi (1971) who tested the survival of seed of temperate subterranean clover and ladino clover species containing higher levels of soft seed in the rumen of goats were confirmed. The low germination of some seeds, mainly the larger seeds of subterranean clover and snail medic was explained by a break down of hard seed in the rumen, that differed between species (Gardener *et al.* 1983).

Compared with tropical legume pasture species (Simao Neto and Jones 1987), the temperate clover and medic species tested in the present studies were more resistant to the effects of digestion in the rumen and were completely tolerant to further digestion in a simulated abomasum.

In the pen-feeding experiment, which was undertaken to test the survival of Paraggio medic seeds differing in the level of hard-seededness and seed size, the recovery of seed voided in faeces by sheep was highest on Day 3 and declined after Day 4. A similar trend has been reported by Yamada and Kawaguchi (1972) and St John-Sweeting and Morris (1990) for temperate pasture legume species also Simao Neto *et al.* (1987) for tropical pasture legume species.

A delay in the output of seed from the small pods compared to large ones, especially under untreated conditions, suggests that smaller, hard seed could be retained longer in the digestive tract of sheep. This is presumably after leaving the rumen through the

reticulo-omasal orifice, given the higher proportion of hard seed with a particle size below the limit of high relative resistance to passage from the rumen (Poppi *et al.* 1980 and 1985). According to Suckling (1952), most of the seed recovered from the digestive tract of slaughtered sheep fed with white clover seeds was found in hard condition and lodged in the omasum.

At the same time, the amount of seed recovered was significantly associated with the level of hard-seededness because, as reported before, only the hard seed escaped the negative influence of initial mastication and rumination. The small seeds in small pods, were less affected by the chewing effects than the more compact and hard larger pods. Jones and Carter (1989) reported that the lower recovery of medic seed after ingestion by sheep, is explained by the extent of the chewing process which breaks down the pods to a level suited to favor the evacuation of solid particles from the rumen.

Based on the dominant seed sizes recovered from sheep fed with small and large pods, it also seems possible that small and hard free seeds had shorter times of retention in the rumen. This would allow them to avoid the continuous process of regurgitation and softening. Thomson *et al.* (1987) stated that 80% of the variability in survival of pasture legume seed ingested by sheep was explained by the size of seed. Similar findings were reported by Carter *et al.* (1988) working with temperate legume pasture species and Simao Neto *et al.* (1987) testing tropical legume pasture species. In general, the passage of seed through the digestive tract of sheep increases with decreasing seed size (Playne 1969) and increasing levels of hard-seededness (Jones and Carter 1989).

The germination percentages of seed recovered in faeces was lower than germination recorded before feeding and was related to the levels of hard-seededness originally in each pod-size category. If all or most of the soft seed that becomes non-viable in the rumen, according to the *in sacco* digestion studies reported previously, have disintegrated (Simao Neto and Jones 1987), it is reasonable to presume that the seed

which leaves the digestive tract has an increased percentage of seed coat impermeability and is less able to germinate.

Higher percentages of germination were obtained after passage of seed originally enclosed in treated pods, especially large ones, compared with untreated pods, suggesting that hard seed enclosed in treated pods, reached more advanced stages of softening. When subjected to digestion these seeds became more susceptible to the natural process of breakdown of seed coat impermeability. This also may explain the higher percentages of emergence of abnormal seedlings from these seeds.

The results of these studies indicate the resistance of hard seed of clover and medic pasture species and the susceptibility of soft seed to digestion in the rumen. Much of the damage to the hard seed ingested by sheep must occur during the chewing process. It is at this level, that the size of the seed is a more relevant factor in seed survival. Also, but to a lesser extent, the lower resistance of smaller seed to passage from the rumen, reduces the change in the permeability of seed coat favouring the survival of seed. However, there is some evidence that smaller seed, after leaving the rumen, could be retained longer than larger seeds, reducing survival, because of digestion or lack of persistence after the faeces are dropped.

It is quite clear, that the use of pasture species with small seed and high rates of survival will ensure a better persistence of seed under grazing (Thomson *et al.* 1987). This is an important factor to be considered, especially when some pasture species (e.g. Paraggio medic) have been selected and developed to give a progressive breakdown of hard-seededness between the stage of seed set and the time when an effective rainfall occurrs (Reed *et al.* 1989). With such a cultivar the risk of over-grazing during the summer-autumn period is higher. This can be difficult to avoid, especially when there are severe shortages of feed due to delays in the opening of the growing season. However, if there are known to be good seed reserves in the surface soil there is no harm in utilizing medic pod or clover burr reserves if there are urgent feed needs. In this respect it is important

for the farmer to be able to predict in advance the probable emergence of medics or clovers as can be done by the method of Carter *et al.* (1989 b) and Carter and Porter (1992).

7 GENERAL DISCUSSION AND CONCLUSIONS

7 GENERAL DISCUSSION AND CONCLUSIONS

7.1 Introduction

This chapter summarises and interprets the results and discussion sections of the thesis, together with proposals for future research, under the following headings:

* The influence of seed size and environmental factors on the germination of annual pasture legume seeds (Section **1**).
* The influence of seed size and environmental and management factors on the emergence and early growth of medic and subterranean clover seedlings (Section **2**).
* The effect of seed size on seed survival under the influence of sheep (Sections **3** and **4**).
* Future research which could be useful in improving the persistence of annual legume pastures in livestock production systems.

7.2 The influence of seed size and environmental factors on germination of annual pasture legume seeds

Despite its importance, the trend in the allocation of dry matter to different seed components, with changes in seed size in pasture legume species has received little attention, when precise relationships between seed size, and germination (McWilliam *et al.* 1970), emergence (Black 1956) and early seedling growth (Taylor 1972) are required. Sava snail medic and Trikkala subterranean clover produce seeds in which the embryonic component (cotyledons + embryonic axis) increases with seed size (3.2 Laboratory Test 1). However, the embryonic axis and the seed coat component decrease with increases in seed size. Both of these seed components positively influence the water-absorption capacity of germinating seeds (Stiles 1948; Calero *et al.* 1981).

The germination of a seed under a wide range of environmental conditions is a major determinant of success in the natural regeneration of an annual legume pasture under a Mediterranean-type climate. Small differences in the ability of a seed to germinate may influence the success or failure of an individual in a population, or a group of individuals in a community of emerged seedlings of annual pasture species (Cocks and Donald 1973).

It is apparent that Sava snail medic and Trikkala subterranean clover have different temperature requirements for germination (3.3 Laboratory Experiment 1). Final germination of Sava occurred in a broader optimum range of temperatures (2.1°C to 33.6°C), but only at temperatures between 6.4°C to 30.1 was germination relatively unaffected. At 336°C germination was reduced, whilst at 38.1°C germination was completely arrested. Trikkala seeds germinated between 2.5 °C and 32.9°C and final germination percentages obtained between 6.8°C and 23.8°C were unaffected by temperature . At 26.5°C germination became very sensitive to temperature, while at higher temperatures germination was almost completely arrested and ceased at 37.9°C. Trikkala showed greater sensitivity to higher temperatures than Sava, but greater tolerance to lower temperatures. The ecological significance of these temperature requirements has important practical implications, because germination is restricted or delayed under certain conditions, because temperature also determines the soil depth from which emergence may occur, since diurnal change in temperature decreases sharply with depth (Cocks and Donald 1973).

Although germination in both species is clearly temperature-dependent, the effect of seed size is only significant in Sava. The rate of germination of Sava tended to decline with increased seed size but this effect was not evident with Trikkala. This is an important trait associated with plant competition, especially under mixed pasture swards where species from seeds germinating more rapidly tend to became dominant, compared with species from seeds that germinate more slowly (Black and Wilkinson 1963).

Both species showed two phases of water uptake during the 24-hour testing period (3.4 Laboratory Experiment 2). The initial phase, regarded as a physical process, is characterized by a rapid hydration due to matric forces at the seed level. The second phase is the lag period of water uptake represented by an almost steady state situation, in which the ability of the seed matrices to be hydrated and bind water decreases. At this stage, the most important metabolic processes commence in preparation for radicle emergence (Bewley and Black 1978). The length of the imbibitional phase was significantly temperature- and seed-size-dependent for both Sava snail medic and Trikkala subterranean clover. In Sava at the highest temperature, virtually all the water required to initiate germination was imbibed in the first five hours for the smallest seed size, and in eight hours for the largest seed size. In contrast, at the lowest temperature none of the seed-size categories, with the apparent exception of the smallest seed size, completed the initial phase of imbibition even after twenty four hours of testing. Under the optimum temperature for germination (19.6°C), the time required by the seed to imbibe most of the water sufficient to start the visible process of germination was about eight hours for all seed-size categories, although there were differences in the total amount of water imbibed. When Trikkala subterranean clover imbibed at the highest temperatures, almost all the water required to initiate the growth of the radicle in all seed-size categories was taken up in the first five hours. However, at the lowest temperature, the smallest and the largest seed-size categories required about nine and twelve hours, respectively. Under the optimum temperature for germination, the imbibition phase lasted about eight hours for all seed-size categories. These studies suggest that if small seed is able to imbibe water at higher rates than large seed, especially under low (unfavourable) temperatures, an early approach to the lag phase of water uptake will facilitate earlier elongation of the radicle and seedling emergence. This should enhance the competitiveness of seedlings from small seeds in a dense pasture sward and is of special ecological significance.

Seeds adapted to the Mediterranean environment in southern Australia, normally germinate and become established after the first effective rain that falls at any time

between April and June. Summer rains followed by cool weather, also result in an early germination of non-dormant seeds (Puckridge and French 1983). Furthermore, germinating rains may also be preceded by rains that are inadequate to ensure germination, but are sufficient to keep the seed wet for a period sufficient to stimulate the germination process. These rains may also be followed by dry periods long enough to cause the desiccation of seed (Cocks 1969). Under these circumstances, the ecological significance of the effect of soaking and drying on the seed of annual pasture species has differing implications that vary according to the extent and frequency of these wetting-drying conditions. The seeds of Sava snail medic and Trikkala subterranean clover started to germinate within the first 12 hours only when wetting periods of at least eight and six hours were pre-imposed respectively (3.5 Laboratory Experiment 3). Final germination was unaffected by the pre-wetting periods, resulting in higher values of germination independent of the species, as well as of the seed-size categories used. The drying treatment imposed on the seed after various periods of pre-wetting was coincident with the desiccation-tolerant state of the embryo, in which the rate of germination is increased and the viability of the seed is unaffected by longer periods of soaking prior to dehydration (McKersie and Tomes 1980). The increased rates of germination were a consequence of the initiation of physiological processes for germination during the soaking period; the subsequent drying of the seed does not alter the metabolic stage achieved (Bewley and Black 1982). The highest rates of germination were associated with smaller seed sizes and were more apparent in Sava snail medic. This should represent an additional advantage for an early germination of the seed at the time of subsequent germination if rains are not followed by a resumption of drought conditions. It is postulated that dehydration after soaking also confers drought-resistance without interfering with germination (Henckel 1964). Both factors may have practical implications in governing germination, emergence and establishment of annual pasture legume species.

Differences in the ability to germinate at lower osmotic potentials were found between Sava snail medic and Trikkala subterranean clover (3.6 Laboratory Experiment 4). The clover seeds showed higher tolerance to lower water potentials and germinated better under slightly reduced water potential of -2 bars than under the control treatment. Differences in the rate of germination observed between seed-size categories of both species also revealed that small seeds have greater tolerance to moisture stress, and suggest that under natural conditions a better seedling emergence should be obtained.

7.3 The influence of seed size and environmental and management factors on the emergence and early growth of medic and subterranean clover seedlings

The critical problem of self-regenerating annual legume pastures is frequently associated with regeneration rather than establishment. The adoption of more-frequent cropping practices, or a shorter pasture phase in the rotations, are factors influencing the persistence of legumes in pasture-crop systems (Wheeler 1987).

The emergence and early vigour of Sava snail medic and Trikkala subterranean clover seedlings were examined (4.2 Glasshouse Experiment 1). The rate of emergence and the final emergence of seedlings were influenced by all factors included in this study (seed size, depth of sowing and soil texture). A positive relationship between embryo weight and emergence was observed, confirming that seed size in these epigeal pasture species limits the potential elongation of the hypocotyl and consequently the ability of seedlings to emerge from the soil (Black 1956). While the emergence force induced by an emerging seedling is positively correlated with the weight of the seed (Williams 1956), the presence of larger cotyledons, such as in Sava snail medic, may reduce or retard emergence (Moore 1943; Carter and Challis 1987). There was also evidence that seedlings from small seeds have higher rates of emergence than seedlings from large seeds under non-constrained situations. The reason for this may be in the ability of small seed to germinate faster than large seed, providing an opportunity for earlier

emergence (3.3-3.6 Laboratory Experiments 1-4) and possibly better seedling establishment.

A direct relationship between seed weight and seedling vigour was also observed during the early autotrophic growth phase of seedlings. This relationship suggests that competition between seedlings was not operating at that stage of plant growth and consequently all seedlings, irrespective of seed size, were still performing with increased rates of growth (Black 1959).

The effect of concentrations of wheat straw residues on emergence and early growth of snail medic seedlings was also studied (4.3 Glasshouse Experiment 2). This additional impediment to seedling emergence found in pasture-crop farming systems has been reported to cause a 16% reduction in dry matter production by annual medic swards (Quigley 1988).

It was noted as before (4.2 Glasshouse Experiment 1) that seedlings produced by small seeds have higher initial rates of emergence than seedlings produced by large seeds. Only when constraining the seedlings by placing pods in a deeper position in the soil, or through increased levels of straw mulch, were there higher rates of seedling emergence from larger pods (containing large seed) than from small pods containing small seed.

The physical barrier imposed on the hypocotyl by the cereal straw residues was also responsible for a reduction in the potential seedling development by affecting the rate of photosynthesis (Robbins *et al.* 1942). Quigley (1988) reported that physical impedance produced by cereal straw *per se* has minor influence on seedling emergence. However, more stressful conditions used in the present study, demonstrates that wheat straw residues above 6 ton DM/ha may be detrimental to seedling emergence and early growth. In common crop-pasture ley-farming systems, normal concentrations of straw residues, range between 5 and 9 t DM/ha. Over the summer-autumn period, residues in the concentrated straw rows become a dense and compact mulch due to trampling by livestock and weathering (Quigley 1988). However, there are some positive effects of

straw up to 4 t DM/ha. This may be due to better micro-environmental conditions produced by the presence of wheat stubble at the soil surface (Aston and Fisher 1986).

It appears that seed size determines the depth from which a seedling can emerge in a particular soil (Black 1955, 1956; Carter and Challis 1987) and influences the initial growth of seedlings (Black 1957a; Williams *et al.* 1968). Under natural conditions, the optimum depth of placement of seed in a specific soil type will not necessarily ensure optimum emergence and early growth of seedlings. Root development is generally correlated with seed weight, and the deepest root penetration occurs at the greatest sowing depth from which a seedling can emerge (Tadmor and Cohen 1968). This has important practical implications, especially when rapid drying of the seedbed layers occurs frequently. Thus, a seedling from a large seed is better suited than a seedling from a small seed to survive or become retarded in growth at that stage of plant development: this may have critical implications on subsequent growth (Black 1955).

In Mediterranean-type climates the availability of forage in rainfed livestock production systems is normally not a limiting factor in spring and summer. However, a frequent combination of low availability and poor nutritive value of forage occurs in autumn and low availability in winter when feed is the critical determinant of animal production and year-round carrying capacity (Purser *et al.* 1987).

It is well recognized that a large seed bank is one of the major requirements to ensure a productive legume pasture sward. For early vegetative growth, there is a positive linear relationship between plant density and yield. The great practical value of a dense sward compared with a sparse one lies in the early production of forage (Donald 1951). Autumn and winter green feed is usually the main determinant of profitable grazing enterprises.

The effects of seed size and plant density on potential forage production during the early vegetative growth stage of Sava snail medic and Trikkala subterranean clover swards were examined (4.4 Raised-bed Experiment 1). The studies show that early production

of a pasture sward depends on the magnitude of embryo weight per unit area of ground (Black 1957a). This relationship can be managed for practical applications either by modifying seed size or plant density (Black 1957b).

Both factors positively influence the leaf area available for the initiation of the autotrophic stage of plant development. Larger seeds produce large cotyledons that expand earlier than those produced by smaller seeds. This allows, an earlier onset of leaf production and an earlier leaf development. All are important factors determining the initial growth of seedlings (Machado *et al.* 1974; Hur and Nelson 1985).

It has been accepted that competition between plants occurs for environmental factors such as water, nutrients and light. These are of main significance in natural plant communities, as well as under agricultural systems (Donald and Black 1958). In the present studies, water and nutrients were not limiting factors in the growth of pasture swards. Therefore, the ultimate capacity of the species to produce dry matter was dependent on the ability of plant canopies of such species to utilize the incident light (Donald 1951).

The onset of inter-plant competition at a critical Leaf Area Index (LAI), was characterized by a decrease in the individual growth rate of plants followed by a reduction of density due to plant mortality. It was initiated earlier at the highest plant density and subsequently appears over time with more advanced stages of plant development at the lower plant densities (Donald 1951).

This situation has important practical implications for grazing management practices which must aim to maximize forage production, especially in winter, rather than to maximize the LAI of the pasture swards. The results of the experiments in this thesis reveal that only at higher plant densities and larger seed sizes is it possible to achieve adequate early pasture availability (e.g. 1200-1600 kg DM/ha) above which no further limitation on sheep growth is expected (Willoughby 1959; McFarlane 1965; Arnold and Dudzinski 1967). At farm level where grazing systems occur, this is of great

significance because the importance of possible losses of pasture productivity through having a LAI above the optimum is minor compared with low levels of LAI that occur through inadequate pasture density. According to Carter and Day (1970) and Smith *et al.* (1972) a maximum forage utilization by a fixed density of grazing animals occurred when the availability of forage was about 1000 kg DM/ha. However, this amount resulted in a low LAI between 1 and 3 and forage yields of only 20-70% of the theoretical potential (Cocks 1974).

7.4 The effects of seed size on seed survival under the influence of sheep

Jones and Carter (1989) proposed that a greater use of legume mixtures combining several cultivars and/or species is a preferred alternative to mono-specific swards. A variety of pasture genetic resources minimises the adverse effects of environmental factors and management on the persistence of a pasture. This strategy combines diverse and efficient plant traits, including seed size, which should have important ecological implications for the survival of the seed under grazing.

The use of high livestock stocking densities during the summer-autumn period is a common practice on many farms using annual legume pastures in ley-cereal rotations, as well as permanent pastures. Poor nutritive value of grassy pastures on offer in summer and the low availability of forage in autumn are the major constraints to animal production (Purser *et al.* 1987). Where the farmer has some good legume pastures these are frequently overgrazed in summer and autumn because of the vastly superior nutritive value of dry legume residues, together with pods of annual medics or burrs of subterranean clover.

The analysis of this part of the discussion will be based on the summer-autumn grazing study (5.2 Field Experiment 1) which was designed to examine the survival of subterranean clover, balansa clover and cluster clover seeds. However, when appropiate, other studies will be considered. It was shown that the ability of sheep to

defoliate selected plant parts in order to improve the nutritive value of their diet was related to the dry forage on offer. The results show a marked early selection by sheep of small-seeded balansa clover and cluster clover heads compared with subterranean clover burrs. Differences in accessibility (Black and Kenney 1984; Kenney and Black 1987) and acceptability (Arnold 1981; Black 1987) both affect the selection of plant structures by sheep.

These differences in accessibility and acceptability may have practical implications for management practices used for colonization of fields where normal cultivation procedures are not possible, or are not recommended, or for recolonization of previously-sown areas where the legume component of the pasture has been partially or completely lost. The probability of successful dissemination is increased if the legumes have high hard seed content and small seed (Simao Neto *et al.* 1987). However, the survival of seed ingested by sheep is also positively correlated with the quality of the diet (Jones and Simao Neto 1987). The marked selection by sheep of balansa clover heads during the early stages of grazing (5.2 Field Experiment 1) may have reduced seed survival and explain the lower recovery of seed compared to that obtained by Carter *et al.* (1988a) in pen-feeding experiments, in which the seed of balansa clover was fed to sheep with a high-quality companion diet.

Further changes in the proportion of plant components during the grazing period are attributed to the progressive reduction in the scope for selection. This also reflects the efficiency of sheep in consuming the subterranean clover burrs, including the buried ones. Similar trends in the selection by sheep of reproductive structures have been reported by Carter (1981) and de Koning (1990) with grazed dry medic and subterranean clover pastures.

Legume species have different mechanisms that permit them to protect the seed from the grazing livestock. An example is the burr-burial (grazing escape) mechanism of subterranean clover (Katznelson and Morley 1965). However, on hard-setting soils, cultivars that are not efficient in burr burial or set more seed above ground are more

likely to lose seed through the summer-autumn period due to grazing (de Koning 1990). This was clearly represented in this thesis study in which 94.4% of the subterranean clover burrs were set above ground and most of the seed was retained in burrs until ingested during the grazing period. In contrast, negligible changes in the subterranean clover seed reserves occurred during the summer-autumn period, when more efficient burr-burial cultivars grew in a sandy-loam textured soil (5.3 Field Survey 1) at Parawa. Grazing during seed set positively affected the ability of subterranean clover to bury burrs by forcing changes in growth habit in response to defoliation (Broom and Arnold 1986).

Another important seed-protective mechanism in small-seeded clovers is the natural shattering of the seed heads after ripening. It was demonstrated that small clover seeds lying at the soil surface were almost completely conserved through the grazing period. In contrast, those seeds enclosed in heads were completely grazed by sheep. The disadvantage of early selection by sheep of seed enclosed in heads may be reduced by using management practices that involve deferment of grazing until shattering of seed heads. Furthermore, the use of larger livestock, e.g. cattle and horses which are less selective and more efficient in trampling, may help to conserve these legume seeds.

However, concentration of seed at the soil surface leads to a rapid accumulation and storage of seed by harvester ants. It has been demonstrated that the presence of grazing sheep, especially under heavily-stocked situations, favours the increase of ant populations in pastures (Hutchinson and King 1980). In the Waite Institute studies about 10% of the balansa clover seed produced in the pasture mixture was taken by ants in a relatively short time. This may also explain the disappearance of cluster clover seed from the grazing area during the summer-autumn period at Parawa (5.3 Field Survey 1). The ease of accessibility of seed at the surface of the soil, and the small seed size relative to the ant (Culver 1972; Briese 1974) seem to be the main factors governing the selection of small-seeded clovers compared with the heavier subterranean clover seeds (McGowan 1969) which are mainly enclosed in inaccessible burrs. Consequently, a

large number of small ants collect many small, easily-carried seeds. This is probably the most efficient strategy for the ants to cope with the food requirements of the colony (Shoener 1969). The results suggest that some attention should be given to this topic in the future, because of its likely biological and economical impact in grazed pasture ecosystems.

Furthermore, the results (5.2 Field Experiment 1) reveal the initial selection by sheep of heavier heads and burrs containing a higher number of heavier seeds. This is probably the most efficient strategy for sheep to cope with their own nutritional requirements. Similar trends were observed by Carter (1981) and de Koning (1990) with dry medic and subterranean clover residues grazed by sheep. However, in the present study sheep were obliged to eat almost all the available forage. This caused the formerly-buried, heavier burrs to become dislodged by trampling and available for grazing by the end of the grazing period. Consequently, there was a progressive decline in the availability of dry pasture residues, leading to the dominance of seed of the small-seeded species in the grazing area.

The progressive removal of dry pasture residues also improved the exposure of subterranean clover burrs and small-seeded clover seeds to the natural variation of temperatures, increasing the rate of hard seed breakdown (Quinlivan 1965; Taylor *et al.* 1984). The same response was also observed at Parawa (5.3 Field Survey 1). In contrast, de Koning (1990) found that increases in germination over a short-term during the summer-autumn period were due to a breakdown of physiological dormancy rather than to a breakdown of hard-seededness. Insufficient exposure of subterranean clover burrs to enviromental conditions may explain this contradictory response: de Koning found that there were 2559 kg DM/ha of dry residues (mulch) at the end of the experiment.

