PHYSIOLOGICAL ATTRIBUTES OF TOLERANCE OF OATS (avena) TO *Heterodera avenae*

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SUMMARY

1. Regression lines relating initial inoculum density and grain yield of four oat cultivars, New Zealand Cape (NZC) Sual, Swan and West, grown under glasshouse conditions, were significantly different, the slopes of NZC and West being less negative than Sual and Swan, indicating a higher level of tolerance to Heterodera avenae in the former two cultivars. Intolerant plants, when heavily infested used water less efficiently than tolerant, infested plants. A higher level of tolerance in NZC appeared to be due to grain development on late formed tillers being less affected by infestation than on intolerant plants.

2. Detillering (leaving only the main culm) enhanced the level of tolerance of Heterodera avenae infested oat cultivars irrespective of whether the cultivars were early maturing, and had a low tiller production capacity (Swan and West) or were the reverse (NZC and Sual). Detillering may have relieved the competition between the main-stem and tillers for a common energy and nutritional source, thereby improving the nutrition of the single-stemmed, infested plants, and enhancing tolerance.

3. Less impaired extension of infested seminal roots of NZC was related to a higher level tolerance to Heterodera avenae infestation. Uninfested lateral root growth was greatest on cultivars most reduced in seminal root growth by infestation (Sual, Stout and Avena fatua). Nodal roots developed earlier on NZC than on the other three cultivars.

4. Reduced extension rate following nematode infestation of axenically cultured, attached and detached roots, was related to increases in endogenous abscisic acid and ethylene concentrations. However, differences in root growth of infested tolerant (NZC) and intolerant (Sual) cultivars were not related to endogenous hormone level.

Root segments of Sual and NZC did not differ in sensitivity to exogenously applied ABA or ethylene.

5. Where water supply was finite and in progressively more limited supply, seedling shoot growth of infested tolerant NZC was reduced more by water deficit stress than intolerant Sual because of its more developed root system.

6. The inability of infested roots of the cultivar Swan to extend beyond a dry soil zone and into a well-watered zone at as fast a rate as uninfested root systems resulted in a reduction in early main-stem yield but not of later total yield. Infestation alone and water stress alone, reduced main-stem yield of Sual, but the reduction was not additive when effects were combined.

Rapid root extension into the well-watered zone of both infested and uninfested cultivar, NZC prevented a decline in main-stem as well as total grain yield.

7. Heterodera avenae infestation had no direct effect on the stomatal conductance or transpiration rates of well-watered Swan and NZC up to 20 days after inoculation, but temporarily enhanced the stomatal conductance of Sual.

8. There was no relation between stomatal conductance and leaf water potential of uninfested seedlings of cultivars NZC, Sual, Swan or West. Heterodera avenae infestation did not alter these findings.

9. Oat cultivars differed in the permeability of their root systems (hydraulic conductivity) to water entry under conditions in which hydrostatic pressure between the root and shoot was varied. NZC and Sual typified the responses observed. Permeability decreased when the gradient was small in NZC, but did not change in Sual at high or low pressure gradients Heterodera avenae infestation enhanced root permeability of NZC and had little or no effect on the other three cultivars.

10. Root extension of infested oat cultivars, Sual and Swan was not enhanced by a 2 week exposure to an enriched atmospheric CO_2 supply (x 3 ambient) but that of NZC was, relative to infested plants grown under ambient CO_2 conditions. Reduced root extension of infested Sual and Swan was therefore not due to a reduced supply of carbohydrates to the root.

11. Heterodera avenae infestation had no effect on the CO_2 assimilation rate of NZC and Sual at ambient CO_2 partial pressure, but reduced the CO_2 assimilation rate under CO_2 saturating conditions, the effect being more noticeable on Sual. An accumulation of triose phosphates arising from impaired root extension was the suggested cause of inhibition of photosynthesis.

12. Main-stem and tiller grain yield of Sual and West were reduced by infestation at 10 and 100 M but not at 1 μM solution phosphorus in sand culture. Phosphorus supply did not influence the response of either cultivar to Heterodera avenae infestation.

13. A decline in P uptake and therefore shoot growth in infested, intolerant oat cultivars Sual and Swan grown in nutrient solution, arose from reduced root length. Infestation had no effect on the tolerant cultivar, NZC. Phosphorus deficiency of infested Swan was alleviated by increasing the solution phosphorus concentration from 10 to 100 μM. This occurred as a consequence of a higher phosphorus utilization efficiency of Swan compared to the other cultivars.

DECLARATION

This thesis contains no material which has been accepted for the award of any Degree or Diploma in any University and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference is made in the text.

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K.M. VOLKMAR

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LIST OF ABBREVIATIONS

a	Proportionality Coefficient
ABA	Abscisic Acid
C	Root Hydraulic Conductance
CO_2	Carbon Dioxide
cm	Centimetre
Cr	Concentration of Ions at Root Surface
cv	Cultivar
E	Transpiration Rate (mg Leaf Area^{-1} s^{-1})
E_R	Transpiration Rate Per Root Surface Area (mg Leaf Area^{-1} Root Surface Area s^{-1})
F	Flux
G	Stomatal Conductance
g	gram
GLC	Gas-liquid Chromatography
h	Hours
IAA	Indole Acetic Acid
Ip	Rate of Phosphorus Absorption
Jv	Volume Flow
K	Hydraulic Conductivity
Kg	Kilograms
l	Litre
Lp	Hydraulic Conductivity Coefficient
LSD	Least Significant Difference
M	Molar
min	Minute
ml	Millilitre
mm	Millimetre
~~um~~ µM	Micromolar
mol	Mole
Mpa	Megapascal
ng	Nanograms
nl	Nanolitres
P	Phosphorus
Δp	Pressure Difference

LIST OF ABBREVIATIONS

$p(CO_2)$	Partial Pressure CO_2
ppm	Parts Per Million
ppb	Parts Per Billion
PEG	Polyethylene Glycol
PVC	Poly Vinyl Chloride
Q	Quantity of P Absorbed
R	Hydraulic Root Resistance
RWC	Relative Water Content
s	Second
spp	Species
uv	Ultra-violet
w	watts
W_i	Mole Fraction of Water Vapour inside Leaf
W_o	Mole Fraction of Water Vapour Outside Leaf
W	Total Plant Dry Mass
Z	Nutrient Uptake

LIST OF SYMBOLS

α	Reflection Coefficient for Solute Movement across a Semi Permeable Membrane
Ψ_l	Leaf Water Potential
Ψ_m	Matric Potential
Ψ_p	Pressure Potential (Turgor Potential)
Ψ_s	Solute Potential (Osmotic Potential)
Ψ_w	Water Potential
$\Delta\Psi_p$	Pressure Potential Difference

CHAPTER 1: INTRODUCTION

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The extent of yield decline caused by nematode infestation varies within plant species (Trudgill and Cotes 1983, Boerma and Hussey 1984, Barr and Dube 1985). Cultivars reduced least in yield have been termed tolerant. The environment often influences the plant-nematode interaction (Baker et al. 1976, Griffin 1981, Edongali 1982). The difficulty in characterizing tolerance independently of the environment has prompted one author to suggest that true disease tolerance may not exist (Gaunt 1981). Nonetheless tolerance is a useful descriptive term when used in comparing the relative yield response of a set of cultivars to nematode infestation within prescribed environmental conditions.

In practical economic terms tolerance to nematodes has obvious benefits by maintaining yield in spite of infestation. To obtain an understanding of the mechanisms involved in this defensive property of the host, the physiological processes which are important determinants of yield and which are primarily influenced by nematode infestation must be ascertained. The identification of these processes requires the examination of the effects of infestation on as many yield limiting factors as possible in cultivars varying in their level of tolerance to nematodes. Those processes that are clearly different between infested cultivars may be the tolerance mechanisms that are being sought.

Heterodera avenae contributes significantly to reduction in grain yield in cereals grown in the grain-belt of south-east Australia (Meagher 1977, Simon and Rovira 1982). Differences in levels of tolerance of oats (*Avena* to *Heterodera avenae* have been observed in field experiments (Barr and Dube 1985).

This thesis describes experiments in which the effect of *Heterodera avenae* infestation on root growth, plant water relations, phosphorus nutrition and carbohydrate utilization and assimilation in tolerant and intolerant oat cultivars was examined. Such data is used in attempting to identify the tolerance mechanisms in this host-parasite system.

CHAPTER 2: LITERATURE REVIEW

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1. Distribution and Lifecycle of Heterodera avenae

Heterodera avenae is a plant parasitic nematode present in most cereal growing areas of the world (Meagher 1977). It has recently become recognized as a serious pathogen on cereals in the south of central regions of Australia (Banyer 1966, Meagher 1968, Rovira and Simon 1982) though its presence in the region was known much earlier (Davidson 1930).

Hatching of larvae from cysts occurs in the field following a dormant period (Banyer and Fisher 1971a). Observation of seasonal fluctuations of Heterodera avenae hatching showed that the maximum population of infective larvae coincided with the period of cereal growth from seed germination to floral initiation, about ten weeks (Meagher 1970). Hatching patterns are strongly influenced by environmental conditions such as temperature (Cotten 1962; Fushtey and Johnston 1966; Banyer and Fisher 1971 a,b) and root exudation (Kerry and Jenkinson 1976; Williams and Beane 1979). Newly hatched larvae infest roots in the region of vascular differentiation behind the root tip. They move into the host cortex where they induce cell enlargement and cell wall breakdown with the resultant formation of a giant cell or syncytium (Dropkin 1979).

Infestation generally occurs within the first 15 days of hatching, root penetration reaching a maximum at 20°C (Davies and Fisher 1976). Competition between larvae for limited feeding sites has been observed to increase with larval density with a resultant decline in the percentage invaded roots (Kerry and Jenkinson 1976; O'Brien and Fisher 1978a). The ratio of male to female larvae developing to maturity is related to feeding site competition and the inherant resistance of the plant species and cultivar (Cook et al. 1974; O'Brien and Fisher 1977; O'Brien and Fisher 1978 b). Development of fertilized females into mature cysts coincides approximately with maturation of the host plant.

2. Effects of Heterodera avenae Infestation on Growth and Physiology of the Host

Plants infested by Heterodera avenae show a progressive impairment of root extension with increasing nematode density (Gair 1965, Kerry and Hague 1974, O'Brien and Fisher 1981, Price et al. 1983). Radial thickening and profuse short lateral root formation at the penetration site is typical (Gair 1965). Shoot development is impaired when a critical density is exceeded but may be enhanced below this density (Seinhorst 1981 a). Shoot impairment is widely believed to be due to the general effects of seminal, lateral and nodal root stunting which limits the volume of soil explored by the plant thereby reducing the supply of water and minerals.

(O'Brien and Fisher 1981, Simon and Rovira 1985). Thus infested plants appear nutrient deprived (Gair 1965; Kort 1972).

3. Plant Tolerance to Nematode Infestation

3.1 Resistance Versus Tolerance

Nematode resistance refers to the varying capacity of the host plant to reduce the number of egg laying females reaching maturity (Robinson 1969). Resistance has the obvious advantage of reducing the number of larvae that will hatch and infest germinating host seedlings the following year. Field studies have demonstrated the effectiveness of using resistant varieties (McLeod 1976). However total resistance is at present unachievable. Meagher and Brown (1974) reported that the best resistance still resulted in sufficient females to cause up to 30% yield reduction the following year. It is considered important therefore to seek ways of reducing the effect of infestation (Fisher 1982).

Nematode tolerance limits the impact of infestation on growth and yield. Tolerance refers to the ability of a plant to sustain heavy infestation while suffering minimal yield loss (Caldwell et al. 1958). Cultivar variations in levels of tolerance have been reported in potatoes (Huisman et al. 1969, Seinhorst and den-Ouden 1971, Evans and Franco 1979, Evans 1982) tobacco (Fox and Spasoff 1976), and wheat (Fisher et al. 1981, Stanton 1983).

Adhering to the definition of tolerance just described, apparent tolerance conferring factors that act by limiting the number of larvae penetrating, establishing or maturing in the root or operate by inhibiting larval hatch, more closely constitute mechanisms of avoidance, not tolerance. This distinction is difficult to adhere to in the field because a precise determination of the ratio of the number of hatching larvae over the number penetrating and establishing cannot be made. For this reason operational definitions of field tolerance include factors that may prevent penetration and establishment such as the absence of hatching stimulation on some potato cultivars (Evans and Franco 1979), or factors that prevent female maturation (Trudgill and Cotes 1983 a). This does not deter the more precise definition being applied in the laboratory, when initial larval density can be controlled.

3.2 The Physiology of Tolerance

Through its effect on root growth, infestation can influence both the nutritional and water status of the plant. Tolerance conferring mechanisms are therefore most likely related to processes that redress the nutritional or water imbalance incurred through infestation. The discussion to follow will address in order, the general principles of plant water relations and mineral nutrition. After each section attention will be focused on how nematode tolerant plants might alleviate stress induced by infestation. Because the nutritional status of a plant is closely allied with its water status (Tinker 1969) points raised in the discussion may be common to both topics.

3.2.1 Water Relations

3.2.1.1 Principles of Plant Water Relations

The requirement for water is probably common to every aspect of metabolic function, and its absence precludes growth. The impact of water stress on various aspects of plant development has received ample attention in several recent books (Kozlowski 1976; Turner and Kramer 1980; Paleg and Aspinall 1981; Lange et al. 1982; Taylor et al. 1982) as well as in several shorter reviews (Boyer and McPherson 1975; Hsiao et al. 1976).

Terms and concepts relevant to water relations will be described. The thermodynamic solute and pressure forces in plants are described by water potential (Ψw) which is the chemical potential of the ~~solute~~ solvent (free energy/mol) divided by the partial molar volume of water (18.0cm^3/mol). It is expressed in various terms applying to pressure, eg. bars and megapascals. Measurements of Ψw are always defined in relation to the water potential of pure water which is equal to 0. Ψw is the sum of the solute and pressure forces on it. This relationship is described by the equation:

$$\Psi_w = \Psi_s + \Psi_p + \Psi_m$$

where s is the osmotic ~~pressure~~ potential, p is the pressure or turgor potential, and m relates to matric forces originating from the cell surfaces. In plants the matric potential component is considered negligible and the equation reduces to:

$$\Psi_w = \Psi_s + \Psi_p$$

the value of Ψs is always negative while Ψp can be positive or negative, though turgor pressure in plant tissue rarely becomes negative in nature. During dehydration, Ψp falls until in a fully wilted plant it equals 0 and contributes ~~almost entirely~~ nothing to the water potential term (Slatyer 1969).

van den Honert (1948) described flow of water through plants as a catenary process analogous to electricity flow through a network with varying resistances. Thus the flow of water across any section of the flow path is proportional to the gradient in water potential between the shoot and root and inversely proportional to the resistance to flow imposed by the soil, root, vascular system and leaves (Cowan and Milthorpe 1968). This can be described by the classical transport equation:

$$J_v = Lp\ (\Delta\Psi\ w) = \frac{R\ root - R\ leaf}{R\ root + R\ stem + R\ leaf}$$

where J_v is the water flow ($cm^3\ cm^{-2}\ sec^{-1}$) and Lp is the hydraulic conductivity ($cm\ sec^{-1}\ Mpa^{-1}$) of the pathway, describing the degree to which hydraulic forces can move water through the frictional resistances of the plant, and R is the resistance ($sec.\ cm^{-1}\ Mpa^{-1}$) (Slatyer 1969; Cowan and Milthorpe 1968). In the transpiring plant, evaporation from the leaf surface results in a fall in hydrostatic pressure within the vascular system which provides the driving force

for water movement to the top of the plant. In this sense $Jv = L_p \Delta p$ where Δp equals the pressure drop between the external medium and the ~~tracheae~~ xylem. The total force driving the water equals the osmotic ~~solute~~ potential Ψs and the transpirationally induced hydrostatic force, (Weatherly 1982), so that the equation describing flow is properly described by:

$$Jv = Lp\ (\Delta p - \sigma \Delta \Psi s)$$

where $\Delta \Psi s$ is the rise in the osmotic pressure (Pa) across a membrane, and σ is the reflection coefficient that accounts for membrane permeability to a given solute (Passioura 1984).

From the equation immediately above it follows that root hydraulic conductivity can be estimated by obtaining values of flow (Jv) at different hydrostatic pressures (Mees and Weatherly 1957; Fiscus and Kramer 1975; Michel 1977). Root resistance values can be derived from known flow rates at a given hydrostatic pressure (Ramos and Kaufman 1979; Syvertsen 1981). This can be done by monitoring exudation rates from the stump of a plant in which the shoot has been excised and pressure applied externally to the root system. The values obtained from this method are based on an assumption that the transpirational force driving water through an intact plant is the same as the hydrostatic force exerted on an excised root system. This may not always be true.

The major resistances to diffusion of water vapor from the leaves to the air are the cuticle, the boundary layer resistance (R_a) and the stomata. The degree of stomatal opening governs plant transpiration rate (Raschke 1979) when Ra is small, i.e. in a well circulated atmosphere.

Stomata are also the major pathway for movement of CO_2 from the atmosphere into the leaf mesophyll. Stomatal conductance therefore determines the rate of both water loss and CO_2 uptake so that conservation of water precludes high rates of CO_2 assimilation (Cowan and Farquhar 1977). Stomata tend to open with changes in the environment which promote carbon fixation and close with changes promoting water loss. Thus their operation has the effect of reducing the average rate of water loss relative to the average rate of carbon fixation (Cowan 1977, Farquhar et al. 1980, Hall and Schulze 1980a).

The dependence of transpiration rate (E) on conductance ~~conductivity~~ of ~~to~~ water vapor ~~diffusion~~ (G) is simply expressed (Cowan, 1977):

$$E = G (W_i - W_o)$$

where E and G are in moles m^{-2} sec^{-1}, and W_i and W_o are mole fractions of water vapour inside and outside the leaf.

3.2.1.2 Tolerance Mechanisms that Alleviate Drought Stress Caused by Nematode Infestation

Inability to procure water deep within the soil during dry conditions is believed to contribute significantly to crop failure of nematode infested plants (Kerry and Jenkinson 1976, Evans et al. 1977). Because the root system is the site where host and the nematode interact, nematode consequent deleterious effects on the water relations of the whole plant are likely to evolve from localized effects on the root. Nematode tolerance is likely to consist of many processes that operate both locally and remotely to reverse or neutralize the impact of infestation on plant water relations. Several plant responses alleviate drought stress associated with nematode infestation. For example, plants able to grow in arid environments may do so because they can either avoid drought, or because they are able to function efficiently at a low plant water potential; that is they may tolerate drought. While the importance of mechanisms conferring drought tolerance may contribute significantly to nematode tolerance, they fall outside the scope of this review.

The most obvious form of drought avoidance applicable to a nematode infested plant is root extension beyond the point of invasion. Because of the relevancy of root growth in mitigating drought stress as well as facilitating mineral access, some attention will be paid to this topic.

Much of the Root stunting associated with infestation by Heterodera spp. has been attributed to the early effects of penetration and establishment of second stage juvenile larvae (Seinhorst and den Ouden 1971). Indeed, proposed mechanisms of tolerance have been equated with the response of early root-growth to infestation (Seinhorst 1961 1965 1981 b Trudgill and Cotes 1981; Wallace 1973). For example it has been suggested that root systems may be able to sustain a larger nematode population at no expense to shoot growth because of their inherently greater size. Alternatively the rate of lateral root proliferation on tolerant plants following infestation may exceed that of intolerant plants thereby serving to compensate for growth impairment of infested roots. Tolerance may be manifest in reduced sensitivity of infested roots to impairment of root extension. Root growth of tolerant cultivars has frequently been recognised as being less impaired by Heterodera infestation eg. on potatoes (Evans et al. 1977) wheat (Meagher 1970; Stanton 1983) and oats (Price and Hague 1983). Tolerant potato cultivars have root systems variously described as being more vigorous (Evans et al. 1977) more active (Trudgill and Cotes 1983 b), having an enhanced capacity to produce extra roots (Trudgill and Cotes 1983 a,b) and having a higher root length/unit root mass ratio (Evans et al. 1977). Stanton (1983) found no evidence to suggest

that tolerance in wheat was related to compensatory root growth or that tolerant plants had inherently larger root systems. In contrast Kerry and Hague (1974) suggested that early nodal root development may have offset the damaging effects of infestation on the seminal roots of cereals.

A second potential tolerance mechanism which could alleviate drought stress arising out of nematode infestation relates to stomatal functioning. Under water limiting conditions, the efficiency with which plants utilize water, that is the volume of water transpired for each unit increase in dry matter, is crucial (Fischer and Turner 1978, Taylor et al. 1982). Since stomata constitute the major barrier to water loss from the shoot the regulation of stomata in a way which maximizes CO_2 uptake with minimal H_2O loss may render an infested plant less prone to water defecit stress. Studies directed toward finding relationships between nematode tolerance and drought tolerance have been revealing. Tolerance to the potato cyst nematode has been attributed, in part, to a higher water use efficiency (WUE) (Evans and Franco 1979, Evans 1982a, Fatemy et al. 1985). WUE of nematode infested potato plants exceeded that of uninfested plants during initial growth (Evans 1982a). However, the mean WUE of intolerant infested plants over the entire growth interval was less than that of the uninfested controls due to a decline in WUE of infested plants after five weeks.

On the other hand, infested nematode-tolerant potato varieties used water more efficiently than did uninfested, intolerant controls (Evans 1982a).

Nematode infestation has been found to cause closure of stomata (Evans, et al. 1975; Kaplan, et al. 1976, Meon et al. 1979). Recently Fatemy et al. (1985) found that an infested nematode tolerant potato variety had a higher daily mean stomatal resistance and that stomatal closure occurred sooner in response to water deficit stress compared to an intolerant variety. They concluded that greater efficiency of water use was achieved by rapid stomatal closure following onset of stress and that properties of the plant that enhance drought tolerance may also confer nematode tolerance.

Evans and co workers have investigated the possible mediative role of abscisic acid (ABA), a plant growth substance involved in regulating stomatal movement of infested plants. ABA is known to cause stomatal closure at physiological concentrations (Wright 1969). Their work has shown that the endogenous concentration of foliar ABA may rise to nearly twice that of controls following infestation but that uninfested potato cultivars, more tolerant to infestation by Heterodera rostochiensis, have levels of ABA above those of less tolerant plants (Evans 1982a). They found that the ABA concentration of these tolerant cultivars showed the least increase when infested by nematodes (Evans

1982b, Fatemy et al. 1985). Exogenously applied ABA was reported to cause more prolonged closure of stomata on the tolerant plants, further supporting the contention that tolerance to nematode infestation may be related to greater sensitivity of stomata to incipient water stress (Fatemy et al. 1985). However, it is unclear from these studies whether elevated ABA levels in tolerant plants were a cause or an effect of tolerance. Given that the endogenous ABA concentration varies inversely with cell turgor (Pierce and Raschke 1980) the rise in ABA may be an early indication of reduced leaf water potential, itself a consequence of factors more likely to be root than shoot related.

Tolerance to nematode infestation may be manifest as an increase in the permeability of the roots to water entry. Larval penetration, establishment and giant cell formation could, through the disruptive effects on cortical cell integrity, reduce the hydraulic conductivity of the root system. There is no work yet to support or refute this claim. Root systems impaired by infestation may benefit from an enhanced root conductance.

3.2.2 Mineral Nutrition with Emphasis on Phosphorus

Root growth is most strongly influenced by Heterodera avenae infestation (Chapter II, Section 2). Because phosphorus is relatively immobile in the soil, (Lewis and Quirk 1966) and its concentration in the available form is

low (Bhat and Nye 1974) the uptake of this element is most sensitive to changes in root morphology and size, such as those caused by nematode infestation.

3.2.2.1 Principles of Phosphorus Nutrition

The relationship between nutrient supply and yield or total dry matter production follows a hyperbolic curve with an optimum supply for growth being followed by growth inhibition at higher concentrations (Moorby and Besford 1983). The effect of limitation of P on plant function has been reviewed from a number of standpoints (Moorby and Besford 1983, Bieleski and Ferguson 1983, Gerloff and Gabelman 1983) and will not be considered here.

An increase in root surface area will improve P nutrition more than other nutrients because of the relative immobility of P in the soil compared to more mobile ions such as K^+ and NO^{-3} (Nye and Tinker 1977). Increased root surface area brings into closer proximity soil regions not depleted of P (Barley 1970). Factors that have the overall effect of increasing the total surface area of the root, such as reduced root radius for the same root mass (Hackett 1969, Christie 1975), increased root length, (Jungk and Barber 1975) and increased root hair formation (Barley and Rovira 1970, Itoh and Barber 1983) have been shown to enhance P absorption.

Deficiencies in phosphorus nutrition can have differential effects on the components of root length. Hackett (1968) observed a decline in the number of nodal roots but not their length, and a decline in lateral root length but not their number on P deficient plants. Localized stimulation of root growth in regions of high P availability has also been observed (Drew and Saker 1978, Hackett 1972).

3.2.2.2 Effect of Infestation on Plant Mineral Nutrition and Related Mechanisms of Nematode Tolerance

Evidence indicates that, by impeding root extension, nematode infestation can seriously influence plant mineral nutrition. In field studies of potato plants, it was found that a decline in size of the root systems of infested plants was correlated with a reduced rate of P and K uptake Trudgill et al. 1975, Evans et al. (1977). These findings corroborated results of studies conducted in the past on a range of plant species Kruger 1925; Neuworth 1930; Paris and Jehle 1943; Oteifa 1952; Jenkins and Malek 1965).

Nematode tolerant plants may have some characteristics in common with plants capable of thriving in mineral deficient soils. Plants tolerant to low phosphorus soils either extract more phosphorus from a given soil volume or may utilize the limited phosphorus available more efficiently, that is, they can produce more dry matter per unit plant phosphorus than low phosphorus-intolerant plants

(Gerloff 1976, Loneragan 1978, Chapin 1980). Greater exploitation of available soil phosphorus could also be the result of a more extensive root network (Barley 1970), (Baldwin et al. 1972), or be due to the release of substances which solubilize an otherwise unavailable form of phosphorus (Silberbush et al. 1981).

In the case of nematode tolerance attention has already been drawn to the relevance of root extension in ameliorating the impact of drough stress brought on by nematode infestation (Chapter II, Section 3, 2.1.2), and similar considerations apply to mineral nutrition.

While it is conceivable that the release of exudates accompanying root nematode penetration might aid in the solubilization of unavailable forms of phosphorus, rendering a larger pool of P available for absorption by the plant, no work has been published on this potential nematode tolerance mechanism.

There is also no evidence in the nematode literature to substantiate or refute the claim that nematode tolerant plants are more efficient in utilizing plant phosphorus.

One compensatory mechanism which may relate more directly to nematode tolerance than to low mineral tolerance is the ability to increase the rate per unit root surface area of mineral uptake to compensate for a growth attenuated root system. Evidence indicates that, in uninfested plants,

a variable ion absorption rate serves to maintain a constant internal low concentration over a wide range of external ion concentrations (Williams 1948, Asher and Loneragan 1967, Drew and Saker 1978).

The observation that declines in plant growth and photosynthesis induced by nematode infestation are reversible by fertilizer amendment (Oteifa 1952, Wallace 1974, Evans et al. 1977) not only confirmed that nematode infestation caused nutrient deprivation but also that such plants were able to compensate for their growth restricted root systems by increasing the rate of mineral uptake when supplied with additional fertilizer.

There is also evidence that indicates that even in the absence of fertilizer amendment infested plants may have higher rates of ion uptake. Hunter (1958) found no indication of elemental deficiency of shoots resulting from infection of tomato plants by Meloidogyne incognita. In contrast often higher levels of P, N, K Mg and Cu were observed in the roots of infested plants. More recently Price et al. (1982) published a paper studying uptake rates of ^{32}P and ^{86}Rb of oat seedlings infested with Heterodera avenae. They reported that the shorter infested roots took up significantly more phosphorus and the shoots acquired more potassium per unit root length than the uninfested controls. The authors interpreted this as demonstrating that infestation did not restrict mineral uptake or transport, but rather that plants could respond to root growth impairment by increasing their uptake of P and K.

A recent study by Price and Sanderson (1984) may partly explain the disproportionately high levels of some elements in roots and shoots of infested plants. They found that eleven times more calcium was translocated to the shoots of Heterodera avenae infested oat plants than in the shoots of uninfested controls. They ascribed the increased rate of uptake to endodermal disruption by the nematode increasing the area free to apoplastic transport.

4. Root Growth and Development

There is an intimate association between root development and Heterodera avenae infestation. (Chapter II, Section 2). Compensatory root growth may be an important component of nematode tolerance (Chapter II, Section 3). This section will provide a framework around which further considerations of the interactions between root growth and nematode infestation may be placed.

4.1 Terminology

The method of nomenclature used to describe the root systems of oat plants in this study is one commonly used (Barley 1970; Hackett 1968; Russel 1977). A root produced from the base, seed or stem of the plant is an axis, those arising from the axes are termed first-order laterals, those arising from first-order laterals are called second-order laterals, and so on. Each axis with its associated laterals is a root.

Root systems of oat plants, like cereal plants in general, have two distinct types of axes: seminal axes, which arise from initials present in the ungerminated embryo and nodal axes, which develop subsequently from nodes on the shoot (Schuurman and de Boer 1970).

4.2 Growth and Differentiation

The oat plant usually has three or four seminal roots. Differentiation of newly divided root cells occurs in a region extending from 0.5cm above the root apex giving rise to the vascular stele, the cortex and the epidermis (Clowes 1961). The innermost layer of cortical cells surrounding the stele, the endodermis, becomes heavily lignified and suberized blocking apoplastic passage of ions between the cortex and stele (Robards et al. 1973).

The distance from the root tip at which lateral root initiation and emergence occurs is dependent on both the rate of growth of the parent root and upon the environment (Russel 1977). For example soil compaction can considerably reduce the distance from the apex to the first primordia (Goss, 1977).

4.3 Hormonal Control of Root Growth

General reviews have been published on this subject (Scott, T.K. 1972; Torrey 1976) and most recently by Feldman (1984). Root growth is controlled by several groups of hormonal substances and most have their origin in the root, though they may be produced elsewhere.

The precise nature of their control and their interaction has not yet been established.

4.3.1 Root Elongation

It is generally agreed (Torrey 1976; Elliott 1977; Pilet 1983) that elongation of the primary root is regulated by the interaction of indole acetic acid (IAA), a growth promoting substance derived principally from the shoot (McDavid *et al.* 1970) transported acropetally in the root (Pilet 1964; Feldman 1981) with abscisic acid (ABA) a root growth inhibitor (Pilet 1970) moving in a basipetal direction from the root cap (Audus 1983). The physiological effect of IAA and ABA varies with concentration when exogenously applied and their promotive or inhibitory effects can be reversed. For example ABA was reported to stimulate root elongation in *Pisum sativum* root tips (Gaither *et al.* 1975), and IAA has long been shown to have inhibitory effects at high concentrations (Burstrom 1957). Ethylene has recently been shown to have regulatory effects on root growth which mimic those of IAA (Chadwick and Burg 1967), its promotive or inhibitory effect being concentration dependent (Lieberman and Kunishi 1975). At 0.1 ppm, ethylene was found to inhibit cell division at the apical tip (Apelbaum and Burg 1972a), increase root swelling, reduce root extension and increase root mass over a 72 hour period (Apelbaum and Burg 1972b). High rates of root elongation of rice, mustard and tomato were correlated with high levels of ethylene (Konings and Jackson 1979; Bucher and Pilet 1981).

Concentrations greater than 1.0 ppm inhibited root elongation, while concentrations less than 0.1 ppm have in some instances stimulated root elongation (Konings and Jackson 1979).

4.3.2 Lateral Root Initiation

Cultured roots of a range of species respond to auxin treatment by initiating additional lateral roots (Torrey 1950, Wightman *et al*. 1980). The induction of lateral root primordia by cytokinins (Torrey 1962) and gibberellins (Butcher and Street 1960) has also been reported. Goldacre (1959) proposed that kinetin produced at active meristems diffused down a concentration gradient inducing lateral root initiation when the active physiological concentration was reached. Later work confirmed the requirement for auxin on cultured roots of *Haplopappus ravenii* (Blakely *et al*. 1972). More recently, Wightman *et al*. (1980) observed that cytokinins inhibited lateral root initiation on pea seedlings and that gibberellins had no effect. Ethylene at concentrations in the range of 5.0 ppb were found to restore lateral root branching on a non-lateral forming tomato mutant, Diageotropica (Zobel 1973). Exposure of barley or radish roots to 2-3ppm ethylene inhibited lateral root development (Radin and Loomis 1971, Crossett and Campbell 1975). It is now believed that ethylene plays no role in lateral root initiation but rather in subsequent root out-growth (Batten and Mullins 1978; Drew *et al*. 1981).

Further striking effects of ethylene on lateral root development were described by Jackson (1983). Temporary ethylene exposure at an early stage gave lateral roots a strong competitive advantage that resulted in faster rates of growth compared to laterals developing later. Ethylene (1-10 ppm) accelerated the emergence of nodal roots (Jackson et al. 1981).

ABA has been shown to inhibit (Street 1969; Bottger 1974) stimulate (Chin et al. 1969), or have no effect on lateral root initiation (Coleman and Greyson 1977) depending on the applied concentration. Applied concentrations as low as 10^{-8}M have been claimed to inhibit lateral root initiation (Bottger 1974) but more recently Goodwin and Morris (1979) and Wightman et al. (1980) found that inhibition only occurred when ABA concentrations exceeded 10^{-4}M. In this case, however, intact plants were used and it has been suggested (Wightman et al. 1980) that growth regulators from the intact shoot may have interacted with the applied ABA.

4.4 Nematode Invasion and Endogenous Hormone Concentration

Hormones originating in both the plant and the nematode have been claimed to be involved in the host-parasite relationship (Veech 1981). Sessile nematodes that move into

host cortical and stelar tissue such as Meloidogyne and Heterodera species cause changes in the invaded root that have been equated to those induced by exogenous application of IAA (Giebel 1973). In the case of invasion by Heterodera spp. cell and nuclear hyperplasia are induced and lateral roots form near the invaded zone (Dropkin 1978). There are several studies reporting the presence of indole compounds in root galls of Meloidogyne species (Bird 1962; Yu and Vigilierchio 1964), and in larvae and egg masses of Meloidogyne spp. (Yu and Viglierchio 1964) and Heterodera schachtii (Johnson and Viglierchio 1969). The absence of reports of the presence of hormones in larval secretions led Dropkin (1979) to suggest that tissue damage in the host may lead to changes in growth regulator concentration, for example by destroying sites of hormone synthesis. Others have suggested that nematodes may secrete glycosidases or proteases that release auxins from conjugates (Giebel et al. 1971, Giebel 1974).

4.5 Ethylene and Pathogenesis

Many plant pathogens have been claimed to stimulate the production of ethylene. Muller et al. (1940) reported increased ethylene production in citrus fruit infected with Penicillium digitatum. Daly et al. (1970) also found that resistant wheat infected with Puccinia graminis showed stimulated levels of ethylene. Ethylene production increases

in a wide variety of plant tissues subjected to stress induced by mechanical injury (Burg and Thimann 1959, McGlasson and Pratt 1964, Saltveit and Dilley 1978). Ethylene produced by infected tissue has been attributed largely to the effects of cell damage (Sequeira 1973, Yang and Pratt 1978). Recently, Glazer et al. (1983) reported elevated ethylene production in excised tomato root cultures infested with Meloidogyne javanica. They found that resistant tomato cultivars produced less ethylene than susceptible plants and related ethylene production to gall development.

These are the possible mechanisms in which tolerance may operate. The remainder of this thesis is concerned with testing these hypotheses.

CHAPTER 3: METHODS AND MATERIALS

CHAPTER 3: METHODS AND MATERIALS

3.1 Materials

Oat (*Avena*) seeds were kindly provided by Mr. A. Barr of the South Australian Department of Agriculture.

Chemicals and reagents were of analytical grade when purity was essential. For general purposes laboratory grade chemicals were used. The source of relevant chemicals are given where they are first mentioned.

3.2 Methods

3.2.1 Seed Preparation and Germination

Seeds were presoaked for 1 hour at room temperature (20-30°C). Hulls were removed using blunt forceps. Seeds were surface sterilized in 1.0% sodium hypochlorite solution for 5 minutes and rinsed about 6 times with 200 ml deionized water. Seeds were then placed dorsal side up onto sterile 1.5% water agar plates (8.5 cm) onto which 2 mls of 2% KNO_3 solution had been earlier applied. Plates were enclosed within black plastic bags and incubated for one week at 2°C. A second incubation interval at 20°C for 35-48 hours gave about 75% germination with the longest seminal root about 15 mm. Seedlings with all three seminal roots emerged at the end of this period were used in experiments.

3.2.2 Plant Culture

3.2.2.1 Soil

For experiments in which plants were soil-grown a steam sterilized one-half strength John Innes soil mixture without peat was used (Table A). Generally this soil was not used earlier than 6 weeks after sterilization. Crushed gravel was placed at the bottom of the pots to facilitate drainage.

Table M1 : Preparation of Half-strength John Innes Soil Mix-Minus Peat

Equal parts by volume of coarse sand and medium loam were steam sterilized at 100°C for 30 minutes.

The following nutrients were added upon cooling:

Nutrient	Amount Added (g m^3)
Blood Meal	600
Potassium Sulphate	300
Super Phosphate	550

In some experiments plants were grown in either a single or double length of 2.7 (I.D.) X 13.0 cm rigid poly vinyl choride electrical conduit. Lightly dried soil was compacted at the base of the tube with a plastic plunger, then further soil increments were added and lightly tamped until the soil was 3.0 cm from the top of the tube. Tubes were arranged onto plastic lined metal trays and supported with a wire grid. Six ml of distilled water was added to the soil surface prior to planting seedlings. A further 2.0 cm of dry soil was added to cover the seed along with 2 ml of distilled water. Subsequent water was added to the floor of the trays when the upper soil appeared dry.

3.2.2.2 Sand

In some experiments a sterile fine grained phosphorus free sand was required. Waikerie river washed sand was dried and sieved to obtain fine grained sand (< 750 μm > 250 μm). The sand was soaked in an 8 N HCL solution for 24 hours on a moving table before repeated rinsing in distilled water until the pH of the sand-water solution was that of distilled water (pH = 6.8). Colorimetric determination (Olsen et al. 1954) of $NaHCO_3$ - soluble phosphorus (Colwell 1965) failed to detect residual phosphorus after the acid washing treatment. Specific details of plant culture are given in Chapter 4, Section 6.

3.2.2.3 Hydroponics

Details of nutrient solutions and culture methods are given in the relevent sections (Chapter 4, Sections 5 and 6).

3.2.3 Environmental Control

3.2.3.1 Conditions of Growth in Glasshouse and in Growth Cabinets

Plants grown in a constant temperature (20 ± 2°C) glasshouse received an average canopy level irradiance that measured 115 to 680 μE $m^{-2}s^{-1}$ at midday.

Controlled environment cabinets were maintained at 20/15 ± 1°C day/night with a 16 hour photoperiod unless otherwise stated. Light was supplied by a combination of high pressure sodium (60W), "cool white" fluorescent tubes (80W) and incandescent bulbs (60W) to provide a total irradiance of 590 $\mu Em^{-2}s^{-1}$ at canopy level.

3.2.3.2 Atmospheric CO_2 Regulation

Plants to be tested were grown inside specially designed mini-chambers. These were constructed from cellulose acetate plastic sheeting superimposed onto 85 x 40 cm metal trays to obtain an enriched atmospheric CO_2 partial pressure within the chamber. Pure CO_2 was mixed with outside air (ambient partial pressure, 310 μbar) pumped into the chamber by 2 diaphragm pumps ($3 l min^{-1}$) linked in parallel. Attenuated CO_2 partial pressures were obtained by pumping air through 10% KOH, carbosorb, a water trap, a cotton and then a charcoal filter before entry into the chamber. Where three CO_2 treatments (attenuated, enriched, ambient) were run concurrently in the same growth room, air from the chamber was directed outdoors via latex tubing running from vents at the top of the chambers. CO_2 concentrations were monitored regularly (hourly during initial trials down to 3 times per day when patterns of CO_2 consumption were better understood). Flow rates controlled by air flow regulators were adjusted to correct for over or under-supply of CO_2. 10 ml air samples, drawn by syringe from different positions along the air flow route and from within the chambers, were analysed for CO_2 using an ADC Mark III infrared gas analyser.

3.2.4 Handling of *Heterodera avenae* Cysts and Larvae

3.2.4.1 Collection and Maintenance of *Heterodera avenae*

Mature cysts of *Heterodera avenae* were collected in February or March of successive years from roots of barley and wheat stubble harvested the previous season in fields reportedly infested with *Heterodera avenae*. Cysts were separated from root material by wet-sieving and decanting and placed with some associated organic debris onto nylon mesh or bolting silk. These were put into glass petri plates (diam. 12.0 cm) and suspended on stainless steel or plastic mesh in shallow water. Incubation temperature was maintained at 5 or 10°C. ~~Larvae were collected daily, counted and incubated at 5°C.~~ Larvae were collected daily, counted and incubated at 5° or 10°C in shallow water until required.