These findings have important practical implications for the persistence of annual pasture legumes under grazing. Pasture management practices are required to ensure that dry pasture residues are reduced to avoid suppression of emergence, yet conserving an

adequate seed bank with proper levels of germinability before the opening of the rainfall season. This allows the establishment of a high plant density sward favouring the early production of forage (4.4 Raised-bed Experiment 1).

The need to ensure an adequate seed bank is especially important for larger seeds of subterranean clover grown on hard-setting soils. They are more susceptible to grazing than small-seeded clovers, also less able to survive the passage through the digestive tract of sheep. Thomson *et al.* (1987), stated that 80% of the variability in survival of pasture legume seeds ingested by sheep is explained by the size of the seed. In general, the passage of seed through the digestive tract of sheep increases with decreasing seed size (Playne 1969) and increasing levels of hard-seededness (Jones and Carter 1989).

Similar responses were obtained in this Waite Institute study. The early preference of sheep for small-seeded clover heads and subterranean clover burrs later, was clearly reflected in the level of seed recovered in faeces through the grazing period. However, the persistence in excretion of seed over time suggests a longer retention period for small, hard seed in the digestive tract of sheep and/or collection of seed from the ground. There was evidence from results obtained by feeding *Medicago truncatula* cv. Paraggio pods selected by seed size and level of hard-seededness to sheep (6.5 Pen Experiment 4) to suggest that small seed tends to be retained longer in the digestive tract of sheep than large seed.

It has been suggested that only hard seed can survive chewing and the subsequent effects of the digestive tract in cattle (Simao Neto and Jones 1987) and goats (Yamada and Kawaguchi 1971). There was evidence (6.2-6.5 Pen Experiments 1-4) to suggest that chewing is the most important factor influencing the survival of seed ingested by sheep. The hard-seeded fraction of the Mediterranean pasture legume seeds tested are highly resistant to the digestion process in the rumen and the acid digestion conditions of the abomasum, compared with tropical legume species (Simao Neto and Jones 1978). In contrast, soft seed is highly susceptible and is completely digested by the rumination

process. Only hard seed that escapes chewing is able to retain viability through the digestive tract of sheep. There is also evidence that the recovery of seed from sheep and goats is affected by the length of the seed (Simao Neto et al. 1987) and the effect of passage through the animal may be dissimilar for seed that is enclosed in pods (Simao Neto and Jones 1987). Small seed enclosed in small pods better survive the chewing effect and passage through the digestive tract of sheep compared with large seed enclosed in large pods (6.5 Pen Experiment 4). Jones and Carter (1989), reported that the lower recovery of medic seed after ingestion by sheep is mostly explained by the extent of the chewing process. The breakdown of pods is a critical pre-requisite to ensure the evacuation of solid particles from the rumen. Thus more small, hard seed is expected to survive the chewing effect.

It is reasonable to assume that the persistence in excretion of small-seeded clovers (5.2 Field Experiment 1) is due to a longer time of retention of seed in the digestive tract of sheep after it leaves the rumen through the reticulo-omasal orifice. According to Suckling (1952), most of the seed recovered from the digestive tract of slaughtered sheep fed with white clover seeds was found in hard condition and lodged in the folds of the omasum.

Findings by Simao Neto and Jones (1986) indicated loss of seed viability in faeces, suggesting that some additional damage to the viability of seed occurred during passage through the intestine and after defaecation (Simao Neto and Jones 1987). While some seed mortality is expected after the seed ultimately leaves the rumen, there is also a natural softening of some hard seed. The increase in the relative number of emerged seedlings recorded from faecal material compared to hard seed through the grazing period, also suggests that the time spent by the hard seed in the digestive tract after leaving the rumen, combined with the susceptibility of seed softening (conferred by the previous impact of the environmental conditions under grazing and the chewing effect) are the main factors that affect seed survival at that stage. It was shown (6.5 Pen Experiment 4) that the natural softening of seed due to variation of temperatures (Taylor

et al. 1984) was initiated first in larger seeds and progressed later to the smaller seeds. This may explain the progressive decrease in the individual weight of hard seed voided in faeces by sheep, especially for small-seeded clovers.

The output of viable seed in faeces (comprising germinating and hard seeds) also leads to the dominance of smaller seed sizes in the grazing area. The germinating seeds, as expected, do not survive the desiccation process which occurrs rapidly after the faeces are dropped by sheep in hot, dry weather and become subjected to other natural environmental conditions (Simao Neto and Jones 1986; de Koning 1990). In the present study (5.2 Field Experiment 1) it was demonstrated that the germinating seed did not survive desiccation. Further softening of hard seed in faecal pellets is a key factor that may favour the capability of a clover species to regenerate.

The establishment of subterranean clover seedlings from sheep pellets has been found by de Koning and Carter (1989) to be unlikely to contribute much to the overall regeneration of the pasture. However in small-seeded clovers, the dissemination of seed by sheep significantly influences their establishment. There was clear evidence (5.2 Field Experiment 1 and 5.3 Field Survey 1) to confirm (especially for cluster clover) that the persistence of small-seeded clovers is improved by heavy stocking practices (Carter 1968b; Carter and Lake 1985). This response, mainly due to the size of seed and level of hard-seededness, may have important practical implications in the dissemination of pasture seeds by sheep. However, if the management procedures adopted consider the grazing of seed by sheep before moving them to a field which is selected for dissemination, a proper period of time after eating the seeds has to be considered. Simao Neto *et al.* (1987) proposed a 3 to 4 day period. On the other hand, in order to avoid further contamination with undesirable seeds, they proposed that animals not be moved onto a new field free of weeds until at least 7 days after grazing the contaminated field. This last aspect is important, because some undesirable species, such as winter grass, chickweed, capeweed, etc. (5.3 Field Survey 1) are disseminated by sheep under grazing (Cameron and Cannon 1970; Reed 1974; Carter and Lake 1985).

The last study (5.3 Field Survey 1) also suggests, that small seedlings of cluster clover are less affected by grazing and trampling by sheep compared with larger subterranean clover seedlings during the earlier stages of plant development (Sivalingam 1973) when plants and animals are normally in a critical situation. Both factors contribute to the persistence of small-seeded cluster clover in grazed pastures.

7.5 Conclusions and proposals for future research

It is apparent from the laboratory studies that small seed has potential advantages in achieving an early and complete germination, giving it more favourable water relations in the vicinity of the seed than in the case of large seed. It is also important to note that the variation in the proportion of seed components with seed size and their different imbibition rates, makes small seed more receptive to these moisture relationships. The ability of small seed to imbibe water at higher rates than large seed is important, especially when unfavourable temperatures occur. This also provides a clear advantage for early germination of small seed when a previous incomplete stimulation of the physiological process for germination occur as a consequence of variable periods of wetting and drying. Greater tolerance to water stress in small seed also enhances the influence of seed size on the ability of seed to become hydrated. These features can improve the chances of smaller seeds achieving better seedling establishment than with larger seeds due to favourable competitive advantages in pasture swards. However, further investigation under natural conditions is required. In particular, the water relationships in the micro-environment of the seed seem to be of special relevance under the Mediterranean-type climate. Seed can be ready for regeneration when enclosed in reproductive structures (e.g. pods and burrs) at the soil surface, or buried naturally or by means of tillage practices, or free at the soil surface, naturally or following sowing practices (e.g. surface-sown seed). Consequently, variations in the relationship between the seed and the surrounding aqueous medium can be significant. The seed-soil contact for proper moisture exchange for germination is vital.

The glasshouse experiments have shown that seed size limits the capacity of an emerging seedling to avoid the constraints on emergence imposed by soil type, depth of sowing and the physical impedance of cereal straw. The presence of heavier soil textures, increased depths of sowing and greater levels of wheat straw all influence potential emergence or delay emergence, affecting the vigour of seedlings. Seed size also determines the cotyledon area of seedlings and consequently early growth. However, the general advantage of large seed is the ability to efficiently avoid the constraints of emergence and to produce a more vigourous seedling. These factors can be negated by the presence of extremely large cotyledons. Because moisture is the main limiting factor in the surface of the soil under a Mediterranean-type climate during plant establishment, it is suggested that these relationship have to be carefully monitored under natural or simulated moisture conditons, in order to estimate to what extent a seedling may become successfully established.

The presence of cereal straw residues may be an impediment for emergence and early seedling vigour, especially when combined with more stressful conditions, such as unfavourable soil type and deeply-placed seed. Cereal straw also affects the self-regeneration of annual pasture medic swards due to allelopathic effects, enhanced pathogenesis and limits the breakdown of hard-seededness (Quigley 1988).

More research is required to determine the proper management of cereal straw residues over the summer-autumn period. In particular, practices aimed at promoting the utilization of this material as fodder by livestock needs attention (Cocks 1987). Lot-feeding rations based on straw are receiving attention in research programs at the Waite Institute. However, excessive removal of straw residues must be prevented to avoid soil erosion (Felton *et al.* 1987) and provide the microenvironmental conditions for better establishment of pasture seedlings (Aston and Fisher 1986).

The examination of early vegetative yield in medic and subterranean clover swards (4.4 Raised-bed Experiment 1) supports the proposition that early forage production is contingent on embryo weight/unit area. This can be managed for practical applications

either by modifying seed size or plant density. Given the practical implications of both factors on early dry matter production and the greater use of legumes in pasture mixtures, further investigation into the competitive relationships of several species and/or cultivars growing together in mixed pasture swards is required. Furthermore, grazing studies can confer more realistic responses that are applicable to specific livestock production systems.

The grazing (5.2 Field Experiment 1 and 5.3 Field Survey 1) and pen studies (6.2-6.5 Pen Experiments 1-4) have demonstrated the importance of seed size and level of hard-seededness on survival of annual legume pasture seeds following ingestion by sheep. Accessibility and acceptability of plant components both influence the selection of seed by grazing sheep. Despite sheep showing a marked initial preference for small-seeded clovers, these small seeds better survive the passage through the digestive tract of sheep than larger seeds of subterranean clover. Both factors, selection and survival following ingestion by sheep, govern the persistence of seed in the soil or faeces. On a hard-setting soil, selection during the summer-autumn period leads to the dominance of small seeds in the grazing area. In contrast, under a sandy loam soil, selection leads to the dominance of large seed in the grazing area. This situation demonstrates the significance of the grazing escape mechanisms. The first situation (shattering of seed) favours the small-seeded clovers while the second (burr-burial capacity) favours subterranean clover. However, in both situations, selection of small, easily-carried balansa clover and cluster clover seeds by harvester ants suggests that some attention should be paid to this subject in the future, given its probable biological and economic impact.

Furthermore, after the seed is ingested by sheep, the digestion mechanisms lead to the survival and dominance of small, hard seed. Chewing plays the main role in seed survival and small seed escape this more easily than large seed. It was demonstrated that only the seed that escapes the chewing effect in the hard condition is able to survive passage through the digestive tract of sheep and the desiccation process in faecal pellets after defaecation.

Previous investigations suggest that further research is required to estimate the proper level of utilization of dry pasture residues by livestock, especially having regard to the differences between mono-specific swards and mixed swards comprising contrasting species, seed sizes, level of hard-seededness, grazing-escape mechanism, etc. Management practices must be focused to achieve the best possible opportunity for persistence of every seed species and/or cultivar in the seed bank, also to allow adequate potential regeneration of pasture species. The implementation and adoption of practical field techniques to monitor the status of seed banks and the consequent impact on the regeneration of the pastures are of crucial importance.

The benefits of survival in the digestive tract of livestock, involve mainly the small, hard seed. This may have important practical implications in the dissemination of small-seeded pasture legumes. Studies to estimate the biological and economic impact of pasture seed dissemination by using different livestock species are required. This could be a useful management tool for pasture improvement, especially in remote areas, in which normal tillage practices are not applicable or are economically unmanageable.

However, more research is required: first, to develop feeding and grazing practices orientated to minimize the impact of chewing on seed survival; second, to estimate the influence of the digestive tract of livestock on survival of seed after leaving the rumen through the reticulo-omasal orifice; third, to examine the behaviour of seed in faeces after dropping and its potential regeneration under natural conditions, and, fourth, to determine the impact of invertebrate animals (e.g. coprophagous insects) on seed survival.

Overall, the findings of the studies described in this thesis show that the seed size of self-regenerating pasture species has differing influences depending on the stage of plant development. Small seeds are more efficient in germination and survive better under grazing. In contrast, large seeds allow emergence from greater depths in the soil and produce more vigourous seedlings with higher initial pasture productivity. In summary, in Mediterranean animal pastures it appears that large legume seeds have advantages

only when sowing new pastures or renovating existing pastures. Thereafter, when grazing naturally-regenerated pasture, small seeds have an ecological advantage.

8 APPENDICES

Appendix 3.1 **Summary of analyses of variance with partitioned interactions made on increased values of germination with time for Sava snail medic and Trikkala subterranean clover.**

Interaction	Partitioned component	Species	
		Sava	Trikkala
		(Significance levels)†	
Time:		***	***
	Linear	***	***
	Quadratic	***	***
	Cubic	***	***
	Quartic	***	***
	Deviations	***	***
Temperature x Time:		***	***
	Temperature, linear	***	***
	Temperature, quadratic	***	***
	Temperature, cubic	***	***
	Deviations	***	***
Seed size x Time:		***	NS
	Seed size, linear	NS	NS
	Seed size, quadratic	NS	NS
	Seed size, cubic	*	NS
	Deviations	***	NS
Temperature x Seed size x Time:		***	***
	Temperature x Seed size, linear	NS	NS
	Temperature x Seed size, quadratic	***	NS
	Deviations	***	***

† NS = Not significant; * = P<0.05; *** = P<0.001

Appendix 3.2 **Mean partial germination of seed-size categories of Sava snail medic held at different temperatures and calculated at 0.5 day intervals after 1.0, 1.5, 2.0 and 2.5 days of testing.**

Seed-size category	Temperature (°C)													
	2.1	6.4	10.1	12.6	15.0	17.2	19.1	20.9	22.8	25.1	27.4	30.1	33.6	38.1
	1.0 day:						(%)							
A	0.0	0.0	0.0	0.0	32.0	93.8	92.3	90.8	90.8	90.3	74.5	5.0	0.3	0.0
B	0.0	0.0	0.0	0.0	37.3	91.5	92.0	91.3	96.3	95.0	82.5	5.5	0.3	0.0
C	0.0	0.0	0.0	0.0	31.5	81.8	96.0	80.5	88.3	84.3	69.5	15.0	1.8	0.0
D	0.0	0.0	0.0	0.0	25.3	63.8	88.0	71.5	78.0	70.0	70.5	19.0	2.0	0.0
E	0.0	0.0	0.0	0.0	35.5	67.8	80.8	69.8	85.0	80.8	70.0	36.8	6.5	0.0
	1.5 days:													
A	0.0	0.0	37.3	96.3	62.3	5.3	1.3	2.5	3.3	3.0	19.8	71.5	9.5	0.0
B	0.0	0.0	23.0	95.0	57.8	6.5	3.8	6.0	1.0	2.5	17.5	72.0	6.5	0.0
C	0.0	0.0	33.3	94.3	62.8	14.8	3.8	16.3	10.3	12.5	26.8	68.0	5.8	0.0
D	0.0	0.0	13.3	88.8	64.5	30.8	9.0	23.5	18.5	23.3	21.3	66.5	7.5	0.0
E	0.0	0.0	18.0	76.2	52.0	22.3	17.3	21.3	5.8	12.0	17.5	40.8	31.0	0.0
	2.0 days:													
A	0.0	0.0	36.8	0.5	1.0	0.0	0.0	0.3	0.0	0.0	0.0	1.8	2.8	0.0
B	0.0	0.0	33.8	1.5	0.8	0.3	1.0	0.0	0.8	0.0	0.0	8.8	5.2	0.0
C	0.0	0.0	32.5	2.5	1.5	0.0	0.3	0.0	0.3	1.3	0.5	3.8	2.5	0.0
D	0.0	0.0	36.5	6.5	4.0	1.8	2.0	4.0	1.5	4.3	3.5	3.0	5.3	0.0
E	0.0	0.0	31.3	5.5	3.3	3.8	0.5	2.0	2.0	1.0	0.8	3.3	3.8	0.0
	2.5 days:													
A	0.0	49.5	17.8	0.8	0.0	0.0	0.3	0.0	1.0	0.3	1.0	12.3	19.3	0.0
B	0.0	47.3	42.0	0.0	0.0	0.0	0.3	0.0	0.0	0.3	0.0	10.3	19.0	0.0
C	0.0	24.8	31.0	1.0	0.5	0.0	0.0	0.0	0.0	0.5	1.5	5.5	26.5	0.0
D	0.0	15.0	38.8	4.8	4.0	1.8	0.8	0.0	0.8	0.5	1.8	8.0	26.0	0.0
E	0.0	8.3	34.5	11.0	3.3	0.8	0.0	0.0	1.5	0.5	1.5	6.3	18.0	0.0

Appendix 3.3 **Mean partial germination of seed-size categories of Sava snail medic held at different temperatures and calculated at 0.5 day intervals after 3.0, 3.5, 4.0 and 4.5 days of testing.**

Seed-size category	Temperature (°C)													
	2.1	6.4	10.1	12.6	15.0	17.2	19.1	20.9	22.8	25.1	27.4	30.1	33.6	38.1
	3.0 days:						(%)							
A	0.0	32.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.8	0.0
B	0.0	28.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.6	0.0
C	0.0	35.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.0
D	0.0	32.0	4.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	0.0
E	0.0	24.3	6.8	0.5	1.3	0.8	0.5	0.0	0.0	0.0	0.0	0.5	1.0	0.0
	3.5 days:													
A	0.0	12.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.5	0.0
B	0.0	21.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	19.8	0.0
C	0.0	25.8	1.3	0.3	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0
D	0.0	35.3	4.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	1.3	3.0	0.0
E	0.0	38.5	3.0	1.0	1.0	0.0	0.0	0.0	0.0	0.5	0.0	1.8	2.0	0.0
	4.0 days:													
A	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.8	0.0	0.5	0.0	0.0	8.3	0.0
B	0.0	1.8	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	3.0	0.0
C	0.0	4.3	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	2.5	1.3	0.0
D	0.0	7.5	0.3	0.0	0.0	0.8	0.0	0.3	0.3	1.0	0.0	0.0	0.8	0.0
E	0.0	14.5	0.5	0.8	1.5	1.8	0.5	3.5	0.5	0.5	0.0	0.0	0.0	0.0
	4.5 days:													
A	0.0	2.3	0.0	0.3	0.0	0.0	0.0	0.0	0.5	0.0	0.0	1.3	24.8	0.0
B	0.0	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.3	0.0	2.0	28.3	0.0
C	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	2.8	36.8	0.0
D	0.0	6.8	1.5	0.0	0.0	0.0	0.3	0.5	0.3	1.0	1.3	0.8	26.5	0.0
E	0.0	5.8	0.0	0.8	0.5	1.0	0.0	0.0	1.8	0.5	1.5	5.5	12.5	0.0

Appendix 3.4 **Mean partial germination of seed-size categories of Sava snail medic held at different temperatures and calculated at 0.5 day intervals after 5.0, 5.5, 6.0 and 6.5 days of testing.**

Seed-size category	Temperature (°C)													
	2.1	6.4	10.1	12.6	15.0	17.2	19.1	20.9	22.8	25.1	27.4	30.1	33.6	38.1
	5.0 days:						(%)							
A	21.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0
B	9.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0
C	5.3	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.3	0.0
D	0.3	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5	0.0
E	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	2.8	0.0
	5.5 days:													
A	37.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0
B	29.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
C	12.5	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0
D	3.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	5.5	0.0
E	2.8	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0
	6.0 days:													
A	20.8	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	19.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
C	8.3	0.3	0.3	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0
D	3.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.3	0.3	0.3	0.0	0.3	0.0
E	2.5	0.5	0.0	0.0	0.0	0.0	0.5	1.0	1.8	0.0	0.0	0.0	0.0	0.0
	6.5 days:													
A	20.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B	19.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
C	8.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D	3.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E	2.5	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.8	0.0	0.0	0.0

Appendix 3.5 **Mean partial germination of seed-size categories of Trikkala subterranean clover held at different temperatures and calculated at 0.5 day intervals after 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 days of testing.**

Seed-size category	Temperature (°C)													
	2.5	6.8	9.3	11.9	14.4	16.2	18.2	20.0	22.0	23.8	26.5	29.2	32.9	37.9
	1.0 day:						(%)							
A	0.0	0.0	0.0	0.0	17.8	53.5	70.0	84.3	66.8	24.3	10.0	0.3	0.3	0.0
B	0.0	0.0	0.0	0.0	7.0	27.3	58.8	88.8	62.3	42.8	8.0	1.3	0.3	0.0
C	0.0	0.0	0.0	0.0	6.3	39.5	46.0	83.0	65.6	37.5	17.0	1.3	0.3	0.0
	1.5 days:													
A	0.0	0.0	0.0	52.3	71.0	33.8	21.0	5.8	25.8	39.8	7.3	1.0	0.0	0.0
B	0.0	0.0	0.0	36.3	72.3	49.8	27.3	5.5	30.8	20.3	18.8	2.0	0.5	0.0
C	0.0	0.0	0.0	20.5	61.3	43.3	32.8	12.3	30.5	35.5	20.3	0.8	0.3	0.0
	2.0 days:													
A	0.0	0.0	54.0	34.5	7.3	8.5	2.8	2.0	3.0	17.8	1.0	1.5	0.8	0.0
B	0.0	0.0	35.3	49.5	12.0	17.3	7.5	2.0	1.0	11.8	4.8	0.5	0.3	0.0
C	0.0	0.0	27.5	63.0	26.5	17.3	17.3	2.3	2.5	7.3	5.3	1.8	0.0	0.0
	2.5 days:													
A	0.0	15.8	38.0	5.3	0.8	1.0	1.0	0.8	0.3	0.3	16.8	0.0	0.0	0.0
B	0.0	14.8	47.3	5.3	0.5	1.8	1.8	0.8	0.5	0.3	0.8	0.0	0.0	0.0
C	0.0	5.0	47.5	13.3	2.8	0.0	1.0	1.0	0.5	0.5	1.0	0.3	0.0	0.0
	3.0 days:													
A	0.0	35.0	5.3	0.5	0.3	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0
B	0.0	27.0	1.8	1.0	1.8	0.0	0.0	1.0	0.0	0.0	0.3	0.0	0.0	0.0
C	0.0	12.3	13.5	0.5	0.3	0.0	0.0	0.0	0.5	0.8	0.0	0.3	0.0	0.0
	3.5 days:													
A	0.0	33.3	0.8	0.5	0.3	0.3	0.0	1.3	0.0	0.0	0.3	0.0	0.0	0.0
B	0.0	30.0	7.3	0.5	0.0	0.3	1.0	0.3	0.0	0.0	0.3	0.0	0.0	0.0
C	0.0	29.8	7.8	0.5	0.0	0.0	0.3	0.3	0.0	0.8	0.5	0.0	0.0	0.0

Appendix 3.6 **Mean partial germination of seed-size categories of Trikkala subterranean clover held at different temperatures and calculated at 0.5 day intervals after 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 days of testing.**

Seed-size category	Temperature (°C)													
	2.5	6.8	9.3	11.9	14.4	16.2	18.2	20.0	22.0	23.8	26.5	29.2	32.9	37.9
	4.0 days:						(%)							
A	0.0	6.3	0.3	0.5	0.0	0.3	1.0	0.5	0.3	3.0	1.0	0.0	0.0	0.0
B	0.0	13.8	0.3	0.5	0.8	0.3	1.3	0.0	2.5	7.3	1.0	0.0	0.0	0.0
C	0.0	21.0	0.3	0.0	1.0	0.0	1.0	1.0	0.5	9.0	0.8	0.0	0.0	0.0
	4.5 days:													
A	0.0	5.0	0.3	0.3	0.0	0.3	0.3	0.0	0.5	3.5	2.0	0.3	0.0	0.0
B	0.0	7.8	0.3	0.0	0.3	0.0	0.3	0.0	1.0	6.8	9.5	0.5	0.5	0.0
C	0.0	11.0	0.5	0.0	0.5	0.0	0.3	0.0	0.0	7.0	13.5	0.3	0.3	0.0
	5.0 days:													
A	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0
B	0.0	0.3	1.0	0.5	0.0	0.0	0.3	0.0	0.0	0.8	0.0	0.0	0.0	0.0
C	0.0	2.3	0.3	0.5	0.0	0.0	0.3	0.0	0.0	0.0	1.8	0.0	0.0	0.0
	5.5 days:													
A	30.8	0.3	0.0	0.0	0.0	0.3	0.5	0.0	0.0	2.5	0.3	0.5	0.0	0.0
B	18.3	2.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0	1.8	0.8	0.0	0.0	0.0
C	4.8	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	2.8	0.0	0.0	0.0
	6.0 days:													
A	16.5	0.3	0.0	0.0	0.0	0.0	0.3	0.5	0.0	0.0	0.0	0.0	0.0	0.0
B	14.8	0.3	0.0	0.0	0.3	0.3	0.3	0.0	0.0	0.0	1.8	0.0	0.0	0.0
C	8.3	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0
	6.5 days:													
A	14.8	0.0	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0
B	16.5	0.0	0.0	0.3	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C	12.8	0.5	0.5	0.0	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix 3.7 **Summary of analyses of variance with partitioned interactions made on increased values of water uptake with time for Sava snail medic and Trikkala subterranean clover seeds.**

Interaction	Partitioned component	Species	
		Sava	Trikkala
		(Significance levels)†	
Time:		***	***
	Linear	***	***
	Quadratic	***	***
	Cubic	NS	***
	Quartic	***	***
	Deviations	***	***
Temperature x Time:		***	***
	Temperature, linear	***	**
	Temperature, quadratic	***	***
	Temperature, cubic	**	***
	Deviations	***	***
Seed size x Time:		***	***
	Seed size, linear	***	*
	Seed size, quadratic	***	***
	Seed size, cubic	NS	*
	Deviations	***	***
Temperature x Seed size x Time:		***	NS
	Temperature x Seed size, linear	NS	NS
	Temperature x Seed size, quadratic	NS	NS
	Deviations	***	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Appendix 3.8 **Mean partial increases of water uptake by seed-size categories of Sava snail medic held at different temperatures and calculated after 1, 2, 3, 4 and 5 hours of testing.**

Seed-size category	Temperature (°C)													
	2.0	5.1	8.0	10.4	12.2	14.2	16.0	17.6	19.6	21.7	24.2	26.7	30.0	34.5
	1 hour:						(% of dry weight)							
A	27	22	25	29	25	30	28	23	42	29	29	42	42	32
B	10	17	24	14	24	20	27	27	26	28	28	28	42	24
C	24	20	32	34	31	24	39	22	39	30	33	35	31	26
D	24	23	29	34	21	33	37	27	36	35	34	35	49	46
	2 hours:													
A	0	6	4	6	17	13	23	22	7	24	35	32	55	86
B	7	6	1	12	10	7	6	5	22	14	27	15	21	49
C	0	0	0	0	9	15	0	13	0	18	6	16	22	35
D	3	5	0	0	8	0	3	21	7	11	18	15	7	15
	3 hours:													
A	7	11	19	15	25	28	17	39	36	31	32	43	47	40
B	15	10	11	11	7	19	16	27	4	15	28	49	45	48
C	12	11	7	16	9	2	6	11	16	10	30	24	54	52
D	3	7	0	5	0	1	6	0	11	11	11	14	15	27
	4 hours:													
A	8	16	12	37	15	16	40	32	47	55	55	49	27	39
B	0	3	5	16	15	10	32	19	38	48	33	23	42	31
C	2	4	9	12	11	18	20	31	37	34	43	53	38	48
D	5	2	11	7	15	25	13	19	16	14	27	60	61	65
	5 hours:													
A	11	13	16	12	30	38	29	31	31	26	15	30	30	20
B	13	16	21	13	34	27	20	35	44	32	39	32	16	31
C	3	10	14	9	18	23	30	24	33	47	21	39	49	15
D	2	16	14	1	22	14	10	18	15	34	14	21	31	17

Appendix 3.9 **Mean partial increases of water uptake by seed-size categories of Sava snail medic held at different temperatures and calculated after 6, 7, 8, 9 and 10 hours of testing.**

Seed-size category	Temperature (°C)													
	2.0	5.1	8.0	10.4	12.2	14.2	16.0	17.6	19.6	21.7	24.2	26.7	30.0	34.5
	6 hours:						(% of dry weight)							
A	10	20	24	11	24	27	19	15	13	22	9	14	13	0
B	8	12	11	26	20	35	22	26	6	22	14	14	16	2
C	13	23	18	17	25	26	26	25	15	16	45	16	1	11
D	18	17	10	20	18	20	35	30	22	32	26	12	25	2
	7 hours:													
A	9	8	25	26	27	17	18	36	17	25	47	9	20	8
B	0	18	30	24	20	25	24	26	28	22	14	19	0	31
C	0	3	8	21	31	36	28	31	23	18	30	12	7	10
D	0	1	5	37	10	21	27	36	39	16	50	9	10	5
	8 hours:													
A	10	18	12	18	0	6	17	0	10	10	0	1	0	9
B	7	0	0	2	15	2	18	11	8	0	15	19	10	7
C	13	7	18	20	3	14	21	5	19	7	0	10	12	17
D	0	5	14	0	26	16	17	7	28	14	0	15	0	27
	9 hours:													
A	16	10	2	25	22	1	0	0	5	0	9	4	4	0
B	18	14	24	13	0	11	14	0	9	2	0	0	3	7
C	0	17	17	20	16	0	0	8	11	0	5	0	0	0
D	11	0	29	17	8	9	2	6	2	11	11	0	4	0
	10 hours:													
A	12	13	12	0	0	9	15	5	0	5	5	0	0	3
B	13	14	3	8	4	8	0	3	2	14	20	1	0	0
C	29	10	16	0	4	4	7	7	0	13	0	12	0	4
D	6	22	0	28	10	25	11	2	0	4	0	4	1	12

Appendix 3.10 **Mean partial increases of water uptake by seed-size categories of Trikkala subterranean clover held at different temperatures and calculated after 1, 2, 3, 4, 5 and 6 hours of testing.**

Seed-size category	Temperature (°C)													
	2.0	5.1	8.0	10.4	12.2	14.2	16.0	17.6	19.6	21.7	24.2	26.7	30.0	34.5
	1 hour:						(% of dry weight)							
A	26	16	21	22	20	19	21	24	37	30	34	42	23	26
B	11	8	17	18	14	23	17	21	23	20	29	26	20	28
C	16	17	21	20	24	21	26	33	29	26	23	27	34	44
	2 hours:													
A	8	17	14	28	41	28	48	33	43	56	60	47	68	83
B	13	19	11	16	29	12	33	18	32	28	18	45	63	71
C	0	2	10	23	11	17	19	15	17	21	18	41	40	38
	3 hours:													
A	4	19	33	31	21	49	30	42	41	33	37	50	41	33
B	12	9	14	23	18	39	25	37	27	50	54	43	26	23
C	15	17	18	13	15	14	20	34	25	36	54	32	27	23
	4 hours:													
A	15	22	22	20	32	21	18	34	4	25	0	0	15	0
B	1	0	19	11	24	12	9	26	24	17	13	2	23	16
C	10	6	13	7	22	30	12	14	34	27	27	11	24	32
	5 hours:													
A	26	34	17	24	4	9	15	5	15	0	24	8	10	10
B	9	38	16	25	15	21	22	25	17	17	18	15	14	19
C	11	3	11	21	18	18	32	17	21	21	1	15	2	5
	6 hours:													
A	18	0	22	9	18	0	5	6	1	19	8	16	0	7
B	25	12	25	31	23	4	31	14	11	5	12	10	0	0
C	1	16	22	29	16	22	13	8	6	0	2	11	7	0

Appendix 3.11 Mean partial increases of water uptake by seed-size categories of Trikkala subterranean clover held at different temperatures and calculated after 7, 8, 9, 10, 11 and 12 hours of testing.

Seed-size category	Temperature (°C)													
	2.0	5.1	8.0	10.4	12.2	14.2	16.0	17.6	19.6	21.7	24.2	26.7	30.0	34.5
	7 hours:					(% of dry weight)								
A	16	13	11	0	3	23	8	6	0	2	0	4	16	0
B	12	10	12	2	11	20	0	1	0	0	1	7	1	5
C	21	36	7	0	14	8	0	6	11	0	15	1	8	6
	8 hours:													
A	5	11	0	26	2	5	0	5	10	0	0	0	0	0
B	3	14	4	2	0	0	2	0	6	10	0	0	8	0
C	1	12	20	13	5	0	9	13	0	0	0	0	0	0
	9 hours:													
A	19	7	2	0	1	4	13	0	0	0	11	19	16	3
B	13	15	4	1	11	7	2	9	0	0	10	2	0	0
C	17	11	0	0	1	2	1	0	5	9	11	4	0	12
	10 hours:													
A	0	11	7	0	8	0	0	0	5	18	0	0	0	0
B	0	2	0	16	0	3	6	0	6	4	0	0	0	3
C	0	0	0	0	15	3	0	0	0	0	0	5	1	0
	11 hours:													
A	5	0	7	5	0	3	0	0	7	0	0	6	13	14
B	0	0	10	0	16	8	0	8	0	4	0	6	0	0
C	1	3	7	11	0	4	6	10	2	3	6	5	0	4
	12 hours:													
A	0	10	0	0	0	5	16	3	0	16	1	0	4	0
B	33	18	6	7	0	0	0	0	8	2	16	4	17	5
C	17	11	9	0	0	1	3	0	4	11	1	0	14	7

Appendix 3.12 **Mean seed weight of the seed-size categories used to test the effects of soaking and drying on subsequent germination of Sava snail medic.**

Wetting period (hours)	Seed-size categories								
	A			B			C		
	Silica gel†	Wet††	Dry†††	Silica gel	Wet	Dry	Silica gel	Wet	Dry
0	10.22	-	-	15.14	-	-	23.40	-	-
2	10.46	14.00	10.49	15.42	18.45	15.47	23.56	27.24	23.39
4	10.45	16.62	10.40	15.34	22.38	15.29	23.68	32.00	23.87
6	10.39	22.22	10.38	15.14	28.63	15.09	23.40	42.41	23.62
8	10.49	24.12	10.49	15.13	33.70	15.16	23.51	47.94	23.86
10	10.29	24.69	10.30	15.31	36.55	15.41	23.88	53.22	24.90
12	10.06	25.70	10.54	15.33	35.79	15.37	23.24	53.36	24.24
14	10.28	26.77	10.25	15.16	37.02	15.26	23.43	53.96	24.60
16	10.42	25.59	10.40	15.47	36.99	15.43	23.42	54.15	24.17
18	10.42	25.98	10.43	15.15	36.99	15.26	23.27	53.92	24.40
20	10.37	26.66	10.14	15.40	38.31	15.11	23.57	55.15	23.77
22	10.42	28.33	10.20	15.38	39.26	15.11	23.29	55.50	23.33
24	10.29	27.34	10.09	15.12	41.13	14.90	23.17	55.41	23.52
Mean	10.34	-	10.39	15.28	-	15.22	23.49	-	23.91
S.D.	0.23	-	0.53	0.29	-	0.39	0.47	-	0.89
C.V. (%)	2.20	-	5.10	1.90	-	2.56	2.00	-	3.72
S.E.	0.06	-	0.15	0.08	-	0.11	0.13	-	0.25

† After storing for 48h; †† After the corresponding wetting period; ††† After drying for 24h

Appendix 3.13 **Mean seed weight of the seed-size categories used to test the effects of soaking and drying on subsequent germination of Trikkala subterranean clover.**

Wetting period (hours)	Seed-size categories								
	A			B			C		
	Silica gel†	Wet††	Dry†††	Silica gel	Wet	Dry	Silica gel	Wet	Dry
0	6.67	-	-	9.74	-	-	13.43	-	-
2	7.06	12.49	6.94	9.78	16.43	9.84	13.45	20.31	13.37
4	6.83	13.41	6.80	10.00	18.02	9.97	13.50	21.77	13.48
6	6.83	13.99	6.80	10.00	20.20	9.95	13.38	24.64	13.41
8	6.61	14.56	6.55	10.12	21.07	10.20	13.33	27.00	13.46
10	6.57	14.64	6.50	10.14	21.27	10.24	13.46	26.57	13.57
12	6.74	15.04	6.79	9.99	20.75	10.10	13.55	26.86	13.73
14	6.60	15.00	6.54	10.03	21.75	10.20	13.35	27.18	13.54
16	6.68	15.01	6.64	9.87	21.43	9.91	13.53	27.80	13.68
18	6.61	14.60	6.55	9.92	21.85	9.98	13.51	27.42	13.68
20	6.53	14.96	6.40	9.92	21.80	9.81	13.61	28.49	13.60
22	6.65	15.54	6.41	10.01	22.14	9.94	13.43	27.87	13.32
24	6.60	15.68	6.42	9.90	22.46	9.71	13.50	28.52	13.26
Mean	6.70	-	6.61	9.97	-	9.97	13.49	-	13.52
S.D.	0.29	-	0.25	0.18	-	0.23	0.28	-	0.30
C.V. (%)	4.33	-	3.78	1.81	-	2.31	2.08	-	2.22
S.E.	0.08	-	0.07	0.05	-	0.06	0.08	-	0.08

† After storing for 48h; †† After the corresponding wetting period; ††† After drying for 24h

Appendix 3.14 Summary of analyses of variance made on cumulative values of germination with time.

Treatment	Cumulative values of germination recorded at 0.5	1.0	1.5	2.0 (days)	2.5	3.0	3.5
	(Significance levels) †						
Species	NS	*	NS	NS	NS	NS	*
Wetting period	***	***	***	*	***	***	NS
Seed size	NS	***	**	***	***	**	NS
Species x Wetting period	NS	*	***	***	***	*	NS
Species x Seed size	***	***	***	***	***	***	***
Wetting period x Seed size	**	NS	NS	NS	*	NS	NS
Species x Wetting period x Seed size	***	NS	NS	*	***	***	NS

Treatment	Cumulative values of germination recorded at 4.0	4.5	5.0	5.5 (days)	6.0	6.5	7.0
	(Significance levels) †						
Species	*	*	*	NS	*	***	***
Wetting period	NS	NS	NS	NS	NS	*	NS
Seed size	NS	**	*	***	***	***	***
Species x Wetting period	NS	NS	NS	*	NS	NS	NS
Species x Seed size	***	***	***	***	*	*	NS
Wetting period x Seed size	NS	*	NS	NS	NS	NS	NS
Species x Wetting period x Seed size	NS	NS	NS	NS	NS	NS	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Appendix 3.15 **Mean partial germination of seed-size categories of Sava snail medic submitted to different pre-wetting periods and calculated at 0.5 day intervals after 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 days of testing.**

Seed-size category	Pre-wetting period (hours) 0	2	4	6	8	10	12	14	16	18	20	22	24
	0.5 day						(%)						
A	0	0	0	0	0	3	6	13	13	43	79	77	71
B	0	0	0	0	2	7	1	22	15	21	84	42	69
C	0	0	0	0	0	3	1	3	7	3	26	18	33
	1.0 day												
A	0	18	58	59	73	76	68	77	44	55	20	21	11
B	2	10	34	61	83	54	27	66	62	41	14	24	23
C	0	3	24	14	46	48	15	52	47	26	33	12	10
	1.5 days												
A	53	78	41	13	8	3	1	5	17	1	0	1	13
B	25	61	47	24	10	22	14	4	8	21	1	20	3
C	29	60	54	25	33	26	6	34	38	21	9	5	4
	2.0 days												
A	35	1	1	18	2	3	1	0	12	0	0	0	2
B	47	27	17	13	2	14	27	1	12	6	1	13	1
C	48	16	7	34	4	10	12	3	2	8	18	15	17
	2.5 days												
A	5	2	0	8	15	11	1	2	2	0	0	0	1
B	12	1	1	1	2	3	15	5	0	1	0	1	1
C	12	13	10	17	11	4	14	1	2	3	10	11	31
	3.0 days												
A	5	2	0	1	0	1	1	1	3	0	0	0	0
B	0	0	0	0	0	0	11	0	1	1	0	0	1
C	1	1	1	2	3	3	15	1	1	3	0	1	1

Appendix 3.16 Mean partial germination of seed-size categories of Trikkala subterranean clover submitted to different pre-wetting periods and calculated at 0.5 day intervals after 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 days of testing.

Seed-size category	Pre-wetting period (hours)												
	0	2	4	6	8	10	12	14	16	18	20	22	24
	0.5 day						(%)						
A	0	0	0	0	2	2	2	8	1	24	7	68	51
B	0	0	0	2	2	5	5	6	8	30	60	70	69
C	0	0	0	2	4	12	11	11	30	39	67	50	70
	1.0 day												
A	20	7	37	37	78	68	78	50	49	4	39	8	6
B	26	38	43	73	91	68	87	88	27	47	33	6	9
C	7	13	18	67	65	78	75	68	49	34	18	32	13
	1.5 days												
A	43	36	30	20	14	8	9	12	2	4	19	5	5
B	56	41	42	21	5	8	4	2	13	8	4	4	7
C	60	43	33	18	19	4	4	10	9	12	3	7	7
	2.0 days												
A	35	43	15	11	0	6	3	4	4	5	7	9	17
B	16	13	10	1	0	3	1	2	34	6	1	14	7
C	25	30	11	5	3	1	2	5	5	7	4	5	4
	2.5 days												
A	1	13	8	9	1	4	3	5	3	4	3	3	11
B	1	3	2	1	0	7	2	0	8	4	1	4	3
C	3	7	20	1	2	1	2	3	3	4	2	3	2
	3.0 days												
A	0	1	3	6	0	2	1	2	19	7	0	1	5
B	0	2	1	1	1	3	0	0	2	1	0	1	1
C	0	1	7	1	3	1	1	1	0	1	1	2	1

Appendix 3.17 Mean final germination of seed-size categories of Sava snail medic and Trikkala subterranean clover submitted to different pre-wetting periods and recorded after 14 days of testing.

Water uptake time (hours)	Sava seed-size categories			Trikkala seed-size categories		
	A	B	C	A	B	C
0	100	100	99	100	99	100
2	100	100	100	100	99	99
4	100	100	100	100	100	99
6	100	100	100	100	100	99
8	100	100	100	100	100	100
10	100	100	100	100	100	100
12	100	100	100	100	100	99
14	100	100	100	100	100	100
16	100	100	100	100	100	100
18	100	100	100	99	100	99
20	100	100	100	100	100	100
22	100	100	100	100	100	100
24	100	100	100	100	100	100

Appendix 3.18 Linear regression equations obtained for the response in microvolts of the water potential of the NaCl solutions at different channels.

Channel	Intercept (A)	Regression coefficient (B)	Determination coefficient (R^2)	Correlation coefficient (r)	Level of significance (P=)
1	1.038	0.650	0.998	0.999	0.001
2	-0.266	0.650	0.966	0.983	0.001
3	4.444	0.549	0.982	0.991	0.001
4	0.320	0.581	0.996	0.998	0.001
5	2.298	0.770	0.996	0.998	0.001
6	0.897	0.683	0.999	0.999	0.001
7	-0.184	0.724	0.994	0.997	0.001
8	4.159	0.707	0.996	0.998	0.001
9	-1.625	0.530	0.973	0.986	0.001
10	1.982	0.660	0.998	0.999	0.001
11	1.670	0.725	0.997	0.998	0.001
12	-1.116	0.643	0.973	0.986	0.001
13	3.244	0.600	0.993	0.996	0.001
14	1.942	0.695	0.997	0.998	0.001
15	-0.699	0.691	0.996	0.998	0.001

Appendix 3.19 **Summary of analyses of variance made on increased germination at each time of evaluation during the first 5.5 days of testing.**

Treatment	Partial increased values of germination at: 1.0	1.5	2.0 (days)	2.5	3.0
	(Significance levels)†				
Species	***	NS	**	NS	NS
Water potential	***	***	***	***	***
Seed size	**	NS	*	NS	*
Species x Water potential	***	***	***	***	***
Species x Seed size	NS	**	NS	NS	NS
Water potential x Seed size	**	NS	NS	NS	NS
Species x Water potential x Seed size	**	***	NS	NS	*

Treatment	Partial increased values of germination at: 3.5	4.0	4.5 (days)	5.0	5.5
	(Significance levels)†				
Species	***	NS	NS	NS	NS
Water potential	***	***	***	***	***
Seed size	**	NS	NS	NS	NS
Species x Water potential	***	**	NS	NS	NS
Species x Seed size	NS	NS	NS	*	NS
Water potential x Seed size	***	NS	NS	NS	NS
Species x Water potential x Seed size	***	NS	***	***	NS

† NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Appendix 3.20 **Mean partial increases of germination by seed-size categories of Sava snail medic held at different water potentials and calculated after 1.0, 1.5, 2.0, 2.5 and 3.0 days of testing.**

Seed-size category	Water potentials (-bars)								
	0	2	4	6	8	10	12	14	16
					(%)				
	1.0 day								
A	52	32	0	0	0	0	0	0	0
B	8	9	0	0	0	0	0	0	0
C	4	4	0	0	0	0	0	0	0
	1.5 days								
A	47	59	1	0	0	0	0	0	0
B	83	66	1	0	0	0	0	0	0
C	55	7	1	0	0	0	0	0	0
	2.0 days								
A	1	4	1	0	0	0	0	0	0
B	4	10	1	0	0	0	0	0	0
C	10	5	4	0	0	0	0	0	0
	2.5 days								
A	0	3	1	0	0	0	0	0	0
B	4	4	1	0	0	0	0	0	0
C	23	8	3	0	0	0	0	0	0
	3.0 days								
A	0	1	8	0	0	0	0	0	0
B	1	4	2	0	0	0	0	0	0
C	3	12	6	0	0	0	0	0	0

Appendix 3.21 **Mean partial increases of germination by seed-size categories of Sava snail medic held at different water potentials and calculated after 3.5, 4.0, 4.5, 5.0 and 5.5 days of testing.**

Seed-size category	Water potentials (- bars)								
	0	2	4	6	8	10	12	14	16
					(%)				
3.5 days									
A	0	1	33	1	0	0	0	0	0
B	0	7	34	1	0	0	0	0	0
C	2	41	7	0	0	0	0	0	0
4.0 days									
A	0	0	5	2	0	0	0	0	0
B	0	0	16	0	0	0	0	0	0
C	0	3	2	0	0	0	0	0	0
4.5 days									
A	0	0	4	1	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0
C	0	0	1	0	0	0	0	0	0
5.0 days									
A	0	0	2	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0
5.5 days									
A	0	0	2	2	0	0	0	0	0
B	0	0	2	2	0	0	0	0	0
C	0	1	0	0	0	0	0	0	0

Appendix 3.22 **Mean partial increases of germination by seed-size categories of Trikkala subterranean clover held at different water potentials and calculated after 1.0, 1.5, 2.0, 2.5 and 3.0 days of testing.**

Seed-size category	Water potentials (-bars)								
	0	2	4	6	8	10	12	14	16
					(%)				
	1.0 day								
A	52	85	28	0	0	0	0	0	0
B	80	63	9	0	0	0	0	0	0
C	46	68	8	0	0	0	0	0	0
	1.5 days								
A	38	12	41	0	0	0	0	0	0
B	14	26	23	0	0	0	0	0	0
C	41	27	21	0	0	0	0	0	0
	2.0 days								
A	3	2	20	0	0	0	0	0	0
B	0	3	17	1	0	0	0	0	0
C	4	3	25	1	0	0	0	0	0
	2.5 days								
A	2	0	0	1	0	0	0	0	0
B	0	4	1	1	0	0	0	0	0
C	2	0	6	1	0	0	0	0	0
	3.0 days								
A	3	0	0	2	0	0	0	0	0
B	0	4	1	3	0	0	0	0	0
C	2	1	6	2	0	0	0	0	0

Appendix 3.23 Mean partial increases of germination by seed-size categories of Trikkala subterranean clover held at different water potentials and calculated after 3.5, 4.0, 4.5, 5.0 and 5.5 days of testing.