Inoculum densities were prepared by water dilution of the highest density. Inoculum was usually applied in 0.5 ml aliquots to the base of the 3 to 10 day old seedlings unless otherwise stated. Densities were confirmed by triplicate counts of known volumes of larval suspension in a modified Doncaster dish (Southy 1970) examined under a dissecting microscope. Inoculum densities described are approximations within an error of no more than 5%.

Estimates of the larval population in root systems were obtained either by direct counting of stained larvae on roots squashed between two glass slides, or by homogenizing the stained root system in a blender, at the highest setting for

1-4 minutes and doing triplicate counts on the number of larvae from a measured homogenate volume in a modified Doncaster dish. Larvae counts were done under a dissecting microscope.

3.2.4.2 Surface Sterilization of Larvae

The procedure of Aist and Riggs (1969) was adopted. Hatched larvae were placed into a 200 ml fritted glass Buchner funnel containing 30 ml penicillin (50%) and 30 ml streptomycin (0.1%) and incubated for 45 minutes at 20°C. Larvae were then sequentially rinsed in 50 ml hexadecyltrimethylammonium bromide (0.05%) for 1 minute, 100 ml sterile deionized water, 50 ml Chlorhexidine Acetate[1] (0.5%) for 6 minutes. Finally they were rinsed 8 times with 150 ml sterilized water.

3.2.5 Sampling of Root and Shoot Material

Plants grown in sand were removed from their containers and roots were washed on a 250 µm screen under a gentle stream of water before separating shoots from roots.

When only root length was to be determined, roots were held at 2°C to be counted within 2-3 days, or were kept in FAA (Formalin-Acetic-Alcohol) for longer term storage.

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1. Supplied as Hibitane^{R}

When both root length and larval population on the root were to be assessed, roots were stored in lactophenol containing 0.1% cotton blue (Hooper, 1970). To hasten staining, vials were incubated for 5 hours at 80°C. Otherwise they were left for one or more weeks before counting.

Root mass was obtained by thoroughly rinsing soil or sand from the roots. If only fresh mass was required, roots were carefully blotted dry on Whatman[2] No. 1 filter paper before weighing. If both fresh and dry mass were required, samples were either returned to their sample vials and freeze-dried or heated for 48 hours at 80°C before storing over silica crystals prior to weighing. Freeze-dried samples could be rehydrated to permit determination of root length or stained in lactophenol with cotton blue to obtain nematode counts and root lengths. Root length was measured using the line intersect method of Newman (1966) modified by Marsh (1971). The root sample was spread out evenly in a round 19 cm diameter glass plate containing a small amount of water. A grid of half an inch squares was laid below the plate. Vertical and horizontal lines were scanned and roots were counted where they intersected a line. Root length in centimetres was calculated according to the formula:

$$R = \frac{\pi A \times n}{2H}$$

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2. Trade name

Where R = total root length, A = area in which the roots are distributed, n = the number of intersections between roots and straight lines and H = total length of straight lines.

In some experiments root surface area was calculated using the $A = 2\pi r l$ where A is root area, r root radius and l, root length. In order to partially account for differences in root radii of main axis seminal and nodal roots and first and higher order laterals, roots were classified as either 500 um or more and 300 um or less in diameter. Lengths of roots with respective diameters were calculated separately.

3.2.6 Estimation of Ethylene

Ethylene concentrations in air surrounding root tissues was measured using a Varian 1400 gas chromatograph fitted with a flame ionization detector. Ethylene was separated on a glass column silanized and packed with Porapak Q (100-120 Mesh; Waters Assoc. Inc., U.S.A.). Flow rates of individual gases through the column were ($ml.min.^{-1}$): N_2, 30; H_2, 30; air, 300. Temperatures at the injector, column and detector were (^oC): 100, 40 and 100, respectively. Gas samples of 1 ml were injected into the column and quantitatively assessed by comparing peak heights of standards prepared by dilution of known C_2H_4 concentrations in 1 L pickling jars fitted with "suba-seals".

3.2.7 Estimation of Abscisic Acid

Abscisic acid was measured using a Varian 2700 gas chromatograph equipped with an electron-capture detector. Free ABA was extracted by a modified method of Coombe and Hale (1973). Samples were rinsed, wrapped in aluminium foil, frozen in liquid N_2, lyophilized and weighed. Tissue was macerated with a glass homogenizer in 2 ml each of acetone and water and titrated to pH 9 with 3 N NH_4OH. Samples were centrifuged twice at 2000 rpm for 10 minutes retaining the supernatant and resuspending the pellet in 1.5 ml 2% NH_4HCO_3. The water soluble fraction was separated by centrifuging twice (2000 rpm) with 3 ml chloroform and acidified with 10% H_3PO_4 to pH 3. The organic fraction was partitioned with 5 and 2.5 ml ethyl acetate and the organic fraction was concentrated by drying under a stream of nitrogen gas.

Samples were further purified by applying the extracts to acid washed Whatman No. 3 mm chromatography papers in 40 mm streaks. Chromatographs were developed in a descending mixture of isopropanol:water:NH_4OH (10:1:1 v/v) and dried for 30 minutes. Known ABA standards co-chromatographed with the samples were identified on the paper under UV light and Rf regions of the corresponding sample were cut out and eluted with 70% methanol. Eluted samples were reduced under N_2 gas, washed with anhydrous petroleum and dried.

Dried extracts were suspended in ethyl acetate (0.5 ml) and methanol (0.5 ml) then methylated with diazomethane. Diazomethane gas, produced by reacting 2 ml carbitol with 2 ml 60% (w/v) KOH and 100 mg P-tolysulphonylmethylnitrosoamide, was bubbled through the sample until it became yellow indicating diazomethane saturation. The extract was dried under N_2 gas and stored at 2°C in the dark until injecting it into the GC.

ABA (methyl-cis trans) extracts from samples were dissolved in ethyl acetate and injected (0.5-2 ul) into a salinized OV-17 Gas Chrom Q (Applied Science Lab., U.S.A.) glass column. Operating temperatures of the injector, column and detector were 210, 180 and 245°C, respectively. Flow rate of the carrier (N_2) gas was 25 ml min^{-1}. Comparison of retention times of sample with those of authentic cis-trans ABA (Sigma Chemical Co. U.S.A.) confirmed the presence of ABA. Quantification of ABA was based on comparison of peak height of known ~~Abs~~ ABA standards with the sample. Losses during extraction and purification were checked by adding 20 ng ABA to the homogenizing medium before macerating. Recovery rates were as low as 45% in some instances but generally were about 75 to 80%. Corrections were made in calculations to account for losses.

3.2.8 Estimation of Phosphorus

Plant material was oven dried at 80°C for 48 hours and after weighing, was coarsely ground and digested in a mixture of nitric and perchloric acid (3:2). Phosphorus was measured by spectrophotometry of a phospho-molybdate blue complex in the presence of ascorbic acid (Murphy and Riley 1962) on an auto analyser. The ascorbic acid concentration was increased to reduce the interference of Fe^{3+} ions (John 1970).

3.2.9 Estimation of Total Soluble Carbohydrates

Total soluble carbohydrates was determined colorimetrically using the phenol sulfuric acid reaction described by Montgomery (1961) using glucose standards.

3.2.10 Measurement of Plant and Soil Water Relations

3.2.10.1 Determination of Field Capacity

Field capacity of John Innes soil was determined according to the method described by Kezdi (1980). 2 litre graduated cylinders were lined with plastic tubing sealed at the bottom. The cylinder was filled with approximately 2 kg oven dried (80°, 48 hours) soil. 175 ml of water were added at once to the soil. Water equilibrated for 2 days. The water content of the soil 5 cm from the wetting front was determined gravimetrically.

3.2.10.2 Leaf Water Potential (Ψ_L)

Ψ_L was measured with a Spanner thermocouple psychrometer (Barrs 1968). Freshly excised leaves were coiled into the thermocouple chamber and equilibrated for 2-4 hours at 25°C in a water bath. Ψ_L was determined from calibration curves prepared from a graded series of NaCl solutions. A Scholander pressure chamber (soil moisture equipment Corp., U.S.A.) was also used to measure Ψ_L. (Scholander et al. 1964). The pressure at which a drop of exudate was forced to the cut end of the leaf was taken as the Ψ_L.

3.2.10.3 Relative Water Content (RWC)

Measurement of RWC was in accordance with the method of Barrs and Weatherly (1962). RWC is calculated using the formula:

$$RWC\ (\%) = \frac{\text{Fresh mass} - \text{dry mass} \times 100}{\text{Turgid mass} - \text{dry mass}}$$

Freshly cut leaves were weighed then floated on water under low light at 20°C for 3 hours. The turgid leaves were weighed after lightly blotting on filter paper, then oven dried (48 hours, 80°C) and weighed again.

3.2.10.4 Stomatal Conductance

Stomatal conductance was measured using a diffusive resistance automatic porometer, model, Mark III (Delta Devices). Calibration of the instrument using a calibration plate with graded pore sizes was done before and after each set of measurements. The time required for a single measurement varied between 20 and 60 seconds depending on the leaf water status.

3.2.11 CO_2 Gas Exchange

Measurements of water vapour and CO_2 exchange of single leaves were performed using an open ended gas exchange system similar to that described by Wong *et al.* (1978). Leaves to be measured were enclosed within an aluminium and glass cuvette maintained at a constant temperature ($26.5 \pm 0.2^{o}C$). Flow rate through the cuvette was 1.0 litre. min^{-1}. Air passing over the leaf was conditioned by removing CO_2 with soda lime and then humidified, with the dewpoint set when the air was passed through a glass condenser at $18^{o}C$. Water vapour content was determined by a Vaisala HM606 (Vaisala, Oy, Helsinki, Finland) capacitive sensor. The CO_2 concentration was esablished by injecting 10% CO_2 in air into the air stream through a mass-flow controller. The CO_2 depletion caused by the leaf was measured with an ADC Mark III infrared gas analyser. Air was circulated inside the cuvette by a small fan. The light souce was a tungsten

halogen projector lamp with an irradiance of 600 μmol $m^{-2}s^{-1}$. Assimilation and evaporation rates, leaf conductance, and intercellular CO_2 concentration were calculated on a Commodore mini-computer. Calculations of results followed those set out by Von Caemmerer and Farquhar (1981). On the day of measurement plants were taken from the growth cabinet to the gas exchange unit. The penultimate leaf was laid across the cuvette so that 9 cm of the central portion of the leaf was inside the chamber. The area of the leaf surface within the chamber was between 10 to 14 cm^2, depending upon the cultivar and the leaf position. The top of the cuvette was then laid over the leaf and tightened with clamp screws until the inside of the cuvette was completely sealed from outside air. After 15 to 30 minutes of equilibration time the partial pressure of CO_2 ($p(CO_2)$) within the cuvette was increased from 330 ubars to 900 ubars pCO_2 and triplicate gas exchange measurements were obtained. Gas exchange measurements were then recorded at progressively lower pCO_2 levels, down to 45 ubar pCO_2. Measurements were taken during the normal daylight hours, corresponding to the photoperiod of the growth cabinet in which the plants were grown.

3.2.12 Chlorophyll Determinations

Three leaf discs were placed immediately into 2 ml of N, N-dimethylformamide (DMF) in glass vials and stored in the dark at 2°C until measurement 2 weeks later (Skeep and Bloom 1985). The resultant chlorophyll extract was analyzed spectrophotometrically (Model CE 303, Cecil Instruments Ltd.

Cambridge) at wave lengths of 665 and 647 nm. The amounts of chlorophyll a and b were calculated using the equations:

$$CHLa = 12.7\ (A665) - 2.79\ (A647)$$

$$CHLb = 20.7\ (A647) - 4.62\ (A665)$$

$$\text{Total CHLa + CHLb} = 17.9\ (A647) - 8.08\ (A665)$$

3.2.13 Leaf Area

Leaf area was determined on freshly harvested leaves using a Paton planimeter. Leaves were placed on a transparent conveyor and passed between a line of photocells at a constant speed. Total area was shown on a digital readout meter.

3.2.14 Experimental Design and Statistical Analysis

Completely randomized or randomized complete block designs were used in the experiments described. Data were analyzed with the assistance of the Biometry section of the Waite Institute on either a Cyber or a Vax computer using Genstat statistical analysis programs.

CHAPTER 4: RESULTS AND DISCUSSION

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SECTION 1: EVALUATION OF TOLERANCE OF FOUR OAT CULTIVARS TO *Heterodera avenae*

1.1 Tolerance in Tillering Plants

1.1.1 Introduction

Most grass species are hosts for *Heterodera avenae* (Meagher 1977, Brown 1984). Differences between oat cultivars in levels of tolerance and resistance to *Heterodera avenae* have been observed in field studies (Barr and Dube 1985).

Plant response to nematode infestation has been shown to be influenced by such factors as soil temperature (Barker *et al*. 1976; Nardacci and Baker 1979, Griffin 1981) irradiance (Fawole and Mai 1979) salinity (Endogali and Ferris 1982) water availability (Evans and Franco 1979; Evans 1982a) and soil mineral composition (Evans *et al*. 1977; Simon and Rovira 1985). Assessment of nematode tolerance under controlled glass house conditions in which the impact of a variable climate on plant response to infestation, is minimized may therefore differ from tolerance assessments in the field.

Field rating of cultivars for tolerance at harvest provides no information on how differences in tolerance originated. Continuous monitoriing of plant growth has greater potential to provide information on how differences

in development may affect final yield response to infestation. For example, early maturing cultivars may have less opportunity to counter infestation through growth of tillers than late maturing cultivars. The production of large root systems by some cultivars may facilitate water and mineral aquisition and neutralize effects of infestation on growth. Such developmental differences between cultivars must be taken into account in assessing nematode tolerance before more subtle physiological traits, such as hormone levels can be assessed.

The present study was undertaken to assess the tolerance and resistance ratings of four oat cultivars previously established in field trials, in response to a range of initial nematode densities under controlled environment conditions. Information was also sought on whether morphological and developmental traits could be related to cultivar response to infestation.

1.1.2 Methods and Materials

Plastic pots, 20 cm diameter were filled with John Innes soil without peat (Chapter III, Section 3.2.2.1). Plastic PVC tubes 5.4 (I.D.) X 13.0 cm were placed 1 tube per pot, vertically into the soil of the larger pot, the bottom 2 cm embedded below the soil surface, and filled with the same soil mixture. Perlite was sprinkled onto the soil surface to reduce evaporation.

Seeds of four oat cultivars, New Zealand Cape (NZC) Sual, Swan and West were pregerminated (Chapter 3.2.1) and planted 4 seedlings per tube. After one week plants were thinned to one plant per pot. Second stage Heterodera avenae larvae were applied at densities of 0, 100, 300, 900, 2000, 5000, 10,000, 20,000 and 40,000 per plant between 7 to 10 days after sowing. There were a sufficient number of replicates to enable destructive sampling on plants 30, 70, 120 (Swan and West) and 182 days after planting (NZC and Sual), with 4 (day 30) 6 (day 70) or 8 replicates (days 120 and 180) per treatment.

Plants were maintained until maturity, at a constant temperatue of 20°C + 3°C in a temperature controlled glasshouse from the months of November to May with an average daylength of 14 h, with night time supplemental lighting provided by "Coolwhite" fluorescent tubes (80w) and incandescent bulbs (60w).

Plants were watered every three to four days to maintain a soil field capacity of 95%, determined gravimetrically. The development of all plants was recorded by measurement of leaf lengths and later through destructive harvests. Because they matured earlier, final harvests of Swan and West were carried out earlier (120 days after planting) than those of Sual and NZC (182 days).

Tolerance was assessed by comparing the grain yield of control plants with that of inoculated treatments and was expressed as the absolute reduction in yield (g) and % yield reduction (tolerance index) (Fisher et al. 1981 Boerma and Hussey 1984).

Larval penetration of roots was measured four weeks after sowing. The number of cysts per root system was measured 70, 120 and 182 days after sowing.

Water consumption was recorded every third or fourth day until day 136, by measuring weight loss of pots with plants over the time period and subracting water loss due to evaporation from control pots without plants.

The experiment was done in 1982 and 1983, lower inoculum densities in the first year followed by higher densities in the second year. For uniformity larval densities of 1 0 and 2000 larvae per pot were included in both experiments, permitting comparison between years.

A randomized complete block design was used in both years.

1.1.3 Results

Variation between the two years in which the experiment was conducted was not significant on the basis of final grain yield and shoot mass (Table 1.1). Differences between cultivars and densities will be discussed in the following sections.

Table 1.1: The Effect of Different Planting Dates on Grain Yield and Shoot Mass of Four Oat Cultivars

CULTIVAR	INITIAL INOCULUM DENSITY (LARVAE/POT)	GRAIN YIELD (g)			SHOOT MASS (g) AT HARVEST		
		1982	1983	MEAN	1982	1983	MEAN
New Zealand Cape	0	10.66	9.48	10.17	41.98	38.72	40.51
	2000	8.22	7.34	7.69	44.80	39.46	42.01
	MEAN	9.41	8.41		43.31	39.14	
West	0	3.80	3.43	3.62	11.03	10.21	10.57
	2000	2.27	1.85	2.18	6.28	5.74	6.10
	MEAN	3.02	2.66		8.64	7.89	
Swan	0	7.99	6.04	7.01	15.49	13.98	14.69
	2000	6.53	5.82	6.19	13.41	14.67	14.13
	MEAN	7.19	5.98		14.45	14.33	
Sual	0	15.80	13.79	14.83	62.86	59.47	61.14
	2000	7.46	8.01	7.76	30.14	27.16	28.72
	MEAN	11.61	10.92		46.43	43.32	

LSD ($P \leq 0.05$)		
Between Years	NS	NS
Between Densities	2.34*	6.74**
Between Cultivars	NS	8.22*

Larval Infestation

Analysis was performed on log transformed data and presented in the original form in the relevant figures. The nematode population on invaded roots increased as inoculum density increased (Figs. 1.1a and 1.1b). No significant cultivar by density interactions with respect to male larvae numbers on roots at 30 days were observed. Males were more numerous on NZC and Swan than on Sual and West. A significant interaction between density and cultivar was noted for female larvae, however Swan had more females in the intermediate density range, while most females were counted on West at the higher densities (Fig. 1.1b) (analysis of log transformed data). By day 70 West had a consistently higher female population while Sual and NZC had significantly fewer females than West over all densities (Fig. 1.1c). Swan was again highly variable. At plant maturity the number of cysts on roots had declined dramatically (Fig. 1.2) in all cultivars, this was significant at initial densities of 5000 larvae or more. By this date differences between cultivars were not significant at lower densities (Fig. 1.1d). At initial larval densities greater than 5000 Sual had significantly fewer cysts.

FIG 1.1a The effect of initial inoculum density of Heterodera avenae larvae on the number of male larvae on four oat cultivars 30 days after planting (mean of four replicates).

FIG 1.1b The effect of initial inoculum density of Heterodera avenae larvae on the number of female larvae on four oat cultivars 30 days after planting (mean of four replicates).

FIG 1.1c The effect of initial inoculum density of Heterodera avenae larvae on the number of cysts on four oat cultivars 120 (Swan and West) and 182 (NZC and Sual) days after planting (mean of eight replicates).

FIG 1.1d The effect of initial inoculum density of Heterodera avenae Larvae on the number of cysts on four oat cultivars 129 (Swan and West) and 152 (NZC and Sual) days after planting (mean of eight replicates).

IDENTICAL SYMBOLS USED IN FIGS. 1.1 to 1.4:

CULTIVAR

NZC ▲——▲

SUAL ●——●

SWAN ■——■

WEST ◆——◆

NOTE: Initial inoculum density in Log_{10} scale in Figures 1.1a-d.

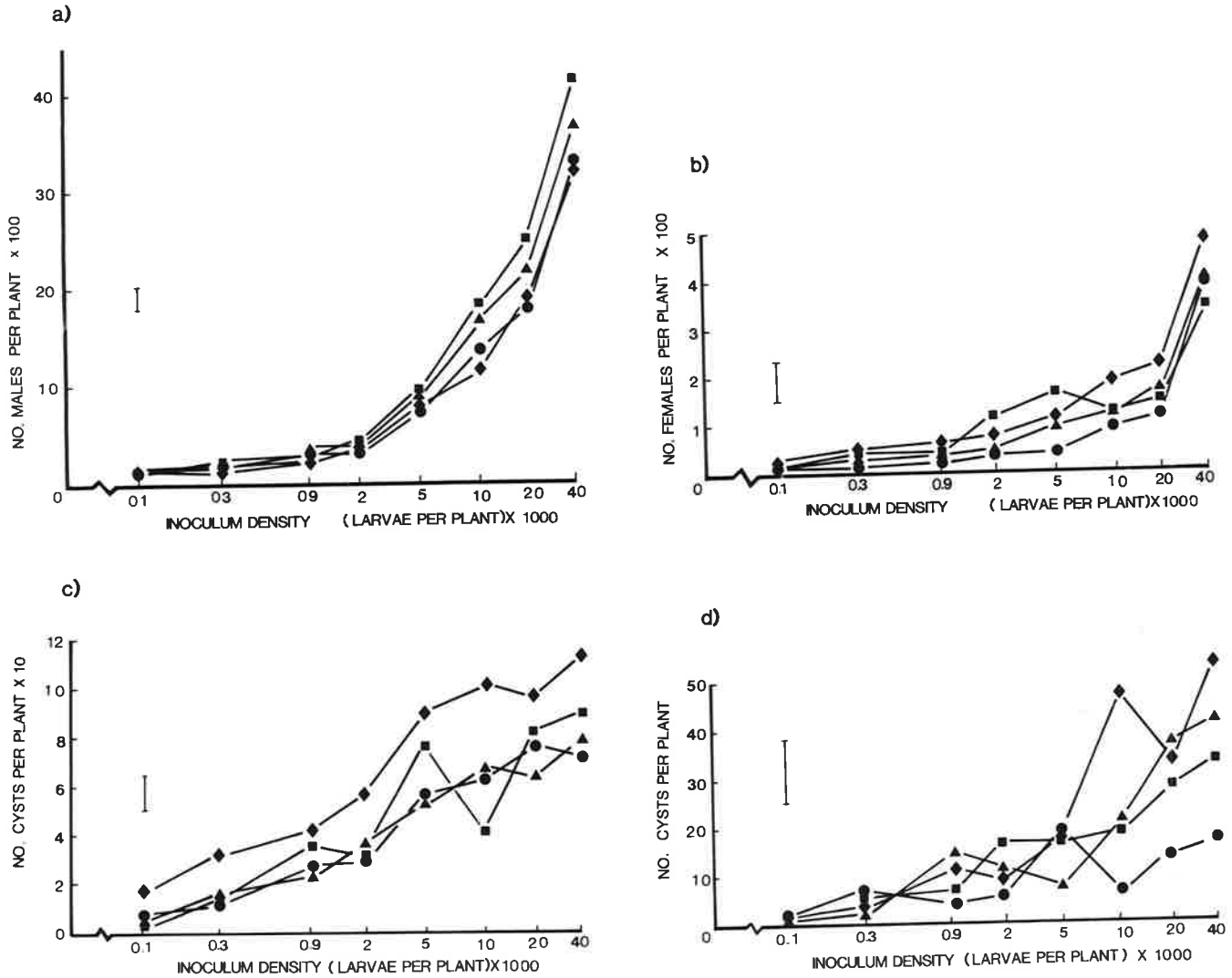
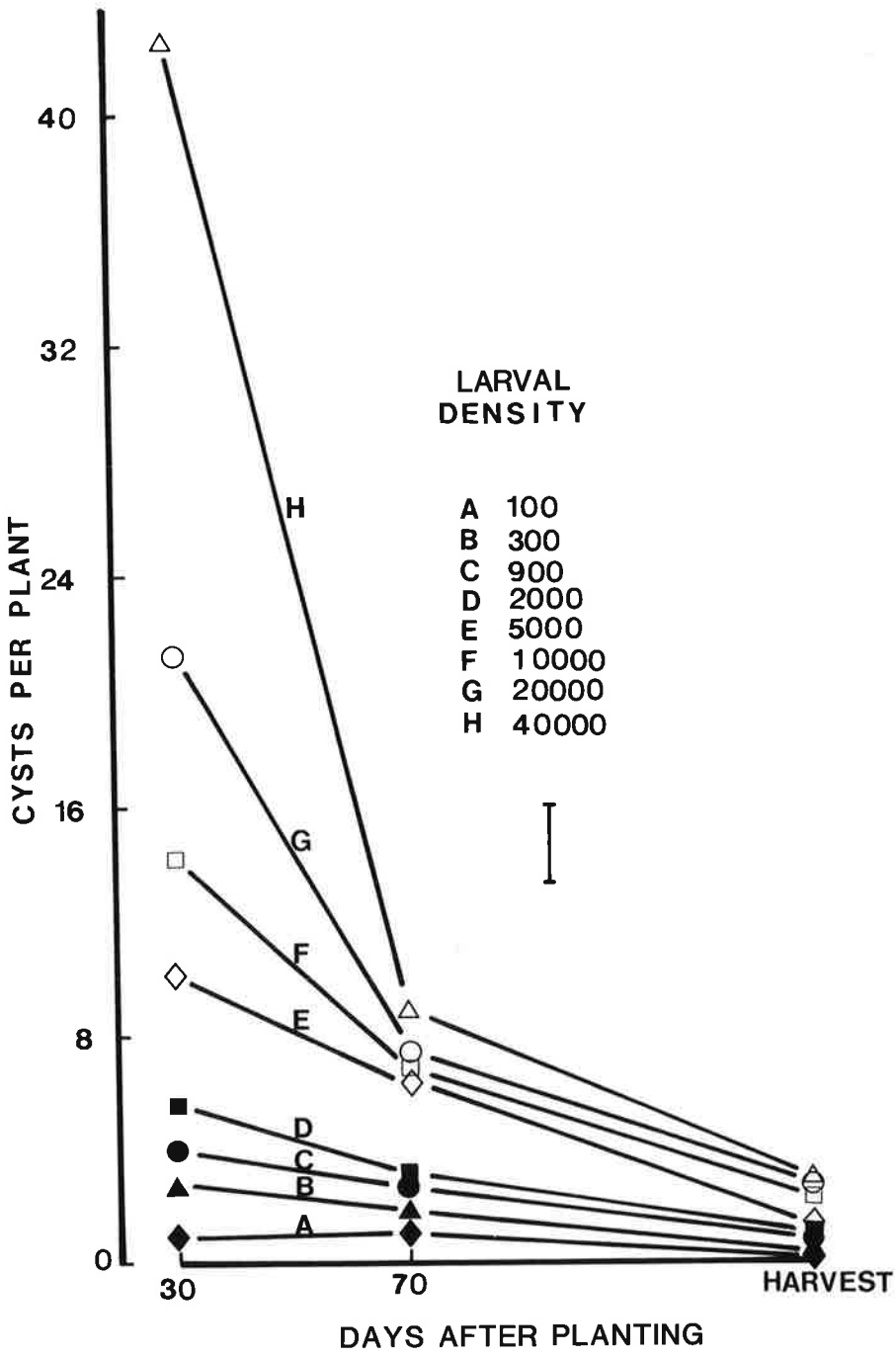

a)
NO. MALES PER PLANT x 100
0
10
20
30
40
0.1
0.3
0.9
2
5
10
20
40
INOCULUM DENSITY (LARVAE PER PLANT)X 1000
b)
NO. FEMALES PER PLANT X 100
0
1
2
3
4
5
0.1
0.3
0.9
2
5
10
20
40
INOCULUM DENSITY (LARVAE PER PLANT)X1000
c)
NO. CYSTS PER PLANT X 10
0
2
4
6
8
10
12
0.1
0.3
0.9
2
5
10
20
40
INOCULUM DENSITY (LARVAE PER PLANT)X1000
d)
NO. CYSTS PER PLANT
0
10
20
30
40
50
0.1
0.3
0.9
2
5
10
20
40
INOCULUM DENSITY (LARVAE PER PLANT) X 1000

FIG 1.2 The effect of plant age (days after planting) on the number of female larvae on cysts on roots of four oat cultivars inoculated with different initial inoculum densities of Heterodera avenae larvae


40
32
24
16
8
0
CYSTS PER PLANT
LARVAL
DENSITY
A 100
B 300
C 900
D 2000
E 5000
F 10000
G 20000
H 40000
H
G
F
E
D
C
B
A
30
70
HARVEST
DAYS AFTER PLANTING

Main-stem Leaf Growth

The effect of nematode density on leaf growth 12, 24 and 40 days after planting is shown in Table 1.2. Analysis of variance was performed on individual sample dates for each leaf. Values for densities under 2000 are not included because infestation had no effect on leaf growth below this density. For clarity no values for the density of 5000 are shown.

In general the effect of infestation on leaf growth was most severe 24 days after planting. At that date infestation reduced leaf length and/or delayed leaf extension at densities of 20,000 larvae or more. However, cultivars differed in their response to infestation at lower densities, and at different dates.

NZC and Swan were unaffected by infestation at any density on day 12. Sual and West showed significant delays in leaf extension on day 12, Sual showing greater sensitivity to infestation than West. By day 24 all cultivars showed evidence of both decline in final leaf length as well as delay in extension. Least affected were NZC, and West and most affected were Sual and Swan. The latter two cultivars were significantly affected by densities of 2000 (Sual) and 10,000 (Swan). After 40 days, differences between cultivars established on day 24 were still evident.

Table 1.2: Effect of Density of *Heterodera avenae* on Lamina Length of Four Oat Cultivars 12 to 40 Days After Planting

a) DAY 12

CULT.	DENSITY	LAMINA LENGTH (mm) LEAF NO. L1	L2	L3	CULT.	DENSITY	LEAF NO. L-1	L2	L3
NZC	0	89	170	82	SWAN	0	104	123	52
	2000	84	182	86		2000	111	126	54
	10000	91	157	64		10000	108	118	65
	20000	87	149	66		20000	103	115	41
	40000	82	147	57		40000	98	109	32
SUAL	0	84	162	52	WEST	0	76	119	44
	2000	87	152	21*		2000	72	111	57
	10000	78	126*	9*		10000	72	91	39
	20000	85	103*	-		20000	79	82	21
	40000	81	84*	-		40000	69	48*	-

b) DAY 24

CULT.	DENSITY	L3	L4	L5	L6	CULT.	DENSITY	L4	L5	L6
NZC	0	-	322	258	58	SWAN	0	326	222	73
	2000	-	309	273	97		2000	321	198	58
	10000	-	314	229	42		10000	299	174*	31*
	20000	-	291	192*	12*		20000	261*	142*	-
	40000	-	247*	163*	-		40000	193*	107*	-
SUAL	0	261	330	232	-	WEST	0	228	203	141
	2000	249	337	176*	-		2000	224	191	113
	10000	204	193*	104*	-		10000	181	176	74
	20000	165*	128*	62*	-		20000	126	92*	-
	40000	142*	84*	-	-		40000	87	59*	-

Table 1.2 cont.

C) DAY 40

CULT.	DENSITY	LEAF NO.				
		L5	L6	L7	L8	L9
NZC	0	338	324	232	196	84
	2000	351	316	289	234	118
	10000	371	309	206	171	22*
	20000	288	321	173	169	8*
	40000	273	276	158*	97*	-
SUAL	0	289	352	324	216	73
	2000	247	292	318	140*	-
	10000	223*	242*	221*	-	-
	20000	182*	266*	193*	-	-
	40000	147*	257*	72*	-	-
SWAN	0	354	341	254	64	-
	2000	366	308	218	96	-
	10000	338	338	137*	-	-
	20000	281*	278	118*	-	-
	40000	227*	239*	34*	-	-
WEST	0	224	208	172	-	-
	2000	207	179	139	-	-
	10000	183	161	110*	-	-
	20000	194	157*	117*	-	-
	40000	176	78*	91*	-	-

a) Leaves are numbered in increasing order of emergence.

* Significantly different from 0 density ($P < 0.05$).

Shoot Mass, Root Mass, Root/Shoot Ratio

Analysis was performed on $\sqrt{n}$ transformed data. Results are presented in their original form.

Cultivar differences were observed in shoot mass after 30 days growth (Fig. 1.3). Swan and NZC had significantly ($P = 0.01$) larger root and shoot masses than Sual and West. Nematode density reduced root and shoot mass. Shoot mass of all cultivars was similarly affected by density of inoculum. A significant ($P = 0.05$) cultivar by nematode density interaction was observed for root mass after 30 days (Fig. 1.4). Root mass of all cultivars except that of NZC was significantly depressed at larval densities of 10,000 or more. No measurements were made on root growth after 30 days. Nematodes had no effect on the root/shoot ratio of NZC. Sual, Swan and West had higher root/shoot ratios at larval densities of 10,000 or more. (Table 1.3).

Table 1.3: The Effect of Nematode Density on Root/Shoot Ratio (Dry Mass) of Four Oat Cultivars 30 Days After Sowing

ROOT/SHOOT RATIO

INITIAL DENSITY	CULTIVAR NZC	SUAL	SWAN	WEST	MEAN
0	0.59	0.56	0.50	0.48	0.54
100	0.66	0.50	0.51	0.41	0.52
300	0.62	0.55	0.56	0.50	0.55
900	0.56	0.58	0.53	0.47	0.53
2000	0.64	0.59	0.53	0.46	0.55
5000	0.72	0.57	0.61	0.53	0.61
10000	0.66	0.67	0.59	0.58	0.62
20000	0.66	0.71	0.65	0.61	0.65
40000	0.68	0.76	0.64	0.67	0.69
MEAN	0.64	0.61	0.56	0.52	

ANALYSIS

SOURCE OF VARIATION	LSD (P = 0.05,*; P = 0.01**)
Cultivar	0.069**
Density	0.072**
Cultivar X Density	0.149*

Early Tiller Development

Cultivars differed in tiller number ($P \leq 0.01$)). NZC and Sual had more tillers than Swan and West (Table 1.4).

Table 1.4: Effect of Heterodera avenae Density on Tiller Number of Four Oat Cultivars 55 Days After Emergence

	TILLER NO.			
DENSITY	CULTIVAR			
	NZC	SUAL	SWAN	WEST
0	16	17	8	6
900	14	13	7	6
5000	9	11	4	4
10000	8	9	3	2
40000	6	5	2	2

ANALYSIS SOURCE OF VARIATION	LSD (P = 0.05,*; P = 0.01,**)
CV	4.2**
Density	3.7**
CV X Density	NS

Infestation reduced the tiller number of all cultivars. Interactions between cultivar and density were not significant.

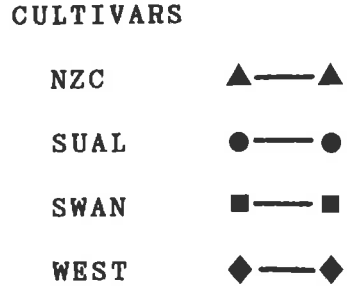
Yield Components and Final Yield

NZC and Sual produced a greater number of tillers at final harvest (day 120, Swan and West; day 182, NZC and Sual) than Swan and West (Table 1.5).

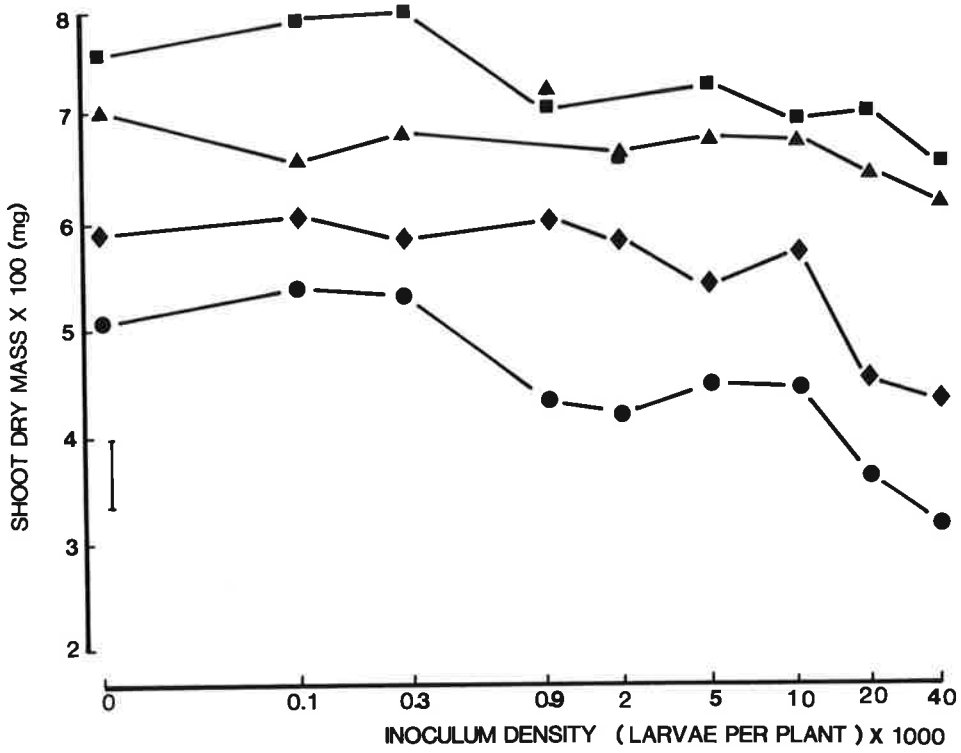
FIG 1.3 The effect of initial inoculum density of Heterodera avenae larvae on shoot dry mass of four oat cultivars 30 days after planting (mean of four replicates)

FIG 1.4 The effect of initial inoculum density of Heterodera avenae on dry root mass of four oat cultivars 30 days after planting (mean of four replicates).

Figs 1.3 and 1.4 have common legends:



NOTES: Initial inoculum density in $\log_{10}$ scale in Figures 1.3 and 1.4


SHOOT DRY MASS X 100 (mg)
8
7
6
5
4
3
2
0
0.1
0.3
0.9
2
5
10
20
40
INOCULUM DENSITY (LARVAE PER PLANT) X 1000

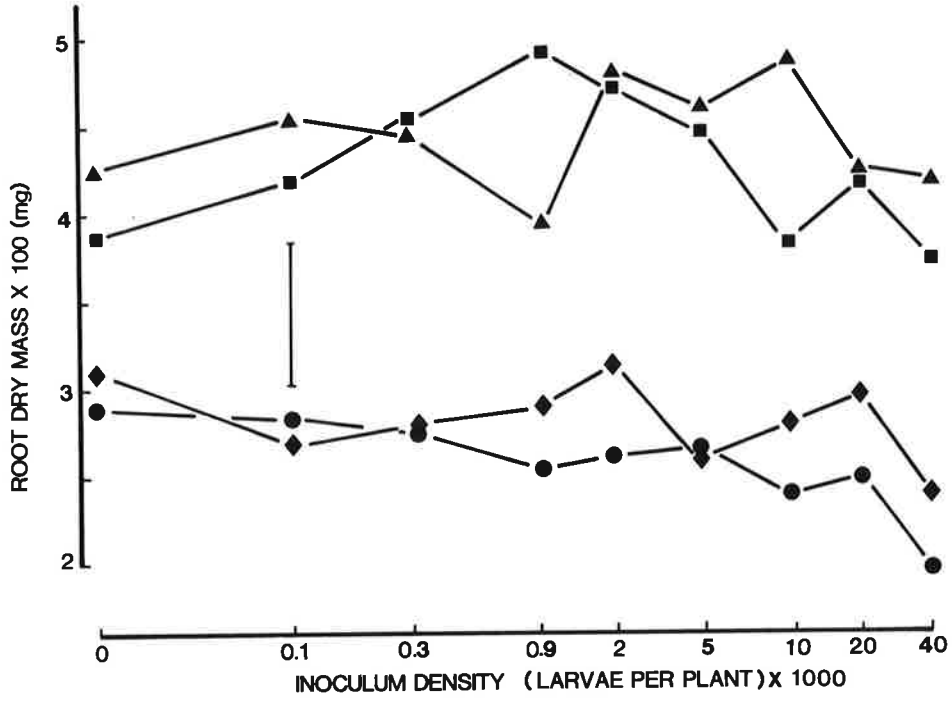

ROOT DRY MASS X 100 (mg)
5
4
3
2
0
0.1
0.3
0.9
2
5
10
20
40
INOCULUM DENSITY (LARVAE PER PLANT) X 1000

Table 1.5: Effect of Density of *Heterodera avenae* on Tiller Number and Number of Panicles on Four Oat Cultivars at Final Harvest

CULT.	LARVAL DENSITY	TILLER NO.	PANICLE NO.	TOTAL GRAIN NUMBER PER PLANT	% DECLINE IN GRAIN NO.	GRAIN N PER PANICL
NZC	0	19	19	471		24
	100	21	15	394	(84)	26
	300	23	23	351	(74)	15
	900	21	20	407	(86)	20
	2000	23	20	392	(83)	19
	5000	24	23	469	(99)	21
	10000	23	22	399	(85)	18
	20000	20	20	353	(75)	18
	40000	18	18	291	(62)	16
SUAL	0	29	22	607		28
	100	28	20	598	(99)	30
	300	27	21	460	(74)	22
	900	32	24	463	(76)	19
	2000	20	15	339	(56)	23
	5000	22	17	317	(52)	19
	10000	17	17	249	(41)	14
	20000	19	16	221	(37)	13
	40000	14	14	103	(17)	7
SWAN	0	9	9	257		29
	100	9	9	274	(107)	30
	300	7	8	253	(98)	31
	900	7	7	199	(77)	28
	2000	8	8	230	(89)	29
	5000	8	8	212	(82)	25
	10000	5	5	115	(44)	21
	20000	5	5	103	(40)	19
	40000	4	4	57	(22)	13
WEST	0	8	8	136		17
	100	7	7	104	(76)	14
	300	7	7	97	(71)	14
	900	6	6	59	(43)	10
	2000	6	6	83	(61)	13
	5000	6	5	63	(46)	13
	10000	4	2	22	(16)	11
	20000	2	2	19	(14)	10
	40000	2	1	7	(5)	8

ANALYSIS LSD ($P \leq .05$)

SOURCE OF VARIATION	TILLER NO.	PANICLE NO.	GRAINS/ PANICLE
Between Cultivars	3.4	3.3	3.9
Between Inoculation	2.8	2.4	3.0
Cultivar X Inoculation	5.9	5.1	5.7

Because plants were grown until no further tillering occurred infestation had an almost identical effect on both total tiller number and panicle number. Infestation markedly reduced the number of tillers on Sual, Swan and West. Inspection of panicles during harvest of grain indicated that fewer grains were harvested per panicle at the higher inoculum densities. This is illustrated in Table 1.5. NZC was least reduced by infestation and Sual was most reduced. Infestation also had a significant effect on single seed mass, presented in Table 1.6 as mass per hundred grains.