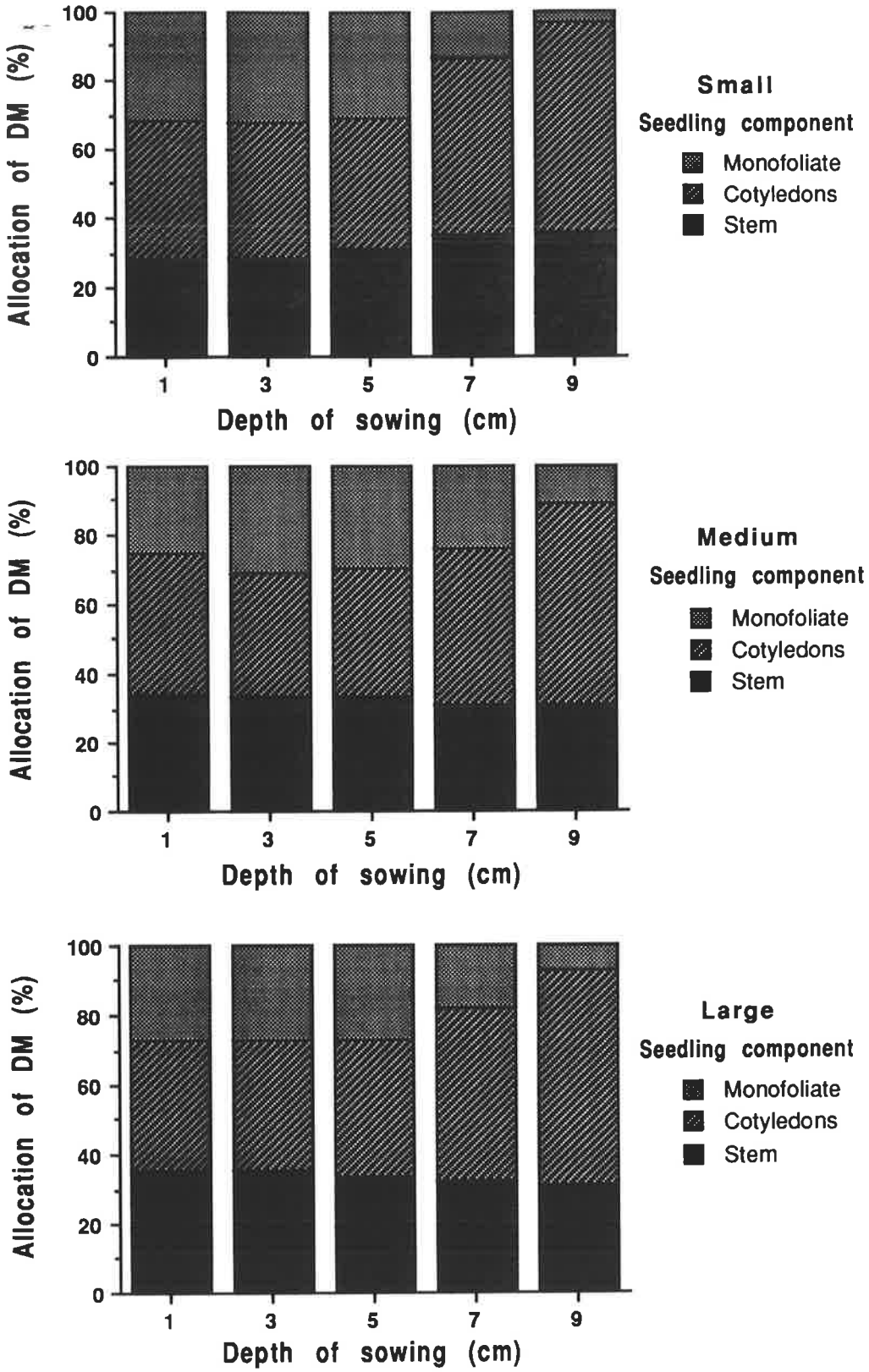
Seed-size category	Water potentials (-bars)								
	0	2	4	6	8	10	12	14	16
					(%)				
	3.5 days								
A	1	1	0	1	0	0	0	0	0
B	2	0	9	1	0	0	0	0	0
C	5	1	5	3	0	0	0	0	0
	4.0 days								
A	0	0	0	2	0	0	0	0	0
B	0	0	2	2	0	0	0	0	0
C	0	0	4	2	0	0	0	0	0
	4.5 days								
A	0	0	0	2	0	0	0	0	0
B	0	0	1	1	0	0	0	0	0
C	0	0	1	0	0	0	0	0	0
	5.0 days								
A	0	0	0	0	0	0	0	0	0
B	0	0	1	0	0	0	0	0	0
C	0	0	1	1	0	0	0	0	0
	5.5 days								
A	0	0	0	2	0	0	0	0	0
B	0	0	1	1	0	0	0	0	0
C	0	0	1	1	0	0	0	0	0

Appendix 4.1 **Cumulative emergence of Sava snail medic and Trikkala subterranean clover seedlings recorded at harvest time (14 days) and at the end of the experimental period (28 days).**

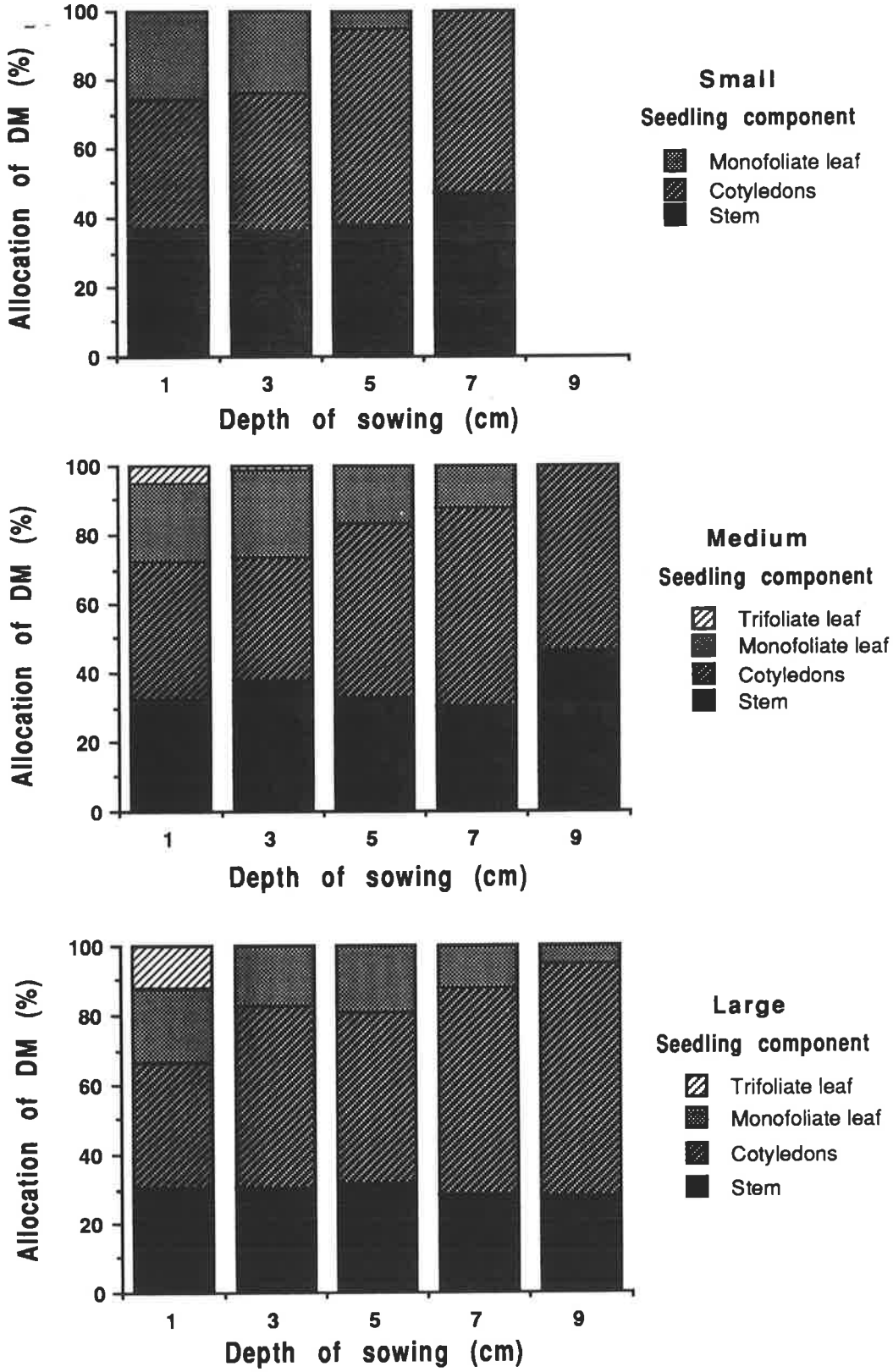
Seed size	Depth of sowing (cm)	Soil texture	Cumulative emergence (%) obtained at: 14 days Sava	14 days Trikkala	28 days Sava	28 days Trikkala
Small	1	Sandy	94.8	88.0	98.5	93.8
	3		94.8	82.3	96.8	99.5
	5		76.8	61.0	84.3	77.3
	7		88.0	0.0	92.8	4.3
	9		47.5	0.0	65.3	0.0
Medium	1	Sandy	99.3	91.3	99.3	97.0
	3		95.5	78.8	96.3	92.0
	5		91.3	64.5	93.8	73.0
	7		74.5	6.0	94.5	22.0
	9		55.3	0.0	78.3	0.0
Large	1	Sandy	98.8	92.8	100.0	96.3
	3		92.5	72.0	95.0	76.0
	5		94.5	73.8	97.5	84.0
	7		89.5	10.5	97.5	32.8
	9		72.5	0.0	94.5	0.0
Small	1	Loamy	89.5	91.8	90.3	98.3
	3		72.5	34.8	73.0	35.3
	5		47.5	9.0	48.5	9.0
	7		4.0	0.0	4.0	0.0
	9		0.0	0.0	0.0	0.0
Medium	1	Loamy	80.0	95.3	82.0	97.0
	3		62.0	36.5	65.3	38.8
	5		54.0	1.8	54.5	1.8
	7		15.8	0.0	15.8	0.0
	9		1.5	0.0	1.5	0.0
Large	1	Loamy	82.5	90.3	86.3	93.0
	3		50.8	42.8	54.0	44.0
	5		27.0	8.0	32.0	8.0
	7		19.0	0.0	21.0	0.0
	9		5.3	0.0	5.3	0.0

Appendix 4.2 **Individual weight of Sava snail medic and Trikkala subterranean clover seedlings estimated at harvest time (14 days).**

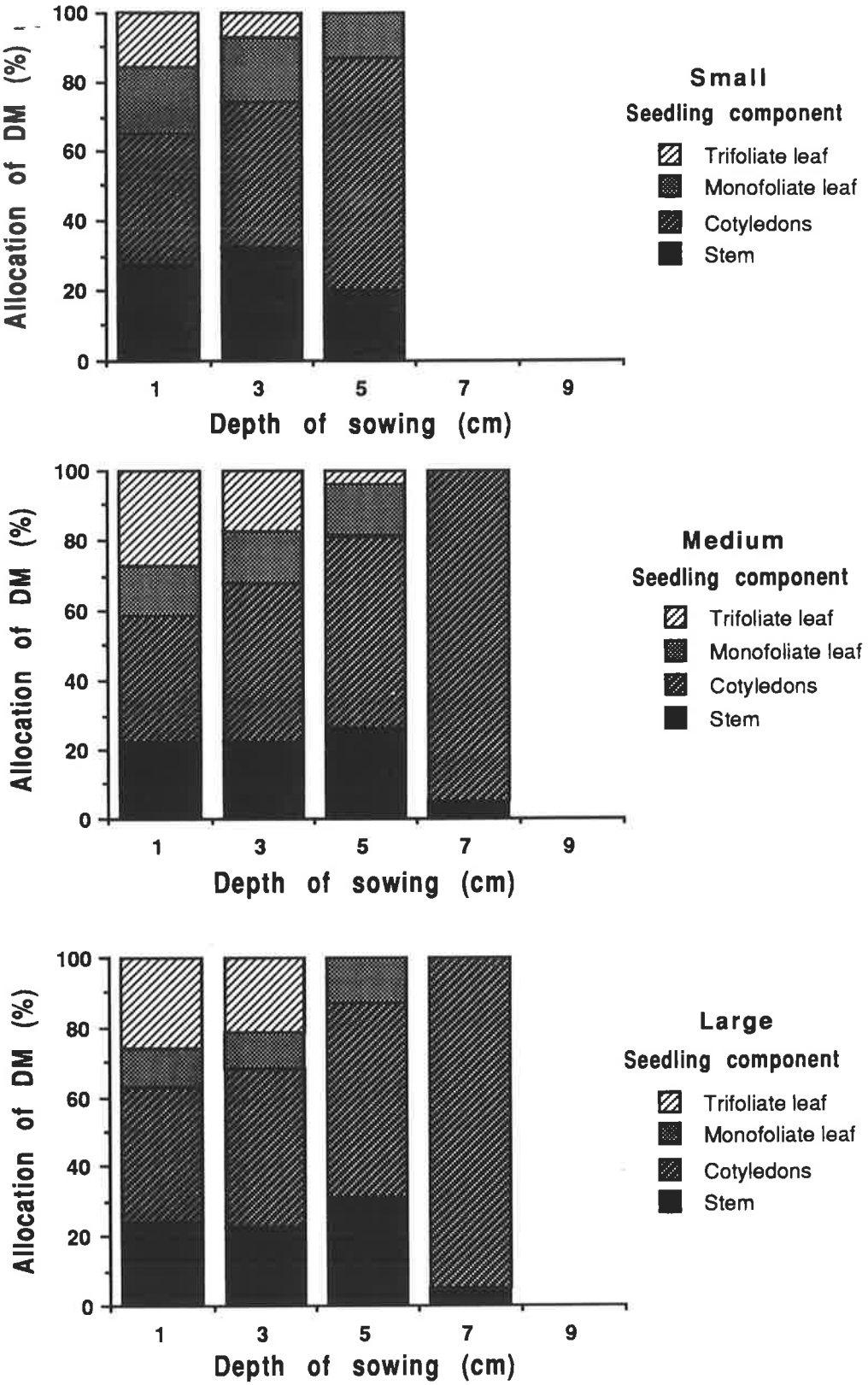
Seed size	Depth of sowing (cm)	Soil texture	Individual seedling weight: Sava (mgDM)	Trikkala (mgDM)
Small	1	Sandy	16.6	10.1
	3		13.7	8.9
	5		12.0	4.1
	7		7.4	-
	9		6.2	-
Medium	1	Sandy	21.0	13.1
	3		21.8	10.3
	5		18.8	8.4
	7		14.3	1.0
	9		8.0	-
Large	1	Sandy	30.8	16.9
	3		29.1	12.0
	5		25.3	10.1
	7		18.1	2.6
	9		13.4	-
Small	1	Loamy	17.0	11.8
	3		13.0	9.6
	5		8.4	2.0
	7		3.7	-
	9		-	-
Medium	1	Loamy	22.1	19.4
	3		19.7	14.3
	5		10.5	4.0
	7		10.8	-
	9		4.5	-
Large	1	Loamy	33.8	23.0
	3		19.7	16.6
	5		17.6	11.1
	7		16.1	-
	9		9.4	-



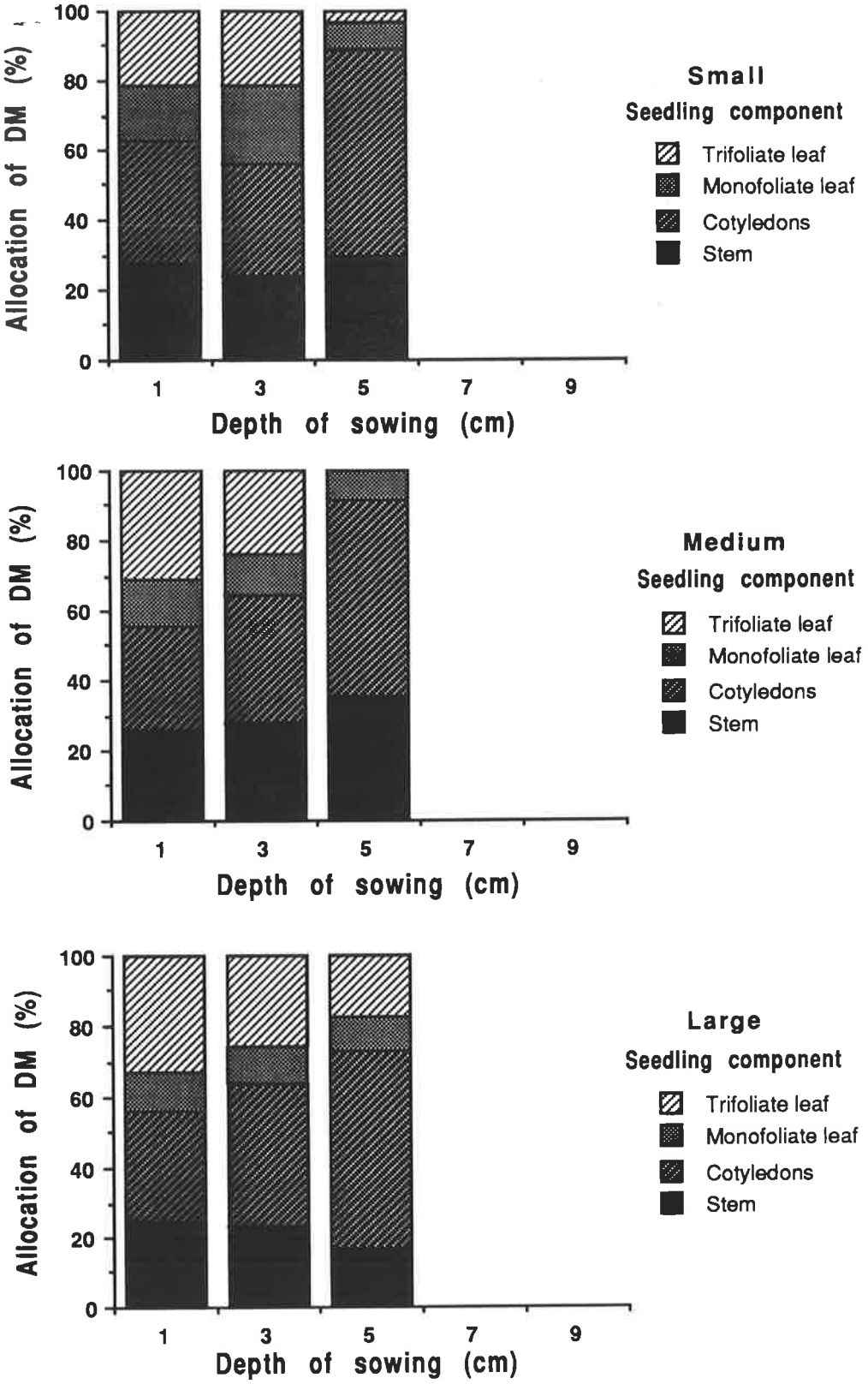
Appendix 4.3 The allocation of dry matter of Sava snail medic seedlings sown in a sandy soil with small, medium and large seed-size categories.



Appendix 4.4 The allocation of dry matter of Sava snail medic seedlings sown in a loamy soil with small, medium and large seed-size categories.



Appendix 4.5 The allocation of dry matter of Trikkala subterranean clover seedlings sown in a sandy soil with small, medium and large seed-size categories.



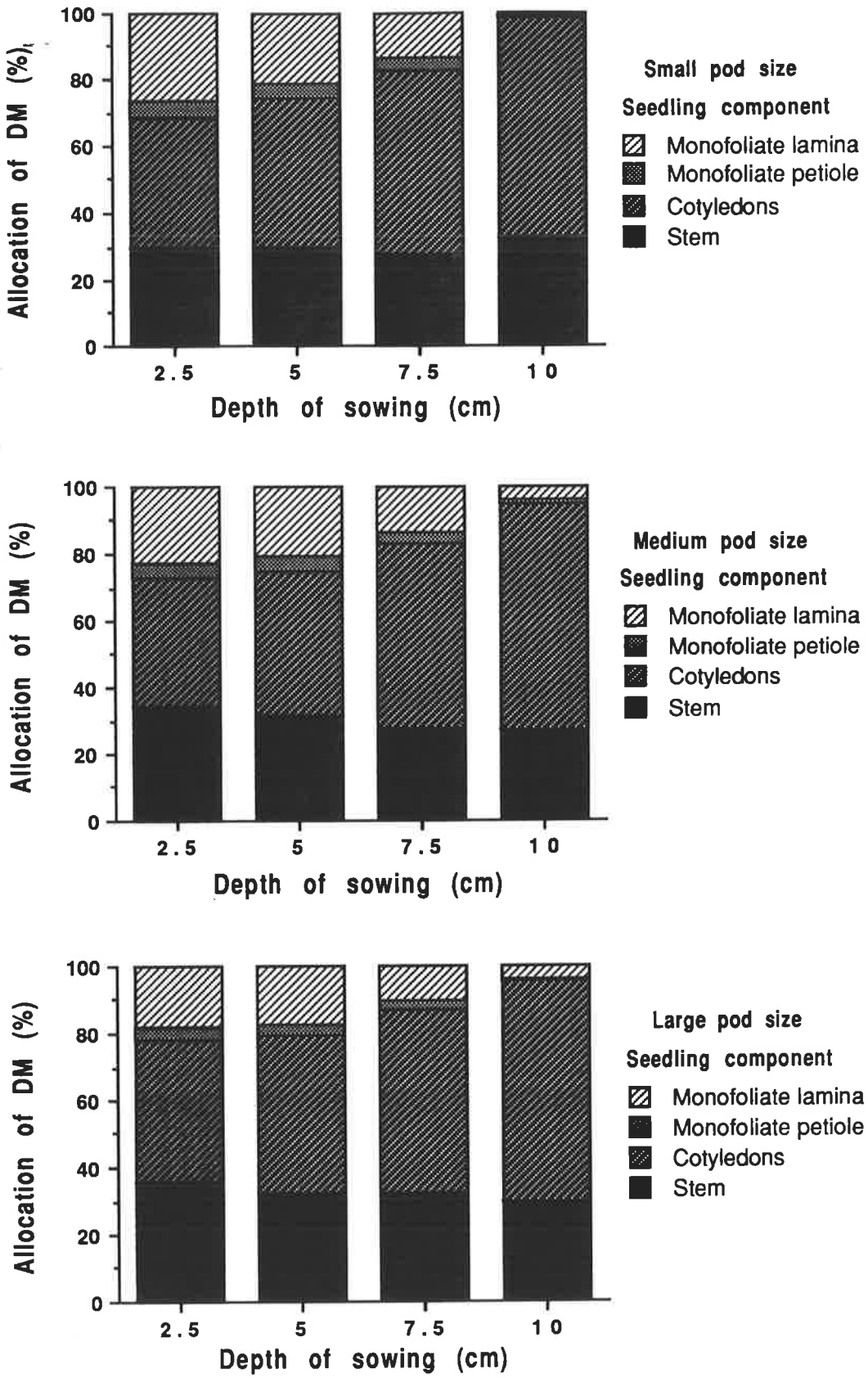
Appendix 4.6 The allocation of dry matter of Trikkala subterranean clover seedlings sown in a loamy soil with small, medium and large seed-size categories.

Appendix 4.7 **Individual lamina area of cotyledon and monofoliate leaf estimated from Sava snail medic and Trikkala subterranean clover seedlings obtained at harvest time (14 days).**

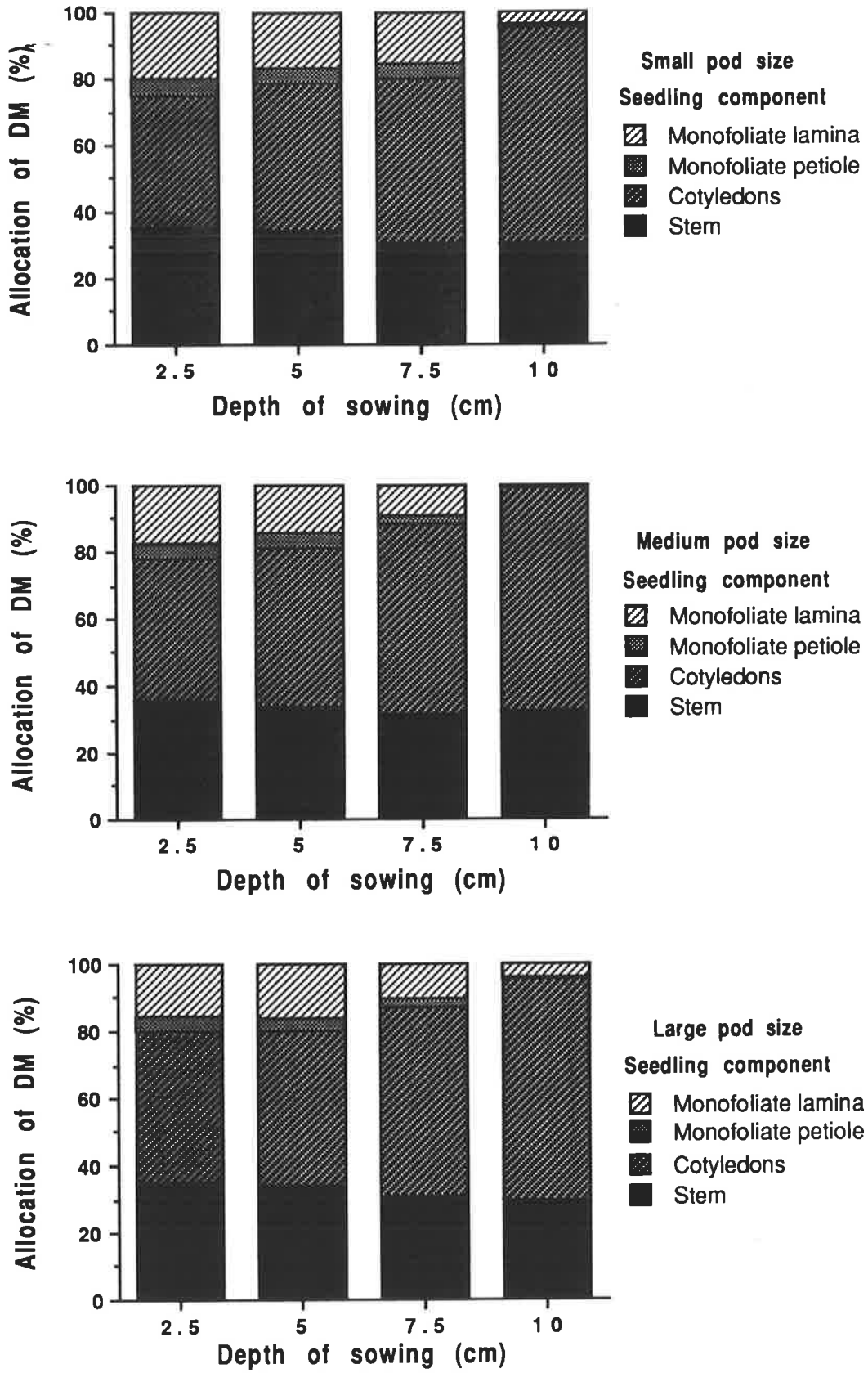
Seed size	Depth of sowing (cm)	Soil texture	Individual lamina area, cm^2: Cotyledon Sava	Cotyledon Trikkala	Monofoliate leaf Sava	Monofoliate leaf Trikkala
Small	1	Sandy	0.806	0.300	1.718	0.545
	3		0.789	0.302	1.646	0.497
	5		0.783	0.232	1.373	0.269
	7		0.571	-	1.119	-
	9		0.497	-	0.607	-
Medium	1	Sandy	1.152	0.378	2.114	0.546
	3		1.169	0.336	2.292	0.562
	5		1.045	0.323	1.886	0.351
	7		1.045	0.170	1.532	-
	9		0.707	-	1.052	-
Large	1	Sandy	1.716	0.506	2.819	0.573
	3		1.611	0.480	2.676	0.491
	5		1.653	0.214	2.317	0.361
	7		1.397	0.203	1.739	-
	9		1.061	-	1.397	-
Small	1	Loamy	1.019	0.370	1.935	0.617
	3		0.863	0.341	1.682	0.518
	5		0.733	0.198	1.379	0.305
	7		0.362	-	-	-
	9		-	-	-	-
Medium	1	Loamy	1.399	0.487	2.807	0.814
	3		1.218	0.442	2.248	0.500
	5		1.185	0.475	1.827	0.235
	7		0.737	-	1.014	-
	9		0.705	-	-	-
Large	1	Loamy	1.773	0.644	3.055	0.719
	3		1.516	0.588	1.669	0.537
	5		1.499	0.449	1.955	0.352
	7		1.327	-	1.582	-
	9		1.131	-	1.230	-

Appendix 4.8 **Cumulative emergence of Sava snail medic seedlings recorded at harvest time (21 days) and at the end of the experimental period (28 days).**

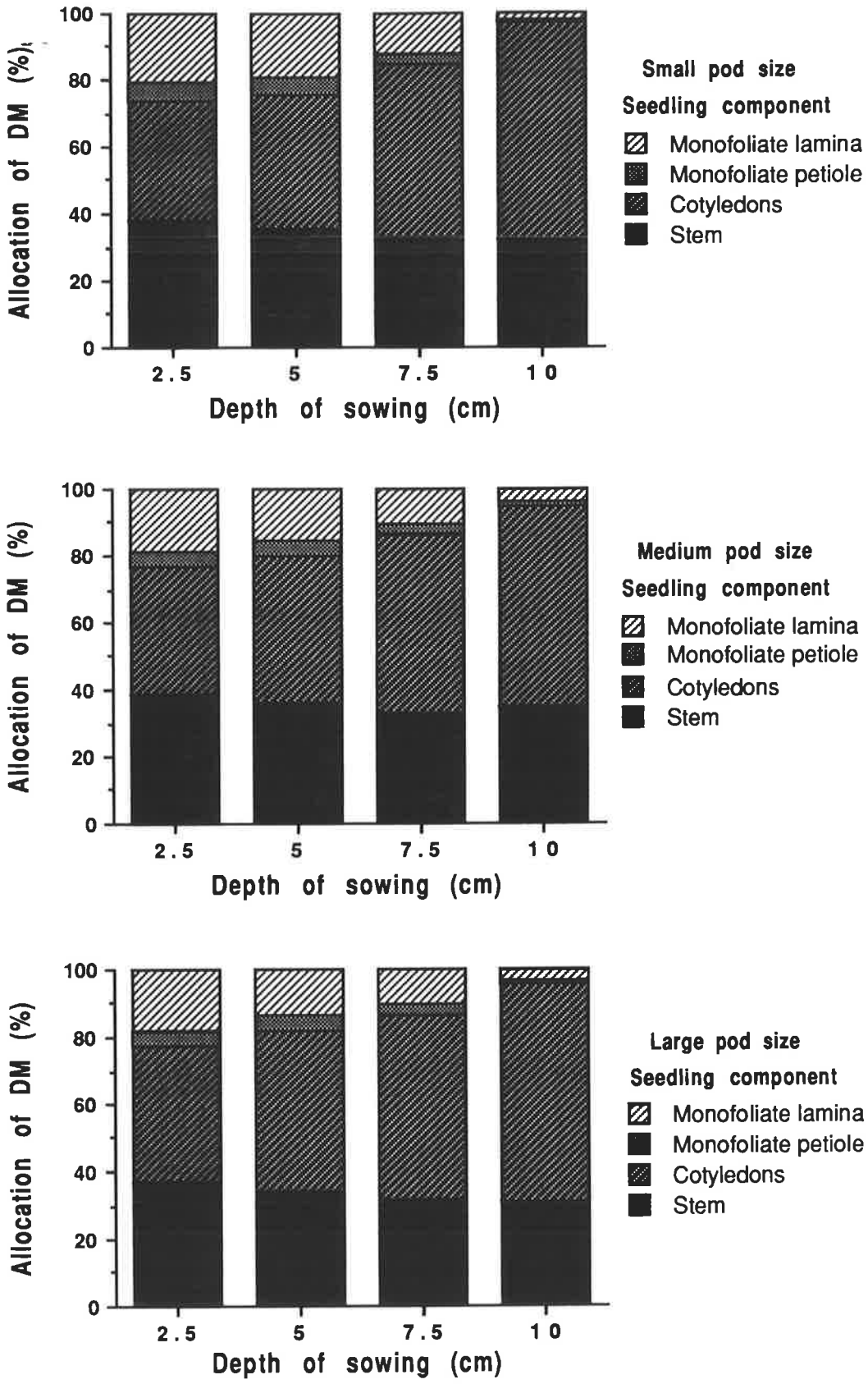
Pod-size	Depth of sowing (cm)	Straw residue (tonne DM/ha) 0	2	4	6	8
				21 days		
Small	2.5	99.0	91.5	89.3	91.5	71.8
	5.0	91.3	90.5	94.8	81.8	65.1
	7.5	82.8	80.8	79.8	58.5	62.5
	10.0	42.0	62.0	47.0	38.5	14.3
Medium	2.5	100.0	92.3	95.3	91.3	99.0
	5.0	93.3	93.5	77.3	77.8	72.3
	7.5	87.3	87.8	78.0	72.0	53.5
	10.0	47.5	59.0	54.8	55.8	20.0
Large	2.5	91.5	92.5	93.3	100.0	90.8
	5.0	88.3	87.0	79.8	70.0	66.5
	7.5	77.8	66.8	60.3	71.0	64.0
	10.0	50.8	64.3	55.3	40.5	39.0
				28 days		
Small	2.5	99.0	91.5	91.8	93.0	73.0
	5.0	91.3	91.0	96.3	82.3	68.1
	7.5	84.0	80.8	80.3	59.5	67.5
	10.0	43.0	62.0	52.3	41.8	18.0
Medium	2.5	100.0	96.5	97.3	91.8	100.0
	5.0	94.5	97.3	79.3	79.5	74.5
	7.5	89.0	87.8	78.0	76.3	53.5
	10.0	50.8	59.5	56.3	57.5	25.3
Large	2.5	93.3	94.8	96.5	100.0	94.3
	5.0	93.5	90.3	84.5	72.5	67.0
	7.5	81.5	71.5	61.3	74.3	66.3
	10.0	53.8	68.3	60.3	44.8	44.3



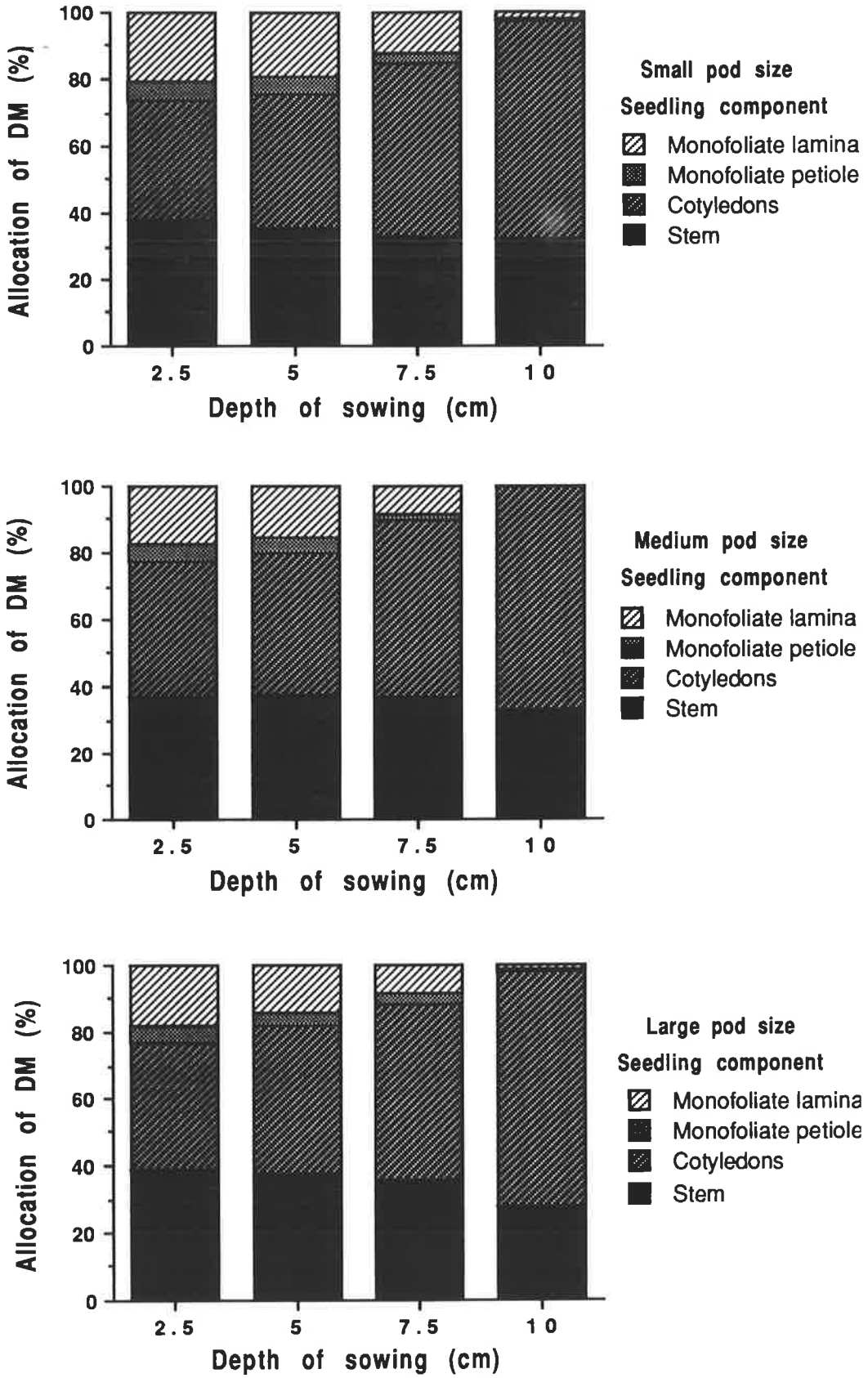
Appendix 4.9 The allocation of dry matter of Sava snail medic seedlings emerged from a soil without cover of wheat straw residues.



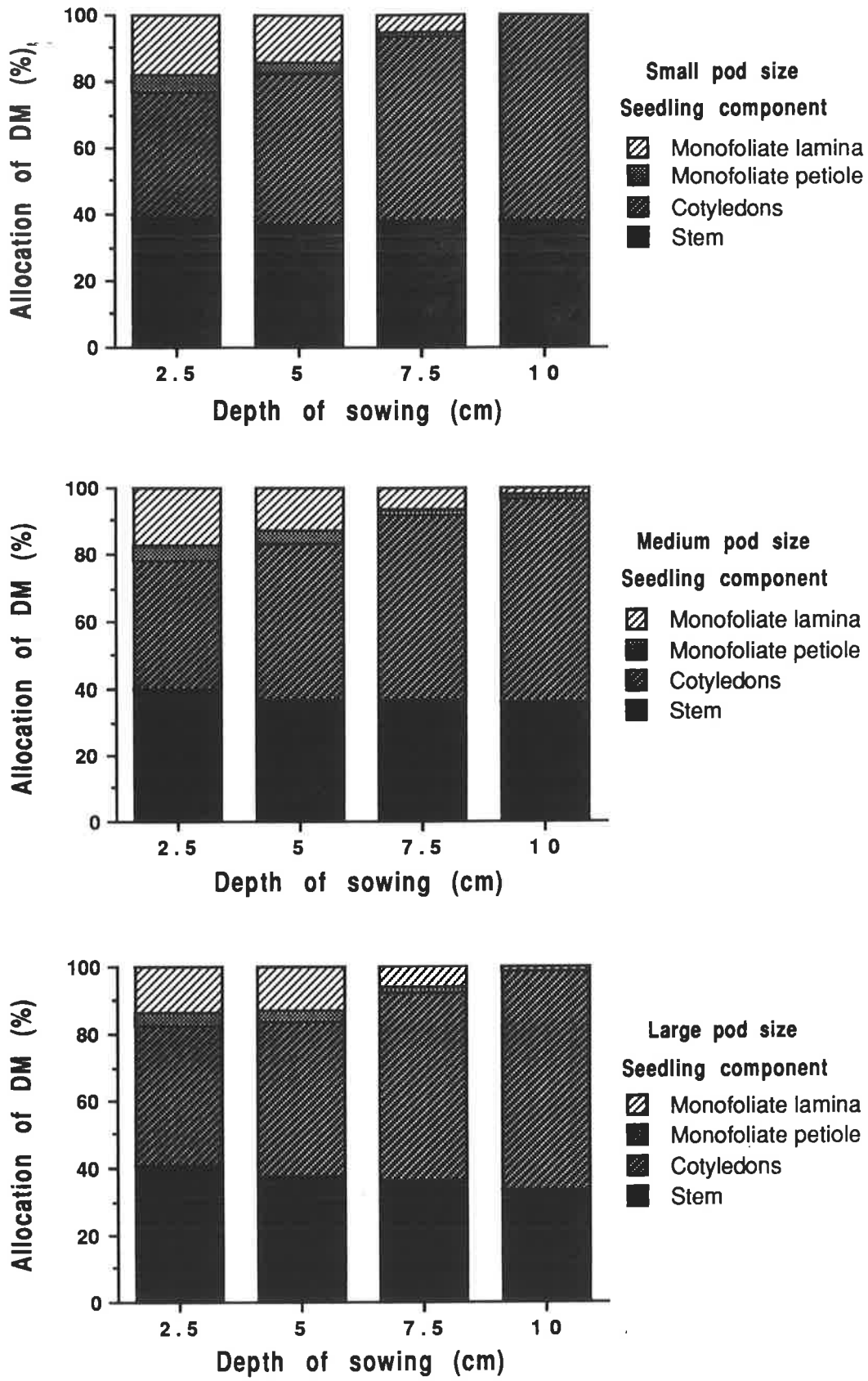
Appendix 4.10 The allocation of dry matter of Sava snail medic seedlings emerged from a soil covered with 2 ton DM/ha of wheat straw residues.



Appendix 4.11 The allocation of dry matter of Sava snail medic seedlings emerged from a soil covered with 4 ton DM/ha of wheat straw residues.



Appendix 4.12 The allocation of dry matter of Sava snail medic seedlings emerged from a soil covered with 6 ton DM/ha of wheat straw residues.



Appendix 4.13 The allocation of dry matter of Sava snail medic seedlings emerged from a soil covered with 8 ton DM/ha of wheat straw residues.

Appendix 4.14 Individual lamina area of cotyledons (cm^2) from Sava snail medic seedlings at harvest time (21 days).

Pod-size	Depth of sowing (cm)	Straw residue (tonne DM/ha) 0	2	4	6	8
Small	2.5	1.556	1.431	1.264	1.158	1.151
	5.0	1.365	1.241	1.210	1.136	1.045
	7.5	1.103	1.060	1.046	1.086	0.959
	10.0	0.852	0.881	0.903	0.930	0.828
Medium	2.5	1.768	1.733	1.425	1.499	1.415
	5.0	1.593	1.727	1.447	1.358	1.335
	7.5	1.301	1.206	1.287	1.224	1.178
	10.0	1.039	0.983	0.998	0.961	1.064
Large	2.5	1.927	1.953	1.865	1.752	1.589
	5.0	1.764	1.703	1.595	1.456	1.535
	7.5	1.527	1.461	1.383	1.458	1.300
	10.0	1.124	1.219	1.191	0.953	1.106

Appendix 4.15 Individual lamina area of monofoliate leaves (cm^2) from Sava snail medic seedlings at harvest time (21 days).

Pod-size	Depth of sowing (cm)	Straw residue (tonne DM/ha) 0	2	4	6	8
Small	2.5	2.009	1.653	1.832	1.578	1.413
	5.0	1.204	1.254	1.354	1.193	1.189
	7.5	0.932	0.727	0.818	0.629	0.587
	10.0	0.470	0.638	0.845	0.620	-
Medium	2.5	2.165	1.893	1.890	1.782	1.899
	5.0	1.561	1.131	1.341	1.297	1.428
	7.5	0.802	0.829	0.713	0.837	0.653
	10.0	0.790	0.730	0.748	-	0.788
Large	2.5	2.246	1.828	2.170	2.025	2.375
	5.0	1.417	1.496	1.401	1.466	1.740
	7.5	0.872	0.723	0.885	0.799	0.700
	10.0	1.002	0.930	1.000	0.710	0.870

Appendix 4.16 **Mean seed weight of Sava snail medic and Trikkala subterranean clover used in the various treatment combinations in the raised-bed experiment.**

Treatment		Seeds / treatment	Block			
Seed size - Plant density		(#)	I	II	III	Mean†
			(mg/seed)			
SAVA						
Small	Low	10	10.02	9.37	9.57	9.65
	Regular	50	10.26	9.96	10.46	10.23
	High	150	10.60	10.68	10.54	10.61
	Very high	660	10.55	10.66	10.57	10.59
Medium	Low	10	14.20	14.20	14.23	14.21
	Regular	50	14.71	14.76	14.52	14.66
	High	150	14.88	14.81	14.35	14.68
	Very high	660	15.04	15.06	15.18	15.09
Large	Low	10	23.00	23.73	23.16	23.30
	Regular	50	22.99	22.95	23.14	23.03
	High	150	23.18	23.24	23.30	23.24
	Very high	660	23.65	23.56	23.60	23.60
TRIKKALA						
Small	Low	10	6.85	6.95	7.17	6.99
	Regular	50	6.05	6.27	6.84	6.39
	High	150	6.75	6.67	6.62	6.68
	Very high	660	6.64	6.77	6.76	6.72
Medium	Low	10	9.37	9.40	10.06	9.61
	Regular	50	9.38	10.04	9.55	9.66
	High	150	10.08	9.87	9.99	9.98
	Very high	660	9.45	9.59	9.62	9.55
Large	Low	10	13.47	12.20	12.97	12.88
	Regular	50	13.25	12.56	13.05	12.95
	High	150	13.21	13.17	13.00	13.13
	Very high	660	13.20	13.50	13.62	13.44

† Represents the mean seed weight of the seed lots used in the six corresponding harvests.