Table 1.6: Effect of Density of Heterodera avenae on 100 Seed Mass of Four Oat Cultivars

	NZC	SUAL	SWAN	WEST
0	2.211	2.497	3.135	2.631
40000	2.148	1.611**	2.742*	2.713

*,** values for inoculated and uninoculated plants significantly different ($P \leq 0.05$, $P \leq 0.01$) respectively.

Infestation at the highest nematode density significantly reduced hundred grain mass of Sual and Swan.

Total grain number per plant of NZC and Sual exceeded that of Swan and West due to more tillers on the former cultivars (Table 1.5). The extent to which grain number of each cultivar was influenced by infestation supported results

already presented. Grain number of NZC was least affected by nematode infestation while that of the remaining 3 cultivars were dramatically reduced, particularly that of West.

Fig 1.5 shows the relation between total grain mass and initial inoculum density, with lines fitted accorded to regression equations presented with the figure. Final grain yield varied between cultivars in the absence of infestation because of the differences in grain number between cultivars. Although Sual had the highest yield in the absence of infestation, its yield was most severely affected by nematode infestation. The relation between initial nematode density with the yields expressed as a percentage of uninfested controls are shown in (Fig. 1.6). The yield of NZC was significantly less affected by nematode infestation than the other 3 cultivars.

Transpiration Rate (Figs. 1.13, Table 1.7)

Figures 1.7 a-d show the transpiration rate of each cultivar at three inoculation densities (0, 5000, 40,000) over the measurement interval. Cultivar, density and date influenced rate of evapotranspiration. Cultivars could be ranked: NZC, Swan, Sual and West in order of decreasing total water use. Nematodes reduced water use of all cultivars. Peak transpiration rate occurred between 40 and 60 days after planting. There were significant interactions between cultivar, density and date of measurement ($P < 0.05$).

FIG 1.5 The effect of initial inoculum density of Heterodera avenae larvae on the final grain yield of four oat cultivars. (Mean of 8 replicates).

FIG 1.6 The effect of initial inoculum density of Heterodera avenae larvae on final yield expressed as percentage of uninfested control plants (tolerance index) of four oat cultivars (mean of 8 replicates).

IDENTICAL SYMBOLS USED IN FIGS 1.5 and 1.6 :

CULTIVAR

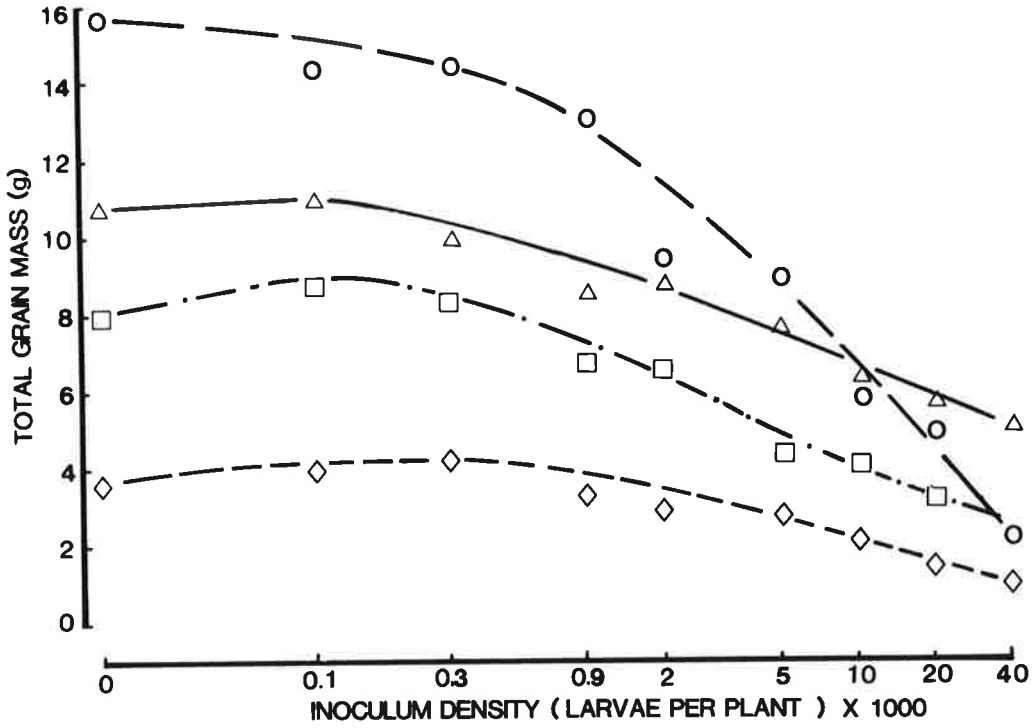
NZC △——△

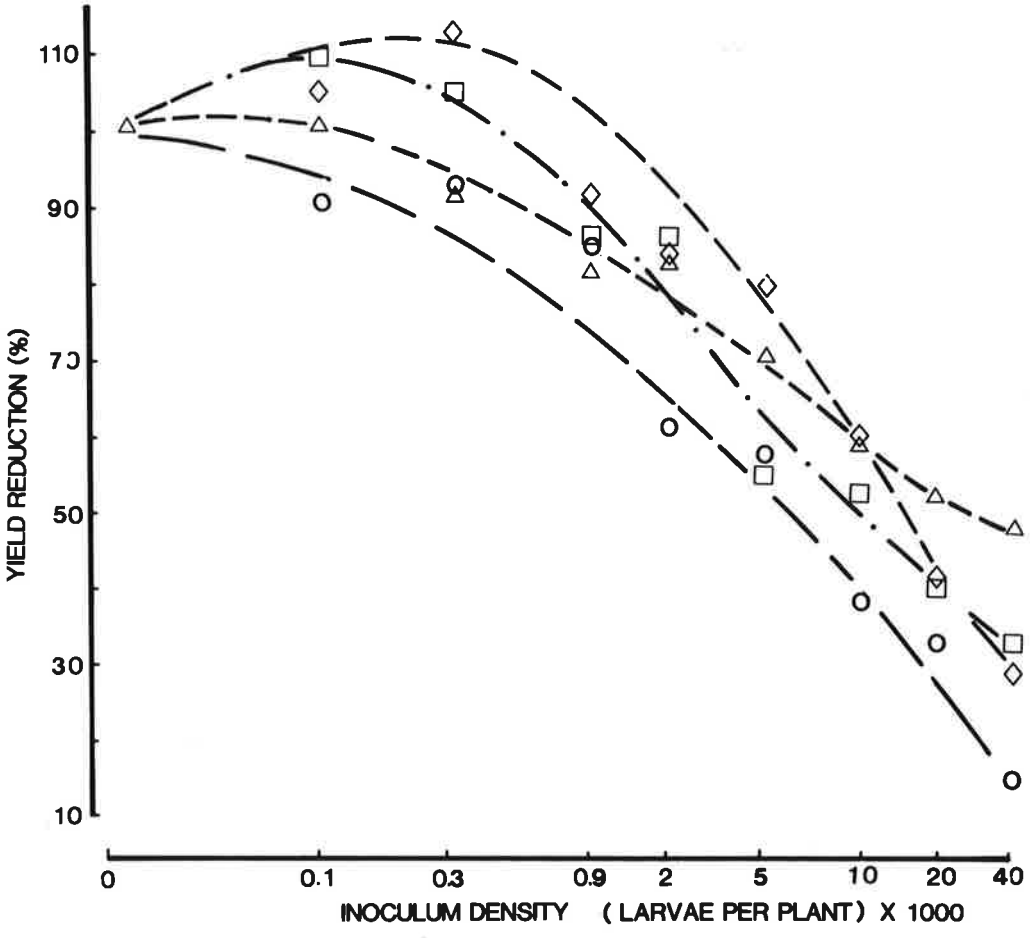
SUAL ○——○

SWAN □——□

WEST ◇——◇

NOTE: Initial inoculum density in $\log_{10}$ in Figs. 1.5 to 1.6 .


TOTAL GRAIN MASS (g)
16
14
12
10
8
6
4
2
0
0
0.1
0.3
0.9
2
5
10
20
40
INOCULUM DENSITY (LARVAE PER PLANT) X 1000


YIELD REDUCTION (%)
110
90
70
50
30
10
0
0.1
0.3
0.9
2
5
10
20
40
INOCULUM DENSITY (LARVAE PER PLANT) X 1000

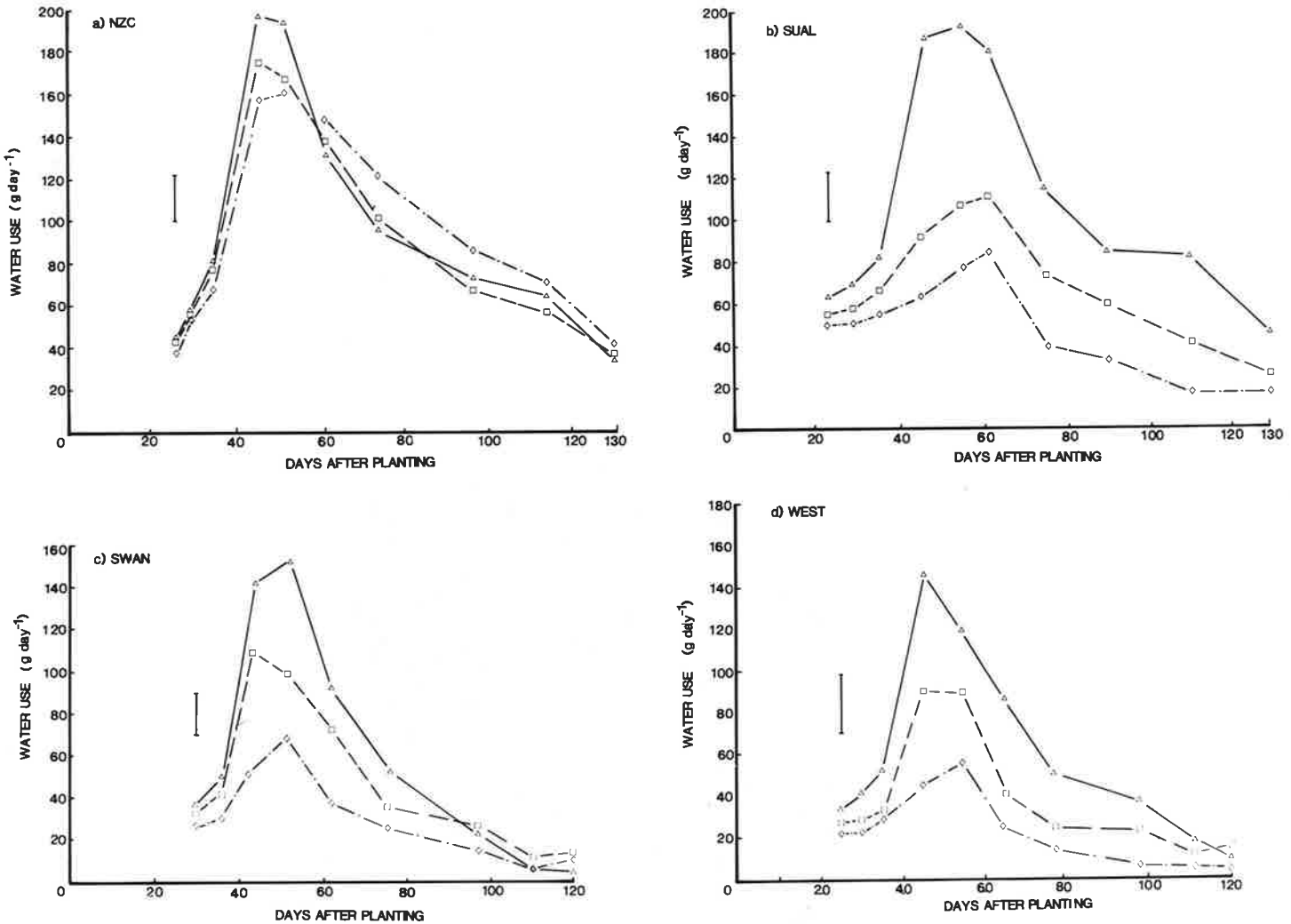
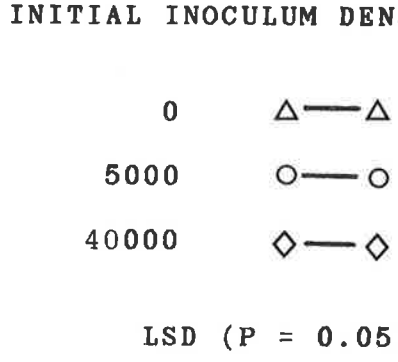

a) NZC
b) SUAL
c) SWAN
d) WEST
WATER USE (g day⁻¹)
DAYS AFTER PLANTING

FIG 1.7 (a-d) The effect of initial inoculum density of *Heterodera avenae* larvae on the water use of a) NZC b) Sual c) Swan and d) West 25 to 130 days after planting



In Table 1.7 the effects of density on daily transpiration over three developmental intervals, with corresponding water use efficiencies are shown. There were significant cultivar, density and measurement interval interactions. Transpiration rate (E) of NZC was higher than that of the other 3 cultivars during the first 70 days but equalled that of uninfested Sual later on. Swan and West had considerably lower E values than NZC at all densities, and of Sual at densities greater than 10,000 larvae. E values of NZC declined steadily with time at all densities but values of Sual, Swan and West declined only after 70 days. Of the four cultivars, greatest and least % decline in transpiration rate due to infestation occurred on Sual and NZC, respectively.

Water use efficiency (WUE), the amount of water used per unit dry matter produced differed between cultivars, densities and days. Overall, WUE of West was lowest during the initial growth interval, with smaller differences noted between the three remaining cultivars. Infestation significantly ($P = 0.05$) reduced WUE of all cultivars. During early growth WUE of NZC improved with increasing density while that of Sual and West declined significantly. Water required per unit grain mass of West was almost twice that of Swan in uninfested plants. Nematodes reduced the WUE of Swan but not of West.

Table 1.7: The Effect of Heterodera avenae Infestation on Transpiration and Water Use Efficiency of Four Oat Cultivars During Three Developmental Stages

		TRANSPIRATION RATE ($g\ DAY^{-1}$)			WATER USE EFFICIENCY			
					$g\ H_2O$/TOTAL ABOVE GROUND MASS			$g\ H_2O$/GRAIN MASS
		DEVELOPMENTAL INTERVAL (DAYS AFTER PLANTING)						
CULT.	DENS. ($x10^{-3}$)	30-50	50-70	70-120	30-120	30-70	70-120	30-120
NZC	0	203	104	70	107	328	323	
	5	178	153	68	112	344	181	
	40	148(73)	106(102)	63(90)	92	386	154	
SUAL	0	135	158	84	112	210	183	
	5	85	105	52	72	227	279	
	40	12(8)	23(15)	20(23)	19	304	1050	
SWAN	0	95	110	34	65	242	91	737
	5	41	119	18	46	288	66	901
	40	39(41)	49(45)	15(44)	30	311	121	1071
WEST	0	65	112	36	59	437	240	1491
	5	47	74	21	38	532	194	1221
	40	32(49)	53(48)	19(53)	23	514	730	1697

a) Parenthesized values indicate % decline due to infestation.

ANALYSIS (P = 0.05)

SOURCE OF VARIATION	TRANSPIRATION RATE	WUE (SHOOT MASS)	WUE (GRAIN MASS)
Cultivar (CV)	14.1**	39.4**	128.6**
Density	11.8**	33.1**	109.5**
Day	13.4**	36.6**	-
CV X Density	29.6*	77.2*	236.0*
CV X Day	31.6*	81.9*	-
Density X Day	24.7*	78.2*	-
CV X Density X Day	39.2*	111.6*	-

1.1.4 Discussion

The cultivars selected for study in this experiment have previously been classified on the basis of field trials with respect to their resistance and tolerance to Heterodera avenae as follows (Barr and Dube, 1985): NZC, resistant/tolerant; Swan, resistant/intolerant; West, susceptible/intolerant; Sual, resistant/intolerant. The absence of an oat cultivar having both susceptibility and tolerance made it impossible to devise a complete resistance/tolerance quadrat. This would have been regrettable had tolerance been associated with an ability to significantly exclude larval establishment. Though there were cultivar differences in the number of larvae that established on the roots by day 30, these appeared small relative to the total numbers of larvae in the root.

There were differences between the four oat cultivars studied in their response to Heterodera avenae and these were evident early in development. Infestation influenced leaf emergence and size of Sual, Swan and West more strongly than NZC. There was little sign of recovery from the initial effects on growth. While differences in growth response did not seem related to differences in actual larval numbers establishing in inoculated roots this possibility could not be entirely discounted. Both NZC and Swan performed better than Sual following inoculation despite there being more larvae detected in roots of the former cultivars than in

Sual. Nonetheless, Sual's root system was smaller than that of NZC and Swan and may have had as many or more larvae per unit root length than NZC or Swan. Unfortunately no root length data were obtained from this study to test this assertion. The poorer growth of Sual relative to West in response to infestation could not be attributed simply to more larvae established per root since West had a greater number of females than Sual on root systems of roughly equivalent dimensions. A more accurate measurement of root size than mass would be required to assess the importance of differences in larval number per plant to differences in growth response to infestation between cultivars.

It is believed that plants resistant to nematode infestation may also be more tolerant because of the fewer number of larvae that develop to maturity on resistant plants (Cotton 1967). All cultivars examined in this study except West have been classified as resistant to Heterodera avenae, yet large differences in tolerance were observed between resistant cultivars in the present pot study and in field studies (Barr and Dube 1985). This suggests that resistance may have no relation to tolerance.

The very large decline in the number of females from 30 days to harvest (from 400 to 30) suggested that either collection and counting efficiency was poor on the older plants or that many females died before plant maturity.

The latter could be indicative of some form of resistance operating in all cultivars. Reports by others support this view (Rao and Peachey 1965, Seinhorst 1967, McClure et al. 1974b). McClure et al. (1974b) found that although similar numbers of Meloidogyne incognita larvae developed, syncytia degenerated and the nematodes died before reaching adulthood. Similar results were reported by O'Brien (1976) on wheat in which increases in inoculation density beyond a critical number resulted in no further female development. This was interpreted as indicating that root resistance was directly proportional to larval density already on the root.

Tolerance in the cultivar NZC seemed to be very general being manifest at every growth stage as less delay in leaf emergence, less reduction in leaf size, no reduction in tiller number or less decline in grain number. Although Sual and NZC closely resembled each other developmentally, both being late maturers, and in growth habit, both being heavy tillering long stemmed cultivars, their response to nematodes differed. The ability of NZC to continue to produce new tillers even at high densities contrasted sharply with that of the other cultivars. Unfortunately the contribution of these later tillers to final yield was not assessed, but it might have largely accounted for the smaller yield decline of NZC compared to the other cultivars.

This regenerative capacity absent in the other cultivars studied could in part be responsible for NZC's higher tolerance limit. Differences between plants in their tolerance limit have been attributed in part to ability to compensate for early setbacks in growth, for example by generation of new roots (Hesling 1957, Seinhorst and den Ouden 1971, Wallace, 1973). The absence of root data after day 30 made it impossible to comment on this aspect of growth compensation. However NZC's tillering capacity may be a further compensatory mechanism.

Increasing tolerance with age has been reported (Seinhorst and den Ouden 1971, Seinhorst and Kozlowska 1977) and has been attributed to the effect of root growth late in development particularly in pot studies (Seinhorst 1981). Seinhorst (1981) found that nematode infested oat plants regained their water requirements as they matured.

In this study a similar delayed water requirement was observed for the two cultivars NZC and Sual that were indeterminate in their tillering ability (Fig. 1.11, Table 7). Continuous emergence of new tillers resulted in the apparent rejuvenation of the inoculated plants by delaying and extending the duration of their water requirements. However, NZC and Sual differed in nematode tolerance because of differences that occurred between their rates of evapotranspiration during the first 60 days. The relevance of the relationship between the apparent excessive water requirements of NZC compared to Sual and NZC's ability to better withstand nematode effects is not obvious.

Results of this study differ from those that have indicated that nematode infestation predominantly delayed plant development without affecting yield, if time was not limiting (Meinl and Stelter 1963; Seinhorst and den Ouden, 1971). Although there was some evidence of developmental delay, eg. panicle emergence, and water consumption, due to infestation. Initial nematode effects persisted to full maturity.

It has been suggested that cultivars with inherently larger root systems may suffer less from nematode infestation than smaller rooted cultivars (Seinhorst 1965; Evans et al. 1977; Trudgill and Cotes 1983 a). Both Swan and NZC had large root systems compared to Sual and West after 30 days growth and yet yield loss of Swan exceeded that of NZC and West. Nematodes reduced the root size of Swan more than NZC. These results suggest that sensitivity of root growth to infestation may be more important than uninfested root size in determining the yield potential of infested cultivars. It should be borne in mind however that root growth in a pot may have no bearing on root growth in the field.

In summary, early effects of infestation are correlated with yield losses at harvest. Early maturation of Swan and West may have precluded recovery from the initial set backs caused by infestation. A greater sensitivity to infestation of Sual compared to NZC with respect to both early leaf development and grain set on late formed tillers was the apparent cause of NZC's better yield at harvest.

More detailed studies are necessary to determine the reasons for differences in tolerance at both early and later growth stages between cultivars.

Conclusions arising from this study are that differences in levels of tolerance to Heterodera avenae between the cultivars examined in this study do exist. In absolute terms, NZC was most tolerant, followed by West, Swan and Sual. If expressed in terms of % yield decline, NZC ranked ahead of West followed by Swan and Sual. These tolerance rankings are in general agreement with those of Barr and Dube (1985).

1.2 Effect of Tiller Removal on Tolerance of Oats to Heterodera avenae

1.2.1 Introduction

In the previous study two heavy tillering cultivars, Sual and NZC, differed markedly in their level of tolerance to nematode infestation. Low tiller numbers on Swan and West conferred no advantage against infestation at least in terms of % yield reduction. These results suggested that tillering capacity was not a relevant factor in determining tolerance to Heterodera avenae. However it was the absence of a nematode effect on tillering capacity of NZC that distinguished it from the other cultivars. The question arose whether unabated tillering was the cause or the result of NZC's tolerance to infestation. The strong growth performance of NZC throughout its development suggested the latter. However, a reciprocal argument also has some substance: the weak performance of Sual may have been further hindered by overabundant tillering resulting in available resources being diverted to tillers at the expense of shoots. There is abundant evidence that suggests that competition for limiting substrates and nutrients occurs between newly developing tillers and the main shoot in cereal plants (Aspinall 1962; Kirby 1973).

Because of the potentially contradictory effects of tiller production on productivity of nematode infested plants the following study was undertaken to test the hypothesis that tiller removal has no effect on the tolerance level of cultivars infested with Heterodera avenae.

1.2.2 Methods

Seedlings of cultivars, NZC, Sual, Swan and West were grown under glass house conditions in open-ended cellulose nitrate tubes, 2.0 x 9.0 cm, imbedded vertically in 20 cm plastic pots previously filled with John Innes soil without peat. 3-day old seedlings were inoculated with suspensions of larvae at densities of 1000 larvae. Subsequent inoculation of plants with 2000 larvae was carried out daily on plants receiving higher densities. Final inoculation densities were 0, 1000, 3000, 7000 and 15000 larvae per plant. Twenty days after planting, the cellulose nitrate tubes were removed to allow expansion of the root system. During growth, all tillers were removed each week with scissors.

Four plants were harvested per treatment 20 and 48 days after planting to assess shoot and root growth and nematode infestation. Final harvest of plants was carried out 120 days after planting on Swan and West and at 176 days on NZC and Sual. All measurements were replicated 8 times. Plants were arranged in a randomized complete block design.

The experiment was conducted between the months of March and August when day length varied between 8 and 10 hours. Glasshouse temperature was maintained at 20° ± 5°C. Fertilizer was applied after some yellowing was noticed 8 weeks after planting and was applied subsequently every 4 weeks. The fertilizer solution was prepared by dissolving 5 g each of monoammonium phosphate, amonium nitrate and potassium chloride in 10 litres of water.

1.2.3 Results

Larval Population

The number of male and female nematodes per root system increased with inoculum density on all measurement dates (Table 1.8). There were also cultivar differences: West had fewest males and the most females and NZC had the fewest females per root system. No significant interactions were observed. The female population generally declined with plant age.

Table 1.8: Effect of Density of *Heterodera avenae* on the Number of Male and Female LARVAE on Four Oat Cultivars 20 and 48 Days After Planting, and at Harvest

CULTIVAR	DENSITY	DAY 20 NO.	DAY 48 NO.	DAY 48 NO. FEMALES	HARVEST NO. FEMALES
NZC	0	0	0	0	0
	1000	389	46	57	26
	7000	1721	182	96	33
	15000	2584	282	114	37
	MEAN	1172	129	68	24
SUAL	0	0	0	0	0
	1000	299	48	42	19
	7000	1847	116	92	29
	15000	2411	337	138	43
	MEAN	1137	125	68	23
SWAN	0	0	0	0	0
	1000	294	27	91	31
	7000	1716	135	118	79
	15000	2897	267	179	87
	MEAN	1223	106	97	49
WEST	0	0	0	0	0
	1000	272	113	93	41
	7000	1426	249	147	93
	15000	1993	484	236	118
	MEAN	926	210	116	66
MEAN (DENSITY)	0	0	0	0	0
	1000	312	58	71	29
	7000	1672	172	116	57
	15000	2468	344	165	72

SOURCE OF VARIATION (LSD P = 0.05,*; P = 0.01,**)				
CV	86*	24*	17*	9**
Density	93**	41**	19**	13**
CV X Density	NS	NS	NS	NS

Leaf Development

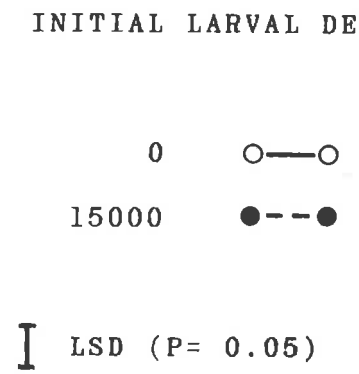
Fig 1.8 shows the effect of infestation at the highest inoculum density on leaf emergence. Infestation generally delayed leaf emergence of Sual, Swan and West, but this effect was not significant. Due to the delay in leaf emergence of infested plants Swan and West had one less leaf than uninfested controls. Final length of leaves L2 to L4 of Swan and West were significantly reduced ($P = 0.05$) by infestation.

The effect of infestation on final leaf length of cultivars NZC and Sual was less consistent (Figure 1.9). Infestation stimulated leaf expansion of NZC during the first 40 days of growth, up to L5, but thereafter leaf length of NZC was consistently but not significantly reduced by infestation. Leaf length of Sual was significantly reduced by infestation only during early and late plant development.

1.2.3.3 Plant Height

Total internode length was equated with plant height. Stems of Swan and West attained final height approximately 60 days sooner than Sual and NCZ (Fig 1.10). Heights of Swan and West were about 50% those of NZC and Sual. Cultivars differed in response to inoculation. Infestation enhanced plant height of NZC, had no effect on West but reduced that of Sual and Swan.

FIG 1.8 (a-d) The effect of Heterodera avenae infestation on the date of leaf emergence of detillered oat cultivars a) NZC, b) Sual and c) Swan and West until the termination of vegetative growth



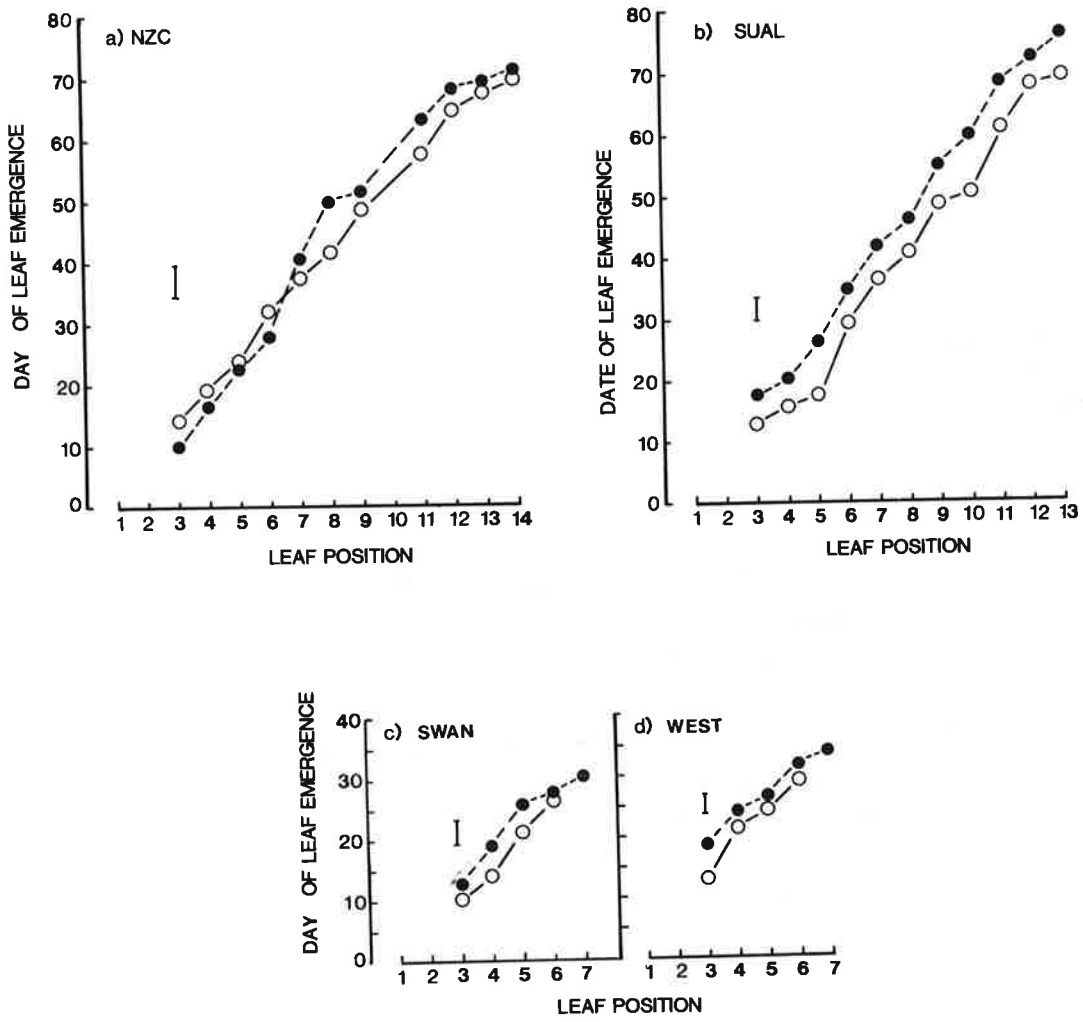
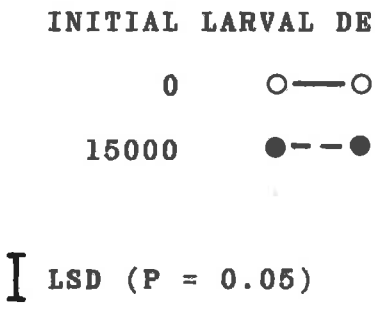

a) NZC
b) SUAL
c) SWAN
d) WEST
DAY OF LEAF EMERGENCE
DATE OF LEAF EMERGENCE
LEAF POSITION

FIG 1.9 (a-d) The effect of *Heterodera avenae* infestation on final leaf length of detillered oat cultivars a) NZC, b) Sual, c) Swan and West until termination of vegetative growth



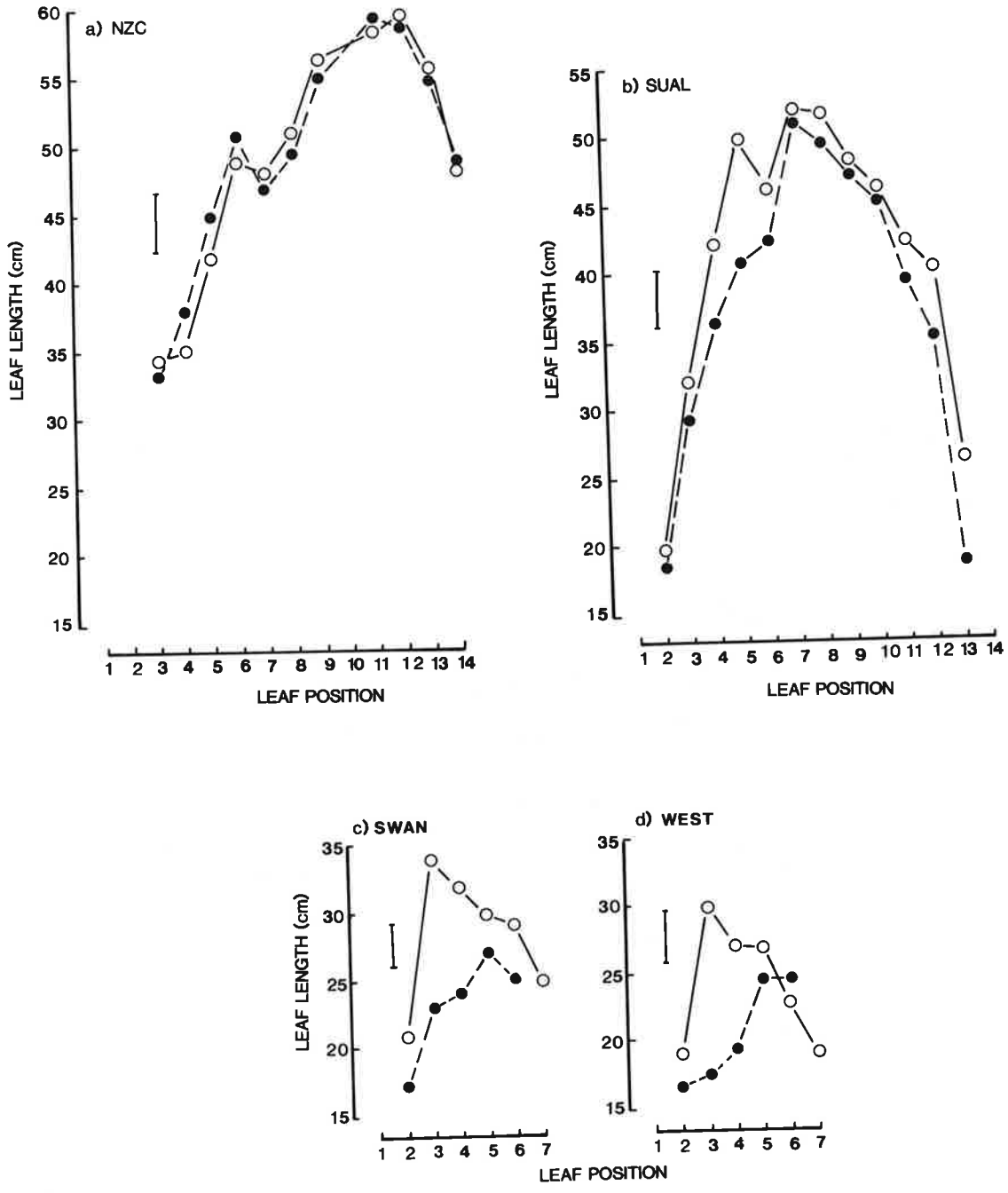
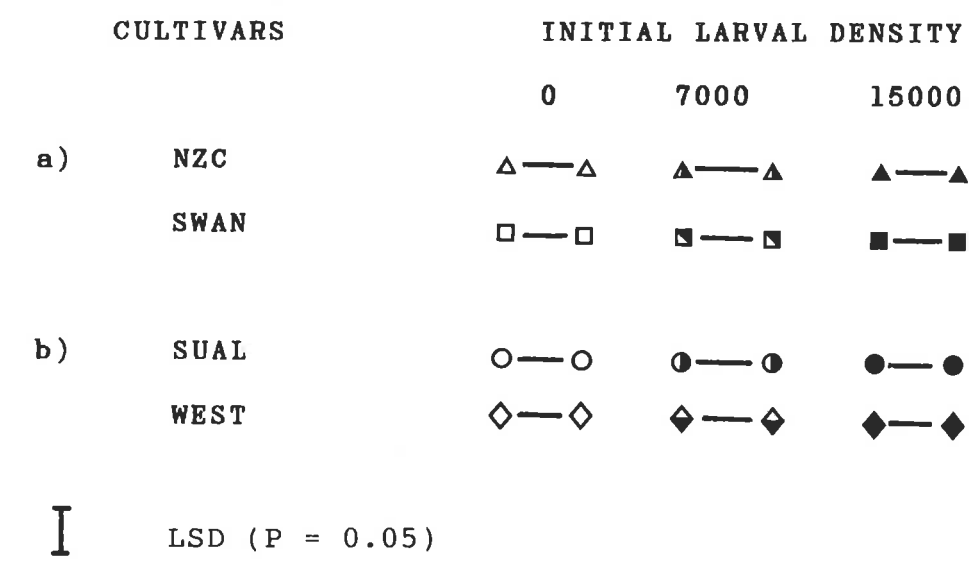
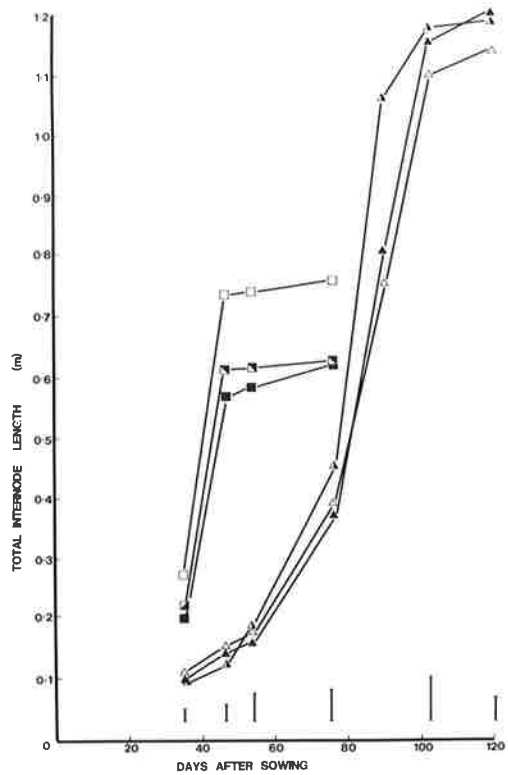

a) NZC
b) SUAL
c) SWAN
d) WEST
LEAF LENGTH (cm)
LEAF POSITION

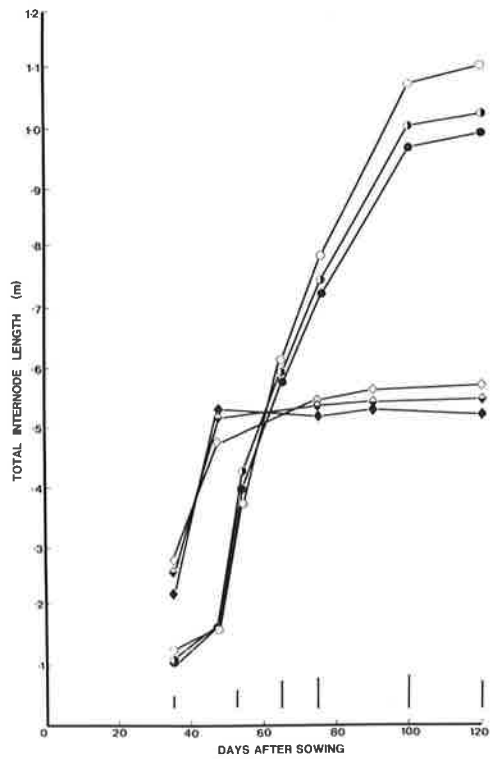
FIG 1.10 a, b The effect of Heterodera avenae infestation on plant height of detillered oat cultivars a) NZC and Swan and b) Sual and West 35 to 12(days after planting



a.