Appendix 4.17 **Seed-size categories and seedling emergence of Sava snail medic and Trikkala subterranean clover in the raised-bed experiment.**

Treatment		Before adjustment			After adjustment
Seed size	Plant density	Potential germinable sowing density (#/m^2)	Emerged Seedling density (#/m^2)	Emergence (%)	Established seedling density (#/m^2)
SAVA					
Small	Low	190	190	100.0	100
	Regular	950	873	91.9	500
	High	2850	2839	99.6	2500
	Very high	12540	12540	100.0	12500
Medium	Low	198	192	97.0	100
	Regular	988	940	95.1	500
	High	2963	2913	98.3	2500
	Very high	13035	12592	96.6	12500
Large	Low	198	182	91.9	100
	Regular	988	964	97.6	500
	High	2963	2880	97.2	2500
	Very high	13035	12800	98.2	12500
TRIKKALA					
Small	Low	208	195	93.8	100
	Regular	1038	947	91.2	500
	High	3113	2839	91.2	2500
	Very high	13695	12627	92.2	12500
Medium	Low	205	192	93.7	100
	Regular	1025	979	95.5	500
	High	3075	2915	94.8	2500
	Very high	13530	12596	93.1	12500
Large	Low	205	182	88.8	100
	Regular	1025	1005	98.0	500
	High	3075	2881	93.7	2500
	Very high	13530	12799	94.6	12500

Appendix 4.18 Climatic data at the Waite Agricultural Research Institute during the raised-bed experiment, 1989 (Meteorological station: 34° 58' S. Lat. and 138° 38' E. Long., 122.5 m.o.s.l.).

Variable	Month			
	April	May	June	Period
Monthly rainfall (mm)	54.0	97.0	104.8	255.8
Monthly evaporation (mm), Standard A class pan	101.0	60.2	35.2	196.4
Mean relative humidity (%), 0900 hours	63.6	76.2	79.0	72.9
Mean of daily air temperatures (°C)				
Maximum	22.2	18.3	13.5	18.0
Minimum	14.0	11.7	7.6	11.1
Media, [0.5 (Max. + Min.)]	18.1	15.0	10.5	14.5
Extremes air temperatures (°C)				
Highest	32.4	21.8	19.4	32.4
Lowest	9.0	8.1	2.7	2.7
Mean daily minimum temperature on the grass (°C)				
Mean	9.6	8.8	4.4	7.6
Lowest	4.1	4.0	-1.3	-1.3
Mean daily hours of bright sunshine per day	6.16	4.07	2.87	4.37
Mean daily solar radiation (Meg J/sq. m/day)	12.59	7.88	5.73	8.37
Mean of daily soil temperatures (°C) at 2.5 cm.				
Maximum	30.3	22.3	16.3	23.0
Minimum	14.3	11.6	7.4	11.1
Mean of daily soil temperatures (°C) at 15.2 cm.				
Maximum	23.0	17.6	12.9	17.8
Minimum	18.5	14.8	10.5	14.6
Mean wind speed at 1.25 metres (km per day)	99.9	87.5	89.1	92.2

Source: Biennial Report, Waite Agricultural Research Institute, The University of Adelaide, 1988-89.

Appendix 4.19 **Trends in shoot yield produced by various sowing rates of small, medium and large seed sizes of Sava snail medic.**

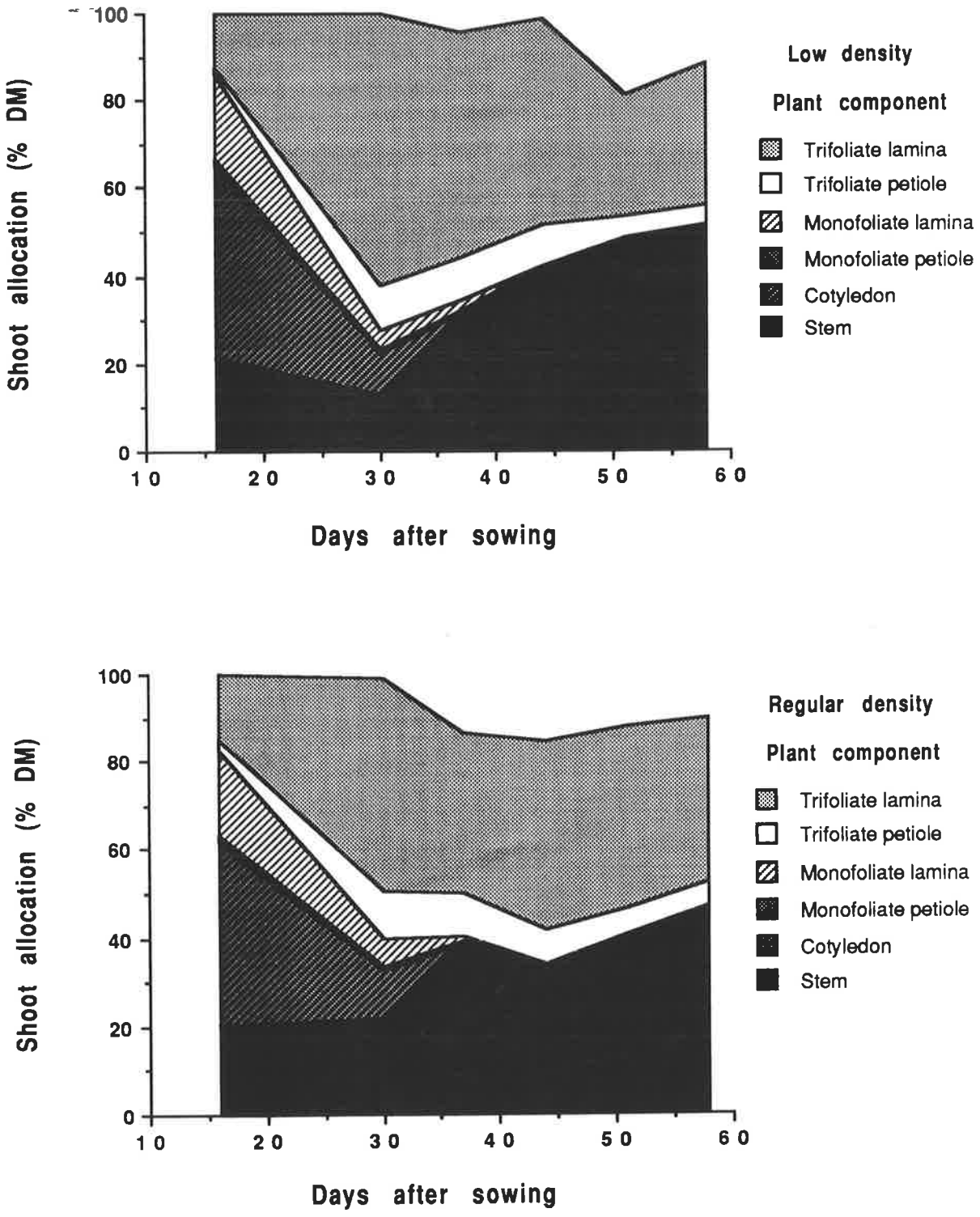
Seed size	Plant Density	Harvest time (days after full emergence) 9	23	30	37	44	51	Polynomial equation order	Correlation coefficient (R)
		(g/DM/m^2)							
Small	Low	1.9	9.2	16.6	17.4	14.9	32.2	2nd	0.909 *
	Regular	8.1	32.7	62.4	57.3	73.9	117.7	2nd	0.963 **
	High	37.9	96.7	124.2	173.0	117.1	272.0	2nd	0.875 NS
	Very High	111.7	205.9	348.2	276.8	313.6	358.5	2nd	0.913 *
Medium	Low	2.3	11.8	18.4	22.0	39.2	35.2	2nd	0.959 **
	Regular	12.0	41.7	54.2	58.4	121.1	112.6	2nd	0.946 *
	High	44.8	111.4	155.2	196.5	299.8	258.9	2nd	0.954 *
	Very High	156.0	257.8	309.8	374.6	390.6	441.2	2nd	0.996 ***
Large	Low	3.2	12.4	22.5	27.0	30.0	59.6	2nd	0.960 **
	Regular	17.6	58.3	70.4	86.8	125.4	169.0	2nd	0.992 ***
	High	60.4	142.5	193.3	276.7	341.5	475.0	2nd	0.997 ***
	Very High	229.6	374.8	633.1	549.4	535.2	549.0	2nd	0.907 *

NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

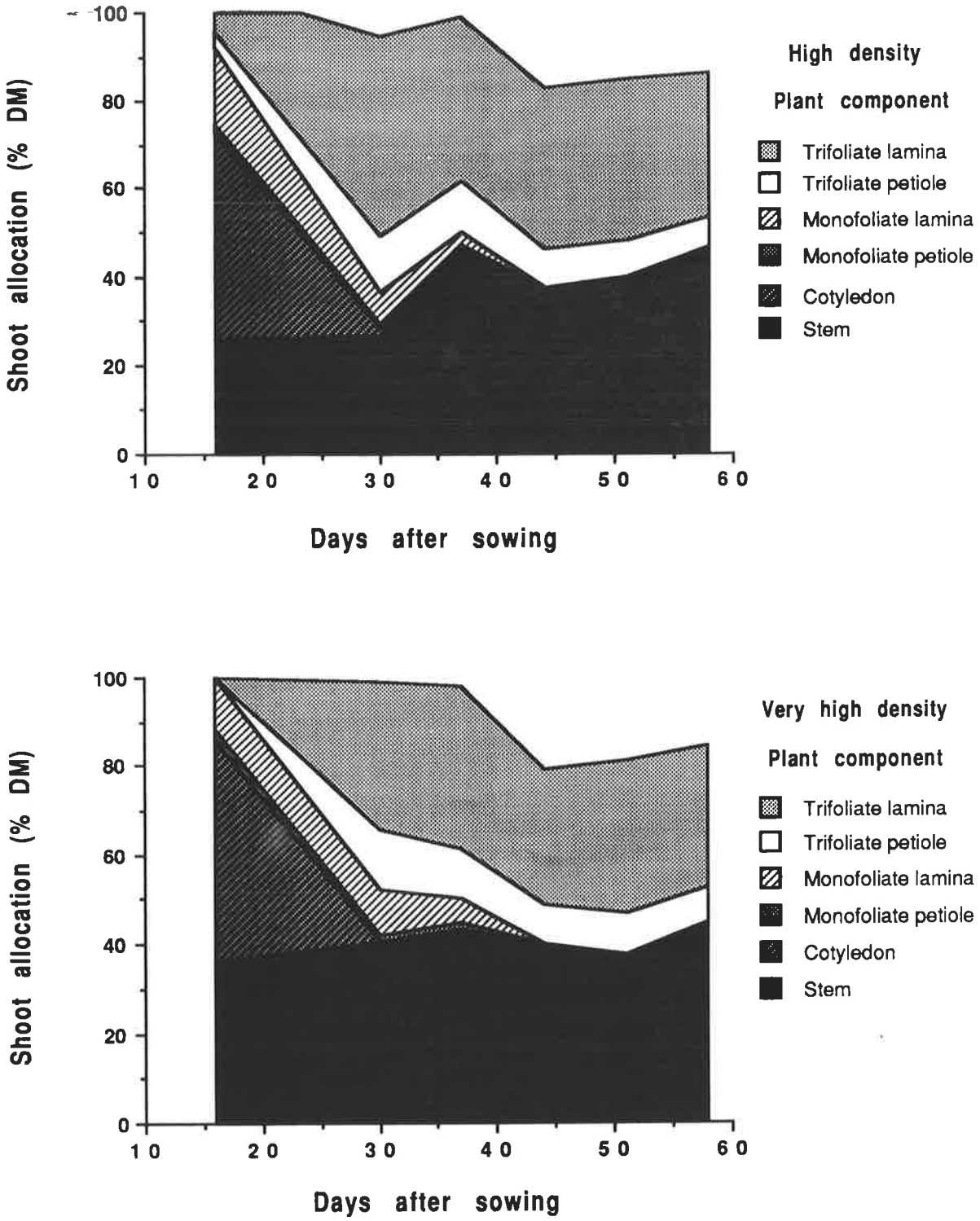
Appendix 4.20 **Trends in shoot yield produced by various sowing rates of small, medium and large seed sizes of Trikkala subterranean clover.**

Seed size	Plant Density	Harvest time (days after full emergence) 9	23	30	37	44	51	Polynomial equation order	Correlation coefficient (R)
		(g/DM/m^2)							
Small	Low	1.3	6.0	13.4	17.3	26.5	39.6	2nd	0.996 ***
	Regular	6.0	27.3	50.8	65.4	108.3	123.8	2nd	0.991 **
	High	23.6	92.6	150.5	227.0	286.8	335.4	2nd	0.996 **
	Very High	99.8	227.5	372.4	427.9	500.0	486.1	2nd	0.982 **
Medium	Low	1.6	7.8	15.5	26.8	38.6	55.2	2nd	0.999 ***
	Regular	7.9	34.7	62.5	83.5	124.0	149.6	2nd	0.997 ***
	High	34.9	122.6	205.8	287.1	354.9	405.4	2nd	0.995 ***
	Very High	128.1	282.0	444.8	511.9	574.3	574.7	2nd	0.985 **
Large	Low	2.0	9.1	18.8	35.6	43.2	56.5	2nd	0.992 ***
	Regular	9.1	37.8	79.1	105.8	163.1	168.1	2nd	0.982 **
	High	42.1	159.2	234.3	300.0	405.9	456.9	2nd	0.997 ***
	Very High	156.4	338.8	502.0	559.4	675.6	664.8	2nd	0.988 **

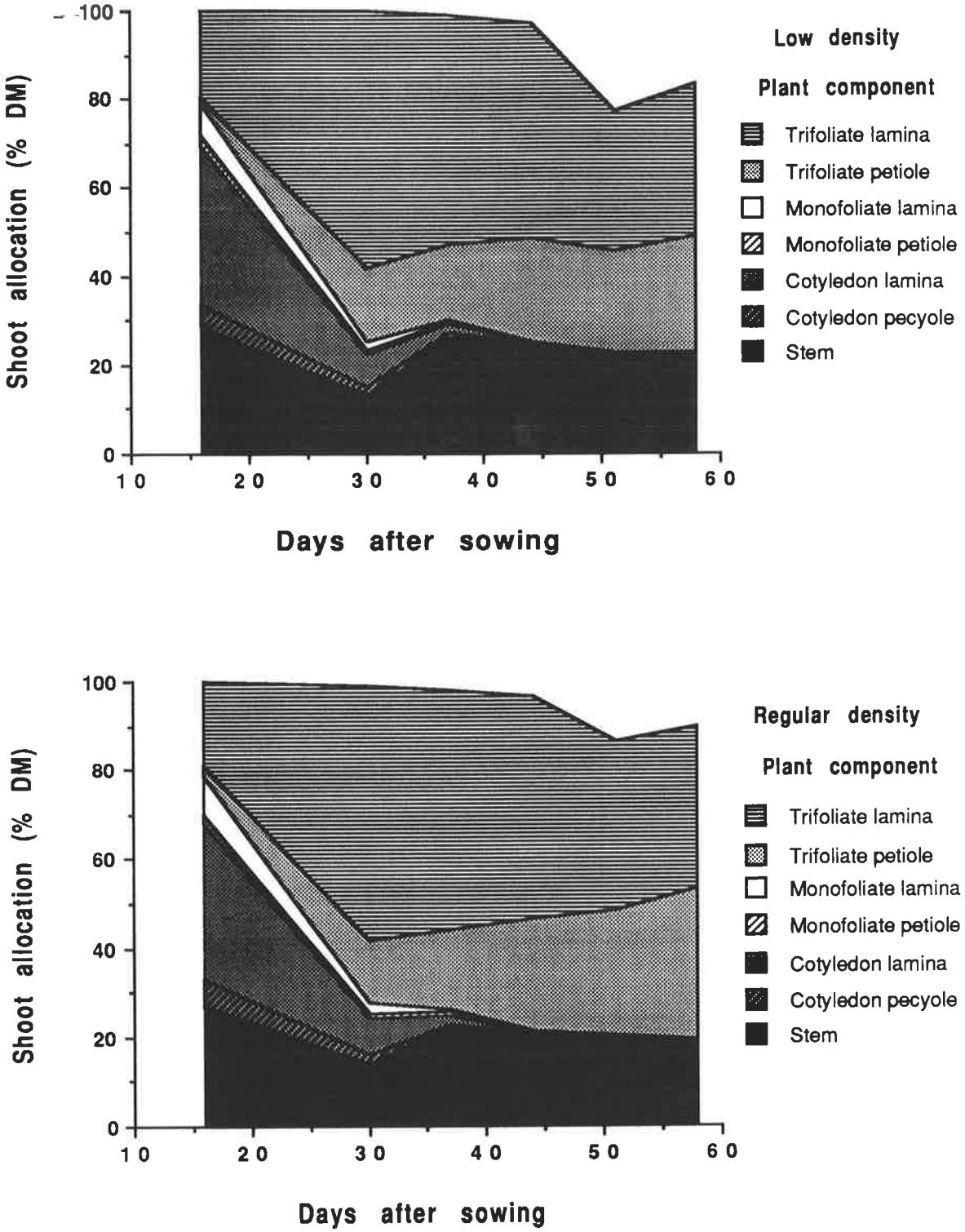
** = P<0.01; *** = P<0.001



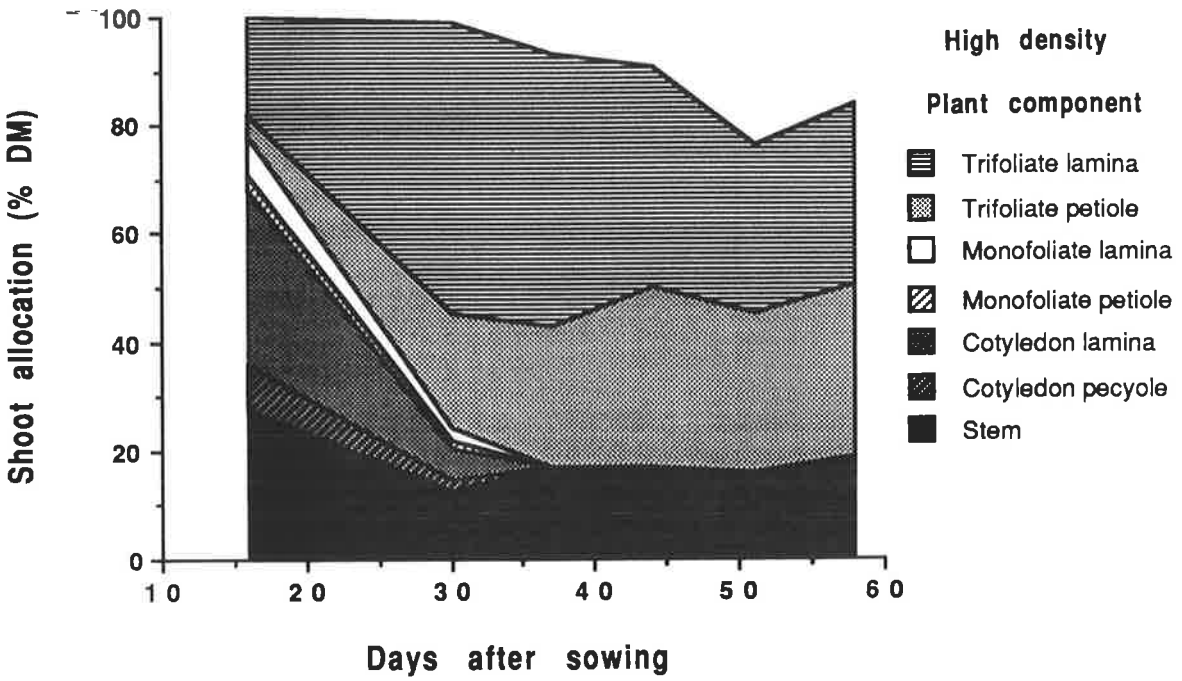
Appendix 4.21 The allocation of dry matter in Sava snail medic plants grown from large seed size sown at low and regular densities.

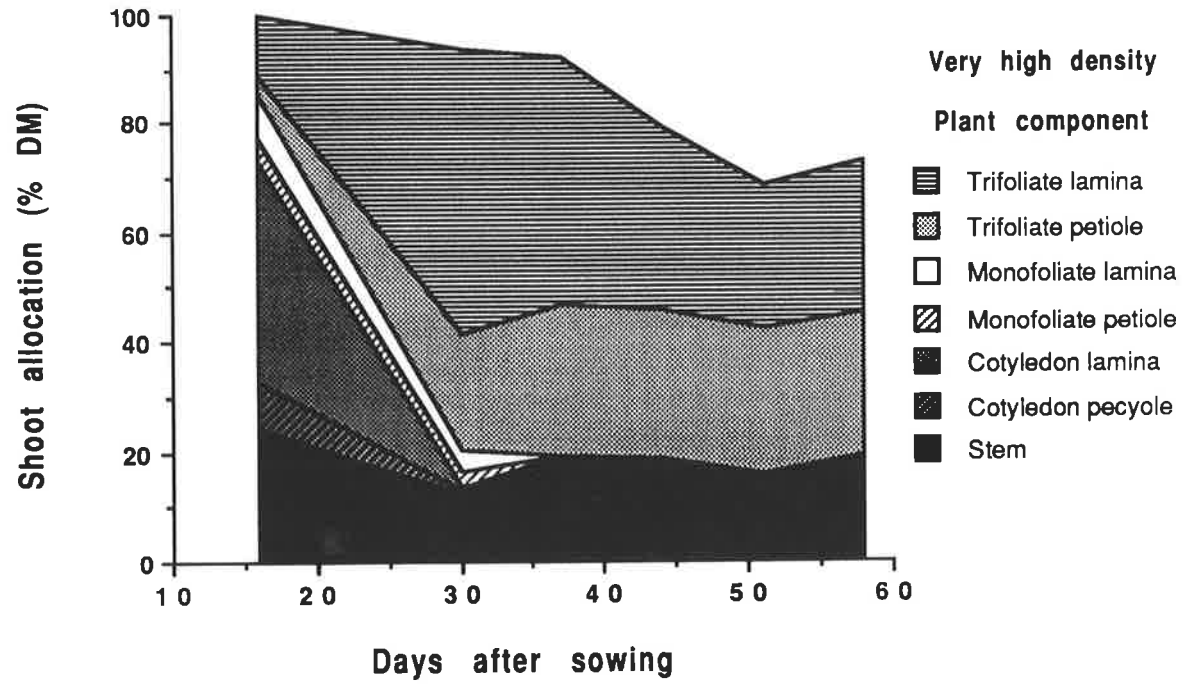


Appendix 4.22 The allocation of dry matter in Sava snail medic plants grown from large seed size sown at high and very high densities.



Appendix 4.23 The allocation of dry matter in Trikkala subterranean clover plants grown from large seed size sown at low and regular densities.





Appendix 4.24 The allocation of dry matter in Trikkala subterranean clover plants grown from large seed size sown at high and very high densities.