TOTAL INTERNODE LENGTH (m)
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
20
40
60
80
100
120
DAYS AFTER SOWING

b.


TOTAL INTERNODE LENGTH (m)
1·2
1·1
1·0
·9
·8
·7
·6
·5
·4
·3
·2
·1
0
20
40
60
80
100
120
DAYS AFTER SOWING

ANALYSIS (LSD P = 0.05,*; P = 0.01,**)			
SOURCE OF VARIATION			
CV	0.018**	0.048**	0.168**
Density	0.014**	0.034**	0.174*
CV X Density	0.021*	NS	0.31*

Shoot and Root Mass, Root Length

Shoot and root mass were influenced by both cultivar and nematode density. Differences between cultivars in shoot growth rate were most distinguishable by day 48, Swan having accumulated the most dry matter, NZC the least (Table 1.9).

Table 1.9: Effect of Density of Heterodera avenae on Shoot Mass of Four Oat Cultivars 20 and 48 Days After Planting and at Harvest

		SHOOT MASS (g)		
		DAYS AFTER PLANTING		
CULTIVAR	DENSITY	20	48	HARVEST
NZC	0	0.21	0.45	2.83
	1000	0.22	0.42	2.86
	7000	0.17	0.36	2.95
	15000	0.16	0.37	3.17
	MEAN	0.19	0.40	2.97
SUAL	0	0.18	0.66	2.42
	1000	0.18	0.62	2.37
	7000	0.11	0.51	2.11
	15000	0.09	0.48	1.81
	MEAN	0.14	0.57	2.18
SWAN	0	0.26	0.82	1.27
	1000	0.25	0.78	1.24
	7000	0.18	0.72	1.03
	15000	0.15	0.55	0.86
	MEAN	0.21	0.71	1.10
WEST	0	0.17	0.69	0.75
	1000	0.18	0.68	0.78
	7000	0.16	0.61	0.75
	15000	0.12	0.42	0.51
	MEAN	0.16	0.60	0.68
MEAN (DENSITY)	0	0.25	0.66	1.82
	1000	0.21	0.63	1.79
	7000	0.16	0.55	1.72
	15000	0.13	0.46	1.59

Nematode infestation reduced shoot mass of all cultivars at densities of 7000 or greater 20 and 48 days after planting. Cultivars varied in their response to infestation. Shoot growth of West was generally reduced only at the highest density. Shoot growth of NZC was reduced 20 and 48 days after planting but had fully recovered by harvest. Other cultivars were reduced in mass by between 25 and 40% ($P \leq 0.01$).

Dry root mass was cultivar and density dependent. Swan displayed the greatest rate of dry mass increase up to day 48 (Table 1.10). By harvest, root mass of NZC and Sual had surpassed that of Swan, followed last by West.

Table 1.10: Effect of Density of Heterodera avenae on Dry Root Mass of Four Oat Cultivars 20 and 48 Days after Planting and at Harvest

Note - Nodal roots aggregated at base of stem were designated basal roots. The rest of the root system was arbitrarily classified as remainder.

		ROOT MASS (g)				
		DAYS AFTER PLANTING				
CULTIVAR	DENSITY	20	48	HARVEST		
				BASAL	REMAINDER	TOTAL
NZC	0	0.18	0.33	0.44	0.70	1.13
	1000	0.18	0.35	0.45	0.68	1.12
	7000	0.15	0.30	0.35	0.87	1.22
	15000	0.15	0.32	0.39	0.74	1.15
	MEAN	0.17	0.33	0.41	0.75	1.15
SUAL	0	0.11	0.43	0.28	0.55	0.83
	1000	0.11	0.38	0.27	0.56	0.84
	7000	0.09	0.34	0.24	0.48	0.71
	15000	0.07	0.36	0.17	0.46	0.65
	MEAN	0.09	0.38	0.24	0.52	0.76
SWAN	0	0.15	0.52	0.32	0.31	0.63
	1000	0.15	0.52	0.28	0.31	0.59
	7000	0.12	0.50	0.23	0.28	0.52
	15000	0.11	0.42	0.17	0.23	0.40
	MEAN	0.13	0.49	0.25	0.29	0.54
WEST	0	0.09	0.31	0.11	0.15	0.26
	1000	0.09	0.32	0.10	0.14	0.24
	7000	0.09	0.35	0.07	0.13	0.19
	15000	0.07	0.38	0.04	0.12	0.16
	MEAN	0.09	0.34	0.08	0.14	0.21
MEANS (DENSITY)	0	0.13	0.40	0.29		
	1000	0.13	0.40	0.28		
	7000	0.11	0.37	0.22		
	15000	0.09	0.36	0.19		
ANALYSIS (LSD P = 0.05,*)						
SOURCE OF VARIATION						
CV		0.01*	0.04*	0.03*	0.05*	0.08*
Density		0.01*	0.03*	0.04*	0.04*	0.06*
CV X Density		NS	NS	NS	0.08*	0.12*

Significant cultivar density interactions were observed only at harvest. The root masses of all cultivars were unaffected by nematodes except at harvest. Root mass of all cultivars except NZC were reduced by infestation at harvest.

Thick, unbranched roots developed from tillers that were subsequently excised and formed a large compact mass of roots immediately below the stem. These roots will be referred to as basal roots. Basal roots comprised between 25 and 30% of the total retrieved root mass (Table 1.10). Because these roots explored very little of the soil column yet their contribution to the overall mass was large a more appropriate guage of root size in which only non-basal roots were measured was used. As it turned out there was very close agreement betwen remainder and total root mass in their response to infestation.

Reduction in shoot growth generally was reflected by similar though smaller declines in root mass in response to infestation. Consequently root/shoot ratios tended to increase with increasing density during early growth (Table 1.11). However, at harvest there were no significant differences between infested and uninfested plants.

Table 1.11: Effect of Density of *Heterodera avenae* on the Dry Mass Root/Shoot Ratio of Four Oat Cultivars 20 and 48 Days After Planting and at Harvest

CULTIVAR	DENSITY	ROOT/SHOOT RATIO		
		DAY 20	DAY 48	HARVEST
NZC	0	0.83	0.73	0.41
	1000	0.79	0.79	0.40
	7000	0.85	0.83	0.41
	15000	0.92	0.86	0.36
	MEAN	0.85	0.81	0.40
SUAL	0	0.64	0.65	0.36
	1000	0.62	0.62	0.37
	7000	0.78	0.68	0.35
	15000	0.76	0.79	0.34
	MEAN	0.70	0.69	0.36
SWAN	0	0.56	0.63	0.48
	1000	0.59	0.66	0.47
	7000	0.67	0.69	0.47
	15000	0.69	0.76	0.50
	MEAN	0.63	0.69	0.48
WEST	0	0.54	0.44	0.35
	1000	0.48	0.47	0.32
	7000	0.53	0.57	0.26
	15000	0.59	0.60	0.31
	MEAN	0.54	0.52	0.31
MEAN (DENSITY)	0	0.64	0.61	0.40
	1000	0.62	0.63	0.39
	7000	0.69	0.69	0.36
	15000	0.74	0.75	0.37

ANALYSIS

SOURCE OF VARIATION	LSD (P = 0.05,*)		
CV	0.076*	0.088*	NS
Density	0.063*	0.079*	NS
CV X Density	NS	NS	NS

ANALYSIS LSD (P = 0.05; P = 0.05,**)

SOURCE OF VARIATION	DAY 20	DAY 48	HARVEST	TOTAL
CV	0.074*	0.17*	-	-
Density	0.056**	0.14**	-	-
CV X Density	NS	NS	0.18*	1.23

The response of total root length to infestation generally paralleled that of root mass (Table 1.12).

Table 1.12: Effect of Density of Heterodera avenae on Total Root Length of Four Oat Cultivars 20 and 48 Days After Planting and at Harvest

		ROOT LENGTH (M)			
CULTIVAR	DENSITY	DAY 20 TOTAL	DAY 48 TOTAL	HARVEST	TOTAL
NZC	0	8.5	31.2	2.3	97.9
	1000	8.5	30.9	2.2	99.2
	7000	6.2	22.6	1.9	141.4
	15000	4.6	17.5	2.0	135.1
	MEAN	6.9	25.4	2.1	118.4
SUAL	0	5.2	35.6	2.4	109.9
	1000	5.2	27.9	2.4	107.4
	7000	2.9	21.4	1.8	105.8
	15000	2.4	16.2	1.5	108.4
	MEAN	3.9	25.3	2.0	107.1
SWAN	0	7.5	42.2	1.7	79.9
	1000	7.0	47.1	1.6	78.6
	7000	4.5	34.9	1.3	75.4
	15000	3.3	29.2	1.3	58.5
	MEAN	5.6	38.4	1.5	73.1
WEST	0	5.2	20.1	0.3	27.9
	1000	5.4	19.4	0.4	26.2
	7000	3.5	24.6	0.5	29.4
	15000	2.4	11.8	0.5	25.0
	MEAN	4.1	18.8	0.4	27.0
MEAN (DENSITY)	0	6.6	32.2		
	1000	6.5	31.3		
	7000	4.3	25.9		
	15000	3.2	18.7		

There was a significant cultivar by density interaction with root length at harvest. Total root length of NZC increased with increase in density whereas that of the other cultivars declined, though the decline on Sual and West was marginal.

Nematode infestation reduced root length more than mass, due to root swelling in response to invasion and cyst formation. This is illustrated in Table 1.3 in which the effect of nematode infestation on the length per unit mass on roots sampled at day 48 and at harvest is shown. Generally nematodes had no effect on the primary root length. Root length per unit root mass of all cultivars was reduced by infestation only on day 48.

Tiller Development

Tiller emergence continued throughout the development of all four cultivars and pruning was necessary on a weekly and later a biweekly basis. To compare differences between cultivars in the effect of nematode density on tiller growth tiller growth during one week was measured (Table 1.14).

Cultivars did not differ significantly in the number of tillers elongating but nematode infestation reduced tiller numbers of all cultivars. The only cultivar having a concomitant decline in total tiller mass was Swan. This suggests that the total regenerating tiller mass was reduced by infestation in Swan, but not in the remaining cultivars.

ANALYSIS LSD (P = 0.05,*)

SOURCE OF VARIATION				
CV	NS	NS	NS	NS
Density	NS	1739*	NS	NS
Density X CV	NS	NS	NS	NS

a Fine roots were designated as roots $\leq$ 250 *um.*

b Course roots designated as $\geq$ 500 *um.*

Table 1.13: Effect of Density of Heterodera avenae on Unit Fresh Root Length (m g^{-1}) of Primary and Secondary Roots of Four Oat Cultivars 48 Days After Planting and at Harvest

CULTIVAR	DENSITY	UNIT ROOT LENGTH (m g^{-1})			
		DAY 48		HARVEST	
		FINEa	COARSEb	FINE	COURSE
NZC	0	473	4978	333	3486
	1000	389	4817	347	3392
	7000	442	3903	304	4116
	15000	518	2914	279	3934
	MEAN	451	4158	316	3739
SUAL	0	529	5472	417	4689
	1000	476	5198	432	4606
	7000	602	3701	483	4635
	15000	584	3246	446	4162
	MEAN	541	4409	443	4528
SWAN	0	413	5159	487	5434
	1000	587	5248	385	–
	7000	498	3986	382	4502
	15000	324	3921	369	4613
	MEAN	449	4581	401	4852
WEST	0	372	6226	455	5242
	1000	429	6469	401	5163
	7000	316	7005	279	4119
	15000	248	5033	321	4997
	MEAN	340	6189	359	4887
MEAN (DENSITY)	0	447	5458	423	4716
	1000	469	5433	394	4389
	7000	462	4641	371	4340
	15000	419	3784	353	4421

Table 1.14: Effect of Density of *Heterodera avenae* on One Week of Late Tiller Growth of Four Oat CV's Restricted in Tillering[a]

CULTIVAR	DENSITY	TILLER NO.	TOTAL TILLER MASS (g)	TILLER MASS per tiller (g)
NZC	0	7	0.14	0.021
	1000	7	0.14	0.023
	7000	5	0.13	0.024
	15000	3	0.14	0.026
SUAL	0	8	0.19	0.027
	1000	8	0.20	0.034
	7000	7	0.27	0.033
	15000	5	0.19	0.026
SWAN	0	9	0.27	0.033
	1000	9	0.26	0.031
	7000	6	0.19	0.032
	15000	7	0.18	0.030
WEST	0	9	0.14	0.017
	1000	8	0.13	0.017
	7000	8	0.14	0.024
	15000	5	0.15	0.035
MEAN (DENSITY)	0	8		
	1000	8		
	7000	7		
	15000	5		

ANALYSIS (LSD $\leq$ 0.05,*; P = 0.01,**)

SOURCE OF VARIATION			
CV	NS	0.019**	0.012*
Density	2.1**	0.028*	0.009**
CV X Density	NS	0.037*	0.018*

a - Date of sampling for Swan and West - 84 days after planting.

Date of sampling for Sual and NZC - 132 days after planting.

Vegetative and Reproductive Growth - Day 48

Cultivars differed in total leaf area. The later maturing, more leaf bearing cultivars, Sual and NZC had larger areas than the early maturing Swan and West (Table 1.15). Infestation reduced leaf area of all cultivars, though in absolute terms declines due to infestation were larger on Sual and Swan.

Unit leaf area per plant of NZC and Sual exceeded that of Swan and West (Table 1.15). Nematode density had no effect on unit leaf area, indicating that shoot mass and leaf area were co-regulated, so that decline in mass due to infestation was coupled to a decline in leaf area. By day 48 flag leaves had already emerged on West and Swan. The flag leaf of Swan was larger than that of West. Infestation did not influence flag leaf area.

Spikelets were well developed by day 48 on Swan and West though not yet emerged. On NZC and Sual only rudimentary floral primordial development had occurred. Mass and number of spikelets were similarly reduced by infestation on Swan and West.

Yield Components at Harvest

The number of spikelets borne by each plant varied between cultivar and inoculation density (Table 1.16). NZC and Sual had equivalent numbers of spikelets. Swan and West had about 40 and 60% fewer spikelets respectively. All cultivars had fewer spikelets as a result of infestation, NZC and West were least affected.

Table 1.15: Effect of Density of Heterodera avenae on Leaf Area, Spikelet Number and Mass of Four Oat Cultivars 48 Days After Planting

CULTIVAR	DENSITY	LEAF AREA TOTAL cm^2	LEAF AREA PER UNIT MASS $cm^2 g^{-1}$	AREA FLAG (cm^2)	No.[a] SPIKELETS	PANICLE MASS (g)
NZC	0	177	387	-	-	-
	1000	172	417	-	-	-
	7000	151	412	-	-	-
	15000	145	394	-	-	-
	MEAN	159	403			
SUAL	0	181	276	-	-	-
	1000	169	271	-	-	-
	7000	136	268	-	-	-
	15000	124	255	-	-	-
	MEAN	154	267			
SWAN	0	126	156	22.2	14	0.221
	1000	114	144	23.7	13	0.209
	7000	102	141	26.1	10	0.179
	15000	77	139	18.3	7	0.088
		104	145	22.1	11	
WEST	0	87	124	12.7	15	0.212
	1000	83	121	14.2	13	0.181
	7000	74	121	16.8	8	0.096
	15000	60	142	12.8	8	0.078
	MEAN	76	127	14.7		
	MEAN (DENSITY)	0	142		15	0.217
		1000	35		13	0.198
		7000	14		9	0.139
		15000	99		8	0.082
SOURCE OF VARIATION		LSD (P = 0.05,*; P = 0.01**)				
CV		27.3**	48.4**	3.42*	NS	NS
Density		19.6**	NS	NS	2.1**	0.019*
CV X Density		NS	NS	NS	NS	NS

a - sampled at boot stage

Table 1.16: Effect of Density of *Heterodera avenae* on Yield Components of Four Oat Cultivars at Harvest

CULTIVAR	INOC. DENSITY	SPIKELET NO.	GRAIN	GRAINS/ SPIKELET	PANICLE MASS	100 SEED MASS (g)
NZC	0	36	44.4	1.23	1.76	2.56
	1000	35	43.2	1.25	1.81	2.84
	7000	32	48.6	1.51	2.16	2.95
	15000	29	45.3	1.57	1.94	2.29
	MEAN	33	45.5	1.35	1.92	2.66
SUAL	0	36	53.5	1.49	3.46	2.57
	1000	34	51.9	1.53	3.39	2.41
	7000	28	40.7	1.42	2.54	2.85
	15000	22	36.7	1.67	2.19	2.86
	MEAN	30	44.9	1.49	2.87	2.67
SWAN	0	20	34.1	1.71	1.54	3.66
	1000	18	31.7	1.76	1.43	3.73
	7000	15	26.5	1.79	1.17	2.99
	15000	10	14.1	1.42	0.67	3.18
	MEAN	16	26.6	1.66	1.20	3.39
WEST	0	15	20.5	1.39	1.96	1.71
	1000	15	19.1	1.26	1.97	1.64
	7000	12	15.2	1.25	1.89	2.18
	15000	11	11.4	1.04	1.59	2.18
	MEAN	14	16.6	1.25	1.83	1.95
MEAN (DENSITY)	0	26				
	1000	26				
	7000	22				
	15000	18				

ANALYSIS (LSD P = 0.05,*; P $\leq$ 0.01,**)

SOURCE OF VARIATION					
CV	3.9*	5.7**	NS	0.18**	0.30**
Density	4.13*	4.6**	NS	0.17**	NS
CV X Density	NS	11.1*	NS	0.23*	NS

Grain number at harvest roughly reflected spikelet number. There was a significant cultivar x nematode density interaction in grain number per plant. Infestation significantly reduced grain number of Sual and Swan but had no effect on NZC and West. This occurred in spite of the absence significant cultivar x nematode density interactions with respect to spikelet number or grain number per spikelet. The effects of infestation on spikelet number and grain number per spikelet became significant when combined into the single variable of grain number.

On the basis of individual seed mass, expressed as one hundred seed mass, Swan was heaviest, Sual and NZC intermediate, and West the lightest. Infection at the applied densities had no effect on 100 seed mass.

There were differences between cultivars in final grain yield (Table 1.17). Yields of NZC, Swan and Sual were similar. In the absence of infestation grain mass of West was only about 30% that of the other cultivars. The yields of all cultivars was reduced at the highest level of nematode infestation. Cultivars differed in their response to nematode density. In absolute terms NZC and West were least reduced in grain mass by infestation. By comparison, Sual and Swan lost between 4 and 7 times more in grain mass, respectively, at the highest density than West and NZC. There was a marked increase in yield of NZC at intermediate densities that was not evident in the remaining cultivars.

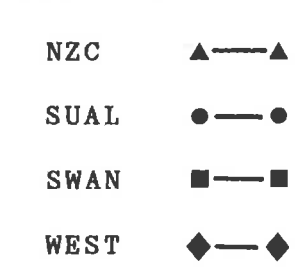
When yield loss was expressed as a percentage of control, Swan was most strongly affected by infestation, Sual and West were intermediate and NZC was least influenced by infestation (Fig 1.11).

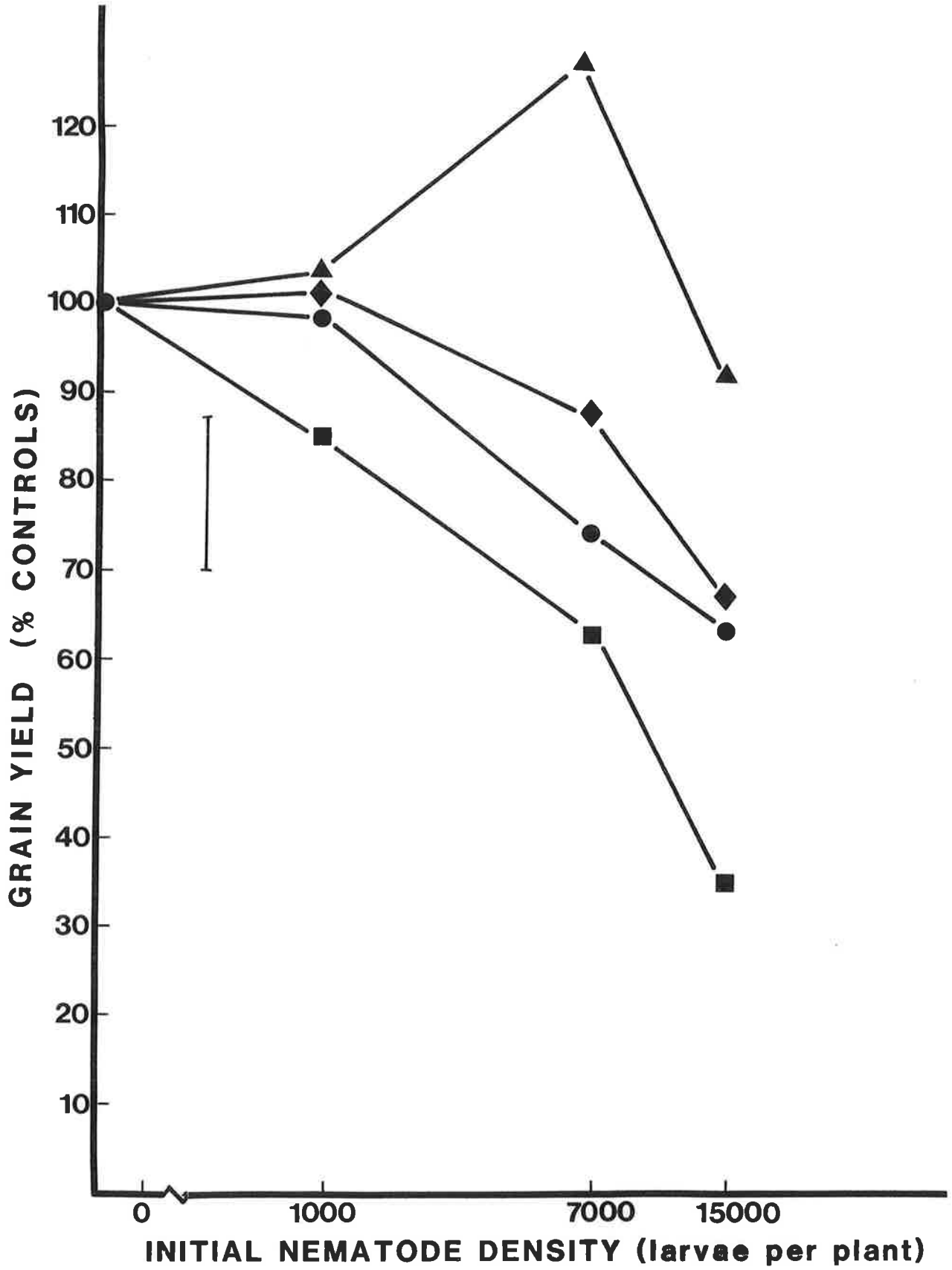
Harvest index, or grain mass per gram of shoot produced, was cultivar and density dependent (Table 1.17). Swan proved to be about twice as efficient as the other cultivars when not infested. Harvest index of Sual and Swan declined as a result of infestation.

Table 1.17: Effect of Density of Heterodera avenae on Final Grain Yield, Grain Yield Reduction and Harvest Index of Four Oat Cultivars

CULTIVAR	DENSITY	GRAIN YIELD (g)	REDUCTION (mg)	HARVEST INDEX ($g\ g^{-1}$)
NZC	0	1.138	0	0.401
	1000	1.155	+ 17	0.404
	7000	1.433	+ 314	0.485
	15000	1.041	- 84	0.326
SUAL	0	1.377	0	0.569
	1000	1.363	- 14	0.575
	7000	0.993	- 384	0.409
	15000	0.878	- 499	0.265
SWAN	0	1.255	0	0.984
	1000	1.072	- 183	0.865
	7000	0.794	- 464	0.772
	15000	0.449	- 814	0.523
WEST	0	0.349	0	0.467
	1000	0.362	+ 13	0.464
	7000	0.332	- 37	0.444
	15000	0.243	- 113	0.480
ANALYSIS (LSD P = 0.05; P = 0.01,**)				
SOURCE OF VARIATION				
CV X Density		0.152*	93*	0.094*

FIG 1.11 The effect of initial inoculum density of *Heterodera avenae* on the final yield expressed as percentage of uninfested controls (mean of 8 replicates). Initial inoculum densit is in Log_{10} scale




GRAIN YIELD (% CONTROLS)
120
110
100
90
80
70
60
50
40
30
20
10
0
1000
7000
15000
INITIAL NEMATODE DENSITY (larvae per plant)

1.2.4 Discussion

Competition for substrates and nutrients is believed to occur between newly developing tillers and the main shoot in cereals (Aspinall 1962; Kirby 1973). When nutrients are not limiting it is considered that main shoot growth can be restricted by continual demands of tillers for carbohydrates (Kirby and Jones 1977; Mohamed and Marshall 1979). Repeated tiller removal as they emerge results in heavier ears on the main shoot compared to control plants (Wheeler 1976). Detillering has also been shown to have a stimulatory effect on rate of leaf emergence, leaf size and number and shoot mass and height (Seiler-Kelbitsch et al. 1974, Kirby and Jones 1977). The impact of tiller production on yield may be accentuated when the ability of the main stem to secure nutrients and water is impaired by a root system reduced in size by nematode infestation. If so, then tiller removal could enhance a plant's tolerance to nematode infestation. Heavy tillering plants might be expected to respond more positively to detillering than plants producing few tillers. On the other hand, detillering may remove one means by which heavy tillering plants can compensate for yield decline of the main-stem caused by infestation

A comparison between the effect of nematode infestation on several important components of growth of tillering (Section 1.1) and detillered (Section 1.2) plants is shown in Table 1.18 to obtain some measure of the impact of detillering on nematode tolerance. In making such a comparison it is acknowledged that differences between the two experiments may be due not only to the presence or absence of tillers but also to differences in the environment.

The results showed that detillering improved the performance of all cultivars in the presence of infestation. Differences in growth response were evident at both low and high larval densities. No adverse affect on shoot growth of any cultivar was observed at the lowest larval density (1000) in this study, whereas there was measureable growth impairment at a density of 900 in the previous experiment. Similarly at high initial densities lamina length of Sual at full extension during the first 40 to 50 days increased on detillered plants but decreased on tillering plants. Reports of growth stimulation following nematode infestation are not uncommon (Lownsberry and Peters 1956, Olthoff and Potter 1972). This stimulating effect on growth has been attributed to an increase in growth regulator synthesis (Doney et al. 1971). Although the pattern of leaf growth of West and Swan was not altered by detillering, all other growth indicators were significantly increased. The tolerance level of NZC was also increased by detillering.

Table 1.18: Comparison of High *H. avenae* Density Effects on Several Growth Parameters for Tillering (+T) and Detillered (-T) Treatments of Four Oat Cultivars[a]

PERCENT OF UNINFESTED CONTROLS

CULTIVAR	SHOOT MASS EARLY GROWTH +T	SHOOT MASS EARLY GROWTH -T	SHOOT MASS LATE GROWTH +T	SHOOT MASS LATE GROWTH -T	ROOT MASS EARLY GROWTH +T	ROOT MASS EARLY GROWTH -T	GRAIN NO. +T/ PANICLE	GRAIN NO. TOTAL	GRAIN NO. -T/ PANICLE	YIELD/PLANT +T	YIELD/PLANT -T
NZC	73	82	71	112	84[b]	81/96[c]	54	52	101	53	92
SUAL	32	73	38	75	43	63/83	35	26	67	36	63
SWAN	50	67	53	68	62	73/80	55	29	41	40	35
WEST	44	60	48	68	54	77/120	52	9	55	51	65

a - Tillering and detillered plants compared at equivalent initial inoculum densities (15,000 larvae). Results from tillering treatments obtained from regression lines (Figure 1.11) values presented are expressed as percentage of uninfested control plants within the relevant experiment.

b - % root mass - day 30.

c - % root mass - days 20 and 48.

These results suggest that when freely tillering plants are confronted with a biotic stress as in the previous study the competition that occurs between main-stem and tillers for nutrients and assimilate makes them more sensitive to infestation, that is, less tolerant. Hormonally mediated growth stimulation arising from tiller removal cannot be discounted.

Better growth of infested Sual and West following detillering indicated that tiller growth may have constituted a metabolic drain on the plant when coupled with the stress of nematode infestation. In contrast, despite the similar tillering properties of Swan and West, Swan responded poorly to detillering, as indicated by almost equivalent reduction in growth and yield of tillering and detillered plants. Apparently the effect that infestation had on growth of Swan was independent of tiller production. While the potential benefits of late tillering cannot be ruled out, no cultivar was disadvantaged by tiller removal and most were advantaged. Late tillering of heavily infested Sual failed to provide adequate compensation whereas detillering was immediately beneficial. The results suggest that tiller production is not essential for tolerance and that tillering can reduce the potential of plants to tolerate nematode infestation. There are a number of reports that have correlated tolerance with vigorous, faster and therefore, larger root systems (Hogger 1972; Evans et al. 1977; Trudgill and Cotes 1983).

The present study shows that cultivars that yielded well at the highest inoculum density also had root systems comparable in length with control plants at harvest. Although the failure of the cultivar Swan to yield as well as controls at the higher densities was associated with reduced root size the root length of infested Swan was over double that of West, a cultivar less affected by nematodes. In fact West had a tolerance level similar to that of Sual which had a root system over four times larger than that of West, though it must be remembered that the final grain yields of Sual and Swan were about 3.5 and 1.9 times that of West at the highest nematode density. These results suggest that absolute root size was not a determinant of tolerance but that relative root size, that is, the percent decline in root length caused by infestation was important. Clearly, an unimpaired root system was a prerequisite for a given cultivar attaining maximum yield potential within the constraints of the experiement. The positive correlation between root size and tolerance does not necessarily infer a causal relationship. Though apparently less likely an unimpaired root system may be the consequence not the cause of vigorous top growth.

In conclusion detillering has been observed to enhance the tolerance level of nematode infested cultivars irrespective of their tiller bearing capacity. It is believed that this effect was a consequence of relieving the early competitive stain between main stem and tillers for a common nutritional source which when coupled with nematode infestation adversely affected plant growth.

SECTION 2: EFFECTS ON ROOT GROWTH OF *Heterodera avenae* INFESTATION

2.1 Introduction

In Section 1, Part 1, it was shown that early root growth was reduced at initial nematode densities between 2000 and 10,000 depending upon the cultivar. The relative sensitivity of early root growth of different cultivars to nematodes was related to final tolerance rating at harvest suggesting that tolerance levels were determined early in growth. It also indicated that root response to infestation may be related directly to tolerance. Section 1, Part 2, though providing some evidence conflicting with this simple theory, contained results in general agreement with those of Part 1.

To the extent that early floral primordium initiation and development is strongly influenced by water and mineral nutrient supply when these are in short supply (Salter and Goode 1967; Rahman and Wilson 1977) unrestricted root development seems mandatory for maximum grain yield. Uptake of water and some minerals occurs most readily through the regions near the root tip (Brouwer 1954; May *et al*. 1965; Ferguson and Clarkson 1976. Impaired ability to form new root tips or to extend old ones, a commonly reported consequence of *Heterodera avenae* infestation (O'Brien 1976; Price *et al*. 1983; Simon and Rovira 1985) would seriously impede water and mineral acquisition, both through impeding extension into undepleted soil zones and reducing the area of active uptake.

Net impairment of root growth by Heterodera avenae may be the result of delay or inhibition of extension of emerged infested roots and/or prevention or of delay in emergence of roots not as yet subject to infestation. A number of studies support the view that tolerance to nematode infestation may be governed by the vigour with which the root system is able to produce new roots to compensate for nematode damage to existing roots (Evans et al. 1977; Evans and Franco 1979; Trudgill and Cotes 1983). Detailed information is required on the relationship between nematode tolerance and patterns of root growth response to nematode infestation.

The aim of the present experiments was to test the following hypotheses.

1. There is no difference between the rate of extension of Heterodera avenae infested and uninfested seminal roots.
2. Growth of uninfested lateral roots on infested root systems does not differ from that on uninfested root systems.

2.2 Methods

2.2.1 Experiments (1) and (2)

Seeds of two oat cultivars, NZC and Stout were pregerminated (Chapter 3.2.1) and sown in 2.7 x 13.0 cm plastic electrical conduit (Chapter 3, 2.2.1).

Second stage Heterodera avenae larvae were applied in 1 ml water suspensions. In experiment (1) 15, 75 and 200 larvae were applied to the soil surface 1 day after sowing In experiment (2) nematode densities of 100, 250, 500 and 900 larvae per plant were applied 1 day after sowing. Plants were grown for 10 days in a growth cabinet under standard conditions (Chapter 3.2.3.1). At harvest total root length and nematode number were assessed (Chapter 3, 2.5). Both expriments were replicated six times. Experimental design was a randomized complete block.

2.2.2 Experiment (3)

Seeds of three oat cultivars, NZC, Sual, Swan and one wild oat species, Avena fatua were planted in 13.0 cm plastic conduit ~~tubes~~ as described previously (2.2.1). One day later seedlings were inoculated with 100, 400 and 900 second stage Heterodera avenae larvae. After 4 days six plants of each treatment were set aside for assessment of growth and infestation. The remaining plants were removed from their tubes, rinsed lightly to remove most sand particles then laid on a styrofoam board overlaid with a synthetic mat (trade name: love sheet) and a sheet of Whatman chromatography paper which served as a wick supplying the transplanted seedlings with aerated one-half strength Hoaglands nutrient solution (Hoagland and Arnon 1950). A second layer of synthetic mat was laid on top of the roots and the whole apparatus was covered with clear plastic to reduce moisture loss through evaporation. An illustration of the system is shown in Fig. 2.1.

The system of plant culture described above was employed to facilitate the assessment of the effect of larval invasion on extension of the invaded root and the subsequent development of uninfested roots. Measurements of indvidual seminal root lengths were obtained using a ruled line with distances marked every 0.5 cm. After staining with 0.1% cotton blue for 24 hours each seminal root was laid straight on a glass plate, its length measured, then scanned under a dissecting microscope at X 250 magnification to detect sites of larval penetration. Larval infestation was confined to a zone starting about 2 cm below the base of the stem and extending a further 2 cm down the root. Each of the three seminal roots per root system was divided into three zones. The portion of the seminal root above the infested region was classified as zone 1, the infested zone itself, zone 2, and the remaining portion below zone 2 was called zone 3 (Fig 2.1). A zone commencing 2 cm below the stem base and extending 2 cm was used for comparison on uninfested control plants. After recording the number of first order lateral roots emerging from each zone the total length of first and higher order lateral roots arising from each zone was estimated using the grid-line intersect method (Newman 1966).

Treatments were replicated 6 times. The experiment was analysed as a completely randomized design.

The choice of cultivars used in this study arose out of an interest in selecting cultivars that were as different as possible in their level of tolerance to Heterodera avenae.

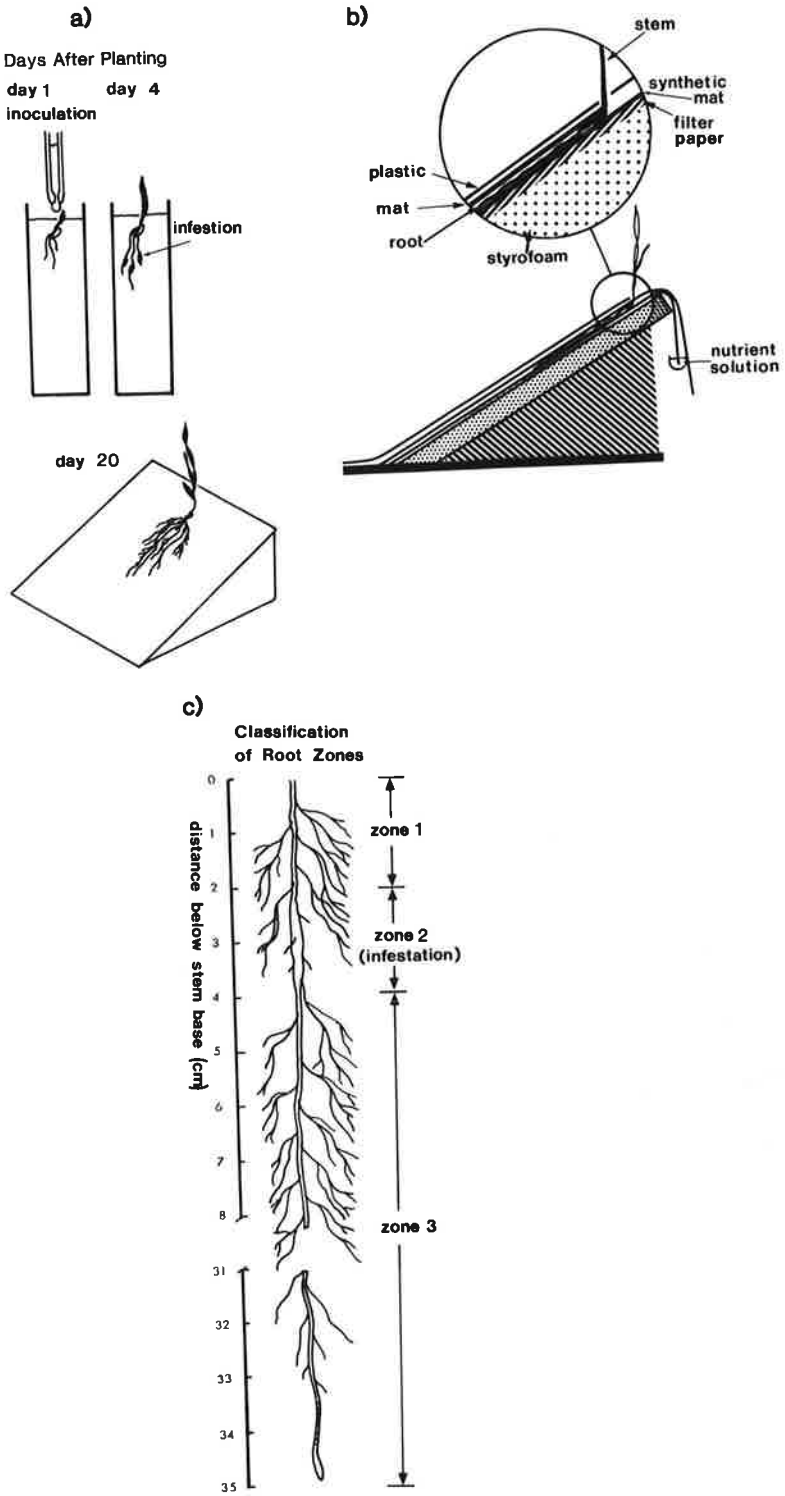
FIG 2.1 Illustration of the Experimental System Described in Chapter 4, Section 2.2.2

a) Plants were inoculated 1 day after transfer of pre-germinated seedlings to plastic tubes.

Three days later infested and uninfested plant roots were transferred to inclined platform supplied with nutrients and grown until 20 days after planting.

b) Illustration of the components of inclined styrofoam platform on which plants were grown.

c) Classification of the three root zones discussed in results.


a)
Days After Planting
day 1
inoculation
day 4
infestion
day 20
b)
stem
synthetic
mat
filter
paper
plastic
mat
root
styrofoam
nutrient
solution
c)
Classification
of Root Zones
distance below stem base (cm)
0
1
2
3
4
5
6
7
8
31
32
33
34
35
zone 1
zone 2
(infestation)
zone 3

Hence the choice of NZC and Stout, a tolerant, resistant and intolerant susceptible oat cultivars respectively. There is some evidence to suggest that wild plant species may possess greater disease tolerance than their cultivated relatives (Ben-Kalio and Clarke 1979) wild oats (Avena fatua) was therefore included in this study to complement NZC, and was tentatively designated tolerant and susceptible.

2.3 Results

2.3.1 Experiments (1) and (2)

These experiments were designed to determine whether there was a nematode density at which changes in root growth, as measured by root length, could be evoked.

In experiment (1) there was no difference between cultivars in root length, at any of the applied inoculation densities (Fig 2.2a). Inoculation had no affect on root length and mass after ten days of growth. Numbers of larvae penetrating resistant and susceptible roots did not differ between cultivars but increased with increasing inoculation density.

In experiment (2), initial larval density was increased to a maximum of 850 larvae per plant. Again the two cultivars responded similarly to infestation (Fig 2.2b). Root length declined with increasing nematode infestation, with cultivars showing no difference in response to infestation at any nematode density.

2.3.2 Experiment (3)

The number of juvenile *Heterodera avenae* larvae detected in individual root systems rose with increasing initial larval densities. There was no significant difference between cultivars in their infectivity (Fig 2.2c), nor did time influence total nematode numbers.

In general, root growth was reduced by nematode infestation. Differences between cultivars in response to infestation were observed in a number of aspects of root growth.

Seminal Roots

Root analysis included assessment of growth of the three seminal roots in each root system. NZC and Stout had the longest total seminal root lengths, Sual and *Avena fatua* being significantly shorter (Table 2.1). Initial nematode densities of at least 400 larvae per plant significantly reduced seminal root length on all plants. Increasing inoculum density delayed the time to attain maximum extension rate within the time span of the study (Fig 2.3). This effect was cultivar dependent. Stout, Sual and *Avena fatua* were all strongly impaired in seminal root growth by nematode infestation.

FIG 2.2a The effect of initial unoculum density of Heterode avenae on total root length and larval number on the oat cultivars NZC and Stout 10 days after inoculation (11 days after planting)

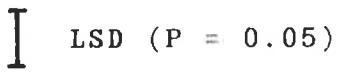


FIG 2.2b The effect of initial inoculum density of Heterode avenae on total root length and larval number on the oat cultivars NZC and Stout 10 days after inoculation (11 days after planting)

FIGS 2.2a and 2.2b have common legends:

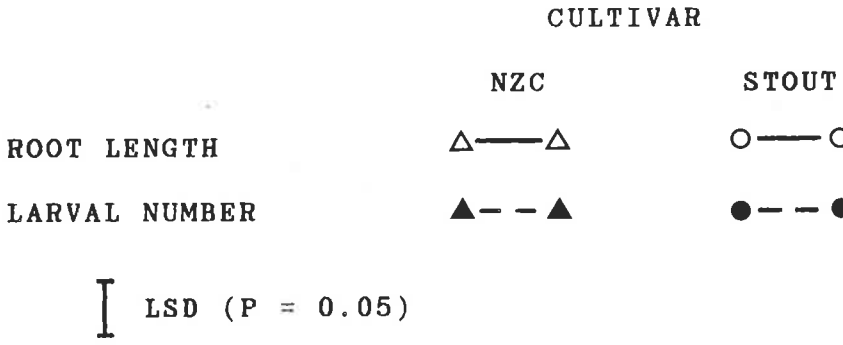
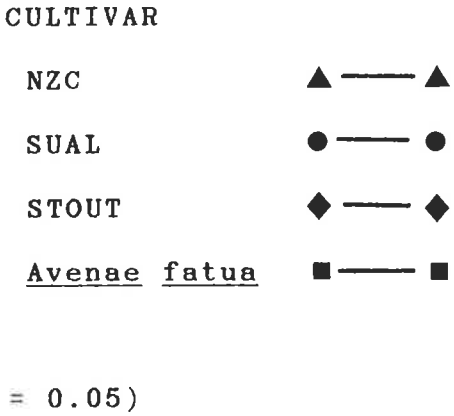


FIG 2.2c The effect of initial inoculum density of Heterod avenae on the mean number of larvae on roots of four oat cultivars averaged over 5 sample dates 3 to 19 days after inoculation (4 to 20 days after planting)



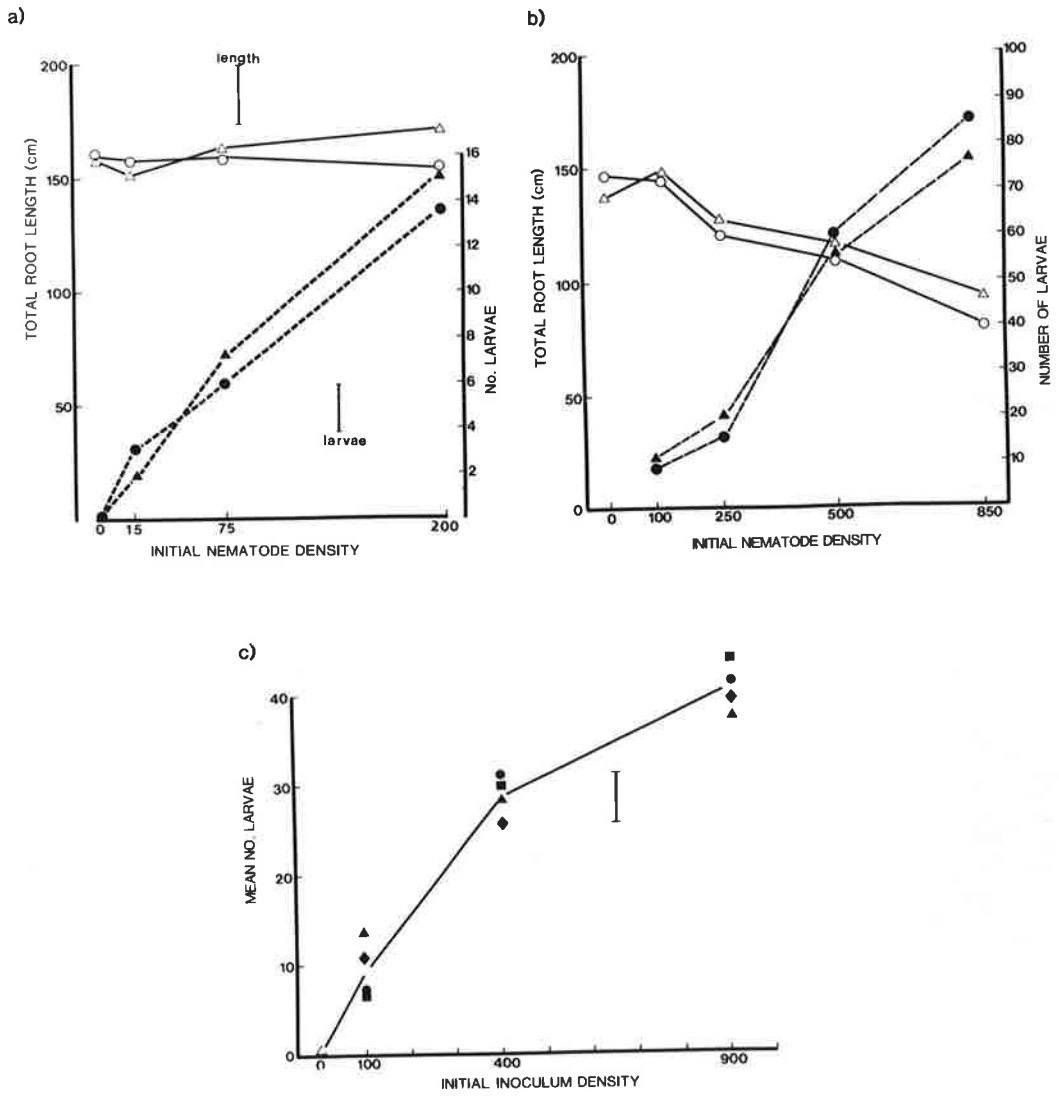
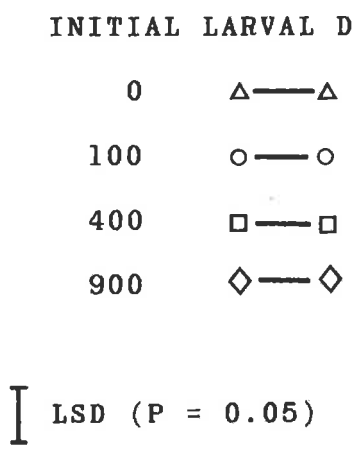

a)
length
larvae
TOTAL ROOT LENGTH (cm)
No. LARVAE
INITIAL NEMATODE DENSITY
0
15
75
200
b)
TOTAL ROOT LENGTH (cm)
NUMBER OF LARVAE
INITIAL NEMATODE DENSITY
0
100
250
500
850
c)
MEAN NO. LARVAE
INITIAL INOCULUM DENSITY
0
100
400
900

FIG 2.3 (a-d) The effect of initial inoculum density of *Heterodera avenae* larvae on the rate of seminal root extension of a) NZC, b) Sto c) *Avenae fatua* and d) Sual 0 to 20 days after planting (3 to 19 days after inoculation)



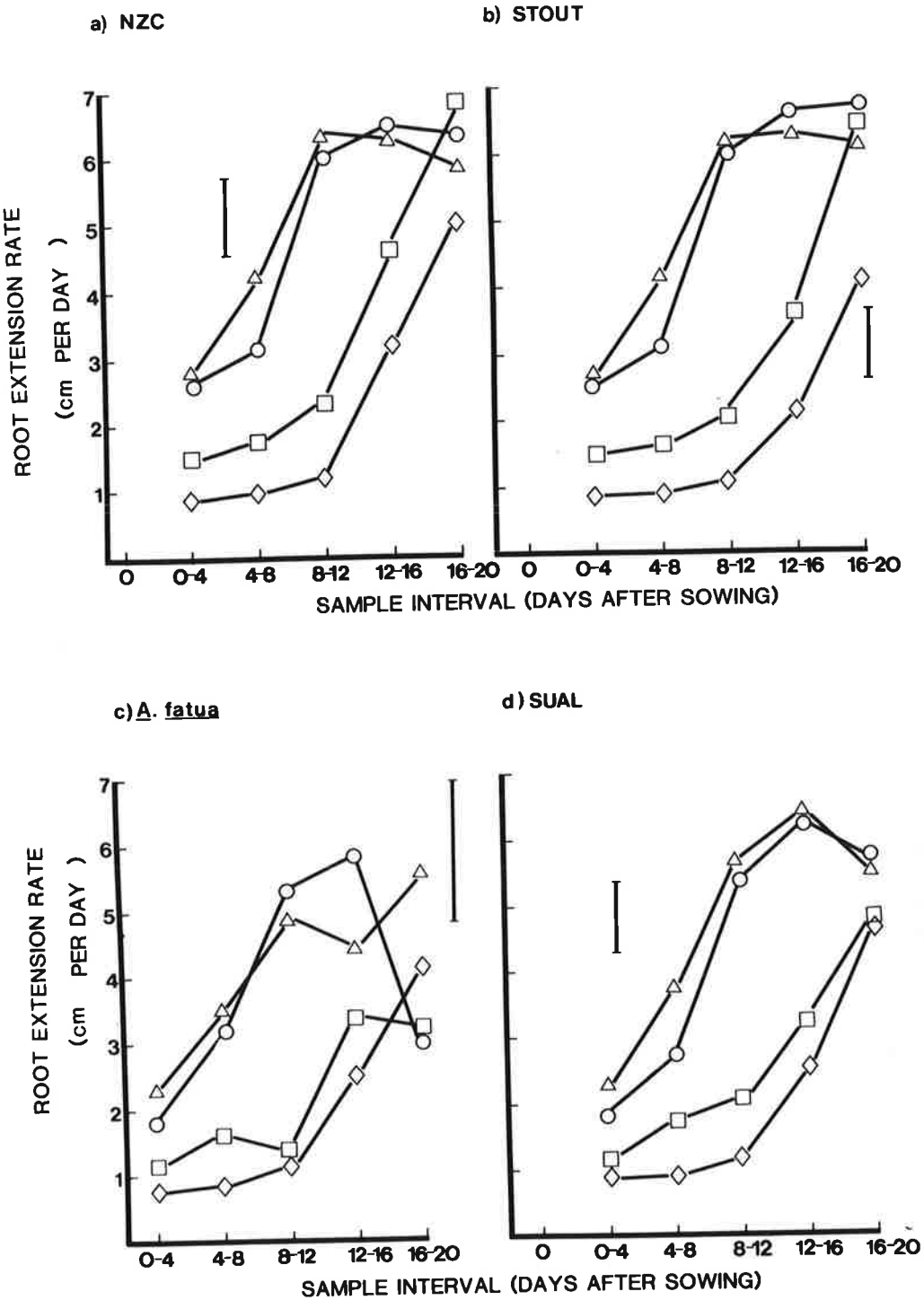

a) NZC
b) STOUT
c) A. fatua
d) SUAL
ROOT EXTENSION RATE
(cm PER DAY)
1
2
3
4
5
6
7
0
0-4
4-8
8-12
12-16
16-20
SAMPLE INTERVAL (DAYS AFTER SOWING)

Table 2.1: The Effect of Heterodera avenae Infestation on the Total Seminal Root Length of NZC, Stout, Sual and Avena fatua 20 Days After Planting

NEMATODE DENSITY	SEMINAL ROOT LENGTH (cm) CULTIVAR				
	NZC	STOUT	SUAL	Avena fatua	MEAN
0	112.4	106.2	92.6	82.6	97.8
100	101.3	100.8	86.2	65.8	89.2
400	76.1	64.6	51.8	42.2	59.4
900	53.9	42.4	39.7	37.6	43.4
MEAN	86.1	78.8	68.4	59.3	

ANALYSIS	LSD (P = 0.05, * ; P ≤ 0.01 **)
CULTIVAR	15.8*
DENSITY	12.7**
CULTIVAR & DENSITY	NS

Number of First Order Lateral Roots

The total number of first order lateral roots was cultivar and nematode density dependent (Table 2.2). Avena fatua had increasingly fewer lateral roots than the other three cultivars as the plants developed.

Infestation significantly (P = 0.05) reduced the number of first order laterals of all cultivars at initial larval densities of 400 or more. The cultivar X density interaction was not significant.

Table 2.2: The Effect of Density of Heterodera avenae on the Total Number of First Order Lateral Roots per Root System Arising from Seminal Roots of NZC, Sual, Stout and Avena fatua During 20 Days of Growth

TOTAL NUMBER OF FIRST ORDER LATERAL ROOTS

		DAYS AFTER PLANTING					
CULT.	DENSITY	4	8	12	16	20	MEAN
NZC	0	5	28	52	80	107	54.4
	100	6	33	60	87	113	59.9
	400	5	28	34	38	49	30.4
	900	8	22	33	32	45	27.0
MEAN		6.0	27.9	44.1	58.3	79.2	42.7
SUAL	0	7	32	62	84	128	62.6
	100	5	36	64	78	118	60.2
	400	9	24	41	31	47	29.1
	900	8	21	30	24	41	23.6
MEAN		7.4	28.1	49.1	54.3	83.6	44.0
SWAN	0	2	33	56	75	98	52.7
	100	3	26	59	81	90	51.8
	400	3	16	34	29	48	26.0
	900	4	16	31	25	42	23.2
MEAN		3.0	22.9	45.1	52.5	69.5	38.4
WEST	0	1	30	35	57	82	41.0
	100	1	13	39	58	81	38.4
	400	3	21	32	25	37	24.1
	900	4	26	33	18	32	22.4
MEAN		2.3	22.8	33.8	39.2	58.6	31.3
MEAN		3.8	31.3	51.2	74.0	103.8	52.7
(DENSITY		3.8	27.1	55.6	76.1	101.5	52.6
X DAY)		5.0	22.3	35.2	30.8	45.3	27.4
		6.0	21.4	30.9	23.9	39.6	24.1

ANALYSIS LSD (P = 0.05,*; P = 0.01,**)

SOURCE OF VARIATION	
CV	10.9*
DENSITY	14.8**
DAY	16.2**
CV X DENSITY	NS
CV X DAY	25.7*
DAY X DENSITY	28.6*
CV X DAY X DENSITY	NS

The number of lateral roots per unit root length did not differ significantly between cultivars (Table 2.3). Infestation reduced the number of first order laterals per unit root length (Table 2.3).

Table 2.3: The Effect of Heterodera avenae Density on the Number of First Order Lateral Roots Per Unit Root Length Arising from Seminal Roots of NZC, Stout, Sual and Avena fatua 20 Days After Planting

NO. FIRST ORDER LATERAL ROOTS cm^{-1}

DENSITY	CULTIVAR				
	NZC	STOUT	SUAL	Avena fatua	MEAN
0	0.95	1.21	1.06	0.99	1.05
100	1.11	1.16	1.04	1.23	1.13
400	0.64	0.63	0.79	0.71	0.68
900	0.79	0.68	0.76	0.58	0.71
MEAN	0.87	0.92	0.91	0.88	

ANALYSIS (LSD P = 0.05)

CULTIVAR	NS
DENSITY	0.29*
CULTIVAR X DENSITY	NS

There were no differences between cultivars or densities in the number of lateral roots produced in zone 1 (Table 2.4a). Larval invasion caused a significant increase in the number of roots emerging from zone 2, the effect being similar on all cultivars (Table 2.4b). The number of lateral roots emerging from zone 3 was significantly less (P = 0.05) on Avena fatua than on the other 3 cultivars after 20 days growth (Table 2.4c). Infestation significantly reduced the

number of laterals emerging from this zone at densities of 400 larvae or more. This appeared to have been a direct consequence of shorter seminal roots on infested plants. All cultivars responded similarly.

Table 2.4: The Effect of Density of Heterodera avenae on the Number of First Order Lateral Roots Initiated Above (zone 1), Within (zone 2), and Below (zone 3) the Zone of Infestation Arising from Seminal Roots of NZC, Sual, Stout and Avena fatua During the First 20 Days of Growth

2.4a) ZONE 1

CULT.	DENSITY	DAYS AFTER PLANTING					
		4	8	12	16	20	MEAN
NZC	0	3	17	16	17	18	14.3
	100	4	15	18	16	20	14.4
	400	3	16	15	16	14	12.4
	900	3	15	17	16	17	13.4
MEAN		3.3	15.8	16.5	16.3	17.3	13.6
STOUT	0	4	15	15	17	21	14.4
	100	3	15	22	19	18	15.4
	400	5	12	13	13	19	12.4
	900	3	14	18	16	17	13.6
MEAN		3.8	14.0	16.9	16.3	18.8	14.0
SUAL	0	0	13	18	16	23	14.0
	100	0	12	15	18	20	13.0
	400	0	10	17	14	17	11.6
	900	0	11	16	14	19	12.0
MEAN		0	11.5	16.5	15.5	19.8	12.7

CULT.	DENSITY	DAYS AFTER PLANTING					
		4	8	12	16	20	MEAN
Avena fatua	0	0	9	17	15	16	11.4
	100	0	4	16	13	18	10.2
	400	0	7	15	13	17	10.4
	900	0	8	14	12	14	9.6
MEAN		0	7.0	15.5	13.3	16.3	10.4
MEANS (DENSITY X DAY)		1.8	13.5	16.4	16.1	19.9	13.5
		1.8	11.1	17.6	16.5	18.9	13.3
		2.0	11.3	15.2	14.4	16.8	11.7
		1.5	12.0	15.9	14.4	16.8	12.2
		1.8	11.9	16.3	15.4	18.1	12.3

2.4b) ZONE 2

CULT.	DENSITY	NO. OF LATERAL ROOTS DAYS AFTER PLANTING					
		4	8	12	16	20	MEAN
NZC	0	1	1	3	1	2	1.6
	100	1	2	3	2	3	2.2
	400	2	4	5	3	4	3.6
	900	5	5	4	6	4	4.8
MEAN		2.3	3.0	3.8	3.0	3.3	3.1
STOUT	0	2	1	2	1	1	1.4
	100	1	2	3	1	2	1.8
	400	4	3	4	4	5	3.6
	900	6	6	5	5	6	5.6
MEAN		3.3	3.0	3.5	2.8	3.5	3.1

NO. OF LATERAL ROOTS

CULT.	DENSITY	DAYS AFTER PLANTING 4	8	12	16	20	MEAN
SUAL	0	2	1	1	2	2	1.6
	100	3	1	2	3	3	2.4
	400	3	2	5	3	5	3.6
	900	4	4	7	5	4	4.8
MEAN		3.0	2.0	3.8	3.3	3.5	3.1
Avena fatua	0→	1	2	1	1	2	1.4
	100→	1	1	3	1	3	1.8
	400	3	3	2	4	3	3.0
	900	5	5	6	4	5	5.0
MEAN		2.5	2.8	3.0	2.5	3.3	2.8
MEANS (DENSITY X DAY)	0	1.5	1.3	1.8	1.3	1.8	1.5
	100	1.5	1.5	2.3	1.8	2.8	2.0
	400	3.0	3.0	4.0	3.5	4.3	3.5
	900	5.0	5.0	5.5	5.0	4.8	5.1
MEAN (DAYS)		2.8	2.7	3.4	2.9	3.4	

2.4c) ZONE 3

NO. LATERAL ROOTS

CULT.	DENSITY	DAYS AFTER PLANTING 4	8	12	16	20	MEAN
NZC	0	1	10	33	62	87	38.1
	100	1	16	39	69	90	43.2
	400	0	8	14	19	31	14.8
	900	0	2	11	10	24	9.4
MEAN		0.5	9.1	24.3	40.2	57.7	26.5
STOUT	0	1	16	45	66	106	46.6
	100	1	19	39	58	99	43.8
	400	0	9	24	14	23	14.0
	900	0	1	7	3	18	5.8
MEAN		0.5	11.3	28.8	35.3	61.4	27.8

NO. LATERAL ROOTS

DAYS AFTER PLANTING

CULT.	DENSITY	4	8	12	16	20	MEAN
SUAL	0	0	18	37	57	73	37.2
	100	0	13	42	61	67	45.8
	400	0	4	12	12	26	10.8
	900	0	1	8	6	19	6.8
MEAN		0	9.4	24.8	34.1	46.3	25.4
Avena fatua	0	0	19	27	41	64	30.2
	100	0	8	20	44	60	26.1
	400	0	11	15	8	17	10.6
	900	0	1	13	2	13	5.5
MEAN		0	9.8	18.8	23.8	38.3	18.1
MEANS (DENSITY X DAY)		0.5	15.6	35.9	55.6	82.9	38.1
		0.5	13.9	34.0	58.2	79.1	37.3
		0	8.0	16.1	13.1	23.6	12.4
		0	1.3	9.8	5.3	18.9	7.1
MEAN (DAYS)		0.3	9.7	23.8	33.1	51.6	

ANALYSIS LSD ($P = 0.05$,*; $P \leq 0.01$**)

SOURCE OF VARIATION	ZONE 1	ZONE 2	ZONE 3
CULTIVAR (CV)	NS	NS	7.4*
DENSITY	NS	2.7*	9.8**
DAY	6.2*	NS	11.3**
DENSITY X DAY	NS	NS	19.2*
CV X DENSITY	NS	NS	NS
CV X DAY	NS	NS	17.9*
CV X DAY X DENSITY	NS	NS	NS

ANALYSIS	LSD (P = 0.05,*; P ≤ 0.01,**)
SOURCE OF VARIATION	
CULTIVAR (CV)	0.11*
DENSITY	0.10**
DAY	0.11**
CV X DENSITY	0.18*
CV X DAY	0.19*
DENSITY X DAY	0.18*
CV X DENSITY X DAY	0.27*

Length of First and Higher Order Lateral Roots

The effect of nematode density on total lateral root length is presented in Table 2.5.

Table 2.5: The Effect of Density of *Heterodera avenae* on the Total Length of Lateral Roots Per Plant Arising from Seminal Roots of NZC, Sual, Stout and *Avena fatua* During 20 Days of Growth

		LOG TOTAL LATERAL ROOT LENGTH PER PLANT (cm)					
CULT.	DENSITY	DAYS AFTER PLANTING					
		4	8	12	16	20	MEAN
NZC	0	1.63	1.79	2.71	2.91	3.02	2.41
	100	1.23	2.33	2.78	2.94	3.06	2.47
	400	1.17	2.22	2.51	2.77	2.87	2.32
	900	1.36	2.13	2.37	2.61	2.78	2.25
MEAN		1.34	1.86	2.59	2.81	2.93	2.37
STOUT	0	1.38	2.24	2.64	2.89	3.03	2.44
	100	1.48	2.33	2.65	2.89	3.01	2.41
	400	1.27	2.05	2.41	2.71	2.84	2.26
	900	1.51	2.17	2.24	2.55	2.71	2.24
MEAN		1.41	2.13	2.49	2.78	2.91	2.34
SUAL	0	0	1.99	2.54	2.79	2.89	2.04
	100	0	1.91	2.46	2.69	2.84	1.98
	400	0	1.79	2.33	2.59	2.78	1.89
	900	0	1.84	2.17	2.46	2.61	1.78
MEAN		0	1.84	2.43	2.73	2.78	1.96
Avena fatua	0	0	1.77	2.35	2.75	2.85	1.93
	100	0	1.84	2.29	2.64	2.92	1.94
	400	0	1.81	2.07	2.31	2.60	1.76
	900	0	1.71	1.99	2.25	2.46	1.68
MEAN		0	1.78	2.18	2.49	2.71	1.82
MEANS (DENSITY X DAY)		0.75	1.95	2.56	2.84	2.95	2.21
		0.60	2.10	2.55	2.79	2.96	2.20
		0.61	1.96	2.33	2.59	2.78	2.05
		0.71	1.98	2.19	2.48	2.64	2.02
MEAN (DAYS)		0.66	2.02	2.41	2.69	2.84	

No lateral root development was observed on Sual and Avena fatua until after 8 days of growth. Infestation significantly reduced (P = 0.01) lateral root growth of all cultivars at the highest initial inoculum density, but had no effect at lower densities. Differences in total lateral root length between cultivars NZC, Stout and Sual within individual densities were not significant after 8 days. Total lateral root length of NZC was significantly larger than Avena fatua at all 3 inoculation rates up until day 16.

The combined length of first order lateral roots and higher order lateral roots arising from them within each seminal root zone was examined. The mean length of lateral roots within zone 1 of uninfested NZC and Stout was significantly (P = 0.05) larger than that of Sual and Avena fatua over the experimental interval (Table 2.6a). This arose out of the absence of lateral root development on the latter two cultivars within zone 1, 4 days after planting. The mean lateral root length of Sual within zone 1 averaged over all densities was equal to NZC and Stout whereas that of Avena fatua was still significantly less than NZC and Stout. Infestation had no effect on the mean length of zone 1 lateral roots averaged over time on NZC, Stout and Avena fatua but significantly enhanced the lateral root length of Sual inoculated with 400 larvae. This was reflected in a significant (P = 0.05) density by day effect, wherein inoculation of 400 larvae caused an increase in lateral root length within zone 1, 16 to 20 days after planting.

Table 2.6: The Effect of Density of Heterodera avenae on the Length of First and Second Order Lateral Roots Above (zone 1) and Below (zone 3) the zone of Infestation on Seminal Roots of NZC, Sual, Swan and Avena fatua During 20 Days Growth

2.6a) ZONE 1

		LOG_{10} LATERAL ROOT LENGTH (cm)					
CULT.	DENSITY	DAYS AFTER PLANTING					
		4	8	12	16	20	MEAN
NZC	0	1.0	2.00	2.23	2.35	2.43	2.19
	100	1.17	2.12	2.32	2.41	2.48	2.11
	400	1.14	2.16	2.43	2.51	2.63	2.21
	900	0.94	1.88	2.29	2.49	2.68	2.08
MEAN		1.06	2.04	2.32	2.48	2.58	2.15
STOUT	0	1.30	2.06	2.27	2.40	2.49	2.10
	100	1.13	2.13	2.25	2.38	2.49	2.07
	400	0.91	2.02	2.36	2.56	2.68	2.11
	900	1.21	1.85	2.16	2.45	2.59	2.03
MEAN		1.13	2.02	2.26	2.35	2.56	2.04
SUAL	0	0	1.77	2.02	2.19	2.32	1.66
	100	0	1.67	1.99	2.22	2.37	1.67
	400	0	1.75	2.23	2.44	2.56	2.23
	900	0	1.65	2.06	2.29	2.45	1.71
MEAN		0	1.70	2.08	2.29	2.48	1.73
Avena fatua	0	0	1.58	1.94	2.16	2.31	1.58
	100	0	1.64	2.06	2.11	2.29	1.71
	400	0	1.76	1.99	2.18	2.42	1.64
	900	0	1.69	1.88	2.15	2.36	1.62
MEAN		0	1.67	1.82	2.16	2.23	1.55
MEANS (DENSITY X DAY)		0.57	1.84	2.11	2.26	2.38	1.88
		0.58	1.86	2.16	2.29	2.43	1.92
		0.51	1.92	2.26	2.44	2.59	2.04
		0.53	1.86	1.76	2.35	2.52	1.85
(DAY)		0.55	1.86	2.12	2.32	2.45	

2.6b) ZONE 3

Log 10 LATERAL ROOT LENGTH (cm)

CULT.	DENSITY	DAYS AFTER PLANTING					
		4	8	12	16	20	MEAN
NZC	0	0.48	1.78	2.53	2.77	2.89	2.09
	100	0.30	1.91	2.57	2.79	2.92	2.10
	400	0.0	1.28	1.74	2.28	2.35	1.53
	900	0.0	0.90	1.61	1.98	2.08	1.31
MEAN		0.20	1.47	2.11	2.46	2.56	1.76
STOUT	0	1.39	1.76	2.39	2.72	2.88	2.23
	100	1.61	1.89	2.43	2.73	2.85	2.30
	400	0	0.90	1.58	2.15	2.35	1.41
	900	0	0.48	1.41	1.89	2.06	1.18
MEAN		0.75	1.30	1.97	2.38	2.54	1.77
SUAL	0	0	1.56	2.38	2.66	2.77	1.88
	100	0	1.54	2.27	2.49	2.67	1.78
	400	0	0.78	1.62	2.03	2.37	1.43
	900	0	0.30	1.53	1.96	2.07	1.17
MEAN			1.05	1.95	2.29	2.47	1.55
Avena fatua	0	0	1.32	2.27	2.58	2.72	1.78
	100	0	1.43	1.91	2.46	2.77	1.71
	400	0	0.78	1.28	1.71	2.15	1.18
	900	0	0.30	1.30	1.57	1.77	0.99
MEAN		0	0.96	1.70	2.09	2.35	1.40
MEAN		0.47	1.61	2.41	2.68	2.82	1.99
(DENSITY		0.48	1.70	2.28	2.62	2.81	1.97
X DAY)		0	0.94	1.54	2.04	2.31	1.39
		0	0.50	1.46	1.85	1.99	1.16
		0.24	1.19	1.92	2.31	2.49	1.63

ANALYSIS (LSD P = 0.05,*; P ≤ 0.01,**)

SOURCE OF VARIATION	ZONE 1	ZONE 3
CULTIVAR (CV)	0.97*	0.08*
DENSITY	0.08*	0.08**
DAY	0.08**	0.08**
CV X DENSITY	0.15*	0.15**
CV X DAY	0.13*	0.15*
DENSITY X DAY	0.14*	0.15*
CV X DENSITY X DAY	NS	0.21**

The length of lateral roots within zone 3 on Stout after 4 days growth was significantly greater than that of the other 3 cultivars when no larvae were applied (Table 2.6b). Zone 3 lateral root length of uninfested Sual, Stout and NZC did not differ 8 to 20 days after planting whereas, up until 20 days after planting the lateral root length within zone 3 of uninfested Avena fatua was less than that of NZC. Inoculation with 400 or more larvae caused a significant reduction ($P = 0.01$) in lateral root length of all cultivars on all days except day 4, where infestation of Sual and Avena fatua had no effect on lateral root length because no lateral roots had yet emerged.

The total length of lateral roots within zone 2 on plants inoculated with 400 or more larvae was significantly ($P = 0.01$) less than that from the same zone on uninfested plants (Table 2.7). No cultivar differences were observed throughout the 20 day experimental interval.

Table 2.7: The Effect of Infestation on Total Length of First and Higher Order Lateral Roots Arising from Zone 2 on Seminal Roots of Four Oat Cultivars 20 Days After Planting

LATERAL ROOT LENGTH (cm)

DENSITY	CULTIVAR				
	NZC	STOUT	SUAL	Avena fatua	MEAN
0	31(2.9)a	19(1.7)	23(2.8)	24(3.4)	24
100	31(2.7)	22(2.1)	17(2.4)	19(2.3)	22
400	21(2.8)	12(1.7)	15(2.5)	18(4.5)	17
900	12(2.0)	9(1.8)	8(2.0)	11(3.9)	11
	24	16	16	18	

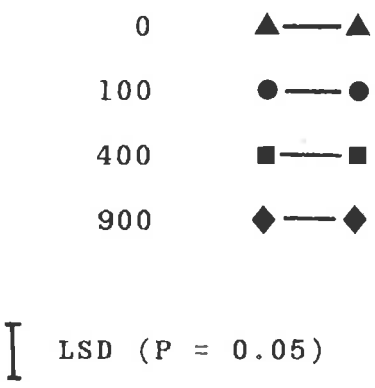
(a) % of total (zones 1 + 2 + 3) lateral root length.

ANALYSIS	LSD (P = 0.01,**)
CULTIVAR	NS
DENSITY	9**
CULTIVAR X DENSITY	NS

The total second and higher order lateral root length deriving from individual first order lateral roots (expressed in Fig 2.4 as unit root length) was greater on moderate to heavily infested plants than on uninfested control plants 16 to 20 days after planting. The unit root length of Stout was higher than that of the other 3 cultivars 16 days after planting at the highest nematode density.

The effect of inoculum density on the total lateral root length/total seminal root length ratio after 20 days is presented in Table 2.8. No effects were significant indicating that the decline in total lateral root length of infested plants was attributable principally to the decline in seminal root length.

FIG 2.4 (a-d) The effect of initial inoculum density of Heterodera avenae larvae on the mean length of second and higher order lateral roots per first order lateral root on cultivars a) NZC, b) Stout c) Avenae fatua and d) Sual 4 to 20 days after planting (3 to 19 days after inoculation)



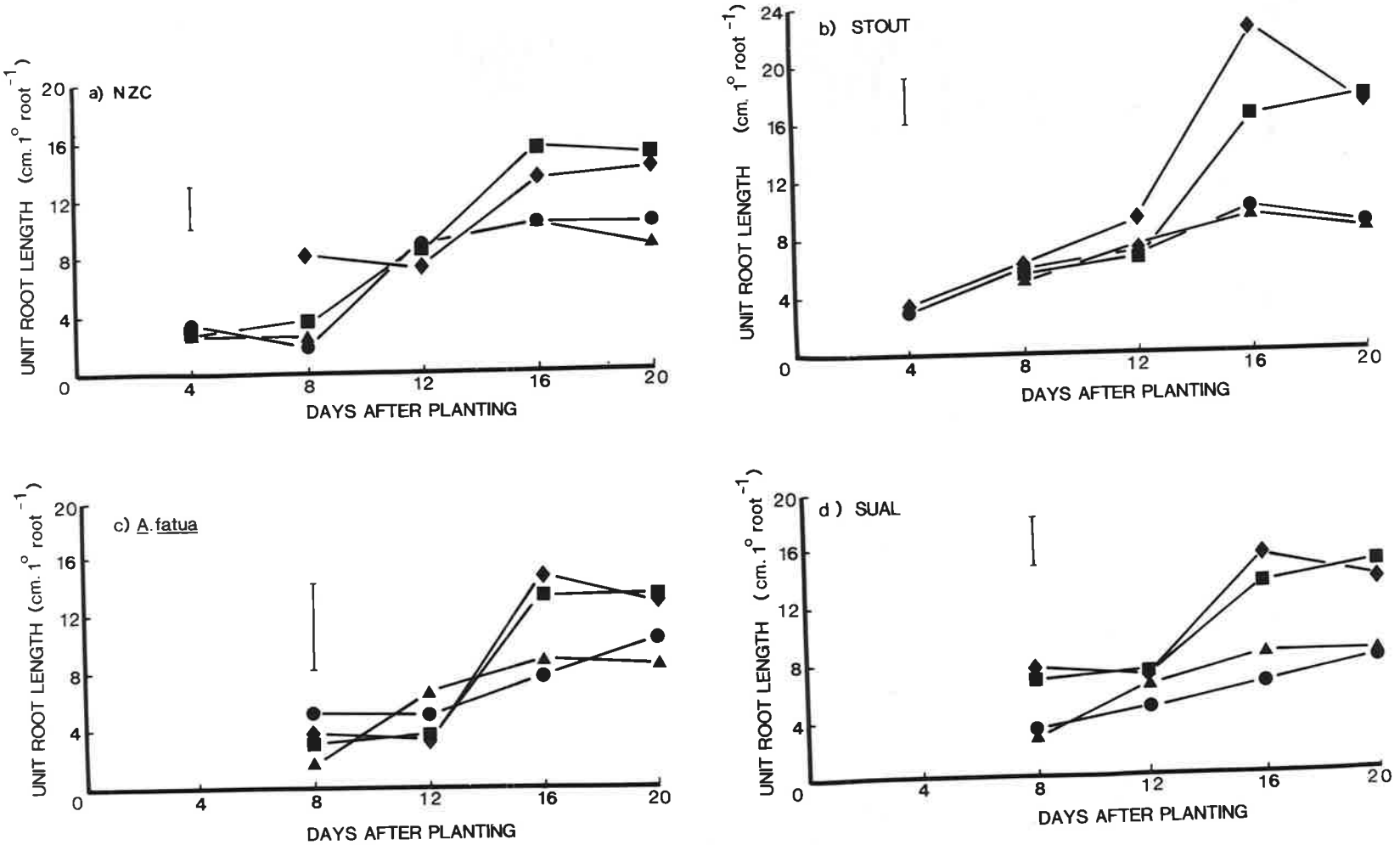

a) NZC
b) STOUT
c) A.fatua
d) SUAL
UNIT ROOT LENGTH (cm. 1° root -1)
DAYS AFTER PLANTING
0
4
8
12
16
20
24

Table 2.8: Effect of Heterodera avenae Infestation on the Lateral Root Length/Seminal Root Length Ratio of Four Oat Cultivars After 20 Days

NEMATODE DENSITY	NZC	STOUT	SUAL	Avena Fatua	MEAN
0	9.35	10.1	8.3	8.6	9.1
100	11.4	10.2	8.0	11.6	10.6
400	9.75	10.8	11.6	9.5	10.4
900	11.4	12.2	10.2	7.6	10.4
MEAN	10.5	10.8	9.5	9.6	

Main and interactive effects were not significant at P = 0.05.

Nodal Root Number and Length

All roots originating above the seminal roots were designated nodal roots. These roots contributed significantly to the total root length within approximately 16 days from planting (about 7%). Only data obtained from the last date of sampling are shown (Table 2.9). By day 20 approximately 4 to 5 nodal roots had emerged. Neither infestation nor cultivar influenced the number of nodal roots. NZC and Stout had the largest total nodal root lengths; Sual and Avena fatua had shorter nodal roots. Nodal root length declined with increasing inoculum density. The length of lateral roots emerging from the nodals was also influenced by cultivar and density in a manner similar to that of nodal root length.

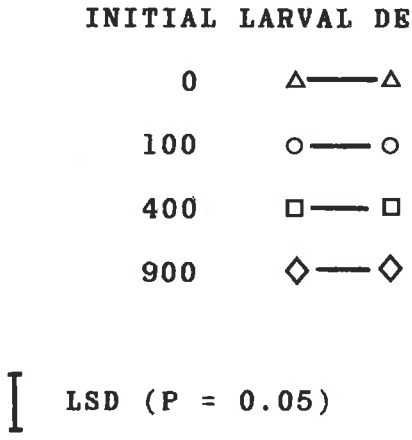
Table 2.9: Effect of Density of Heterodera avenae on Total Nodal Root Number and Length, and First and second Order Lateral Root Length Emerging from Nodal Roots of NZC, Sual, Stout and Avena fatua 20 Days After Sowing

CV	DENSITY	No. NODAL AXES	LENGTH TOTAL NODAL ROOT AXES	LENGTH 1°,2° NODAL LATERALS	TOTAL LENGTH NODAL
			(cm)	(cm)	(cm)
NZC	0	4.2	57	81	138
	100	3.9	56	94	150
	400	6.3	113	267	380
	900	5.7	156	314	470
MEAN			95.5	189.7	283.7
STOUT	0	5.6	53	34	87
	100	5.2	48	19	67
	400	4.8	97	133	233
	900	5.1	121	189	310
MEAN			79.8	93.8	174.3
SUAL	0	3.6	46	0	46
	100	5.8	52	0	52
	400	4.2	69	54	123
	900	4.0	91	113	204
MEAN			64.5	42.1	105.3
Avena fatua	0	2.1	27	21	48
	100	1.8	32	16	48
	400	2.9	63	128	191
	900	3.4	72	76	148
MEAN			48.5	60.7	109.6
MEAN (DENSITY)			31.5	34.1	79.6
			47.0	33.7	79.1
			85.2	14.42	232.8
			110.0	173.0	281.9
ANALYSIS					
SOURCE OF VARIATION			LSD (P= 0.05*; P≤ 0.01**)		
CV		NS	7.1*	13.4**	19.2*
DENSITY		NS	6.8**	11.6**	16.4**
CV X DENSITY		ns	NS	21.3*	39.1*

Total Root Length

Effects on total root length of larval density on the four cultivars tested are shown in Fig. 2.5. Uninfested total root lengths of Stout and NZC were larger (P=0.05) than those of Sual and *Avena fatua*. Density of nematode infestation had less effect on total root system length than on seminal and lateral root lengths (Table 2.10) but all cultivars except NZC had root system lengths significantly less (P = 0.5) than control plants at initial nematode densities of 400 larvae.