Appendix 4.25 **Impact of seed-size and sowing rate on Leaf Area Index of Sava snail medic.**

Seed size	Plant Density	Harvest time (days after full emergence) 9	23	30	37	44	51	Polynomial equation order	Correlation coefficient (R)
Small	Low	0.05	0.19	0.27	0.27	0.25	0.44	3rd	0.966 *
	Regular	0.25	0.78	1.00	0.96	1.24	2.09	3rd	0.993 **
	High	1.15	3.35	2.36	2.41	1.71	3.41	3rd	0.955 *
	Very High	3.26	6.31	5.82	4.09	4.59	5.18	3rd	0.962 *
Medium	Low	0.06	0.25	0.42	0.25	0.35	0.56	3rd	0.931 NS
	Regular	0.34	0.93	0.90	0.99	1.93	2.52	3rd	0.982 *
	High	1.24	4.01	3.61	3.75	4.43	3.43	3rd	0.939 NS
	Very High	3.48	5.70	5.32	4.37	4.54	6.06	3rd	0.995 **
Large	Low	0.08	0.26	0.48	0.33	0.46	1.04	3rd	0.963 *
	Regular	0.47	1.40	1.53	1.57	2.10	3.28	3rd	0.999 ***
	High	1.84	4.02	4.16	5.05	4.53	5.31	3rd	0.977 *
	Very High	6.09	8.28	7.83	7.67	5.92	5.71	3rd	0.969 *

NS = Not significant; * = P<0.05; ** = P<0.01; *** = P<0.001

Appendix 4.26 **Impact of seed-size and sowing rate on Leaf Area Index of Trikkala subterranean clover.**

Seed size	Plant Density	Harvest time (days after full emergence) 9	23	30	37	44	51	Polynomial equation order	Correlation coefficient (R)
Small	Low	0.02	0.12	0.21	0.32	0.36	0.62	2nd	0.984 **
	Regular	0.11	0.53	0.76	1.47	2.00	1.96	2nd	0.970 **
	High	0.55	2.15	2.70	2.47	2.62	2.64	3rd	0.991 **
	Very High	2.29	7.19	6.87	6.54	4.83	4.82	3rd	0.991 **
Medium	Low	0.03	0.16	0.28	0.41	0.49	0.84	2nd	0.986 **
	Regular	0.16	0.57	1.13	1.71	2.13	3.02	2nd	0.997 ***
	High	0.76	2.32	3.26	3.57	3.11	3.55	2nd	0.975 **
	Very High	3.22	8.11	7.40	7.83	5.13	4.91	3rd	0.963 *
Large	Low	0.03	0.18	0.32	0.60	0.70	0.80	2nd	0.983 **
	Regular	0.16	0.86	1.35	2.36	2.49	3.18	2nd	0.987 **
	High	0.90	3.72	4.24	3.86	3.37	3.51	3rd	0.994 **
	Very High	3.50	8.14	8.05	7.02	5.52	5.72	3rd	0.995 **

* = P<0.05; ** = P<0.01; *** = P<0.001

Appendix 5.1 **Monthly rainfall recorded at Waite Agriculture Research Institute meteorological station during the period 1989-1990. (34° 58' S. Lat. and 138° 38' E. Long., 122.5 m.o.s.l.).**

Month	Mean (1925-89) (mm)	Year 1989 (mm)	Year 1990 (mm)
January	23.4	2.6	22.4
February	24.3	1.6	13.6
March	23.8	2.8	1.2
April	54.9	54.0	17.0
May	80.3	97.0	18.4
June	74.6	104.8	99.2
July	87.9	87.0	110.0
August	74.8	88.8	98.8
September	61.9	51.8	50.0
October	52.6	38.2	46.6
November	36.9	33.8	16.4
December	29.9	13.4	50.4
Year	625.3	575.8	544.0

Source: Biennial Report 1989-90, The University of Adelaide; Waite Agricultural Research Institute.

Appendix 5.2 **Bodyweight of the sheep-flock used to test the survival of clover seeds grazing dry pasture residues at the Waite Institute.**

Sheep (#)	Pre-experimental period Initial (kg/head)	Pre-experimental period Final (kg/head)	Change of weight (kg/head)	Experimental period Final (kg/head)	Change of weight (kg/head)
1	47.2	47.3	0.1	40.0	-7.3
2	44.2	48.3	4.1	36.3	-12.0
3	43.0	46.3	3.3	43.2	-3.1
4	53.0	52.3	-0.7	53.0	0.7
5	41.6	45.3	3.7	47.1	1.8
6	49.0	50.3	1.3	49.0	-1.3
7	48.6	48.4	-0.2	48.0	-0.4
8	42.2	45.3	3.1	44.2	-1.1
9	46.0	48.4	2.4	46.0	-2.4
10	45.6	46.4	0.4	50.0	3.6
Sum	460.4	478.3	17.5	456.8	-21.5
Mean	46.0	47.8	1.8	45.7	-2.1
S.D.	3.35	2.11	-	4.68	-
C.V. (%)	7.3	4.4	-	10.2	-
S.E.	1.06	0.67	-	1.48	-

Appendix 5.3 **Characteristics of the feed pellets * used for penned sheep during the pre-experimental period.**

Ingredients:	%	iu/kg	mg/kg	Analysis specifications:	kcal/kg	%
Oat hulls	40	-	-	Digestible Energy	2160	-
Oats	39	-	-	Crude Protein	-	7.8
Triticale	15	-	-	Crude Fibre	-	17
Bentonite	2	-	-	Calcium	-	0.66
Salt	1	-	-	Phosphorus	-	0.24
Limestone	2	-	-			
Urea	0.5	-	-			
Vitamin and Minerals**	1	-	-			
** Vitamin A	-	8250	-			
Vitamin D3	-	1650	-			
Iron	-	-	93	as Ferrous Sulphate		
Manganese	-	-	48	as Manganous oxide		
Zinc	-	-	52	as Zinc oxide		
Cobalt	-	-	1	as Cobalt Sulphate		
Molybdenum	-	-	0.4	as Sodium Molybdate		
Iodine	-	-	0.6	as Potassium Iodide		

* Australasian Feed Services Pty. Ltd., South Australia, Australia.

Appendix 5.4 **Body-weight and fat score of the sheep flock used to test the rumen degradability of the forage components of the summer-autumn grazing experiment and the survival of the seed exposed in the rumen.**

Sheep (#)	Body weight (kg)		Fat score	
	Initial	Final	Initial	Final
1	31.5	30.0	3	3
2	31.5	30.8	3	3
3	35.0	35.8	3	3
4	34.0	35.4	3	4
Sum	132.0	132.0	12.0	13.0
Mean	33.0	33.0	3.0	3.3
S.D.	1.54	2.62	0.0	0.43
C.V. (%)	4.7	7.9	0.0	13.03
S.E.	0.77	1.31	0.0	0.22

Appendix 5.5 **Availability of dry herbage residues and level of bare ground estimated in a subterranean clover-balansa clover pasture during the grazing period.**

Species component	Cumulative days of grazing											Fitted equation	Correlation coefficient (R)†
	0	7	14	21	28	35	42	49	56	63	70		
SOWN	(kg DM/ha)												
Subterranean clover, burrs	1555	1771	1453	1190	1115	1028	544	310	185	107	91	Linear	0.973***
Balansa clover, heads	439	165	77	27	24	8	1	0	0	0	0	Polynomial (3rd)	0.979***
Pasture, stems and leaves	3438	2514	1919	1386	1412	1171	770	379	210	33	6	Polynomial (3rd)	0.991***
Total pasture:	5432	4450	3449	2603	2551	2207	1315	689	395	140	97	Polynomial (2nd)	0.992***
DESIRABLE VOLUNTEERS													
Cluster clover, heads	20	20	17	8	5	3	2	0	0	0	0	Polynomial (2nd)	0.977***
Cluster clover, stems and leaves	9	7	5	2	1	1	0	0	0	0	0	Polynomial (2nd)	0.988***
Total Cluster clover:	29	27	22	10	6	4	1	0	0	0	0	Polynomial (2nd)	0.984***
UNDESIRABLE VOLUNTEERS													
Weeds	10	1	0	1	3	3	2	0	0	0	0	-	-
Total herbage	5471	4478	3471	2614	2560	2214	1318	689	395	140	97	Polynomial (2nd)	0.992***
Bare ground (%)	0.0	16.0	18.8	33.6	37.3	41.8	50.0	59.4	61.3	80.3	94.9	Polynomial (3rd)	0.995***

† Significance level *** = P<0.001

Note: Seeds of balansa clover and cluster clover available at soil surface level, and clover seeds harvested by ants are not included.

Appendix 5.6 Characteristic of the trend in burrs and seeds of subterranean clover during the grazing period.

Variable	Cumulative days of grazing											Fitted equation	Correlation coefficient (R)†
	0	7	14	21	28	35	42	49	56	63	70		
AT PLANT AND SOIL LEVEL													
Burr density (#/m^2)	4472	5400	4704	3964	3643	3478	2201	1210	746	339	253	Polynomial (3rd)	0.989***
Burr weight (mg)	35.1	32.8	30.9	30.0	30.6	29.6	24.7	25.6	24.8	31.6	35.9	Polynomial (5th)	0.960***
Seeds / burr (#)	3.26	3.13	3.06	2.85	3.03	2.86	2.98	2.53	2.60	2.65	2.64	Polynomial (4th)	0.915**
Seed density (#/m^2)	14586	16902	14394	11289	11038	9947	6559	3061	1940	898	667	Polynomial (3rd)	0.988***
Seed weight (mg)	7.20	7.12	7.20	6.84	6.56	6.43	5.92	6.60	6.53	7.03	6.74	Polynomial (5th)	0.932**
Seed reserve (kg/ha)	1050	1203	1036	773	724	640	388	202	127	63	45	Polynomial (3rd)	0.990***
Seed weight/burr (%)	50.9	47.9	50.6	48.6	49.2	48.9	49.5	51.0	53.8	51.7	54.3	-	-

† Significance level ** = P<0.01; *** = P<0.001

Appendix 5.7 **Characteristic of buried and surface burrs and seeds of subterranean clover before grazing and at the end of the grazing period.**

	Grazing period (days)			
	0	0	70	70
Variable	**Buried**	**Surface**	**Buried**	**Surface**
Burr density (#/m^2)	251	4221	65	188
Burr weight (mg)	38.7	34.9	38.4	36.0
Seeds/burr (#)	3.29	3.26	3.20	2.44
Seed density (#/m^2)	826	13760	208	459
Seed weight (mg)	7.46	7.18	8.13	6.12
Seed reserve (kg/ha)	62	988	17	28
Seed/burr weight (%)	52.5	50.8	53.8	55.1
Buried/surface (%)	5.6	94.4	25.7	74.3

Appendix 5.8 Characteristic of the trend in heads and seeds of balansa clover during the grazing period.

Variable	Cumulative days of grazing											Fitted equation	Correlation coefficient (R)†
	0	7	14	21	28	35	42	49	56	63	70		
AT PLANT LEVEL													
Head density (#/m^2)	1059	498	256	148	150	53	7	0	0	0	0	Polynomial (4th)	0.995***
Head weight (mg)	41.5	33.1	30.1	18.0	15.7	14.7	15.6	-	-	-	-	Polynomial (3rd)	0.987***
Seeds/head (#)	18.36	12.19	11.80	4.30	4.46	3.53	4.00	-	-	-	-	Polynomial (2nd)	0.971***
Seed density (#/m^2)	19442	6072	3020	636	669	187	28	0	0	0	0	Polynomial (5th)	0.997***
Seed weight (mg)	0.771	0.746	0.745	0.739	0.747	0.695	0.714	-	-	-	-	Linear	0.842***
Seed reserve (kg/ha)	149.9	45.3	22.5	4.7	5.0	1.3	0.2	0.0	0.0	0.0	0.0	Polynomial (5th)	0.997***
Seed weight/head (%)	34.1	27.5	29.2	17.4	20.8	16.3	20.0	-	-	-	-	-	-
AT SOIL SURFACE LEVEL													
Seed density (#/m^2)	9756	8862	8298	10961	10538	11471	10409	11705	12580	12496	12683	Linear	0.879***
Seed weight (mg)	0.779	0.738	0.712	0.718	0.732	0.724	0.717	0.713	0.727	0.725	0.724	Polynomial (5th)	0.962***
Seed reserve (kg/ha)	76.0	65.4	59.1	78.7	77.1	83.1	74.6	83.5	91.5	90.6	91.8	Polynomial (2nd)	0.826**
Total seed density (#/m^2)	29198	14934	11318	11597	11207	11658	10437	11705	12580	12496	12683	Polynomial (4th)	0.979***
Total seed reserve (kg/ha)	225.9	110.7	86.6	83.4	82.1	84.4	74.8	83.5	91.5	90.6	91.8	Polynomial (5th)	0.996***

† Significance level ** = P<0.01; *** = P<0.001

Appendix 5.9 Characteristics of the trend in heads and seeds of cluster clover during the grazing period.

Variable	Cumulative days of grazing											Fitted equation	Correlation coefficient (R)†
	0	7	14	21	28	35	42	49	56	63	70		
AT PLANT LEVEL													
Head density (#/m^2)	89	89	95	45	32	18	7	0	0	0	0	Polynomial (5th)	0.987***
Head weight (mg)	22.5	22.5	17.9	17.8	15.6	16.7	14.3	-	-	-	-	Polynomial (2nd)	0.944***
Seeds/head (#)	29.20	27.44	19.57	20.96	16.72	16.33	14.00	-	-	-	-	Polynomial (2nd)	0.964***
Seed density (#/m^2)	2599	2442	1859	943	535	294	70	0	0	0	0	Polynomial (2nd	0.987***
Seed weight (mg)	0.412	0.424	0.442	0.406	0.434	0.435	0.420	-	-	-	-	-	-
Seed reserve (kg/ha)	10.7	10.4	8.2	3.8	2.3	1.3	0.3	0.0	0.0	0.0	0.0	Polynomial (2nd)	0.982***
Seed weight/head (%)	53.5	52.0	48.2	47.5	46.0	43.3	30.0	-	-	-	-	-	-
AT SOIL SURFACE LEVEL													
Seed density (#/m^2)	509	760	789	962	814	994	1059	1150	1040	1064	914	Polynomial (3rd)	0.928***
Seed weight (mg)	0.432	0.447	0.443	0.384	0.444	0.405	0.389	0.416	0.439	0.411	0.420	-	-
Seed reserve (kg/ha)	2.2	3.4	3.5	3.7	3.6	4.0	4.1	3.6	4.6	4.4	3.8	Polynomial (3rd)	0.863**
Total seed density (#/m^2)	3108	3202	2648	1905	1349	1288	1129	1150	1140	1064	914	Polynomial (2nd)	0.972***
Total seed reserve (kg/ha)	12.9	13.8	11.7	7.5	5.9	5.3	4.4	3.6	4.6	4.4	3.8	Polynomial (2nd)	0.962***

† Significance level ** = P<0.01; *** = P<0.001

Appendix 5.10 **Germination test done on seeds of buried and surface burrrs of subterranean clover collected during the grazing period.**

Seed condition	Cumulative days of grazing												
	0	0	7	14	21	28	35	42	49	56	63	70	70
	Buried	Surface											Buried
	(%)												
Germinated	23.0	28.5	33.0	32.0	30.0	32.5	37.0	36.5	37.0	36.5	38.5	52.5	42.5
Dormant	0.0	0.5	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0
Dead	0.0	0.5	0.5	0.5	1.0	0.5	1.5	0.8	0.5	0.3	0.0	0.3	0.5
SOFT	23.0	29.5	33.5	32.5	31.3	33.0	38.5	37.3	37.5	36.8	38.8	52.8	43.0
HARD	77.0	70.5	66.5	67.5	68.7	67.0	61.5	62.7	62.5	63.2	61.2	47.2	57.0
Hard seed weight (mg)	6.54	6.12	6.46	6.62	6.23	6.11	6.08	6.24	6.18	6.31	6.35	6.47	7.46

Appendix 5.11 **Germination test done on seeds of balansa clover present in heads and at soil surface level and collected during the grazing period.**

Seed condition	Cumulative days of grazing										
	0	7	14	21	28	35	42	49	56	63	70
AT PLANT LEVEL					(%)						
Germinated	8.0	11.0	13.0	10.0	11.5	14.0	13.0	-	-	-	-
Dormant	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
Dead	2.0	1.8	3.5	2.8	2.5	1.0	0.0	-	-	-	-
SOFT	10.0	12.8	16.5	12.8	14.0	15.0	13.0	-	-	-	-
HARD	90.0	87.2	83.5	87.2	86.0	85.0	87.0	-	-	-	-
Hard seed weight (mg)	0.783	0.752	0.729	0.747	0.754	0.731	0.691	-	-	-	-
AT SOIL SURFACE LEVEL											
Germinated	6.8	9.0	9.0	7.5	7.0	10.8	11.3	12.3	11.5	20.8	32.8
Dormant	0.0	0.0	0.3	0.5	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Dead	1.0	1.8	0.8	0.3	1.0	1.0	1.3	0.8	1.8	0.3	0.5
SOFT	7.8	10.8	10.1	8.3	8.0	11.8	12.6	13.1	13.8	21.1	33.3
HARD	92.2	89.2	89.9	91.7	92.0	88.2	87.4	86.9	86.2	78.9	66.7
Hard seed weight (mg)	0.827	0.726	0.722	0.724	0.737	0.730	0.700	0.735	0.717	0.714	0.723

Appendix 5.12 **Germination test done on seeds of cluster clover present in heads and at soil surface level and collected during the grazing period.**

Seed condition	Cumulative days of grazing										
	0	7	14	21	28	35	42	49	56	63	70
AT PLANT LEVEL					(%)						
Germinated	3.0	4.0	3.0	2.0	2.0	4.0	3.0	-	-	-	-
Dormant	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
Dead	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-	-	-	-
SOFT	3.0	4.0	3.0	2.0	2.0	4.0	3.0	-	-	-	-
HARD	97.0	96.0	97.0	98.0	98.0	96.0	97.0	-	-	-	-
Hard seed weight (mg)	0.455	0.436	0.458	0.410	0.424	0.458	0.475	-	-	-	-
AT SOIL SURFACE LEVEL											
Germinated	1.0	0.0	2.0	0.0	0.0	0.0	3.5	2.5	4.0	14.0	18.0
Dormant	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Dead	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SOFT	1.0	0.0	2.0	0.0	1.0	0.0	3.5	2.5	4.0	14.0	18.0
HARD	99.0	100.0	98.0	100.0	99.0	100.0	96.5	97.5	96.0	86.0	82.0
Hard seed weight (mg)	0.442	0.465	0.460	0.445	0.455	0.414	0.388	0.485	0.409	0.428	0.427

Appendix 5.13 Total output of fresh faeces and dry faeces, and percentage of dry matter in faeces from the sheep flock during the grazing experiment at Waite Institute.

	Fresh faeces		Dry faeces		Dry matter (%)
Day of grazing (#)	Daily (g)	Cumulative (g)	Daily (g)	Cumulative (g)	
0	5857	5857	2071	2071	35.4
7	11031	74845	4704	32943	42.6
14	10744	145631	5075	66220	47.2
21	10035	213801	4868	99450	48.5
28	9180	285056	4688	135093	51.1
35	9549	352652	4598	167911	48.2
42	9284	420510	4407	200710	47.5
49	8843	482948	4073	229889	46.1
56	7430	543642	3524	257600	47.4
63	7278	597995	3682	283691	50.6
70	5121	644007	2854	307484	55.7

Appendix 5.14 **Total output of seeds of subterranean clover voided in faeces by the sheep flock during the grazing experiment at Waite Institute.**

Days of Grazing	Seed output (#) that:						Seed output		Hard seed output		
	Became normal seedling		Remain hard		Total		Seedling	Hard	Hard seed weight	Daily	Cumulative
(#)	Daily	Cumulative	Daily	Cumulative	Daily	Cumulative	(%)		(mg)	(g)	
0	42	42	72	72	114	114	36.8	63.2	3.94	0.284	0.284
7	1533	8293	3108	1369	4641	21932	33.0	67.0	3.82	11.873	51.254
14	2481	21283	6491	49284	8972	70567	27.7	72.3	3.87	25.120	189.114
21	2723	39841	9208	99001	11931	138852	22.8	77.2	3.81	35.082	377.253
28	2413	64802	6227	151374	8640	216176	27.9	72.1	3.72	23.164	574.963
35	4530	95147	4973	198317	9503	293464	47.7	52.3	3.91	19.444	750.115
42	2874	120382	4232	233995	7106	354377	40.4	59.6	4.09	17.309	886.528
49	2466	139479	3677	261719	6143	401198	40.1	59.9	3.79	13.936	993.846
56	1515	151717	2253	282467	3768	434184	40.2	59.8	3.38	7.615	1072.196
63	1877	167029	2253	302667	4130	469695	45.4	54.6	4.33	9.755	1151.957
70	1148	179237	2230	321421	3378	500658	34.0	66.0	4.45	9.924	1229.830

Appendix 5.15 Total output of seeds of balansa clover voided in faeces by the sheep flock during the grazing experiment at Waite Institute.

Days of Grazing	Seed output (#) that:						Seed output		Hard seed output		
	Became normal seedling		Remain hard		Total		Seedling	Hard	Hard seed weight	Daily	Cumulative
(#)	Daily	Cumulative	Daily	Cumulative	Daily	Cumulative	(%)		(mg)	(g)	
0	1346	1346	11791	11791	13137	13137	10.2	89.8	0.754	8.890	8.890
7	20066	175086	161311	1425581	181377	1600667	11.1	88.9	0.737	118.886	1068.152
14	16262	281998	173277	2398589	189539	2680587	8.6	91.4	0.740	128.225	1787.767
21	12995	379921	102637	3221629	115632	3601550	11.2	88.8	0.722	74.104	2396.978
28	5594	438537	36801	3709869	42395	4148406	13.2	86.8	0.699	25.724	2750.995
35	3685	478166	28864	3978150	32549	4456316	11.3	88.7	0.699	20.176	2939.942
42	2567	505505	15818	4145541	18385	4651046	14.0	86.0	0.704	11.136	3057.223
49	1896	520711	9304	4238913	11200	4759624	16.9	83.1	0.690	6.420	3120.693
56	1376	532850	4917	4291517	6293	4824367	21.9	78.1	0.636	3.127	3155.326
63	1468	546193	5043	4327793	6511	4873986	22.5	77.5	0.629	3.172	3177.808
70	1540	558036	5433	4378005	6973	4936041	22.1	77.9	0.703	3.819	3210.696

Appendix 5.16 Total output of seeds of cluster clover voided in faeces by the sheep flock during the grazing experiment at Waite Institute.

Days of Grazing	Seed output (#) that:						Seed output		Hard seed output		
	Became normal seedling		Remain hard		Total		Seedling	Hard	Hard seed weight	Daily	Cumulative
(#)	Daily	Cumulative	Daily	Cumulative	Daily	Cumulative	(%)		(mg)	(g)	
0	112	112	5521	5521	5633	5633	2.0	98.0	0.436	2.407	2.407
7	2221	14778	129850	641061	132071	655839	1.7	98.3	0.430	55.836	279.449
14	1721	25577	94985	1320492	96706	1346069	1.8	98.2	0.429	40.749	58.185
21	1300	35615	60184	1809150	61484	1844765	2.1	97.9	0.427	25.699	784.650
28	590	41417	50614	2144478	51204	2185895	1.2	98.8	0.416	21.055	925.791
35	468	45577	30753	2439148	31221	2484725	1.5	98.5	0.409	12.578	1044.778
42	300	48431	23646	2647925	23946	2696356	1.3	98.7	0.415	9.813	1130.157
49	215	50037	17355	2784937	17570	2834974	1.2	98.8	0.397	6.900	1185.983
56	230	51496	13830	2906054	14060	2957550	1.6	98.4	0.398	5.504	1234.546
63	162	52789	15996	3035306	16158	3088095	1.0	99.0	0.383	6.126	1285.380
70	151	53914	7888	3131439	8039	3185353	1.9	98.1	0.389	3.068	1322.198

Appendix 5.17 Total monthly rainfall recorded at 'Maylands' farm, Parawa in the period 1973 - 1991.