FIG 2.5a-d The effect of initial inoculum sensity of Heteroder avenae larvae on the total root length of a) NZC, b) Stout, c) Sual and d) Avenae fatua 0 to 20 days after planting (-1 to 19 days after inoculation)



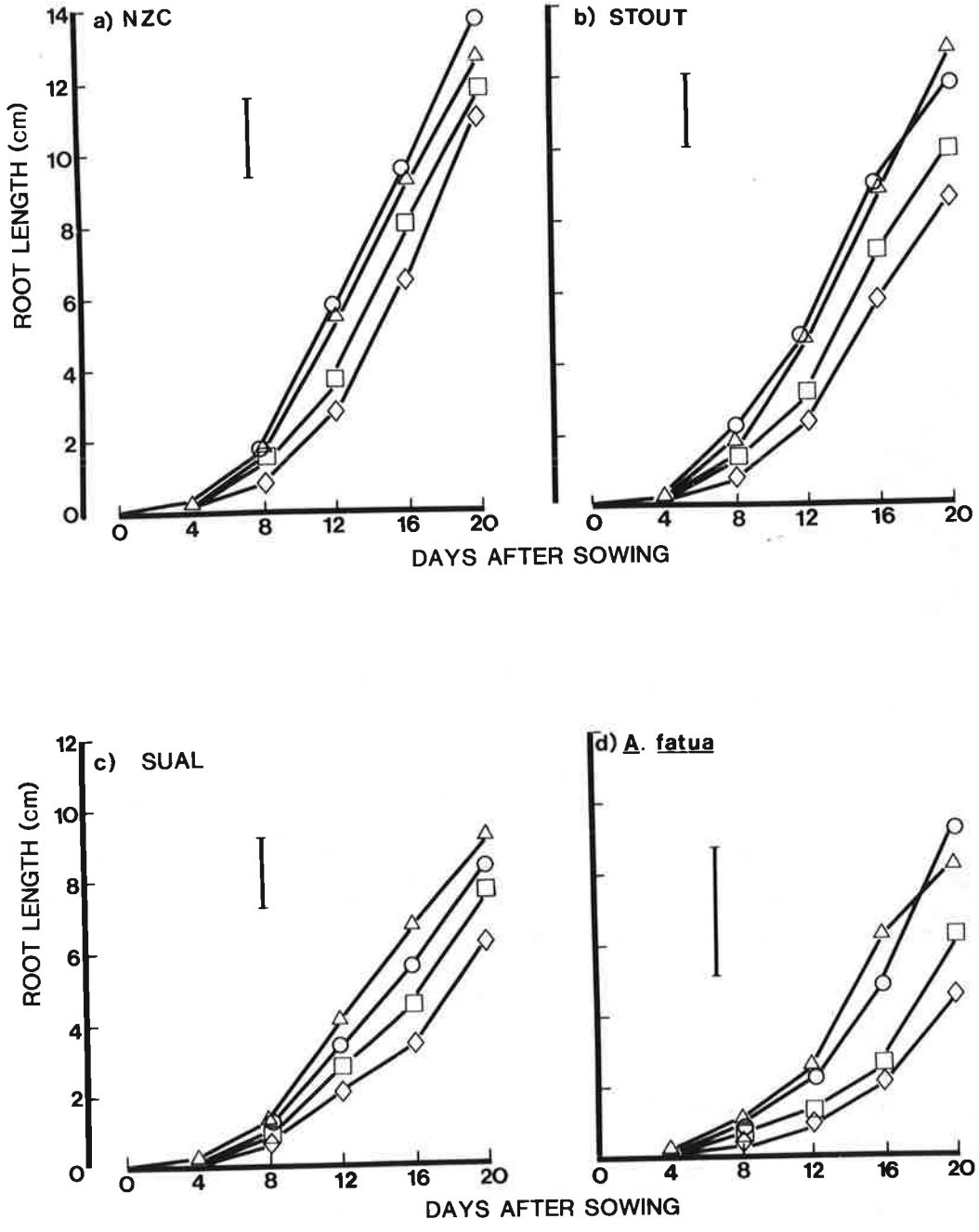

a) NZC
b) STOUT
c) SUAL
d) A. fatua
ROOT LENGTH (cm)
DAYS AFTER SOWING
0
2
4
6
8
10
12
14
16
20

Table 2.10: Summary of Effects of Density of *Heterodera avenae* on Components of Root Growth After 20 Days

CV	DENSITY	% OF UNINFESTED CONTROLS				
		SEMINAL ROOTS	No. 1° LATERALS	LENGTH 1°,2°,3° LATERALS	LENGTH NODAL (AXES & LATERALS)	TOTAL
NZC	100	93	105	109	108	107
	400	78**	47**	71**	275**	93
	900	55**	39**	57**	340**	87
STOUT	100	95	92	94	77**	92
	400	57**	32**	64**	260**	78**
	900	41**	23**	47**	356**	68**
SUAL	100	93	92	88	113	90*
	400	53**	42**	75**	267**	84*
	900	36**	31**	50**	443**	68**
Avena fatua	100	91	98	116	100	112
	400	54**	37**	56**	398**	75*
	900	46**	27**	40**	308**	54**

*, ** significantly different from controls (P=0.05), (P=0.001)

2.4 Discussion

2.4.1 Experiments (1) and (2)

Previous studies have indicated that root growth may be inhibited at densities lower than those applied in the first experiment (O'Brien 1976, Stanton 1983). Inability to obtain a significant root growth decline was likely due to inefficient inoculation resulting in reduced numbers of larvae penetrating the root system. O'Brien (1976) applied larvae directly to the root tip of germinated wheat and barley seedlings and achieved a 98% reduction in root extension of a single root over 24 hours following penetration by 35 larvae. Inoculation efficiency (number of larvae applied divided by the number of larvae penetration X 100) was 12% in O'Briens experiment. The method employed in the present studies reduced the chance of larvae coming in contact with the root and thereby reduced inoculation efficiency to about 7%.

In the second experiment higher inoculum densities resulted in improved inoculation efficiency (10-12%) and depressed root extension to about 35% of controls. The apparent absence of difference in root extension between a tolerant and intolerant cultivar after ten days growth at any of the densities applied indicated firstly that tolerant and intolerant cultivars both suffered the same degree of initial stunting in root growth caused by larval penetration. Secondly it suggested that a longer experimental duration would be necessary to detect differences in root growth following infestation between cultivars if such differences existed.

2.4.2 Experiment (3)

The number of larvae present in roots in this study was lower than in experiments 1 and 2 of this section. This may have been due to some larvae being rinsed off prior to transfer of the root system onto the mat on day four, to a shorter exposure of the root system to larvae, or to larvae leaving the root after transplanting. Because there were still significant differences in root nematode populations between applied densities, determining the cause of the overall reduction in the population of nematodes in the roots in this study was considered unnecessary. The absence of differences between cultivars in numbers of juvenile larvae infesting roots ruled out one possible tolerance mechanism, namely, larval exclusion. It also enabled evaluation of responses to infestation to be based on equivalent infestation rates.

The primary effect of nematode infestation was to reduce the rate of seminal root extension. Impairment of seminal root extension by Heterodera avenae has been reported elsewhere (e.g. O'Brien 1976, Price et al. 1983). As a consequence of shorter seminal roots plants inoculated at densities of 400 to 900 larvae also had fewer first order seminal roots within zone 3. This in turn resulted in shorter root systems on moderately to heavily infested plants.

The smaller number of first order lateral roots on infested plants apeared to be partly compensated for by an increase in the length of second and higher order lateral roots arising from individual first order roots (i.e. increase in unit root length). However, the increase in unit root length with increasing inoculum density was mainly the result of a decline in the number of first order laterals per unit length of seminal root at the higher densities of inoculum.

Because of a significant decline in lateral root growth within zone 3 at moderate to high inoculum densities unabated lateral root growth within zone 1 contributed significantly to the total root length of infested plants. The enhanced lateral root growth within zone 1 of Sual at the inoculum density of 400 larvae was indicative of a possible compensatory root growth mechanism. These findings were reminiscent of a study conducted by Hackett (1971) who reported that 22 days after partial excision of seminal roots of barley, lateral roots growing behind the point of excision more than compensated for the decline in total root length. However, factors responsible for inhibiting seminal root extension of infested plants may also impair the development of uninfested lateral roots. Lateral root extension within zone 2 was significantly reduced in this study. In at least one cultivar, Sual, enhanced zone 1 lateral root growth at the moderate inoculum density (400) did not occur at the higher density, suggesting that at even higher inoculum

densities, zone 2 lateral root growth would have been significantly less than in uninfested plants. On the basis of the above discussion, equating root excision with the effects of nematode infestation may be misleading.

Although there were significant differences between cultivars in the effects of infestation on seminal root extension, differences in total lateral root growth following infestation were smaller, and in all cases except for Avena fatua were not significantly different after 8 days. This was unusual given the close relationship between seminal root growth and lateral root length (Table 2.10). The inconsistancy may be partly explained by the variability in the root measurement data within treatments, wherein differences as large as 175 cm were not significant when the total root length was only 4-5 times that.

It has been suggested that nematode tolerance may be related to vigorous root growth (e.g. Evans et al. 1977). Although rapid seminal root extension was a characteristic of NZC, the cultivar least impaired by nematode invasion, another cultivar, Stout had a comparable seminal extension rate to that of NZC in the absence of infestation, but was significantly more reduced by infestation than NZC. Subsequent lateral root development of the two cultivars did not differ significantly after 8 days in this study. However under conditions in which both seminal and lateral roots would be hosts to larval invasion, as would occur under field

conditions, Stout, Sual and Avena fatua may be more reduced in root length by infestation than NZC. This is assuming that seminal and lateral roots are equally sensitive to nematode invasion as indicated by reduced root growth. Under such circumstances both larval density and duration of exposure to infestation would determine whether lateral root development could compensate for declines in growth on infested roots.

Under field conditions nodal root development may therefore be an important determinant of tolerance.

Nodal roots, which emerged late in the study, contributed significantly to the final root length of infested plants. Because of the large absolute contribution to total root system length of nodal roots in the case of NZC, the overall effect of infestation at the highest density was to reduce total length by only 13% ($P = 0.05$), compared to 39% when nodal roots are excluded (Table 2.10). Nodal root development had a similar but less dramatic effect on root length of the remaining cultivars.

In summary, the results of this study indicate that the higher level of tolerance in NZC to Heterodera avenae, described in Chapter 4, Section 1, may be related in part to the lesser impact that nematode invasion has on seminal root extension, as well as an earlier and more rapidly growing nodal root system when compared to the other cultivars.

Conversely, if it is assumed that lateral and seminal roots respond similarly to nematode infestation, then, because of greater sensitivity to growth inhibition by nematode invasion. The cultivars Sual, Stout and Avena fatua, may be less tolerant than NZC to infestation by Heterodera avenae.

Glasshouse studies have demonstrated an intolerance of Sual to Heterodera avenae (section 1) and therefore support the above statements.

This experiment demonstrates that infestation significantly impairs seminal root growth at inoculum densities of 400 to 900 larvae and that cultivars differ in the extent of impairment. It also shows that infestation can reduce the total length of uninfested lateral roots emerging below the infested region on the seminal root by reducing the length of the seminal root. Lateral root growth above the infested zone is enhanced at moderate inoculum densities, but may be reduced at higher inoculum densities.

These differences could be related to the ability of the least affected cultivar, NZC, to be more tolerant to high infestations of Heterodera avenae than the cultivar Sual. No glasshouse studies were performed to test the tolerance of Stout and Avena fatua and therefore it is not valid to extrapolate the response of these plants observed in this study to possible tolerance levels at maturity.

Further studies are required to determine why root growth of the cultivars studied differed in reaction to nematode invasion. In the next section hormonal involvement is investigated.

SECTION 3: GROWTH OF *Heterodera avenae* INFESTED ROOTS - REGULATION BY ETHYLENE AND ABSCISIC ACID

3.1 Regulation of Growth of Aseptically Cultured Roots

3.1.1 Introduction

The previous study showed that at sufficiently high densities *Heterodera avenae* infestation reduced the rate of seminal root extension and delayed the extension of lateral roots emerging behind the invasion zone. Rates of root extension and lateral root development can be influenced by a number of exogenously supplied plant growth hormones but it is generally accepted that the principle hormones involved in regulation of root growth are indole acetic acid (IAA), abscisic acid (ABA) and ethylene (Street 1969; Scott 1972; Torrey 1976; Feldman 1984). At physiological concentrations (10^{-6}-10^{-8}M) ABA, produced in the root apex, inhibits the promotive effect of IAA on root extension and lateral root initiation (Street 1969; Pillet 1970; Bottger 1974; Wightman *et al*. 1980; Audus 1983). Low levels (0.01 ppm) of ethylene promotes root elongation and lateral root outgrowth (Smith and Robertson 1971; Zobel 1973; Konings and Jackson 1979, Drew *et al*. 1981). At higher concentrations the effects of ethylene are reversed (Radin and Loomis 1971; Apelbaum and Burg 1972a; Crossett and Campbell 1975), and nodal root emergence is stimulated (Jackson *et al*. 1981). The similarity of root developmental responses to ABA and ethylene at elevated concentrations on the one hand and

nematode invasion on the other (Section 2) suggests that infestation may induce a rise in rates of production or release of one or both of these hormones causing the alteration in growth.

The purpose of this study was to test the hypothesis that changing patterns of root growth following Heterodera avenae infestation are not due to localized effects of infestation on ABA and/or ethylene levels in infested oat roots. The associated corollary, that differences in root response to infestation between a tolerant and an intolerant cultivar are not due to differences in ethylene or ABA production or release, was also tested.

Tissue and organ culture techniques have been invaluable in assisting identification of nutritional and hormonal factors regulating root growth (Street 1969b). Gnotobiotic studies have also proven effective in elucidating plant-nematode relationships by eliminating the confounding effects of other micro-organisms (Zuckerman 1970). In the present study, biotic influences on the host-nematode interaction were eliminated by employing organ culture techniques.

3.1.2 Methods

Experiments were conducted with two oat cultivars, Sual and NZC. Seeds were prepared for germination on water agar as described in Chapter 3, 2.1 with an additional precaution that all rinse water was presterilized. When the seminal roots were 3-4 cm long the terminal 2 cm was excised and transferred to nutrient agar medium in 9.0 cm petri dishes, 10 root segments per plate. The nutrient composition of the agar medium was a modified version of that used by McClure and Viglierchio (1966) (Table 3.1). The root segments were incubated in the dark at 22°C. After 10 days segments of the main root, 2 cm in length, bearing emerged lateral roots, were excised using fine sharp scissors and cultured singly for a further 10 days on similar medium. From these cultures 2 cm root tips were excised and used as experimental material in the following experiment.

Nutrient agar slants as above were prepared in 100 ml erlenmeyer flasks, 50 ml flask^{-1}. Ten 2 cm long root apices were transferred to the flasks, tip downward, and incubated at 22°C in the dark. Flasks were stoppered with non-absorbent cotten plugs. One day later about 0.15 g of autoclaved sand, grain size less than 150 μm, was placed on the apices of the root segments. Second stage Heterodera avenae larvae maintained as described in Chapter 3, 2.4.1 and surface sterilized as outlined in Chapter 3, 2.4.2 were then transferred under aseptic conditions to the tips of the root

apices in 0.2 ml sterile water at a rate of 50 and 200 larvae per root. During the following 9 days, at 3 day intervals, root apices were monitored for ethylene and abscisic acid production. Ethylene was measured at the end of a 24 hour period during which time the cotton plugs were replaced with "suba-seals". One ml gas samples withdrawn through the seal from the flask with an hypodermic syringe were injected into a gas chromatograph and analysed as outlined in Chapter 3, 2.6. Estimation of ABA content in root tissue was as described in Chapter 3, 2.7, on rinsed, snap frozen and lyophylized samples.

Measurements of root length, numbers of unemerged root lateral primordia and emerged lateral roots and larval number were made on apices stained in 0.1% cotton blue in lactophenol (Chapter 3, 2.5).

Ethylene samples were replicated 6 times and ABA samples were replicated 3-4 times on bulked samples of 10 apices. Root and nematode measurements were replicated 3 times and 2 times, rspectively, on batches of 10 apices each. Analysis of variance was performed for each parameter measured.

3.1.3 Results

Infestation

Larvae invaded cultured root tips of both cultivars to a similar extent (Table 3.2).

Table 3.1: Composition of Agar Medium Used in Section 3.1

Stock Solution I	(Macro Nutrients)
$Ca(NO_3)_2 .4H_2O$	1.4g
KNO_3	0.25g
$Mg5O_4.7H_2O$	0.34g
KH_2PO_4	0.25g
Water	To 1 litre
Stock Solution II	(Micro Nutrients)
$Mn5O_4.H_2O$	225 mg
KI	50 mg
$Zn5O_4$	10 mg
N_i5O_4	5.0 mg
$Cu5O_4$	5.0 mg
MoO_3	5.0 mg
Water	To 1 litre
Stock Solution III	(Vitamins and Amino Acids)
Glycine	300 mg
i Inositol	1000 mg
Thiamine - HCI	10 mg
Niacin	50 mg
Pyridoxine - HCL	10 mg
Biotin	1.0 mg
Water	To 100 ml.
Iron Stock Solution	
$Fe_2(5O_4)_3$	0.93 g
$EDTA.Na_2$	0.66 g
Water	To 100 ml
Sucrose	20g/l nutrient solution

Final Composition of Nutrient Agar	
Stock Solution I	100
Stock Solution II	10 ml
Stock Solution III	10 ml
Iron Solution	10 ml
Sucrose	20 g
Agar	10 g

There were significantly more (P = 0.05) larvae in the roots of NZC on day 3 at the higher, than at the lower inoculum density. No other differences were observed.

Table 3.2: Effect of Density of Heterodera avenae on the Number of Larvae Infesting Asepically Cultured Root Segments of Two Oat Cultivars

CULTIVAR	DENSITY	LARVAE NUMBER DAYS AFTER INOCULATION	
		3	9
NZC	50	4.1B	5.4^{AB}
	200	8.7A	7.2^{A}
SUAL	50	3.9B	4.2^{B}
	200	6.6AB	7.5^{A}

Significantly different values are marked with different letters (P = 0.05)

Root Growth

The effects of density on main axis root length, the number of first order laterals and the number of unemerged lateral root primordia on aseptically cultured root segments over 9 days are shown in Tables 3.3 to 3.4, respectively.

Root growth of cultured segments was slower than that on intact plants (compare Table 3.3 with Fig. 2.7). No cultivar differences in root length over the trial period were observed. At both densities controls were significantly longer (P=0.05) than infested plants within three days after inoculation. Infestation reduced the number of laterals emerging (Table 3.4) with no effects of cultivar or number of nematodes. There were no significant effects on numbers of lateral root primordia (Table 3.5) but it cannot be concluded that the difference in numbers emerged was or was not due to effects on elongation alone as there were also differences in total lateral root numbers (primordia and emerged).

Table 3.3: Effect of Density of Heterodera avenae on the Length of the Main Root Axis of Aseptically Cultured Root Segments of Two Oat Cultivars

CULTIVAR	DENSITY	AXIS ROOT LENGTH (mm) DAYS AFTER INOCULATION			
		0	3	6	9
NZC	0	34	45B	56A	65A
	50	33	37C	46B (82)	49B (76)
	200	36	35C	41B (73)	43B (66)
SUAL	0	35	41B	53AB	59A
	50	35	36C	43B (81)	48B (81)
	200	34	35C	37C (70)	40B (67)

Significantly different values are denoted by different letters (P = 0.05)

Values in parentheses are percentages of uninoculated control on same day.

Table 3.4: Effect of Density of Heterodera avenae on the Number of First Order Lateral Roots on Aseptically Cultured Root Segments of Two Oat Cultivars

CULTIVAR	DENSITY	FIRST ORDER LATERAL ROOT NUMBER DAYS AFTER INOCULATION			
		0	3	6	9
NZC	0	0	2.2	5.7B	10.9A
	50	0	0.8	2.3C	4.7BC
	200	0	1.1	1.4CD	3.6C
SUAL	0	0	0.4	4.8BC	11.1A
	50	0	0.6	2.0C	5.9B
	200	0	0.5	1.1CD	4.2BC

Significantly different values denoted by different letters (P = 0.05).

Table 3.5: Effect of Density of Heterodera avenae on the Number of Unemerged Lateral Root Primordia on Aseptically Cultured Root Segments of Two Oat Cultivars

		PRIMORDIA NUMBER			
CULTIVAR	DENSITY	DAYS AFTER INOCULATION			
		0	3	6	9
NZC	0	2	7	17B	29A
	50	1	5	16B	21AB
	200	2	5	13BC	23AB
SUAL	0	0	10	18AB	23A
	50	1	7	16B	24A
	200	1	8	13BC	17AB

Significantly different values are denoted by different letters (P = 0.05)

ABA and Ethylene

Levels of ABA and ethylene were higher (P = 0.05) in roots infested with Heterodera avenae (Tables 3.6 and 3.7). Cultivar differences were not significant. Ethylene levels were highest two days after inoculation, then declined.

Table 3.6: Effect of Density of Heterodera avenae on the Concentration of ABA in Aseptically Cultured Root Segments of Two Oat Cultivars

CULTIVAR	NEMATODE DENSITY	ABA CONCENTRATION ($\mu g.g^{-1}$ D.W)		
		DAYS AFTER SOWING		
		3a	6	9
NZC	0	0.087	0.094	0.076
	50	0.361	0.482	0.190
	200	0.421	0.421	0.236
SUAL	0	0.072	0.074	0.081
	50	0.407	0.518	0.406
	200	0.373	0.500	0.333

ANALYSIS

SOURCE OF VARIATION	LSD (P = 0.01)
Cultivar	NS
Density	0.0612**
Day	NS
Cultivar X Density	NS
Density X Day	NS
Cultivar X Day	NS
Cultivar X Density X Day	NS

a - Two days after inoculation

Table 3.7 : Effect of Density of *Heterodera avenae* on Ethylene Production by Aseptically Cultured Root Segments of Two Oat Cultivars

CULTIVAR	NEMATODE DENSITY	ETHYLENE PRODUCTION ($nl.g.^{-1}h.^{-1}$) DAYS AFTER SOWING		
		3[a]	6	9
NZC	0	-	0.13	0.17
	50	1.68	1.09	0.58
	200	2.59	2.06	0.61
SUAL	0	-	0.27	-
	50	1.41	0.93	0.39
	200	2.18	1.21	0.70
MEAN (DENSITY X DAY)	-	0.20	0.086	
	1.55	1.01	0.49	
	2.39	1.64	0.66	

ANALYSIS

SOURCE OF VARIATION	LSD (P = 0.05*)
Cultivar	NS
Density	0.201*
Day	0.165*
Cultivar X Density	NS
Cultivar X Day	NS
Density X Day	0.413*
Cultivar X Density X Day	NS

a Two days after inoculation

- No Ethylene detected

3.1.4 Discussion

Prolonged aseptic root culture of monocots and particularly of Avena has been difficult (Scott et al. 1961, Almestrand 1949). However the modified medium of Gautheret (1942) used by McClure and Viglierchio (1966) promoted satisfactory root segment culture in the present study.

The method used to surface sterilize Heterodera avenae larvae resulted in about 75% recovery of treated larvae in a preliminary trial. Contamination of cultures of nematode inoculated cultures did not differ from controls, (about 10%), a testimony to the adequacy of the method.

The impact of infestation on root growth observed in this study, namely reduced main axis extension, inhibition of lateral root outgrowth and possibly inhibition of lateral root initiation should be compared with reports of effects of elevated levels of ABA and Ethylene on intact roots. Wightman et al. (1980) reported that ABA (10^{-6}M) inhibited lateral root emergence but had no effects on primordia induction except at much higher concentrations (10^{-3} M). Significant inhibition of lateral root induction was observed at 2×10^{-7}M ABA on excised pea roots (Bottger 1974). Ethylene is known to be a strong inhibitor of lateral root outgrowth with little or no effect on primordial initiation (Batten and Mullins 1978, Drew et al. 1981).

ABA and ethylene concentrations measured in the present study were in close agreement with other studies. The concentration of endogenous ABA in a root has been found to vary from around 0.4 $\mu g.g^{-1}$ dry weight (DW) in decapitated pea plants (Bottger 1978) to about 1.1 $\mu g.g^{-1}$ DW in root cultures on Agar (Hartung and Abou-Mandour (1980) and to as high as 2.2 $\mu g.g^{-1}$ DW on sunflower and Sycamore roots (Cohen et al. 1978).

Ethylene production by intact unstressed seedlings of a range of plant species varied between 2 to 6.5 $nlg^{-1}h^{-1}$ (Konings and Jackson 1979). Ethylene production rose from 1.0 $nlg^{-1}h^{-1}$ up to 2.5 $nlg^{-1}h^{-1}$ following Meloidogyne infestation of root cultures of tomato plants (Orion et al. 1983).

Inconsistencies were found in the results of this study compared with results in Section 2. No differences in growth between the two cultivars were observed in either infested or control plants in this study, unlike that in Section 2. Either root excision and/or aseptic root culture rendered NZC more sensitive to nematode attack, or else Sual was made less sensitive. The question arose whether similar amounts of ethylene and ABA detected in both NZC and Sual may have been an artefact of the root culture technique just as growth patterns of Sual and NZC were identical. If so, amounts of ABA and/or ethylene given off by NZC in response to nematodes in intact plants may be less than by Sual.

In conclusion there is a strong indication that changing patterns of root growth following nematode invasion are consequential to nematode-induced increases in endogenous ABA and ethylene concentrations. Although the present experiment uncovered no differences in response of a tolerant and intolerant cultivar this may have been due to the special circumstances of axenic culture.

3.2 Regulation of Root Growth of the Intact Plant

3.2.1 Introduction

In Section 3.1 it was found that rates of root extension and lateral root outgrowth did not differ significantly between aseptically cultured root apices of the nematode tolerant cultivar NZC and the intolerant cultivar Sual, regardless of their infestation status. These findings did not agree with those reported in Section 2 in which it was observed that root growth of NZC was less affected by nematodes than was Sual.

No significant differences in production of ethylene or concentration of abscisic acid was observed between the two cultivars although amounts detected were significantly higher in infested roots compared to controls.

The contradictory results obtained in Section 3.1 and Section 2 may have been due to the shoot: shoot-derived factors possibly accounting for the better growth of NZC than Sual in response to infestation.

The following study was conducted to determine if differences in root growth response to infestation between intact plants having contrasting tolerance levels to Heterodera avenae are due to differences in production of ABA and/or ethylene before or after infestation.

3.2.2 Methods

Seedling preparation was carried out as outlined in Section 3.2.1 on the two oat cultivars Sual and NZC. Between 36 and 48 hours after germination at 20°C coleoptiles 15 mm long were directed bottom upward through 2 mm holes drilled into 2.4 cm rubber suba-seal caps. Caps with seedlings were fitted onto 2.4 cm (ID) X 110.0 cm open-ended plastic tubes, lined with plastic bags of similar dimensions. Bags had been filled with steam sterilized sandy loam as described in Chapter 4, Section 2 but minus John Innes nutrients (Fig. 3.1). Three holes about 3.0 mm diameter were made at the bottom of the planting tubes to assist drainage. Seedlings received 10 ml of half strength nutrient solution (Hoagland and Arnon 1950) at planting. The plants were grown in a cabinet in conditions previously described (Chapter 3.2.3.1). One day after planting, second stage Heterodera avenae larvae cultured as described in Chapter 3, 2.4.1 and

subsequently surface sterilized (Chapter 3.2.4.2) were applied to the surface of the soil at a density of 500 and 1500 larvae per plant in 1 ml aliquots through a slit in the suba seal. The hole was self-sealing. Controls received no larvae.

Every three days, over the course of the next 15 days, plants were sampled in the following manner: plants were lifted from the plastic tubes by extracting the plastic bags. The bags were opened using a sterile scalpel and roots were rinsed free of soil under a gentle stream of deionized water. The root system of the intact plant, still attached to the suba-seal, was then inserted into a dark glass specimen tube 2.35 (ID) X 9.6 cm, into which had been laid a sterile agar slant containing nutrients at the following concentrations (μM): KNO_3, 200;p $CaNO_3$, 200; $Mg50_4$, 100; KH_2PO_4, 50; MICRO nutrients 0.25 strength Hoagland and Arnon (1950), and Fe NaEDTA. Tubes were tightly sealed by inserting the suba-seal into the specimen tube, then returned to the growth cabinet where they were placed at a 45 degree angle to facilitate contact of the root with the agar. Ethylene samples were taken from the test tube using a syringe at three 18 hour intervals and ethylene concentration was estimated (Chapter 3.2.6). Means of the three readings were calculated. After each sampling fresh air, sterilized by passing through conc. H_2SO_4, conc NaOH, sterile water and 2 cotton filters, was introduced into the specimen tube through a syringe after first removing an equivalent amount of air. Ethylene samples were replicated 6 times.

After each sampling period plants were divided into blocks of three and six; the first block was set aside for measurement of root infestation, length and number (Chapter 3.2.5). ABA determinations were performed on the second block (Chapter 3, 2.7) after roots were separated from their stems using a sterile scalpel, snap frozen in liquid N_2 and freeze dried.

3.2.3 Results

Infestation

No significant differences between Sual and NZC were observed in the number of larvae in roots during the three sample dates (Table 3.8). Larval numbers within the root increased with time and density.

Table 3.8: Effect of Initial Larval Density on Number of Larvae in Root Systems of Two Oat Cultivars 2, 8 and 14 Days After Inoculation

		NEMATODE NUMBER		
CULTIVAR	DENSITY	DAYS AFTER INOCULATION		
		2	8	14
NZC	0	-	-	-
	500	31	74	93
	1500	81	207	241
SUAL	0	-	-	-
	500	46	86	102
	1500	69	218	267

No significant differences between cultivars within densities ($P = 0.05$)

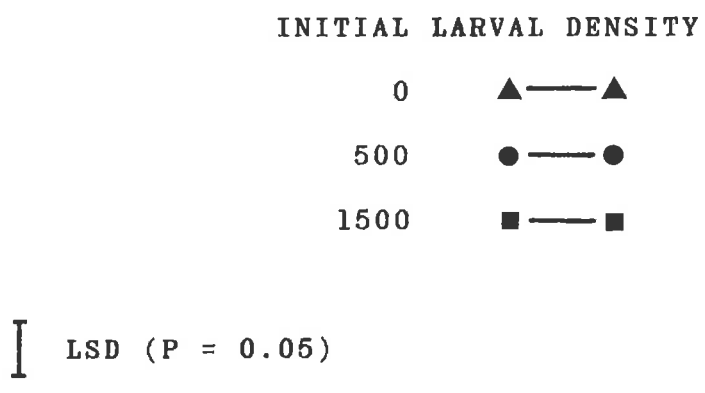
Root Growth

Fig. 3.1 shows the effect of density on total seminal root length over time. Seminal root lengths of Sual and Swan were reduced with increasing nematode density, but only Sual was significantly affected ($P = 0.01$) at the lowest inoculum density.

Total lateral root length and number were reduced by infestation with nematode effects becoming more noticable with time (Fig. 3.2) but there were cultivar differences: root length of Sual was significantly ($P = 0.05$) reduced at a density of 500 larvae, whereas NZC was not affected except at the highest density (analysis of Ln transformed data). Both cultivars showed a significant decline in lateral root number at both initial nematode densities, though Sual's decline was larger.

First order lateral root number per unit seminal root length on Sual was significantly greater than controls at the highest nematode density (Table 3.9). This was offset by a reduction ($P = 0.01$) in the unit lateral root length. (Total length of first and higher order lateral roots arising from a first order lateral root).

FIG 3.1 (a,b.) The effect of *Heterodera avenae* infestation on total seminal root length (~~Log~~ $\ln$ (root length) of a) NZC and b) Sual 3 - 15 days after planting (plants inoculated one day after planting)



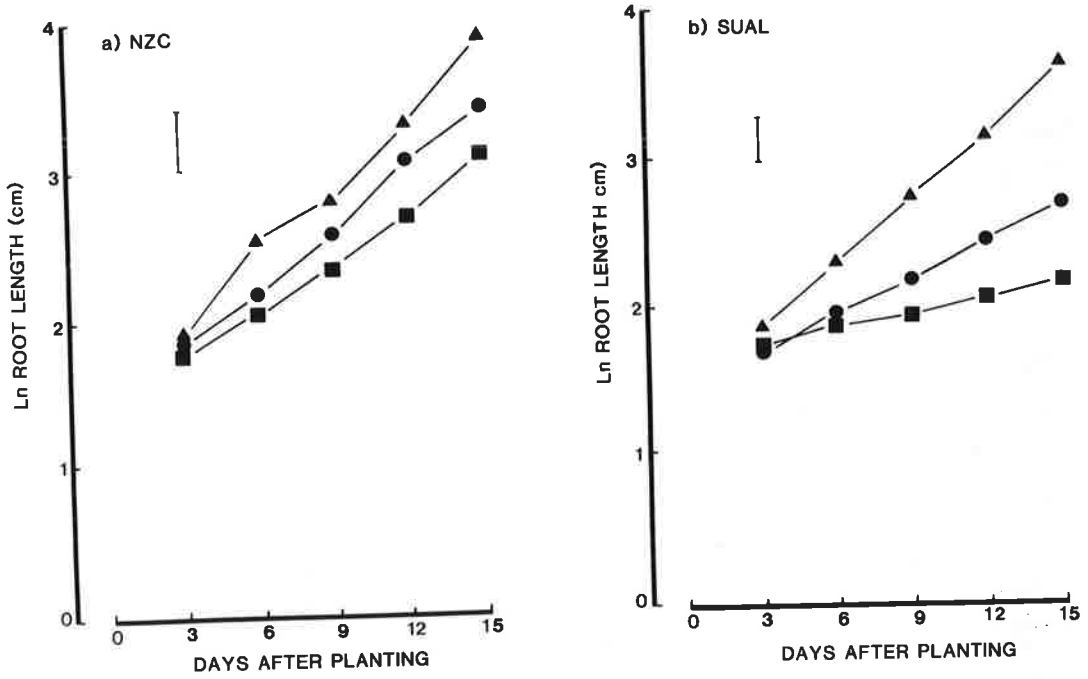
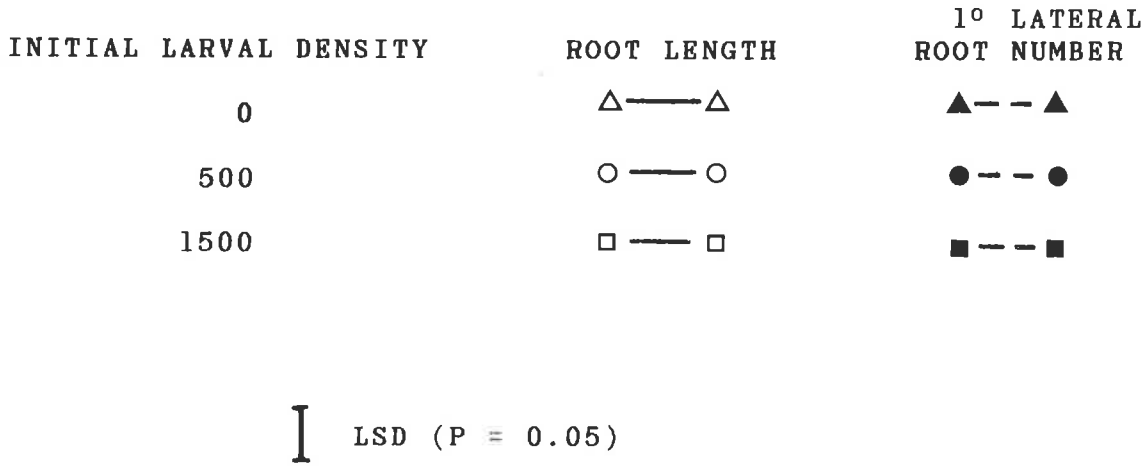

a) NZC
b) SUAL
Ln ROOT LENGTH (cm)
Ln ROOT LENGTH cm)
DAYS AFTER PLANTING
DAYS AFTER PLANTING
0
1
2
3
4
0
3
6
9
12
15

FIG 3.2 (a,b.) The effect of *Heterodera avenae* density on total lateral root length (~~Loge~~ ln root length) and the number of first order lateral roots on a) NZ and b) Sual 6 - 15 days after planting



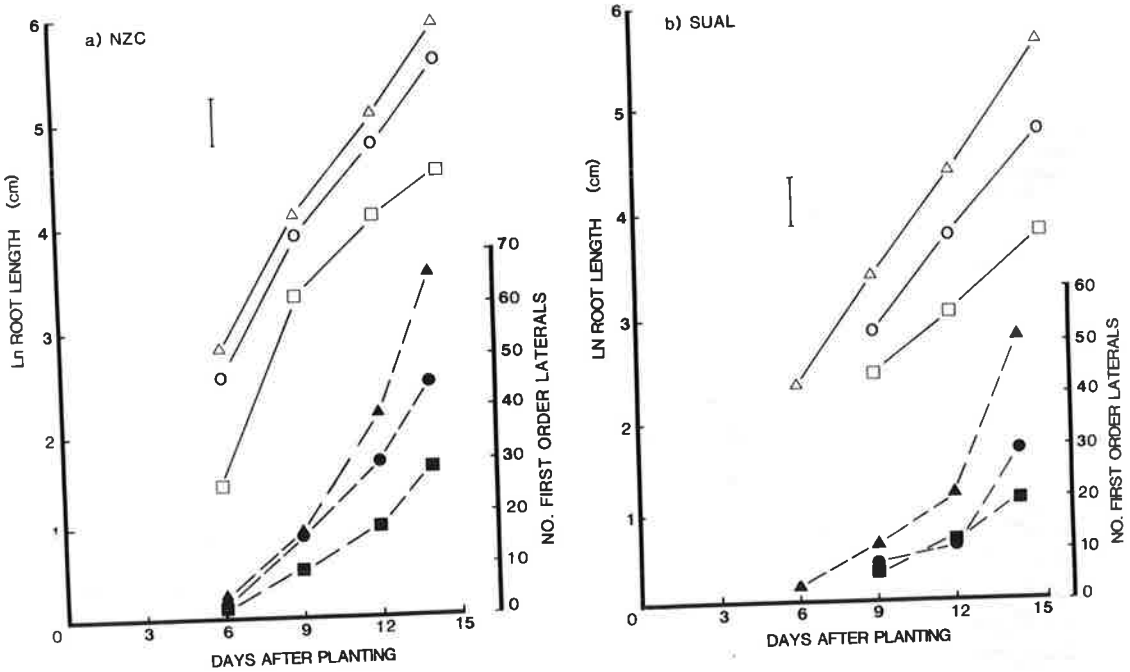

a) NZC
Ln ROOT LENGTH (cm)
NO. FIRST ORDER LATERALS
DAYS AFTER PLANTING
b) SUAL
LN ROOT LENGTH (cm)
NO. FIRST ORDER LATERALS
DAYS AFTER PLANTING

Table 3.9: Effects of Heterodera avenae on Unit Lateral Root Number (No. First Order Laterals per cm Seminal Root Length) and Unit Lateral Root Length (Total Length of 1st and 2nd Order Laterals Per Primary Lateral Root) of Two Oat Cultivars at the End of the Trial Period

		CULTIVAR	
	DENSITY	NZC	SUAL
Unit Lat. Root No. ($No.\ cm^{-1}$)	0	1.51	1.44
	500	1.25	2.02
	1500	1.25	2.30*(159)
Unit Lat. Root Length (cm per 1° Root)	0	5.57	5.21
	500	6.14	4.10*(78)
	1500	3.17*(56)	2.23**(42)

*,** significantly different from control, P = 0.05, P = 0.01, respectively.

( ) percent uninoculated control.

Ethylene and ABA Production

Infestation significantly (P = 0.01) increased the levels of ABA and ethylene (Tables 3.10 and 3.11). Differences between controls and infested plants diminished with time. Differences in hormone levels between initial densities of 500 and 1500 nematodes were not significant, nor were cultivar differences.

Table 3.10: Effect of Density of Heterodera avenae on Level of ABA in Roots of Two Oat Cultivars

CULTIVAR	NEMATODE DENSITY	ABA CONCENTRATION ($\mu g/g^{-1}$.D.W				
		DAYS AFTER SOWING				
		3[a]	6	9	12	15
NZC	0	0.102	0.144	0.192	0.156	0.174
	500	0.822	0.234	0.312	0.246	0.258
	1500	0.644	0.564	0.516	0.446	0.458
		0.523	0.314	0.340	0.283	0.295
SUAL	0	0.090	0.186	0.120	0.211	0.069
	500	0.318	0.396	0.216	0.054	0.114
	1500	0.773	0.414	0.408	0.421	0.378
		0.394	0.332	0.248	0.229	0.187
MEAN (DENSITY X DAY)		0.096	0.166	0.156	0.184	0.122
		0.570	0.315	0.264	0.150	0.186
		0.709	0.489	0.462	0.434	0.418

ANALYSIS LSD (P = 0.05; P ≤ 0.01,**)

SOURCE OF VARIATION	
Cultivar	NS
Density	0.0252**
Day	0.0311*
Cultivar X Density	NS
Cultivar X Day	NS
Density X Day	0.0558*
Cultivar X Density X Day	NS

Table 3.11: Effect of Density of *Heterodera avenae* on Ethylene Production by Roots of Two Oat Cultivars

CULTIVAR	NEMATODE DENSITY	ETHYLENE PRODUCTION ($nlg^{-1}h^{-1}$) DAYS AFTER SOWING				
		3[a]	6	9	12	15
NZC	0	1.12	1.47	1.03	0.86	1.27
	500	4.27	1.32	1.61	1.21	1.72
	1500	4.41	2.53	2.89	1.64	2.06
	MEAN	3.26	1.77	1.84	1.24	1.68
SUAL	0	0.89	0.67	1.04	1.16	0.78
	500	2.19	1.55	1.83	1.19	1.12
	1500	3.68	2.72	1.97	1.13	1.36
	MEAN	2.25	1.65	1.61	1.16	1.08
	MEAN	1.05	1.07	1.04	1.01	1.03
	(DENSITY	3.23	1.44	1.72	1.20	1.42
	x AGE)	4.05	2.63	2.43	1.39	1.71

ANALYSIS -

SOURCE OF VARIATION	LSD (P = 0.05,*; P ≤ 0.01,**)
Cultivar	NS
Density	0.146**
Age	0.183*
Cultivar X Age	NS
Cultivar X Density	NS
Density X Age	0.374*
Cultivar X /Density X Age	NS

3.2.4 Discussion

More nematodes invaded and became established in the roots per unit initial inoculum density than observed in Section 2 possibly because of the longer exposure period. This was supported by the rise with time in larval population retrieved from the root.

Root growth in this study was considerably reduced compared to that observed in Section 2 in which roots grew for an extended period of time with little or no impedance. Longer exposure time to nematode invasion and higher initial densities are the two factors probably accounting for the very large reduction in root length at the highest initial nematode density. At lower densities the duration of exposure had little or no impact on the relative decline in root length. This was indicated by similar percentage reductions in root length for each cultivar at 400 (short duration) or 500 (long duration) larvae per plant in Section 2 (Table 2.7) and in this study, respectively.
This highlighted the significance of the effect of initial invasion on subsequent root development. The increase in the number of first order laterals per unit seminal root length and the reduced total lateral root length per first order lateral on the cultivar Sual at the highest density relative to controls suggested that infestation had little inhibitory effect on lateral root formation but significantly slowed their extension.

Higher levels of ethylene evolution in this study were associated with nematode infestation and reduced root elongation. These findings support those of the previous study (Section 3.1). The very early rise in ethylene production observed in the present study resembled that elicited by that of mechanical injury (McGlasson and Pratt 1964; Saltveit and Dilley 1978) or pathogen invasion (Lund and Mapson 1970; Nakagaki et al. 1970). Though these results suggested a relationship between nematode invasion, ethylene and ABA release and reduced root elongation a causal association could not be inferred on the basis of these results alone. There was reason to question the involvement of these two hormones in the differences in response of the root systems of the two cultivars to nematode infestation. Despite significant differences between cultivars in root extension and lateral root development following infestation, no clear differences in levels of ethylene or of ABA production were observed.

It is possible that the sensitivity of Sual to a given concentration of ethylene was greater than that of NZC, resulting in a larger decline in root growth of that cultivar.

The results of this study provided further evidence of an association between altered root development patterns of nematode infested plants and nematode induced stimulation of ABA and ethylene synthesis and/or release. There is still

no reason to relate the differential response of NZC and Sual to infestation to differing levels of ABA or Ethylene production.

3.3 Response of Root Segments to ABA and Ethylene

3.3.1 Introduction

The previous study showed that differences between cultivars in the proportional decline in root growth in response to nematode infestation were not necessarily related to differences in the rate of ethylene or ABA production. The greater decline in root growth by Sual following nematode infestation compared to NZC may have been due to a higher sensitivity to given concentrations of these two plant growth regulators. The following study tested this hypothesis.

3.3.2 Methods

Experiments were conducted on seedlings of oats (cvs. Sual and NZC). Seed pregermination procedures were as outlined in methods Chapter 3.2.1. When root radical length was 3 cm, one cm long root segments were cut 3 mm behind the tip of the main root. For growth experiments using ABA 10 segments were placed in 50 ml erlenmeyer flasks containing 10 ml of a nutrient solution [1 mM $Ca(NO_3)_2$, 0.5 mM KCl, 0.5 mM $MgSO_4$, 0.5 mM Na_2 $H.PO_4/NaH_2PO_4$, pH 5.6] with or without ABA and shaken gently in the dark at 22°C for 20 hours. At the end of the incubation time the length of segments was recorded. Treatments were replicated 6 - 8 times.

In experiments to examine the effect of ethylene on root elongation ten 1 cm long root segments were incubated in 50 mm plastic petri dishes containing the nutrient solution described above but minus ABA.

Uncovered dishes were placed inside a perspex chamber into which a stream of air containing ethylene at known concentrations was supplied (flow rate 1.0 l min^{-1}). The assembly was enclosed in black plastic and maintained at 22°C for 20 hours after which time the length of segments was recorded. Each treatment was repeated six times.

3.3.3 Results

Growth of excised root segments was stimulated significantly (P = 0.05) by low concentrations of ABA (+24% NZC, + 29% Sual; optimum concentration 10^{-7}M) (Fig 3.3, Table 3.12). Only the root segment length of Sual was significantly increased by ethylene (+18%, concentration 0.05 ppm) although differences between cultivars were small (Fig 3.4, Table 3.12). Root segment extension was inhibited at higher concentrations of ABA and ethylene.

Table 3.12: The Effect of ABA and Ethylene on Growth (% of Controls) of Apical Oat (CVS, Sual and NZC) Root Segments After 24 Hours

		ROOT ELONGATION (% OF CONTROLS)*	
HORMONE	APPLIED CONC.	NZC	SUAL
ABA	0 M	100	100
	10^{-8}	102	105
	10^{-7}	124*	129*
	10^{-6}	111	127*
	10^{-5}	88	97
	10^{-4}	79*	82*
ETHYLENE	0 ppm	100	100
	0.01	97	106
	0.05	108	118*
	0.15	113	111
	1.0	88	84
	10.0	67*	72*

Treatment means were not significantly different between cultivars (P = 0.05)

Significantly different from controls (P= 0.05,*)

3.3.4 Discussion

This study confirmed work of other studies reporting both stimulatory and inhibitory effects on root segment elongation of ABA and ethylene. Abou-Mandour and Hartung (1980) and Pilet (1983) found optimum ABA concentrations at 10^{-7} and 10^{-8}M ABA respectively. Ethylene concentrations were reported as optimal for rice and tomato at 0.15 and 0.02 ppm respectively (Konings and Jackson 1979). These workers observed that plant species having rapid rates of elongation had higher rates of ethylene production than slower growing species. It has already been observed that the rate of root extension of Sual lagged behind that of NZC. The previous study showed that both ABA and ethylene concentrations of NZC tended to be higher than those of Sual (Section 3.2), though not significantly so supporting the observations of Konings and Jackson (1979). These workers also noted that greater sensitivity to low ethylene concentration was associated with low endogenous ethylene production. They suggested that under conditions which resulted in a build up of high ethylene concentrations, plants with greatest ethylene sensitivity would suffer the most from the inhibitory effects of ethylene on root growth. Sual had a greater sensitivity to ethylene than NZC, though differences were small. The greater decline in root growth of Sual compared to NZC following infestation may therefore have been in part due to the effects of nematode invasion on ethylene and perhaps ABA production.

However, in conclusion, it seems unlikely that the differences in growth response between Sual and NZC to nematode infestation observed previously can be attributed to a difference in sensitivity to these two growth regulators.

SECTION 4: THE INFLUENCE OF *Heterodera avenae* INFESTATION ON THE WATER RELATIONS OF TOLERANT AND INTOLERANT OAT PLANTS

4.1 Root Growth and Water Stress

4.1.1 Introduction

Root systems reduced in size by *Heterodera avenae* infestation may suffer the effects of mild water stress more acutely than healthy plants (eg. Kerry and Jenkinson 1976). Several studies have associated increased nematode tolerance with properties of the plant that either enhance water acquisition (Evans *et al*. 1977) or reduce water loss (Kaplan *et al*. 1976; Evans and Franco 1979; Evans 1982; Fatemy *et al*. 1985). On the other hand at least one study has failed to find any effect of water stress on tolerance to *Heterodera avenae* (Seinhorst 1981).

Information is required on the effect of infestation on plant water status. The aim of the following study was twofold: first, to test the hypothesis that infestation at levels above and below that sufficient to induce root stunting has no effect on the plant water status of oat seedlings; secondly, that infestation has no influence on the plant water status of seedlings subjected to short term water stress. If the above hypotheses are correct then it is unlikely that tolerance to *Heterodera avenae* is influenced by water stress within the parameters chosen in this study.

4.1.2 Methods

Two oat cultivars, NZC and Sual were germinated as described in Chapter 3, 2.1 on agar plates. When the shortest of the three seminal roots was 2 cm long, nematode suspensions (0.25 ml) were pipetted onto 0.6 g preautoclaved sand mounds (grain size $\leq$ 250) placed at the apex of all three seminal roots. Nematode densities were 100 and 300 larvae per root tip. After 3 days incubation in the dark at 10°C seedlings selected for uniformity in growth were tranferred to plastic tubes (5.4 cm ID x 26 cm) containing autoclaved modified inert solid medium ("Turface"). A sufficient volume of one-quarter strength Hoagland's (1950) solution with NaFeEDTA to allow free drainage was supplied at planting and every second day for the first 12 days. After 12 days one-half of control and inoculated plants continued to receive nutrient solution while the other half received none. Measurements of water potential (Ψ_L) of the penultimate leaf, (Chapter 3, 2.10.1), leaf length, shoot mass, root length and number of larvae were made on plants every 2 days during the next 8 days. Plants were arranged in a randomized complete block, with 6 to 8 replicates.

A second experiment was performed in which only the method of plant inoculation was modified. Instead of inoculating seminal root tips on agar, seedlings in which all three seminal roots had emerged were planted into 2.7 x 13.0 cm tubes as described in Section 3, 2.2.1 and inoculated with

250 or 1000 larvae after three days. Six days later seedlings were transplanted as described above into taller tubes filled with Turface.

Analysis of variance was performed on all parameters listed in both experiments.

4.1.3 Results

A sufficient interval (12 days) was allowed before imposing water stress to minimize transplant effects and to enable nematodes to become established.

There were more larvae in roots inoculated with 300 than with 100 larvae (Table. 4.1.1). At the highest density the number of larvae on both NZC and Sual decreased significantly after 8 days of water stress. However infestation did not influence root length of any cultivar.

Table 4.1.1: The Effect of Initial Heterodera avenae Inoculum Density on the Number of Larvae on Roots of Plants Preinoculated and Transplanted into Turface 3 Days Later. Larval Numbers Measured 12 and 20 Days After Transplant (Day 0, Day 8 of Water Deficit Stress)

CULTIVAR	DENSITY	NO. LARVAE	
		DAY 0	DAY 8
NZC	100	3	2
	300	7	4
SUAL	100	2	2
	300	7	5

LSD (P = 0.05) = 2

The method of inoculation was therefore changed in the second study and had the desired effect of inhibiting root development.

Larval Number

Both cultivars had significantly more larvae in the roots inoculated with 1000 than with 250 larvae (Table 4.1.2). There were significantly more larvae in NZC roots after 8 days of water stress than prior to stress at the highest inoculum density.

Table 4.1.2 Effect of Initial *Heterodera avenae* Inoculum Density on the Number of Larvae in Roots Before and After 8 Days of Increasingly Severe Water Deficit Stress

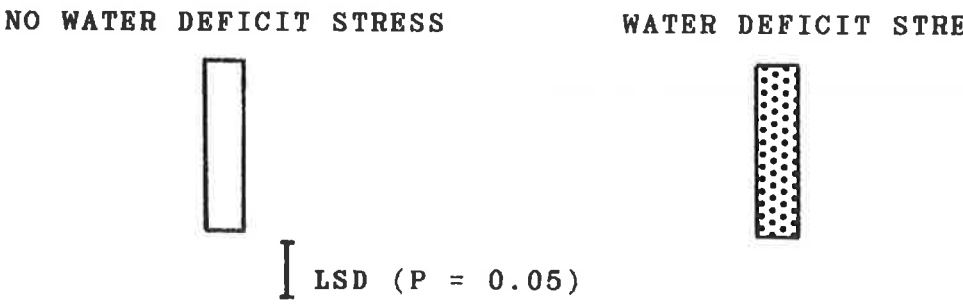
CULTIVAR	INITIAL DENSITY	NO. LARVAE BEFORE STRESS	AFTER STRESS
NZC	250	22	19
	1000	43	56
SUAL	250	18	20
	1000	50	58

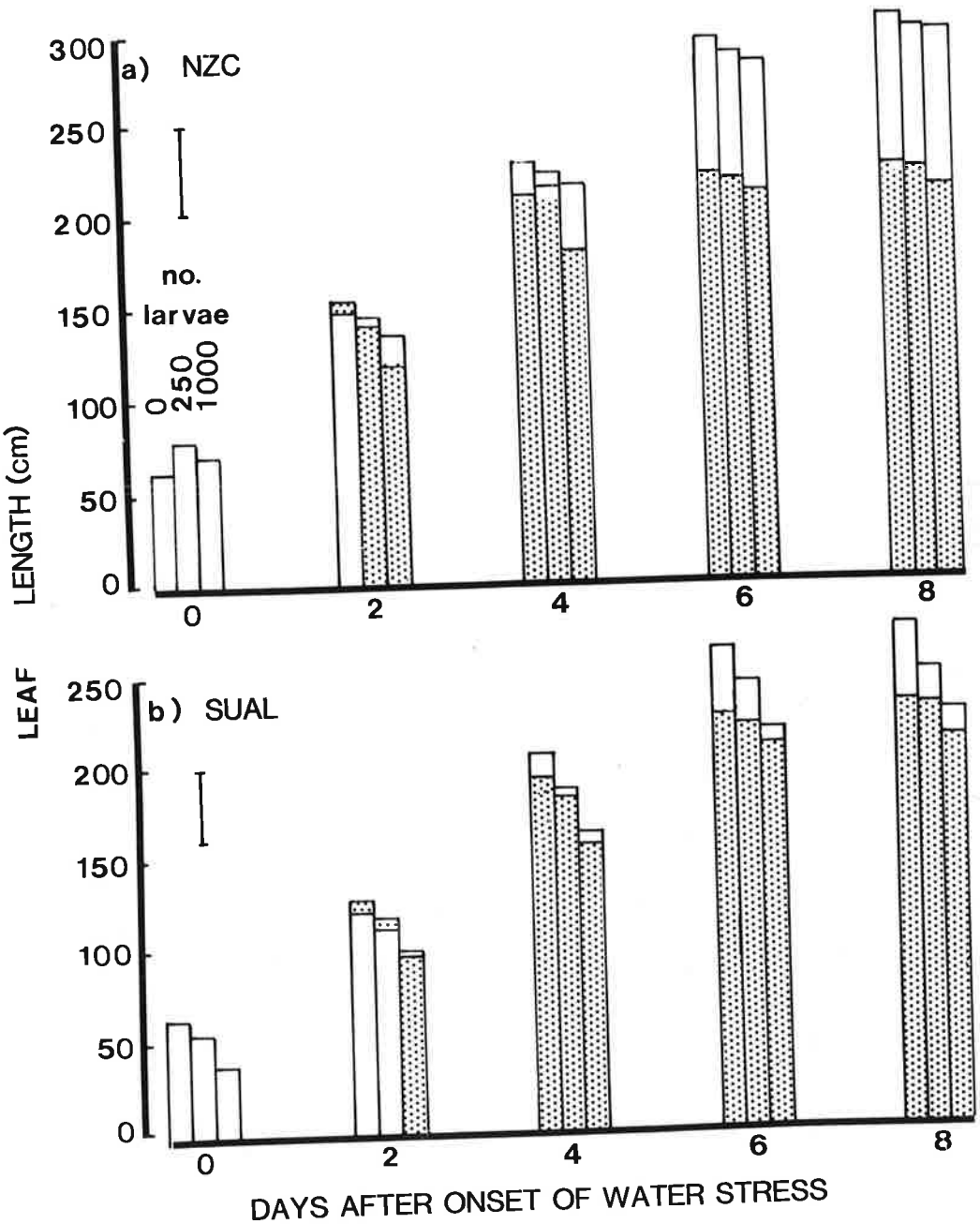
LSD P = 0.05 = 11

Leaf Length

The effect of water stress on length of the third leaf of infested and uninfested oat plants is shown in Fig. 4.1.1. Water stress reduced the length of the third leaf of both control and infested plants equally on NZC. Length of the

FIG 4.1.1 (a,b) The effect of Heterodera avenae infestatio on the length of the third leaf of a) NZC and b) Sual 0 - 8 days after onset of wate stress




a) NZC
300
250
200
150
100
50
0
no. larvae
0
250
1000
LENGTH (cm)
0
2
4
6
8
b) SUAL
LEAF
250
200
150
100
50
0
0
2
4
6
8
DAYS AFTER ONSET OF WATER STRESS

third leaf of NZC was not affected by infestation. Water stress reduced the leaf length of uninfested Sual plants but had no significant effect on the most heavily infested plants. Leaf length of Sual infested plants was significantly shorter than control plants on all sampling days.

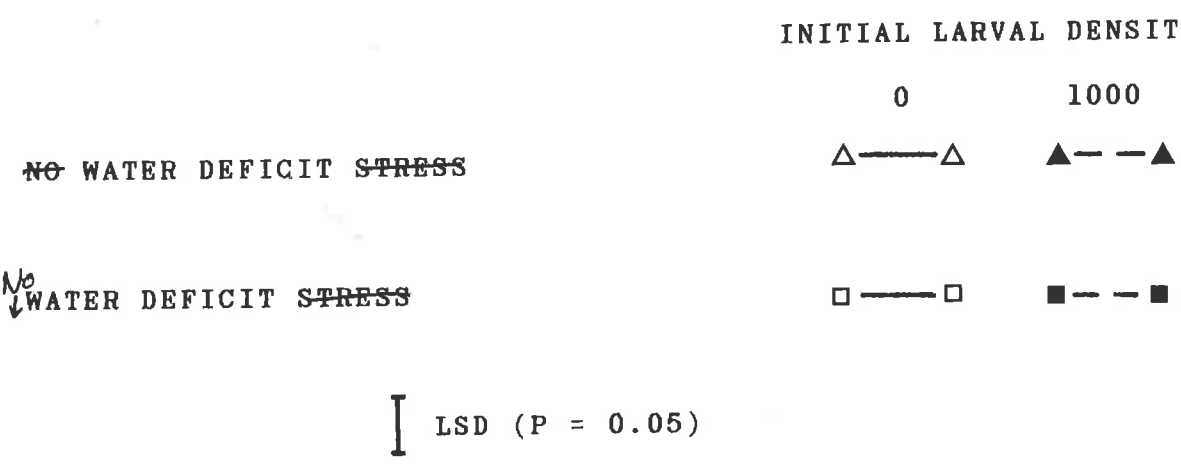
Shoot Mass

Infestation had no effect on shoot mass of either cultivar. Only effects of the highest inoculum density and controls are shown (Fig. 4.1.2). Water stress significantly P = 0.05) reduced shoot mass of NZC by the end of the stress treatment.

Root Length

Total root length of Sual was significantly less (P = 0.01) than that of NZC on all sample days (Fig. 4.1.3). Infestation reduced the root length of Sual but had no significant effect on NZC. Root lengths of plants inoculated with 250 larvae did not differ from that of uninfested control plants and for clarity have been omitted from Fig. 4.3. Water stress enhanced root length of both inoculated and uninoculated cultivars, particularly that of Sual, with the effect being more noticeable with time.

FIG 4.1.2 (a,b) The effect of Heterodera avenae infestation on the shoot mass of a) NZC and b) Sual 0 - 8 days after onset of water stress



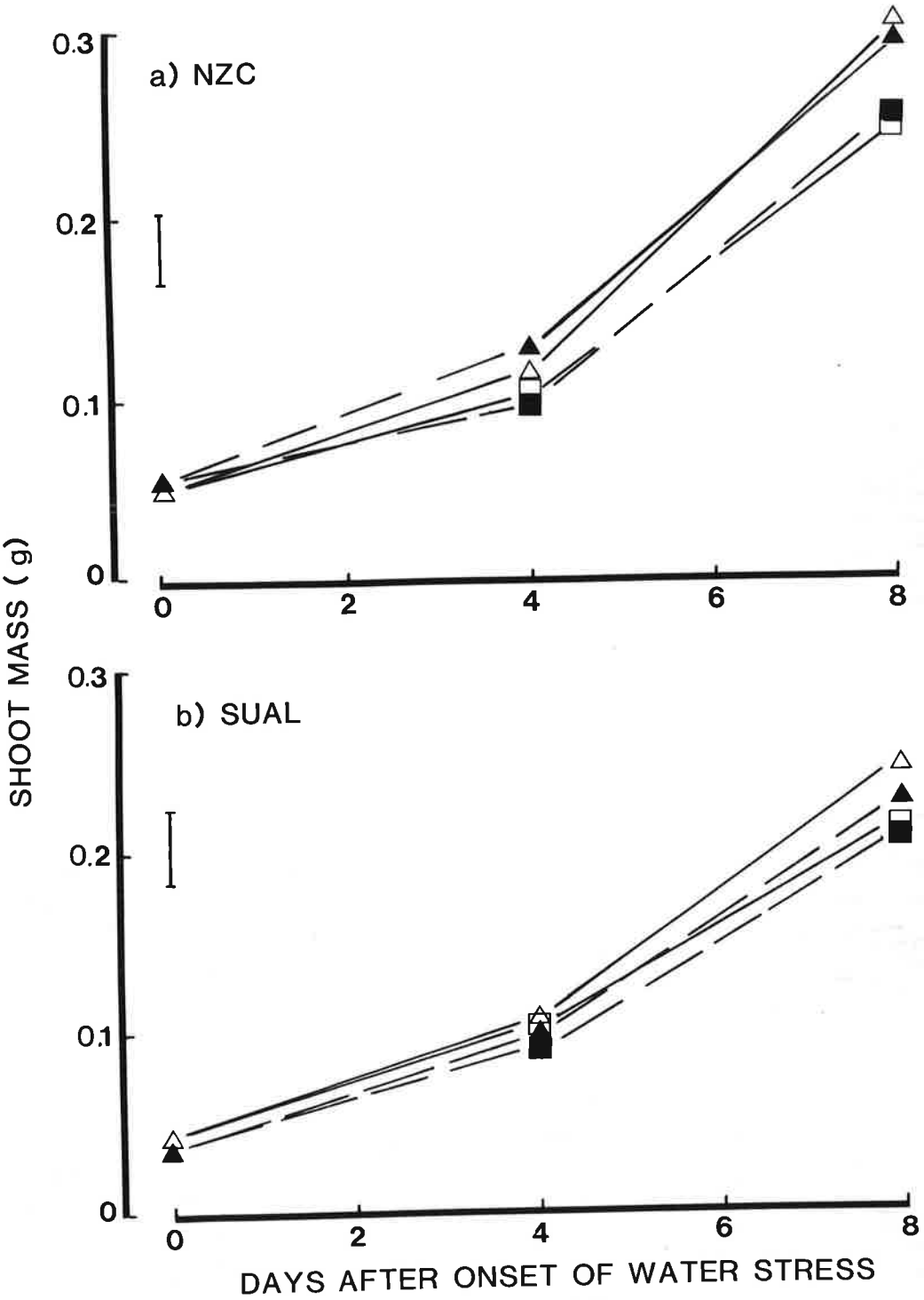
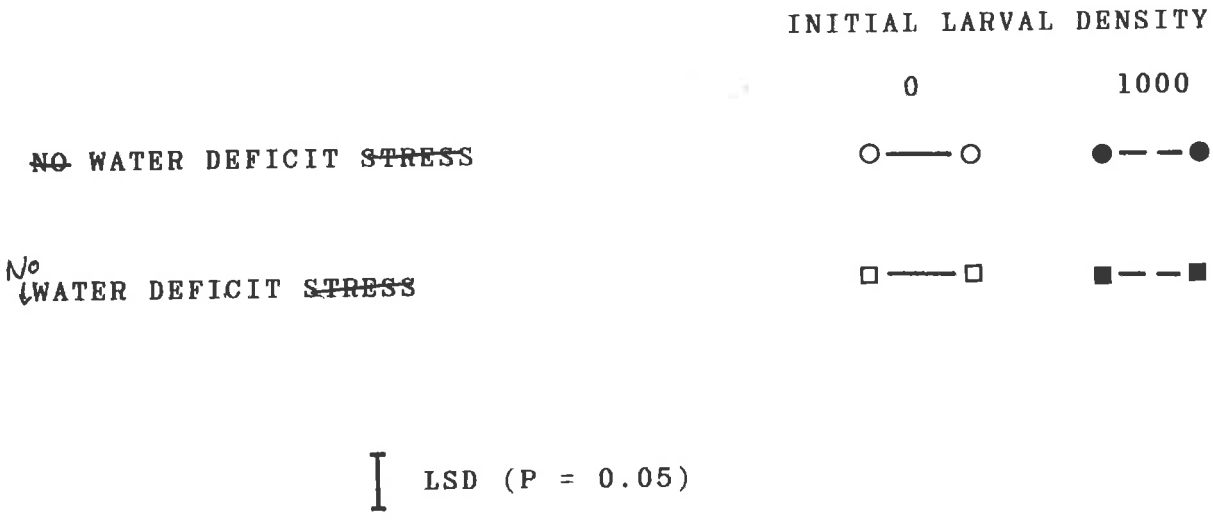
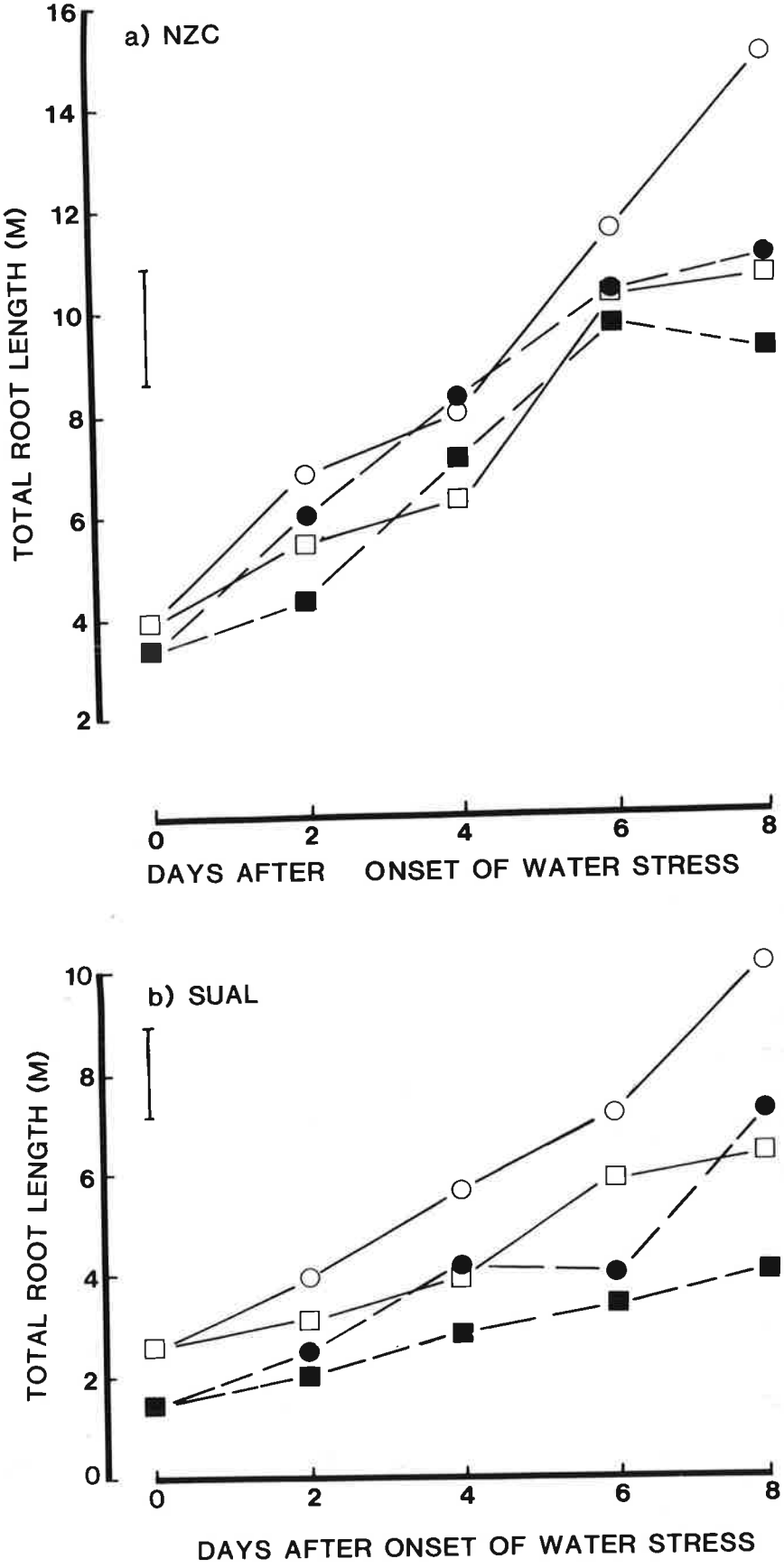

a) NZC
b) SUAL
SHOOT MASS (g)
0.3
0.2
0.1
0
0
2
4
6
8
DAYS AFTER ONSET OF WATER STRESS

FIG 4.1.3 (a,b) The effect of Heterodera avenae infestation on total root length of a) NZC and b) Sual 0 - 8 days after onset of water stress




a) NZC
TOTAL ROOT LENGTH (M)
16
14
12
10
8
6
4
2
0
2
4
6
8
DAYS AFTER ONSET OF WATER STRESS
b) SUAL
TOTAL ROOT LENGTH (M)
10
8
6
4
2
0
2
4
6
8
DAYS AFTER ONSET OF WATER STRESS

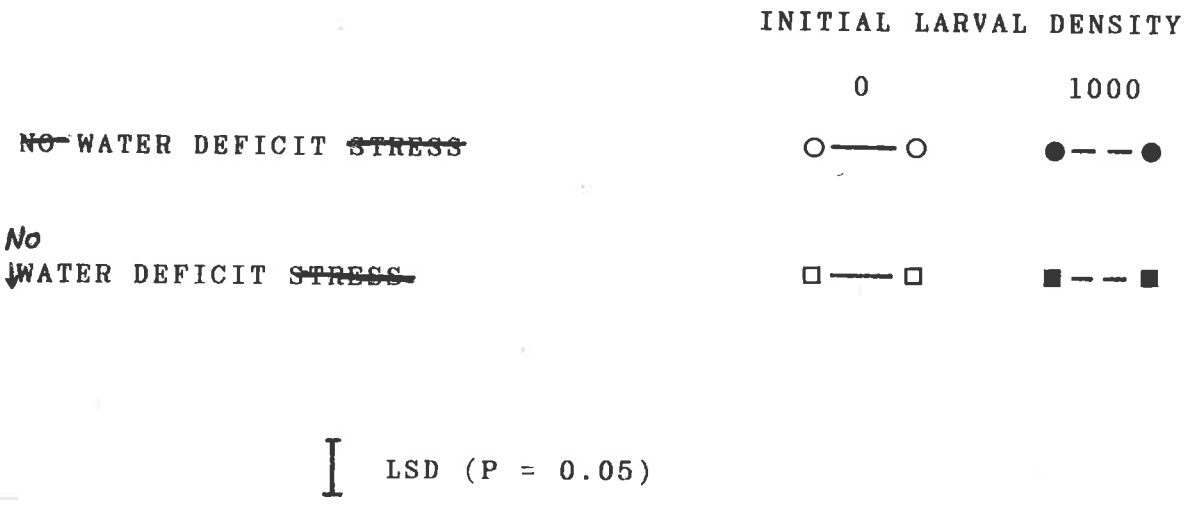
Leaf Water Potential (Ψ_L)

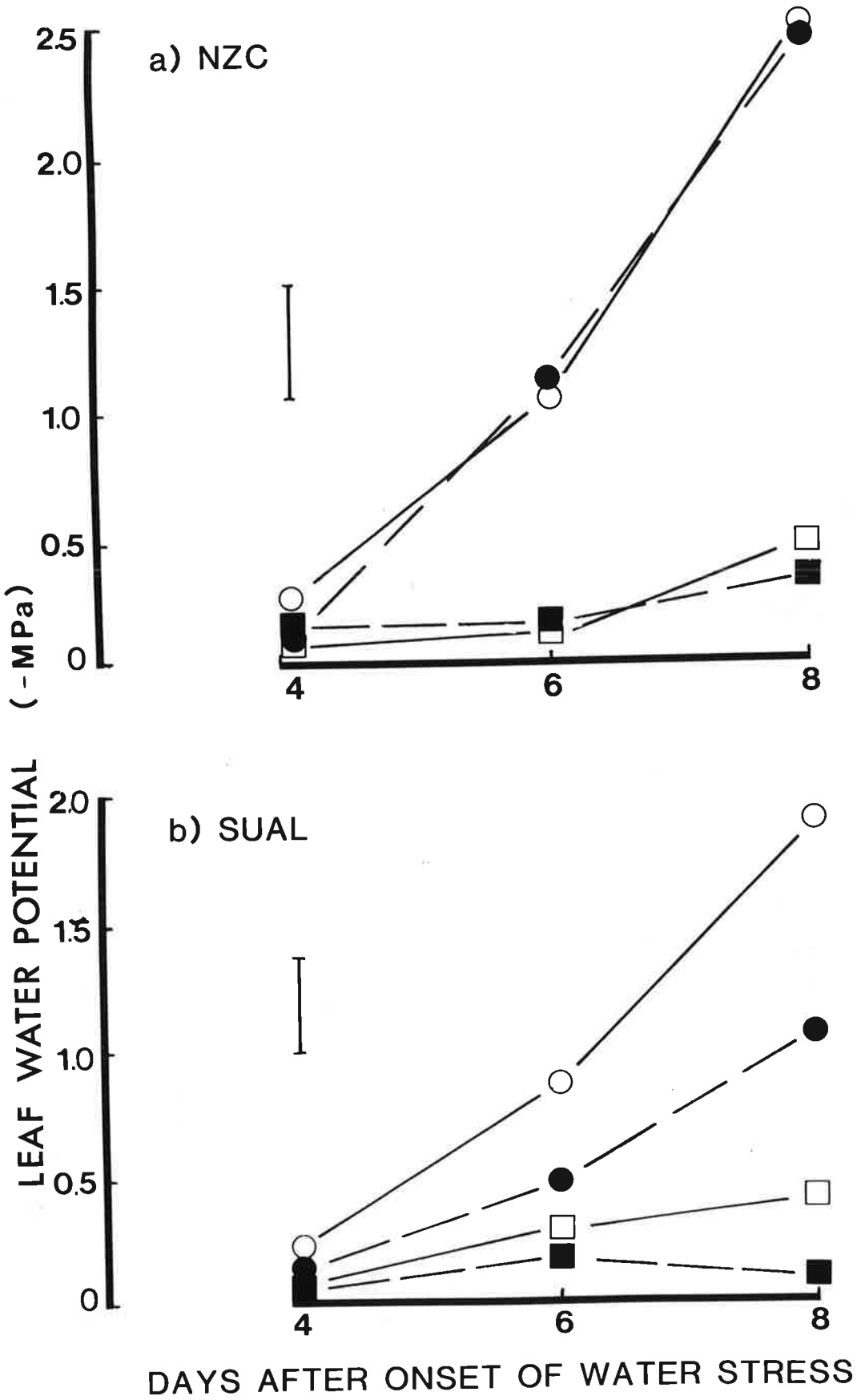
The Ψ_L of NZC declined from about -0.2 to -2.5 MPa during the course of water stress (Fig. 4.1.4). There was a significant three-way interaction between cultivar nematode density and day ($P = 0.01$). Dehydration of leaves of control and infested NZC plants became more severe with time. Infestation of Sual resulted in significantly less dehydration than that of uninfested plants by the eighth day of water stress. Differences between infested and uninfested plants not exposed to water stress were not significant. The intermediate density did not differ from controls with respect to either cultivar and have been omitted.

4.1.4 Discussion

This study demonstrated that root system length can strongly influence the effect of short-term severe water deficit stress on shoot growth of container grown seedlings. The results confirmed findings already reported (Chapter 4, Section 2) that infestation has less impact on root growth of NZC than on Sual. This feature coupled with NZC's larger root system enabled both infested and control plants to extract the limited supply of water remaining in the tubes at a faster rate than did Sual, as indicated by a more rapid decline in Ψ_L following onset of water stress. Water stress was by far the dominant factor limiting leaf growth of NZC. While both control and infested root systems of Sual

FIG 4.1.4 (a,b) The effect of Heterodera avenae infestation on water potential (ψ_L) of the penultimate leaf of a) NZC and b) Sual 0 - 8 days after onset of water deficit stress




a) NZC
2.5
2.0
1.5
1.0
0.5
0
4
6
8
b) SUAL
2.0
1.5
1.0
0.5
0
4
6
8
LEAF WATER POTENTIAL (-MPa)
DAYS AFTER ONSET OF WATER STRESS

apparently depleted water from the tubes at a slower rate than NZC, infested plants must have used significantly less water than control plants, using the more gradual decline in Ψ_L as a reference. The net effect was that by the end of the trial, water stress and infestation individually reduced leaf extension and shoot mass, but in combination water stress contributed only slightly to the overall decline in top growth.

There was no indication that infestation affected water uptake in any way other than by reducing the total root length. This was evident from results of the first study which showed no effect of infestation on growth or water potential of water stressed plants when root length was not significantly affected.

In conclusion the relatively minor effect of nematode infestation on root growth of NZC resulted in more severe growth impairment following rapid onset of water stress compared to Sual. Nematode invasion does not affect the plant water status of well watered plants but can reduce water uptake and thereby facilitate survival of plants under short-term water stress.

4.2 Effects of Heterodera avenae infestation on the Ability of Oat Plants to Extract Water from Soil Zones Differing in Depth

4.2.1 Introduction

The slower rate of growth and the greater sensitivity to nematode infestation of Sual's root system compared to that

of NZC delayed the effects of rapid and severe water stress on plant dehydration and shoot growth (Chapter 4, Section 4.1). Under field conditions the slower growing and ~~greater~~ more nematode-sensitive root system of Sual may be at a disadvantage if unable to extend beyond the zone of water deficiency.

The impact of early drought stress on floral primordium development and subsequent spikelet and grain number on cereals has been reviewed (Salter and Goode 1967; Slatyer 1973). Water stress during inflorescence development has been found to reduce the number of primordia and the development of these into fertile florets (Van der Paauw 1949; Nicholls and May 1963; Aspinall et al. 1964). Early water stress has also been found to reduce the number of grain bearing ears per plant due to suppression of late tillering (Aspinall et al. 1964).

The purpose of the following study was to determine if the effects of drought stress on grain yield would be exacerbated by infestation as a result of the delayed extension of roots from a soil zone deficient in water to one well supplied.

A comparison was made between cultivars having fast growing and slow growing root systems, NZC and Sual, respectively. A comparison was also made between two cultivars having fast growing root systems (NZC and Swan) but varying in their relative sensitivity to root stunting by nematode invasion (NZC, low-, Swan, high-sensitivity).

4.2.2 Methods

Seeds of NZC, Sual and Swan were germinated on agar as previously described (Chapter 3.2.1). Seedlings were selected for uniformity of root emergence and planted into flexible plastic cylinders (2.5 x 5.0 cm) containing John Innes soil. These cylinders had previously been placed into taller rigid plastic tubes (9.0 x 40.0 cm) closed at one end with a water permeable synthetic cloth (love sheet). These were filled with John Innes soil separated about two-thirds (27.0 cm) down the tube with a 5 cm layer of autoclaved crushed gravel ($\geq$ 1 cm $\leq$ 1.5 cm) held in place with plastic mesh. Soil in the lower compartment had a water content just in excess of field capacity (14.0%) at the time of sowing. The soil in the upper section had a water content of 9.5% at planting. Two days after planting seedlings were inoculated with 4000 larvae in 1.0 ml water suspensions. No further water was added to the top compartment during the course of the study. Plants stood in trays and obtained distilled water containing one half strength Hoagland solution (1950) supplied to the base of the pots. Two further treatments were devised. In an arrangement, identical to that described above, basal irrigation was omitted. Water was supplied to the top compartment to maintain a constant soil water content as gauged by decline in pot weight with time. In a third treatment plants went without water from either the base or the top. In the above two trials one-half of the plants received nematodes (4000/plant). Treatments were designated as follows: water supplied only to base, D/W; water supplied to top, W; no water supplied, D.