	Monthly rainfall (mm)												
Year	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**Yearly total**
1973	27.8	32.8	65.5	93.5	88.5	172.0	139.5	154.0	95.0	64.8	25.3	47.0	1005.7
1974	30.5	24.8	19.8	132.0	108.8	65.3	254.0	92.0	160.0	167.5	11.5	21.5	1087.7
1975	47.5	11.3	88.8	39.8	102.0	117.5	119.8	110.3	82.0	170.0	43.3	21.3	953.6
1976	41.5	31.0	6.8	46.3	67.3	119.5	46.5	126.3	80.5	86.3	34.5	49.5	736.0
1977	54.5	36.0	46.8	43.5	136.3	140.5	50.3	78.5	91.8	51.8	28.0	15.0	773.0
1978	22.8	7.0	74.0	106.3	94.5	187.3	162.3	140.0	164.0	40.8	60.5	28.8	1088.3
1979	11.0	143.3	26.8	117.3	116.8	102.8	137.0	125.8	164.3	98.8	60.5	16.0	1120.4
1980	12.0	4.8	4.8	88.5	57.3	137.8	149.3	69.0	41.3	119.3	51.3	45.5	780.9
1981	98.5	24.5	67.5	4.0	144.0	230.5	136.8	222.5	38.3	49.5	46.0	16.5	1078.6
1982	23.5	5.0	84.3	96.0	85.8	132.3	49.5	24.5	73.0	43.8	2.0	14.0	633.7
1983	15.5	3.0	102.8	207.0	92.5	93.8	201.3	144.3	94.3	75.3	41.5	29.3	1100.6
1984	9.5	17.3	86.0	45.8	108.8	66.5	202.0	164.3	121.8	63.3	20.0	10.3	915.6
1985	6.5	2.0	56.5	67.0	73.8	121.0	135.3	206.3	117.8	69.0	32.0	83.3	970.5
1986	9.5	4.5	4.0	127.8	62.3	68.0	201.8	131.3	90.8	76.3	23.5	50.8	850.6
1987	25.0	23.0	19.8	50.8	205.3	157.5	145.8	102.5	37.3	68.3	47.8	23.5	906.6
1988	28.5	21.5	62.5	24.5	150.5	116.3	154.0	97.0	95.5	24.5	83.5	25.8	884.1
1989	15.3	5.8	29.5	30.5	128.0	180.3	190.5	160.0	86.8	40.3	51.0	3.3	921.3
1990	14.0	67.6	4.0	33.8	34.8	157.0	154.0	185.0	79.5	81.5	12.0	57.0	880.2
1991	30.0	0.0	36.5	40.5	48.5†	-	-	-	-	-	-	-	-
n:18 (1973 - 1990)													
Sum	493.4	465.2	850.2	1354.4	1857.3	2365.9	2629.7	2333.6	1714.0	1391.1	674.2	558.4	16687.4
Mean	27.4	25.8	47.2	75.2	103.2	131.4	146.1	129.6	95.2	77.3	37.5	31.0	927.1
S.D.	21.7	32.7	32.4	48.9	39.2	43.4	54.1	48.1	37.8	39.2	19.9	19.8	135.2
C.V. (%)	79.2	126.7	68.6	65.0	38.0	33.0	37.0	37.1	39.7	50.7	53.1	63.9	14.6

† Cumulative amount of rainfall up to 16 May, 1991.
Source: Mr. Grant Mayfield, proprietor of 'Maylands' farm at Parawa, Fleurieu Peninsula, South Australia.

Appendix 5.18 List of species, varieties and cultivars recorded in a subterranean clover-perennial ryegrass pasture at Parawa during 1990 - 1991.

Scientific name of species	Variety (var.)	Cultivar (cv.)	Common name
A. SOWN			
Trifolium subterraneum L.	subterraneum	Mt. Barker Karridale	subterranean clover
Trifolium subterraneum L.	yanninicum	Yarloop Larisa Trikkala	subterranean clover
Lolium perenne L.		Victorian	perennial ryegrass
B. VOLUNTEER DESIRABLES			
Trifolium glomeratum L.		-	cluster clover
Dactylis glomerata L.		(Unknown)	cocksfoot
C. VOLUNTEER UNDESIRABLES			
Poa annua L.		-	winter grass
Hordeum leporinum Link.		-	barley grass
Bromus mollis L.		-	soft brome
Vulpia bromoides (L.) S.F. Gray		-	silver grass
Erodium cicutarium (L.) Ait.		-	common storksbill
Arctotheca calendula (L.)		-	capeweed
Cerastium glomeratum Thuill.		-	mouse ear chickweed
Rumex acetosella L.		-	sorrel

Appendix 5.19 **Abundance and distribution of sheep faeces in January, 1991 at different sites along the main slope of Paddock I at Parawa.**

	Site								
	Low			Intermediate			High		
Sample (#)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)
1	641.0	563.5	87.9	858.0	742.0	86.5	831.0	719.0	86.5
2	645.5	555.0	86.0	1106.5	968.0	87.5	973.0	856.0	88.0
3	574.0	493.5	86.0	566.5	498.5	88.0	907.5	798.5	88.0
4	786.0	691.5	88.0	1029.5	906.0	88.0	583.5	502.0	86.0
5	816.5	706.5	86.5	1292.5	1131.0	87.5	1003.5	878.0	87.5
6	452.5	339.5	87.0	813.5	715.0	87.9	384.5	336.5	87.5
7	805.5	721.0	89.5	812.5	711.0	87.5	756.5	662.0	87.5
8	967.5	841.5	87.0	387.0	344.5	89.0	399.0	351.0	88.0
9	1269.5	1130.0	89.0	645.0	561.0	87.0	234.5	204.0	87.0
10	921.0	824.5	89.5	711.5	622.5	87.5	644.5	560.5	87.0
(n:10)									
Sum	7879.0	6920.5	876.4	8222.5	7199.5	876.4	6717.5	5867.5	873.0
Mean	787.9	692.1	87.6	822.3	720.0	87.6	671.8	586.8	87.3
S.D.	219.8	199.1	1.3	253.9	221.5	0.6	254.1	223.1	0.6
C.V. (%)	27.9	28.8	1.5	30.9	30.8	0.7	37.8	38.0	0.7
(n:30)									
Sum	22819.0	19987.5	2625.8						
Mean	760.6	666.3	87.5						
S.D.	251.5	222.4	0.9						
C.V. (%)	33.1	33.4	1.0						

Appendix 5.20 **Abundance and distribution of sheep faeces in January, 1991 at different sites along the main slope of Paddock II at Parawa.**

	Site								
	Low			Intermediate			High		
Sample (#)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)
1	577.5	508.0	88.0	387.5	347.0	89.5	176.0	153.0	86.9
2	338.5	299.5	88.5	225.5	199.5	88.5	90.0	79.5	88.3
3	586.0	510.0	87.0	716.0	644.5	90.0	413.0	363.5	88.0
4	600.0	531.0	88.5	581.5	506.0	87.0	762.0	670.5	88.0
5	309.0	278.0	90.0	529.0	460.0	87.0	468.5	414.5	88.5
6	251.5	222.5	88.5	307.0	268.5	87.5	822.5	732.0	89.0
7	258.0	229.5	89.0	561.5	491.5	87.5	659.0	586.5	89.0
8	381.5	339.5	89.0	190.0	164.5	86.6	902.5	807.5	89.5
9	277.5	243.0	87.6	218.5	193.5	88.6	686.0	603.5	88.0
10	685.5	596.5	87.0	202.0	178.0	88.1	468.0	416.5	89.0
(n:10)									
Sum	4265.0	3757.5	883.1	3918.5	3453.0	880.3	5447.5	4827.0	884.2
Mean	426.5	375.8	88.3	391.9	345.3	88.0	544.8	482.7	88.4
S.D.	158.2	136.9	0.9	181.8	161.5	1.1	256.4	228.6	0.7
C.V. (%)	37.1	36.4	1.0	46.4	46.8	1.3	47.1	47.4	0.8
(n:30)									
Sum	13639.0	12037.5	2647.6						
Mean	454.6	401.3	88.3						
S.D.	213.0	189.3	0.9						
C.V. (%)	46.9	47.2	1.0						

Appendix 5.21 Abundance and distribution of sheep faeces in March, 1991 at different sites along the main slope of Paddock I at Parawa.

	Site								
	Low			Intermediate			High		
Sample (#)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)
1	924.0	859.3	93.0	1869.5	1683.6	90.0	1707.5	1502.6	88.0
2	900.5	815.0	90.5	1940.0	1755.7	90.5	2100.0	1879.5	89.5
3	1169.0	1052.1	90.0	1285.5	1144.1	89.0	1002.5	887.2	88.5
4	867.5	793.8	91.5	1178.5	1072.4	91.0	1168.0	1045.4	89.5
5	817.5	743.9	91.0	650.5	592.0	91.0	1594.5	1443.0	90.5
6	758.5	690.2	91.0	1154.0	1038.6	90.0	600.5	549.5	91.5
7	787.0	716.2	91.0	796.5	720.8	90.5	443.5	401.4	90.5
8	634.0	573.8	90.5	722.0	646.2	89.5	911.5	824.9	90.5
9	907.0	820.8	90.5	1117.0	1010.9	90.5	832.0	740.5	89.0
10	841.5	761.6	90.5	1066.5	975.8	91.5	547.5	495.5	90.5
(n:10)									
Sum	8606.5	7826.7	909.5	11780.0	10640.1	903.5	10907.0	9769.5	898.0
Mean	860.7	782.7	91.0	1178.0	1064.0	90.4	1090.7	977.0	89.8
S.D.	131.3	118.2	0.8	414.6	373.5	0.7	521.6	463.2	1.0
C.V. (%)	15.3	15.1	0.9	35.2	35.1	0.8	47.8	47.4	1.1
(n:30)									
Sum	31294.0	28236.3	2711.0						
Mean	1043.1	941.2	90.4						
S.D.	414.3	369.5	1.0						
C.V. (%)	39.7	39.3	1.1						

Appendix 5.22 **Abundance and distribution of sheep faeces in March, 1991 at different sites along the main slope of Paddock II at Parawa.**

	Site								
	Low			Intermediate			High		
Sample (#)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)	As collected (kg/ha)	DM basis (kg/ha)	DM (%)
1	351.0	312.4	89.0	438.5	394.7	90.0	860.5	761.5	88.5
2	487.0	435.9	89.5	788.5	717.5	91.0	613.5	546.0	89.0
3	487.5	436.3	89.5	633.5	570.2	90.0	641.0	570.5	89.0
4	941.0	846.9	90.0	540.5	486.5	90.0	1127.5	992.2	88.0
5	573.5	516.2	90.0	521.0	466.3	89.5	412.5	363.0	88.0
6	739.0	657.7	89.0	538.5	473.9	88.0	1157.5	1018.6	88.0
7	547.0	484.1	88.5	763.5	683.3	89.5	1316.0	1164.7	88.5
8	582.0	515.1	88.5	732.0	651.5	89.0	1280.0	1139.2	89.0
9	432.5	380.6	88.0	258.0	232.2	90.0	433.5	377.1	87.0
10	438.0	387.6	88.5	743.0	653.8	88.0	726.5	646.6	89.0
(n:10)									
Sum	5578.5	4972.8	890.5	5957.0	5329.9	895.0	8568.5	7579.4	884.0
Mean	557.9	497.3	89.1	595.7	533.0	89.5	856.9	757.9	88.4
S.D.	162.3	147.0	0.7	160.5	143.7	0.9	324.5	287.6	0.6
C.V. (%)	29.1	29.6	0.8	26.9	27.0	1.0	37.9	37.9	0.7
(n:30)									
Sum	20104.0	17882.1	2669.5						
Mean	670.1	596.1	89.0						
S.D.	264.8	234.5	0.9						
C.V. (%)	39.5	39.3	1.0						

Appendix 5.23 **Seedling emergence from sheep faeces collected during early summer in both paddocks at Parawa and placed on raised-beds.**

Species	Paddock I		Paddock II	
	(#/m²)	(%)	(#/m²)	(%)
A. SOWN				
Subterranean clover	1	1.6	2	8.0
var. *subterraneum*	0	0.0	2	8.0
var. *yanninicum*	1	1.6	0	0.0
Perennial ryegrass	1	1.6	1	4.0
B. VOLUNTEER DESIRABLES				
Cluster clover	25	39.7	3	12.0
C. VOLUNTEER UNDESIRABLES				
Winter grass	25	39.7	11	44.0
Barley grass	0	0.0	0	0.0
Storksbill	0	0.0	2	8.0
Capeweed	4	6.3	1	4.0
Chickweed	7	11.1	5	20.0
Total	63	100.0	25	100.0

Appendix 5.24 **Seedling emergence from sheep faeces collected during early autumn in both paddocks at Parawa and placed on raised-beds.**

Species	Paddock I		Paddock II	
	(#/m^2)	(%)	(#/m^2)	(%)
A. SOWN				
Subterranean clover	3	1.7	11	13.3
var. *subterraneum*	1	0.6	9	10.8
var. *yanninicum*	2	1.1	2	2.5
Perennial ryegrass	2	1.1	1	1.2
B. VOLUNTEER DESIRABLES				
Cluster clover	101	57.1	32	38.6
C. VOLUNTEER UNDESIRABLES				
Winter grass	55	31.1	30	36.1
Barley grass	1	0.6	0	0.0
Storksbill	0	0.0	2	2.5
Capeweed	6	3.4	1	1.2
Chickweed	9	5.1	6	7.1
Total	177	100.0	83	100.0

Appendix 6.1 **Germination trend of Paraggio medic seeds during the process of softening in an alternating-temperature incubator.**

Pod size	Mean value (%) at week :										
	0	1	2	3	4	6	8	10	12	14	16
SMALL											
Germinated	14.0	13.8	22.9	19.7	19.0	13.7	14.1	24.4	15.8	23.4	25.1
Swollen	4.0	6.3	2.5	3.3	4.8	3.2	8.6	3.8	14.3	8.8	6.3
Dead	0.5	0.5	0.3	0.5	0.0	0.0	1.5	0.0	1.5	0.0	1.3
SOFT	18.5	20.6	25.8	23.3	23.8	16.9	24.2	28.2	31.6	32.2	32.7
HARD	81.5	79.4	74.2	76.7	76.2	83.1	75.8	71.8	68.4	67.7	67.3
Hard / soft ratio	4.41	3.85	2.88	3.29	3.20	4.92	3.13	2.55	2.16	2.10	2.06
Hard seed weight (mg)	2.20	2.15	2.21	2.21	2.22	2.25	2.32	2.24	2.15	2.10	2.12
LARGE											
Germinated	12.9	14.8	20.9	19.1	21.8	19.4	16.8	22.2	15.7	20.7	22.7
Swollen	9.5	6.2	4.1	2.5	6.1	6.8	5.5	5.8	9.0	5.3	6.5
Dead	0.3	0.0	0.3	0.4	0.0	1.3	0.3	0.0	0.0	0.5	0.0
SOFT	22.7	21.0	25.3	22.0	27.9	27.5	22.6	28.0	24.7	26.5	29.2
HARD	77.3	79.0	74.7	78.0	72.1	72.5	77.4	72.0	75.3	73.5	75.3
Hard / soft ratio	3.41	3.76	2.95	3.55	2.58	2.64	3.42	2.57	3.05	2.77	2.58
Hard seed weight (mg)	3.34	3.25	3.33	3.26	3.24	3.27	3.29	3.19	3.24	3.20	3.25

Appendix 6.2 **Germination trend of Paraggio medic seeds during the process of softening exposed on the raised beds.**

	Mean value (%) at week :						
Pod size	**18**	**20**	**22**	**24**	**26**	**28**	**30**
SMALL							
Germinated	30.6	38.4	47.4	56.7	62.1	65.8	66.4
Swollen	10.2	12.5	19.8	12.2	8.6	7.2	8.6
Dead	0.0	0.0	0.8	0.5	0.8	1.5	0.7
SOFT	40.8	50.9	68.0	69.4	71.5	74.5	75.7
HARD	59.2	49.1	32.0	30.6	28.5	25.5	24.3
Hard / soft ratio	1.45	0.96	0.47	0.44	0.39	0.34	0.32
Hard seed weight (mg)	2.10	2.09	2.05	2.01	1.90	1.90	1.82
LARGE							
Germinated	25.3	36.7	37.8	48.2	55.2	57.3	59.3
Swollen	8.3	15.8	27.4	15.2	8.3	6.8	5.7
Dead	1.5	2.5	1.2	1.5	0.0	1.2	2.2
SOFT	35.1	55.0	66.4	64.9	63.5	65.3	67.2
HARD	64.9	45.0	33.6	35.1	36.5	34.7	32.8
Hard / soft ratio	1.85	1.22	0.51	0.54	0.57	0.53	0.49
Hard seed weight (mg)	3.18	3.19	3.22	3.12	2.95	2.97	2.89

Appendix 6.3 **Germination trend of Paraggio medic seeds kept at relatively constant temperature during the entire period.**

Pod size	Mean value (%) at week : 0	22	30
SMALL			
Germinated	16.0	18.5	24.0
Swollen	4.3	6.5	2.0
Dead	1.5	1.0	0.5
SOFT	21.8	26.0	26.5
HARD	78.2	74.0	73.5
Hard / soft ratio	3.59	2.85	2.78
Hard seed weight (mg)	2.22	2.25	2.28
LARGE			
Germinated	14.2	20.5	25.0
Swollen	7.0	2.5	1.3
Dead	0.0	0.0	0.5
SOFT	21.2	23.0	26.8
HARD	78.8	77.0	73.2
Hard / soft ratio	3.72	3.35	2.73
Hard seed weight (mg)	3.33	3.29	3.38

Appendix 6.4 Body-weights of sheep used in the pen feeding experiment.

Sheep No.	Pre-experimental period	Experimental period		
	1 May, 1990 (kg/head)	11 May, 1990 (kg/head)	8 June, 1990 (kg/head	Change (kg/head)
1	45.7	45.6	46.2	0.6
2	46.6	46.2	47.5	1.3
3	59.8	58.7	57.9	-0.8
4	49.7	50.0	50.2	0.2
5	44.8 (49.3)†	45.0 (49.1)	43.5 (49.1)	0.0
6	45.7	45.1	45.5	0.4
7	46.6	46.5	47.8	1.3
8	49.7	49.4	51.3	1.9
9	51.6	50.9	52.0	1.1
10	53.6 (49.4)	53.0 (49.0)	52.6 (49.8)	-0.4
11	53.6	53.5	53.5	-1.5
12	46.6	45.8	47.8	2.0
13	41.0	41.0	41.7	0.7
14	50.7	50.2	50.4	0.2
15	55.5 (49.5)	55.0 (49.1)	55.8 (49.8)	0.8
16	44.6	44.0	43.9	-0.1
17	47.8	47.6	46.5	-1.1
18	49.5	50.0	50.3	0.3
19	51.5	51.3	53.3	2.0
20	53.8 (49.4)	52.4 (49.1)	51.8 (49.2)	-0.6
Sum (n:20)	988.4	981.2	989.5	8.3
Mean	49.4	49.1	49.5	0.4
S.D. (n-1)	4.36	4.17	4.13	-
C.V. (%)	8.83	8.49	8.34	-

† Values in parenthesis represent the mean body-weights of the corresponding groups of five wethers.

Appendix 6.5 **Daily dry matter content of the diet components offered to penned sheep in the 28-day feeding experiment.**

Day No.	Diet component			Day	Diet component		
	Pellets†	Oaten hay (DM,%)	Lucerne hay		Pellets	Oaten hay (DM,%)	Lucerne hay
1	89.4	88.1	86.7	15	88.7	86.9	85.5
2	89.1	88.9	84.9	16	88.5	87.8	83.0
3	89.2	90.2	85.2	17	88.5	86.9	86.7
4	89.5	87.4	85.8	18	88.7	87.7	82.5
5	89.6	89.8	86.5	19	88.7	88.0	83.0
6	89.1	88.5	87.0	20	88.5	88.2	83.7
7	89.6	88.5	87.4	21	88.7	88.1	82.8
8	89.5	88.8	85.9	22	88.6	87.5	81.5
9	88.9	88.0	86.5	23	88.5	86.4	81.0
10	89.3	88.8	86.9	24	88.3	87.4	81.6
11	89.2	88.6	86.0	25	88.4	86.5	81.9
12	89.8	89.6	86.8	26	88.4	87.3	82.8
13	89.2	87.6	86.2	27	88.7	86.6	82.0
14	89.2	88.2	84.9	28	88.3	87.2	82.5
Sum (n=28)	2490.1	2460.5	2367.2				
Mean	88.9	87.9	84.5				
S.D.	0.44	1.20	2.03				
C.V. (%)	0.49	1.37	2.40				

† Australasian Feed Services Pty Ltd., South Australia, Australia.

Appendix 6.6 **Mean daily output of faeces per sheep and dry matter content of faeces in the pen-feeding experiment.**

Day No.	Fresh faeces (g)	Dry faeces (g DM)	Dry matter (%)	Day No.	Fresh faeces (g)	Dry faeces (g DM)	Dry matter (%)
1	445.1	193.6	43.5	15	625.2	270.7	43.3
2	485.3	222.3	45.8	16	600.5	268.4	44.7
3	550.5	251.6	45.7	17	613.2	275.9	45.0
4	506.2	221.2	43.7	18	612.3	274.3	44.8
5	487.0	217.2	44.6	19	615.3	273.8	44.5
6	499.1	211.1	42.3	20	595.4	256.0	43.0
7	587.9	251.6	42.8	21	620.9	271.3	43.7
8	615.0	249.1	40.5	22	607.7	271.6	44.7
9	566.7	242.5	42.8	23	557.7	249.8	44.8
10	594.2	256.1	43.1	24	530.9	231.5	43.6
11	622.5	267.0	42.9	25	567.7	252.1	44.4
12	535.8	226.1	42.2	26	643.3	285.0	44.3
13	674.7	290.1	43.0	27	536.5	242.5	45.2
14	623.8	268.9	43.1	28	589.0	251.5	42.7
Sum (n:28)	16109.4	7042.8	1224.7				
Mean	575.3	251.5	43.7				
S.D.	54.10	23.46	1.17				
C.V. (%)	9.40	9.33	2.68				

Appendix 6.7 **Estimation of *in vivo* apparent digestibility of the diet used in the penned-sheep feeding experiment.**

Sheep No.	Diet			Faeces	Material	
	Offered	Rejected	Consumed	Voided	Digested	Digestibility of DM (%)
	(g DM/sheep / 28 days)			(g DM/sheep / 28 days)		
1	20192.5	773.2	19419.3	6941.8	12477.5	64.3
2	19656.2	1091.0	18565.2	7672.5	10892.7	58.7
3	20192.5	0.0	20192.5	7340.0	12852.5	63.6
4	20192.5	0.0	20192.5	7556.2	12636.3	62.6
5	14270.1	607.0	13663.1	4965.8	8697.3	63.7
6	20192.5	79.2	20113.3	8487.5	11625.8	57.8
7	11134.5	4926.9	6207.6	1938.9	4268.7	68.8
8	20192.5	0.0	20192.5	8231.9	11960.6	59.2
9	20192.5	0.0	20192.5	7829.0	12363.5	61.2
10	20192.5	8.7	20183.8	8102.2	12081.6	59.9
11	20190.5	558.2	19634.3	7386.7	12247.6	62.4
12	20190.5	2.1	20188.4	7080.5	13107.9	64.9
13	18851.9	490.7	18361.2	7186.3	11174.9	60.9
14	20190.5	76.6	20115.9	7651.8	12464.1	62.0
15	18849.2	919.3	17929.9	6789.0	11140.9	62.1
16	17869.9	2473.3	15396.6	5765.4	9631.2	62.6
17	20189.5	453.7	19735.8	7425.3	12310.5	62.4
18	18939.3	587.6	18351.7	6994.9	11356.8	61.9
19	20189.5	0.0	20192.5	7458.1	12734.4	63.1
20	20189.5	558.3	19634.2	7668.4	11965.8	60.9
Sum (n:20)	382058.6	13605.8	368462.8	140472.2	227990.6	1243.0
Mean	19102.9	680.3	18423.1	7023.6	11399.5	62.2
S.D.	2342.2	1160.0	3360.4	1437.8	1999.0	2.4
C.V. (%)	12.3	170.5	18.2	20.5	17.5	3.9
Daily values / sheep	682.2	24.3	657.9	250.8	407.1	61.9

Appendix 6.8 **Total viable seed voided in faeces by the sheep flock during the pen-feeding experiment.**

	Treatment			
Sheep No.	**Small-untreated**	**Small-treated**	**Large-untreated**	**Large-treated**
		(%)		
1	7.13	1.23	6.24	2.26
2	4.50	1.13	1.14	1.18
3	3.24	2.44	1.62	1.12
4	7.68	3.50	4.48	0.19
5	7.81	1.73	5.10	0.19
6	7.07	2.70	4.07	0.76
7	5.61	1.12	2.85	0.62
8	4.21	2.22	1.82	2.16
9	6.27	1.91	5.66	0.31
10	12.25	2.19	8.81	2.18
Sum	65.77	20.17	41.79	10.97
Mean	6.58	2.04	4.18	1.10
S.D.	2.40	0.72	2.28	0.79
C.V. (%)	36.48	35.30	54.55	71.82
S.E.	0.76	0.23	0.72	0.25

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