Plants were grown in a glasshouse (mean air temperature was 22°C ± 5) and arranged in a randomized complete block design. Plants were harvested 14, 24, 40 and 100 days after planting, 4-6 replicates per harvest date. Root length in the upper soil compartment, the crushed gravel zone and the lower soil compartment was measured separately. Yield components and final grain yield were measured on days 40 and 100. Ψ_L of the penultimate leaf on days 13, 23 and 45 was obtained using a Spanner psychrometer (Chapter 3.2.10.1. Stomatal conductivity (G) was assessed from days 10-12 onward every 5 days until day 45 on the distal abaxial and adaxial surfaces of the penultimate leaf (Chapter 3.2.10.5). Stomatal conductances presented are the mean of Ab-and Ad-axial values. Ψ_L and G measurements were replicated 4 and 3 times, respectively. Analysis of variance was performed on growth and yield parameters on individual harvest days. G and Ψ_L values were analysed for differences between treatments over time.

4.2.3 Results

Infestation and Root Length

Cultivars did not differ significantly in nematode numbers in roots sampled 2 days after inoculation (Table 4.2.1). Water treatments also had no effect on infestation up to this date. No other measurements of larvael numbers were taken.

Table 4.2.1: No. of *Heterodera avenae* Larvae in Roots of Plants 22 Days After Inoculation With 4000 Larvae

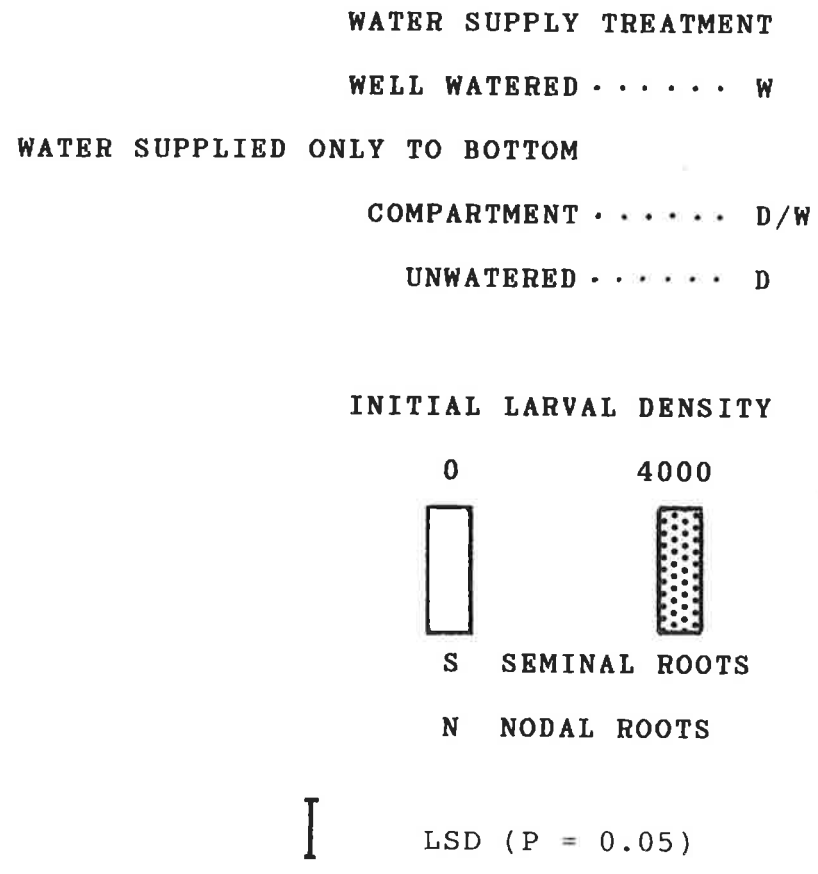
CULTIVAR	LARVAE NO. WATER TREATMENT		
	W	D/W	D
NZC	1929	2241	2128
SUAL	1792	1769	1947
SWAN	1997	2077	2083

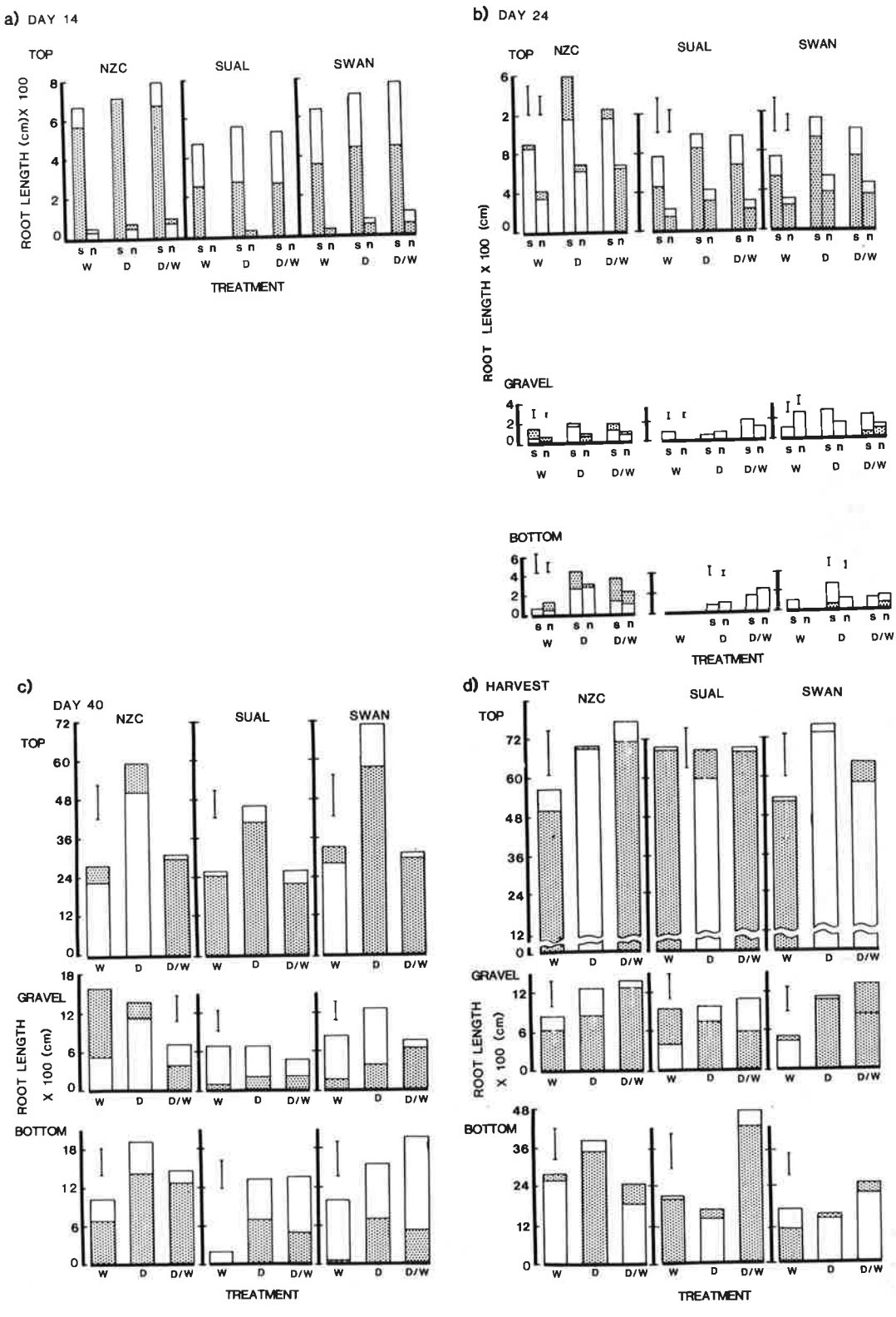
ANALYSIS	LSD (P = 0.05)
CV	394 NS
Water Treatment	418 NS
CV X Water Treatment	622 NS

The effects of water supply treatment and nematode infestation on root growth of NZC, Sual and Swan 14, 24, 40 and 100 days after planting are presented in figs. 4.2.1 a-d. The root systems of all three uninfested cultivars had extended to the bottom soil compartment within 24 days after planting irrespective of water supply treatment.

During the first 40 days of growth seminal and nodal root length (days 14 and 24) and root system length (day 40) of NZC was not reduced by infestation. In contrast seminal root extension within the top soil compartment (day 14) and later, seminal and nodal root growth into the two lower soil compartments (days 24 and 40) of Sual and Swan were significantly impaired by infestation, in all water supply

FIG 4.2.1 (a-d) The effect of Heterodera avenae infestation and water supply treatment on the root length in the top, gravel and bottom soil compartments of NZC, Sual and Swan a) 14, b) 24, c) 40 and d) 100 days after planting




a) DAY 14
TOP
NZC
SUAL
SWAN
ROOT LENGTH (cm)X 100
TREATMENT
b) DAY 24
TOP
NZC
SUAL
SWAN
ROOT LENGTH X 100 (cm)
GRAVEL
BOTTOM
TREATMENT
c)
DAY 40
TOP
NZC
SUAL
SWAN
GRAVEL
ROOT LENGTH X 100 (cm)
BOTTOM
TREATMENT
d) HARVEST
TOP
NZC
SUAL
SWAN
GRAVEL
ROOT LENGTH X 100 (cm)
BOTTOM
TREATMENT

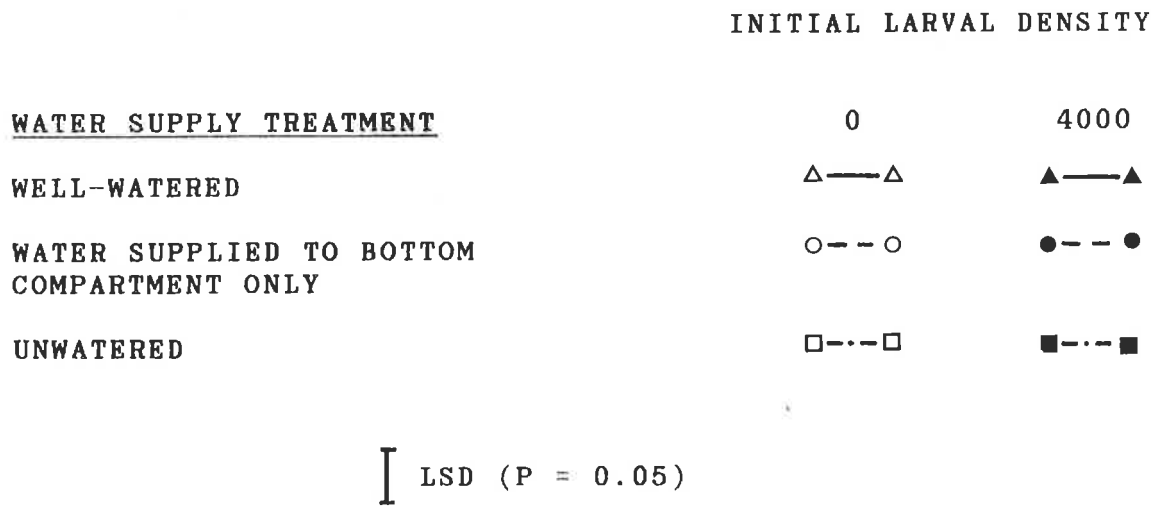
treatments except the D treatment on day 24. After 100 days (harvest) infested and uninfested roots of NZC, Sual and Swan did not differ in length within any of the water supply treatments.

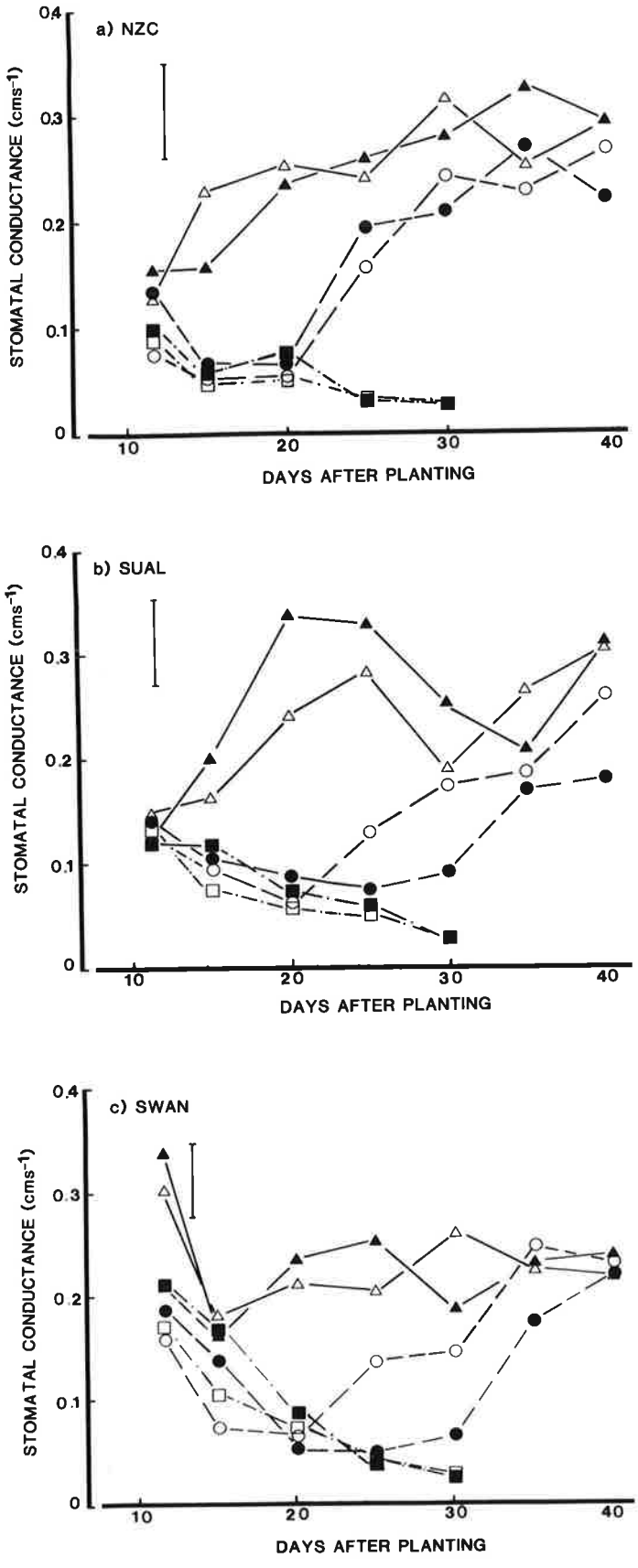
The three cultivars responded similarly to water supply treatment. The dry soil treatment (D) and the mildly water deficit stress treatment (D/W) led to the growth of longer roots than the well-watered treatment (W) within 24 days of planting. After 40 days the D treated plants had considerably longer roots than the D/W and W treatments within the top soil compartment. Longer roots on D treated plants continued until harvest in the case of NZC and Sual, but not in Swan.

Leaf Water Potential (Ψ_L) and Stomatal ~~Conductivity~~ Conductance (G)

Values of G of all three cultivars followed broadly similar patterns (Fig. 4.2.2) and reflected the course of root development. The stomatal conductances (G) of well-watered plants (W) tended to be higher than those of the other two water stress treatments 15 days after planting, and were significantly higher ($P = 0.05$) after a further 5 days.

FIG 4.2.2 (a-c) The effect of Heterodera avenae infestation and water supply treatment on the stomatal conductance of a) NZC, b) Sual and c) Swan during the first 40 days of growth

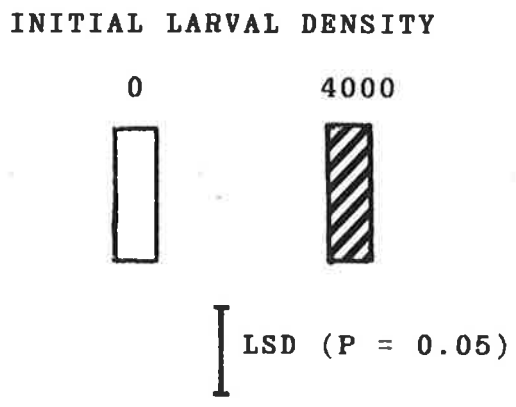


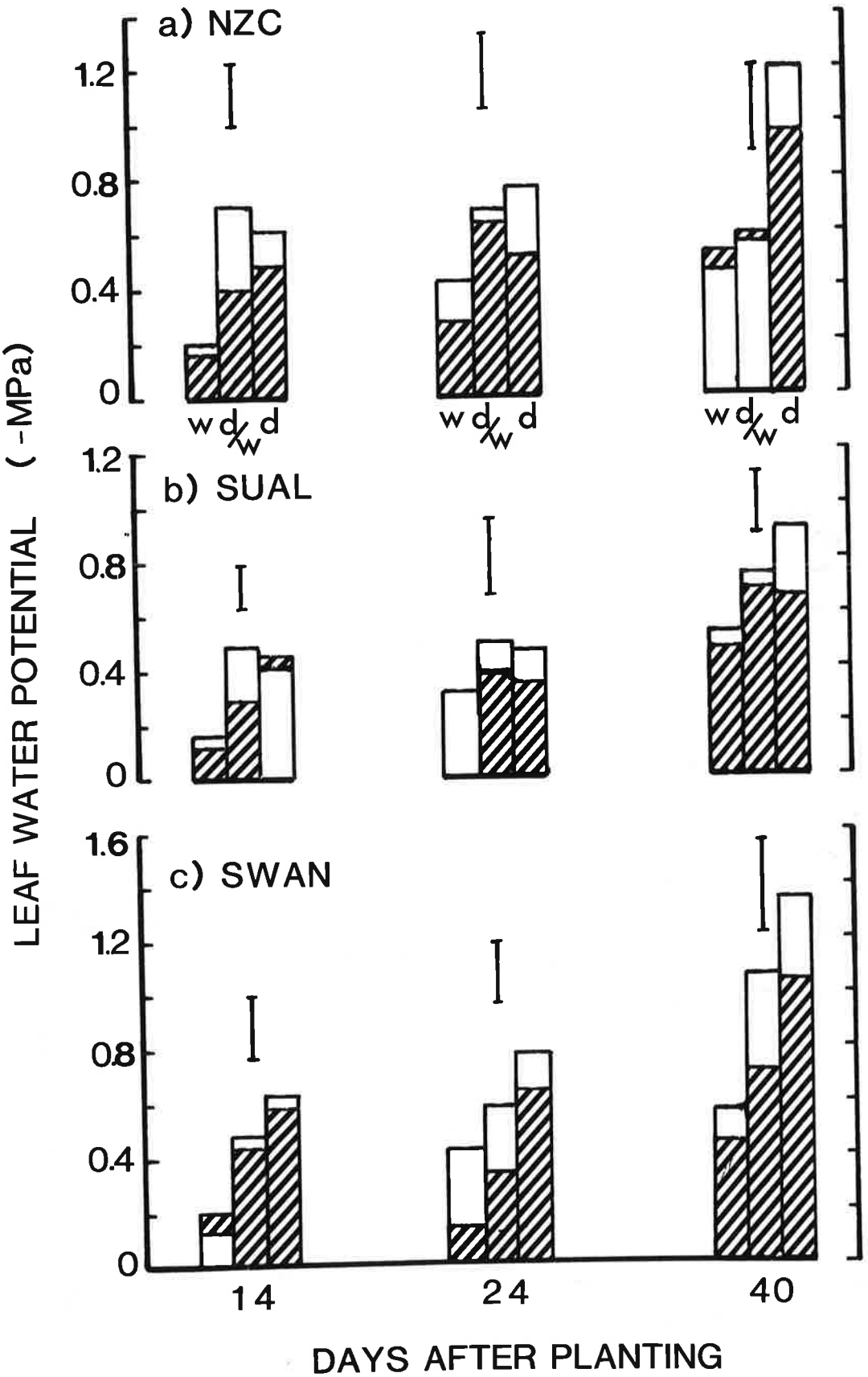

a) NZC
b) SUAL
c) SWAN
STOMATAL CONDUCTANCE (cms⁻¹)
0.4
0.3
0.2
0.1
0
10
20
30
40
DAYS AFTER PLANTING

This was the result of rising (NZC and Sual) or steady (Swan following an initial decline) values of G 10 to 20 days after planting in W-treated plants compared to a decline in the D and D/W plants during the same interval. The D-treated plants never recovered from depressed G values, but those of the D/W-treated plants increased significantly between days 20 to 35, depending on the cultivar and on the presence or absence of infestation. Stomatal conductances of infested and uninfested NZC, treatment D/W, increased significantly between days 20 to 25 and uninfested NZC continued to increase up to day 30. G values of uninfested Sual, treatment D/W, increased significantly from day 20 to day 30 while those of infested Sual, did not differ from day 20 values until a further 15 days. Stomatal conductances of Swan D/W treated plants behaved in a way similar to Sual between 20 and 45 days after planting. From 35 to 45 days after planting, stomatal conductances of infested and uninfested W and D/W treatments did not differ, irrespective of cultivar.

Leaf water potentials (Ψ_L) of the penultimate leaves of each of the cultivars subjected to different water stress treatments are shown in Fig. 4.2.3. Ψ_L values of plants subjected to water stress (D/W and D) were significantly lower 13 days after planting than well watered plants. Infestation had no effect on Ψ_L of well-watered NZC and Sual over the entire measurement interval, whereas infestation reduced Ψ_L

FIG 4.2.3 (a-c) The effect of Heterodera avenae infestation and water supply treatment on the leaf wate potential of a) NZC, b) Sual and c) Swan 13 24 and 40 days after planting




a) NZC
b) SUAL
c) SWAN
1.2
0.8
0.4
0
1.6
w d/w d
14
24
40
LEAF WATER POTENTIAL (-MPa)
DAYS AFTER PLANTING

of Swan on day 23. Differences between uninfested and infested NZC and Sual plants within and between D/W and D treatments were not significant 13 and 23 days after planting but D treatment values of NZC were significantly lower than D/W and W values 45 days after planting. In Swan, D treatments had significantly lower Ψ_L values than D/W and W treatments 23 and 42 days after planting.

Tiller Development

Swan had fewer tillers than the other 2 cultivars (Table 4.2.2). The severe water stress treatment (D) had restricted tillering in all cultivars. Nematode infestation had no effect on tillering in any cultivar or water-supply treatment.

Table 4.2.2: Effect of Infestation and Water Supply Treatment on Tiller Production of 3 Oat Cultivars 100 Days After Planting

		TILLER NUMBER				
		DAYS AFTER PLANTING				
CULTIVAR	NEMATODE	100				
		WATER STRESS TREATMENT				
		W	D	D/W	MEAN (CV X DENSITY	MEAN (CV)
NZC	0	11.5	7.3	12.5	10.4	10.9
	4000	12.3	6.7	14.9	11.3	
SUAL	0	11.4	6.2	13.1	10.2	10.1
	4000	10.2	6.9	12.7	9.9	
SWAN	0	7.7	5.8	8.2	7.2	7.3
	4000	7.5	5.3	9.3	7.4	
MEAN (WT)		10.1	6.4	11.8		

ANALYSIS LSD ($P \leq 0.05$)

SOURCE OF VARIATION

SIGNIFICANT EFFECTS: LSD ($P = 0.05$)

Cultivar (CV) 1.38

Water Treatment (WT) 1.38

(No significant interactions)

Tillering of the 3 cultivars was the same in W and D/W treatments but in the D treatment Swan had significantly more tillers than NZC or Sual. Tillering of NZC and Sual W and D/W treatments was the same but in Swan there were significantly more tillers in D/W than in W.

Grain Yield

The effect of infestation and water supply treatment on total grain number and grain mass is presented in TABLE 4.2.3. Severe water deficit stress significantly reduced grain number and grain mass of all cultivars. No differences were found between infested and uninfested D-treated plants.

Mild water stress had no effect on either total grain number or grain mass of Sual and Swan and increased the total grain mass of NZC (Table 4.2.3). Total grain mass of Sual and Swan was reduced by infestation in both the D/W and on treatments but NZC was not affected.

Table 4.2.3: Effect of Heterodera avenae Infestation and Water Supply Treatment on Yield of Three Oat Cultivars 100 Days After Planting

		TOTAL GRAIN NO.			TOTAL GRAIN MASS		
		NEMATODE DENSITY					
		0	4000	MEAN	0	4000	MEAN
NZC	W	74.2	83.6	78.9	1.48	1.59	1.54
	D	31.7	34.9	33.3	0.45	0.49	0.47
	D/W	89.1	90.7	89.9	1.83	1.71	1.77
	MEAN	65.0	69.7		1.25	1.26	
SUAL	W	96.1	66.9	81.5	2.15	1.49	1.82
	D	35.8	31.4	67.2	0.47	0.47	0.47
	D/W	92.7	61.8	77.3	1.97	1.46	1.72
	MEAN	74.9	53.4		1.53	1.14	
SWAN	W	101.7	87.4	94.6	3.41	2.93	3.17
	D	52.6	51.6	52.2	1.64	1.53	1.59
	D/W	97.3	79.2	88.3	3.19	2.89	3.04
MEANS (DENSITY X WT)	W	83.9	72.8		2.74	2.45	
	W	90.7	79.3		2.35	2.00	
	D	40.0	39.3		0.85	0.83	
	D/W	93.0	77.2		2.33	2.02	
MEAN (DENSITY)		74.6	65.3		1.84	1.62	

ANALYSIS LSD

SOURCE OF VARIATION		
CV	8.97**	0.187*
Density	7.03*	0.131**
W.T.	8.97**	0.187**
CV X Density	13.14*	0.284*
CV X W.T.	16.28*	0.209*
Density X W.T.	13.14*	0.284*
CV X Density X W.T.	NS	NS

*,** - significant P = 0.05, P $\leq$ 0.01 respectively.

Although mild water stress had no effect on total yield, the individual components of yield of Sual and Swan were influenced by the D/W treatment (*TABLE 4.2.4*).

Table 4.2.4: Effect of Infestation and Water Stress on Main Stem Grain Number and Mass of 3 Oat Cultivars 100 Days After Planting

		GRAIN NO.			GRAIN MASS (g)		
CV	W.T.	0	+	MEAN	0	+	MEAN
NZC	W	21.5	23.5	22.5	0.49	0.55	0.52
	D	8.7	10.1	9.4	0.16	0.18	0.17
	D/W	24.3	25.3	24.8	0.54	0.53	0.54
MEAN		18.2	19.6	18.9	0.39	0.42	0.41
SUAL	W	31.2	23.2	27.2	0.86	0.55	0.71
	D	13.9	12.8	13.4	0.17	0.18	0.18
	D/W	26.0	22.2	24.1	0.58	0.54	0.56
MEAN		23.7	19.4	21.5	0.53	0.42	0.48
SWAN	W	25.5	23.0	24.3	0.91	0.83	0.87
	D	13.0	9.4	11.2	0.41	0.25	0.33
	D/W	21.5	15.5	18.5	0.72	0.55	0.54
MEAN		20.0	15.9	17.9	0.68	0.54	0.61
MEAN	W	26.0	23.2	24.7	0.75	0.64	0.70
CV X	D	11.9	10.8	11.3	0.25	0.20	0.23
DENS.)	D/W	24.9	21.0	22.5	0.61	0.54	0.50

	MAIN STEM	
SOURCE OF VARIATION	GRAIN NO.	GRAIN MASS
CULTIVAR (CV)	3.06*	0.09**
DENSITY	NS	0.07*
WATER TREATMENT (WT)	3.06**	0.09*
CV X DENSITY	NS	0.13*
CV X WT	5.31*	0.14*
DENSITY X WT	NS	NS
CV X DENSITY X WT	NS	NS

Main-stem grain mass and number in Sual and main-stem grain number in Swan were reduced on D/W treated uninfested plants. Infestation had no effect on grain mass or number of well-watered Swan but further reduced the grain mass of D/W treated plants. Decline in infested D/W treated, main-stem grain mass of swan was due to a reduction in grain number with no effect on single grain mass (Table 4.2.5).

Table 4.2.5: Effect of Heterodera avenae Infestation and Water Supply Treatment on Single Grain Mass Deriving from the Main Stem and Tillers of 3 Cultivars 100 Days After Planting

		SINGLE GRAIN MASS (mg)					
		MAIN STEM			TILLERS		
		0	+	MEAN	0	+	MEAN
NZC	W	23	23	23	18	19	19
	D	18	18	18	15	15	15
	D/W	22	21	22	19	18	19
MEAN		21	21	21	17	17	18
SUAL	W	28	24	26	19	18	19
	D	12	14	13	13	16	15
	D/W	22	24	23	17	16	17
MEAN		21	21	21	16	17	17
SWAN	W	36	36	36	33	32	33
	D	31	26	29	26	27	27
	D/W	33	35	34	30	35	33
MEAN		33	32	33	30	31	31

ANALYSIS	LSD (P = 0.05,* ; P = 0.01**)	
CULTIVAR	2**	2**
DENSITY	NS	NS
WATER TREATMENT (W.T.)	2*	2*
CV X DENSITY	NS	NS
CV X W.T.	NS	NS
DENSITY X W.T.	NS	NS
CV X DENSITY X W.T.	7*	NS

a) o, o larvae per plant; + 4000 larvae per plant.

In contrast the main-stem grain mass of infested Sual was only reduced in well-watered plants, there being no effect on grain number. Main-stem grain number and mass of NZC were not reduced by mild water stress and infestation alone or in combination.

The effect of infestation on tiller grain yield of Swan and Sual mirrored that of infestation on main-stem yield of Sual (Table 4.2.6). Infestation only reduced grain mass and number of Sual, and grain mass of Swan when plants were not water stressed. Water stress alone had no effect on tiller yield of Swan, but reduced both mass and number of tiller grains of Sual. Tiller yield of NZC was unaffected by infestation but mild water stress enhanced grain number and mass of NZC.

The increase in tiller grain number and mass of D/W treated NZCwas due to an increase in tiller number. The decline in tiller grain mass of infested Swan was due to fewer grains per tiller.

The decline in tiller grain mass and number of well-watered, infested Sual was due to both a decline in tiller number and grains/tiller. The decline in tiller grain mass and number of Sual response to mild water deficit was due to fewer grains per panicle, and slightly smaller grain size. In all cultivars the effects of water stress and infestation on the individual components of tiller grain yield were in themselves insignificant but had a significant effect on tiller yield, with combined.

Table 4.2.6: The Effect of Infestation and Water Supply Treatment on Tiller Grain Number, Grain Number per Tiller and Grain Mass of 3 Oat Cultivars 100 days After Planting

		GRAIN NO.			GRAINS/TILLER			GRAIN MASS (g)		
CV	W.T.	0	+	MEAN	0	+	MEAN	0	+	MEAN
NZC	W	54.3	56.1	55.2	4.7	4.6	4.7	0.99	1.09	1.04
	D	18.1	19.7	18.9	2.2	1.1	1.7	0.28	0.29	0.29
	D/W	68.2	66.1	67.5	5.3	4.4	4.9	1.29	1.22	1.3
MEAN		46.9	47.3	47.1	4.1	3.4	3.8	0.85	0.86	0.86
SUAL	W	66.2	47.5	56.9	5.8	4.6	5.2	1.29	0.85	1.07
	D	23.6	18.4	21.0	3.8	2.4	3.1	0.30	0.29	0.29
	D/W	57.3	49.2	53.3	4.5	3.8	4.2	0.99	0.78	0.89
MEAN		49.0	38.3	43.7	4.7	3.6	4.2	0.86	0.64	0.75
SWAN	W	76.8	67.0	71.9	10.1	8.9	9.5	2.52	2.14	2.33
	D	38.0	42.3	40.2	6.6	7.9	7.3	0.97	1.15	1.06
	D/W	83.3	71.3	76.0	10.2	7.4	8.8	2.47	2.34	2.41
MEAN		65.9	59.3	62.6	8.9	8.1	8.5	1.99	1.21	1.60
MEAN	W	66.8	56.9	61.3	6.9	6.0	6.5	1.60	1.36	1.48
(WT X D DENSITY)		27.6	26.8	31.3	4.2	3.8	4.0	0.51	0.57	0.54
(D/W)		69.6	61.3	65.6	6.7	5.3	6.0	1.58	1.45	1.53
MEAN (DENS.)		54.7	48.1		5.9	5.0		1.23	1.29	

a) 0, 0 larvae per plant; +, 4000 larvae per plant

ANALYSIS LSD (P = 0.05,*; P < 0.01,**)

TILLERS

GRAIN NO.	GRAIN MASS	GRAIN/ TILLER
6.11*	0.11**	0.48*
4.82*	0.09*	0.35*
6.11**	0.11**	0.48**
8.72*	0.16*	NS
10.72*	0.19*	0.87*
8.72*	0.16*	NS
NS	NS	NS

4.2.4 Discussion

The purpose of this study was to examine the effects on grain yield of water stress of nematode infested plants to determine whether water stress imposed during early plant development predisposed a plant to increased damage by nematode infestation. The experiment was designed in such a way that it would test the hypothesis that the cultivar(s) most seriously affected in root growth by nematode infestation would most likely be affected by a soil water deficit. Early water deficit stress has been reported to inhibit or delay floral primordial development (Husin and Aspinall 1970, Fischer 1973) thereby reducing grain number. Tillering can also be suppressed during water stress (Aspinall *et al*. 1964). Mild water deficit during grain filling can reduce final mass per grain (Aspinall 1965, Wardlaw 1971, Brocklehurst *et al*. 1978) and severe water deficit during grain filling has been reported to reduce the rate of starch deposition (Brooks *et al* 1978) and hasten the termination of grain filling (Wardlaw 1971). In this study the decline in tiller number, grain number, total grain mass, and single grain mass of the severely water stressed plants (D) can be explained on the basis of the above.

In these plants the effects of water stress outweighed those of infestation on yield. However, mild water deficit stress influenced the response of main stem yield to infestation. Differences in the way that infestation and the D/W treatment influenced the main stem yield of the three cultivars could be related to differences in root development, and the extent to which root growth was impeded by nematode infestation. The cultivar NZC had a faster growing root system and was less impaired by nematode infestation than Sual and Swan.

Because infested and uninfested roots of NZC extended into the well watered root zone at about the same time no differences in Ψ_L and G were observed between infested and uninfested plants and recovery from stress occurred sooner than on Sual and Swan.

Although slower development of roots within the well-watered bottom soil compartment may account for the deleterious effect of mild water stress on uninfested Sual and Swan compared to NZC, it does not explain why mild water stress had no effect on infested Sual but reduced the yield of otherwise unaffected, infested Swan. The effect of infestation on Swan is understandable, infestation further delaying the time for roots to extend into the bottom root compartment, thereby accentuating the effects of water stress

on main stem yield. Sual should have been similarly affected given the similarity in infested root sizes of the two cultivars. Instead in Sual, only the well-watered treatment was reduced by infestation. These findings indicated that infestation influenced the processes of grain filling of Sual independent of the effects of internal and external water stress. Measurements of stomatal conductance and Ψ_L for Sual supported this observation. For example, stomatal conductance of infested Sual was significantly higher than on uninfested plants after 20 days and Ψ_L values between infested and control plants did not differ. Since root length of Sual was significantly reduced on infested plants the decline in grain mass can be attributed to the inability of the smaller root system to support the same growth as the larger uninfested root system. The absence of such an effect on Swan may be a reflection of a less severely nematode-impaired root system at the density applied. Mild water stress may have had no further effect on main-stem grain yield of Sual because the small stature of the infested root system may have reduced the plants water requirements, thereby in part, cancelling the effect of a drier soil.

The negative effects of infestation on tiller yield of Sual and Swan did not appear to be associated with either reduced root length or with plant water deficit stress during later growth. In both cultivars, root development of well-watered infested plants within the top soil compartment was not significantly different from that of uninfested plants.

The results on main stem and tiller yield responses of Sual and Swan to infestation indicated that water deficiency may not be the primary factor responsible for the decline in yield of infested plants. Nonetheless the extent to which roots were impeded in growth by nematode infestation was related to how severely yield was reduced by infestation.

Because mineral nutrition is also dependent on root extension it is possible that access to minerals and not water was the principle limiting factor in determining the yield of infested plants. The rate of diffusion of ions to the root surface is more rapid when the soil is moist than when it is dry (Reisenauler 1966, Nye and Tinker 1977). Both infested and uninfested plants would have been subject to this constraint in the D treatment, and differences in root length may not have been sufficiently large to benefit the uninfested root system. With increasing soil moisture (D/W and W treatments) the soil conductance to ion movement may have been a less limiting factor than root length. In that case infested plants would be disadvantaged compared to uninfested plants. This could explain why the grain yield of Sual, a cultivar considerably reduced in root length by infestation, was reduced only when water stress was mild or absent. The relationship between root growth of infested plants and mineral nutrition will be examined in more detail in section 6 of this chapter.

To conclude, while an analysis of total yield failed to detect interactions between infestation and water stress, water deficit stress accentuated the effect of infestation on main stem grain mass of Swan. These two factors acted independently on the main stem grain yield of Sual and tiller grain yield of Sual and Swan. The cultivar NZC was largely unaffected by infestation and water supply treatment.

4.3 Direct Effect of Infestation on Stomatal Conductivity

4.3.1 Introduction

In section 4.1, rapid dehydration of the three cultivars exposed to drought stress appeared to be prevented by stomatal closure. Differences between infested and control plants in stomatal conductance could be satisfactorily ascribed to the indirect effect of root stunting that slowed water consumption, thereby delaying onset as well as relief from water stress. Direct effects of nematode invasion on stomatal aperture if they occurred were therefore difficult to identify.

Stomatal closure has been associated with elevated leaf ABA concentrations (Mittelheuser and Steveninck 1969, Jones and Mansfield 1970). It has already been shown that nematode infestation elevated the level of ABA in root tips (section 3). It is possible that nematode invasion may also induce a rise in ABA levels in the leaves of infested plants, thereby directly causing stomatal closure and reducing transpiration.

Since there is little information about the specific effects of nematode invasion on stomatal aperture the following study was undertaken to determine whether nematode infestation has an effect on stomatal aperture independent of root length effects. Cultivar differences were also examined.

4.3.2 Methods

Seeds of four oat cultivars (NZC, Sual, Swan and West) were prepared for germination as described (Chapter 3.2.1). Three day-old seedlings were planted into 2.7 x 26 cm tubes containing John Innes soil with a volumetric soil moisture content of 14.0 + 0.8%. Standard growth cabinet conditions applied. Photoperiod was 16 hours starting at 600 hours. After 12 days the plants were selected for uniformity and soil surfaces, top and bottom, were sealed with tightly fitting Parafilm^{R} and plastic Clipsal^{R} caps respectively, to minimize water loss from the soil. Daily transpirational water loss was monitored gravimetrically during a 10 hour period from 800 to 1800 hours for three days. Total leaf area approximations were obtained from leaf length measurements, enabling transpiration rates to be expressed on a leaf area basis. Distilled water was added to the soil surface to compensate for daily water loss after unsealing the parafilm. Tubes were left unsealed overnight. Stomatal conductance was monitored as described (Chapter 3, 2.10.5) at 1000 and 1600 hours. During the same interval plants having either conductance ~~conductivity~~ or transpirational values that deviated more than ~~significantly (+ -)~~ ± 10% from the mean were removed on the fourteenth day. At 20.00 hours of the fourteenth day after planting one-half of the plants were inoculated with a 1.0 ml water suspension containing 3000 Heterodera avenae nematode larvae, while the other half received 1.0 ml of the same water, minus nematodes.

R - Registered Trade Mark

Stomatal conductance measurements were taken the following day and for the next 5 days, 2 or 3 times daily, and then 10, 15 and 20 days later on control and treated plants. Transpirational losses were monitored until the twentieth day of planting. After that time the Clipsal caps were removed from the bottom of the tubes and plants accessed water from the bottom of plastic lined metal trays.

Ten days after inoculation five control and inoculated plants were destructively harvested to obtain information on root length and larval establishment.

Conductance ~~Conductivity~~ and transpiration measurements were obtained by random subsampling of four plants per treatment from a total population of 16 plants per treatment. The experiment was analysed as a completely randomized block design.

4.3.3 Results

Infestation of Sual by Heterodera avenae resulted in significantly higher ($P = 0.01$) conductivity values than controls on leaf 3 (day 15) and leaf 4 (day 16, 1800 hours to day 19,900 hours) (Fig. 4.3.1). Stomatal conductance ~~conductivity~~ of the remaining three cultivars was not consistently influenced by nematode infestation, although nematode infestation resulted in conductance ~~conductivity~~ values of NZC being significantly less than controls 2 days after infestation but becoming insignificant on the eighteenth day. Transpiration rates of all cultivars remained unaffected by nematodes throughout the experimental period.

FIG 4.3.1 (a-d) The effect of Heterodera avenae infestation on stomatal conductance (G) and transpiration rate (E) of a) NZC, b) Sual, c) Swan and d) West 0 - 36 days after inoculation (12 - 40 days after planting)

Fig. 4.3 a) NZC

L3 to L6 Leaf Position 3 to 6

 - indicates date of larval application

 LSD (P = 0.05)

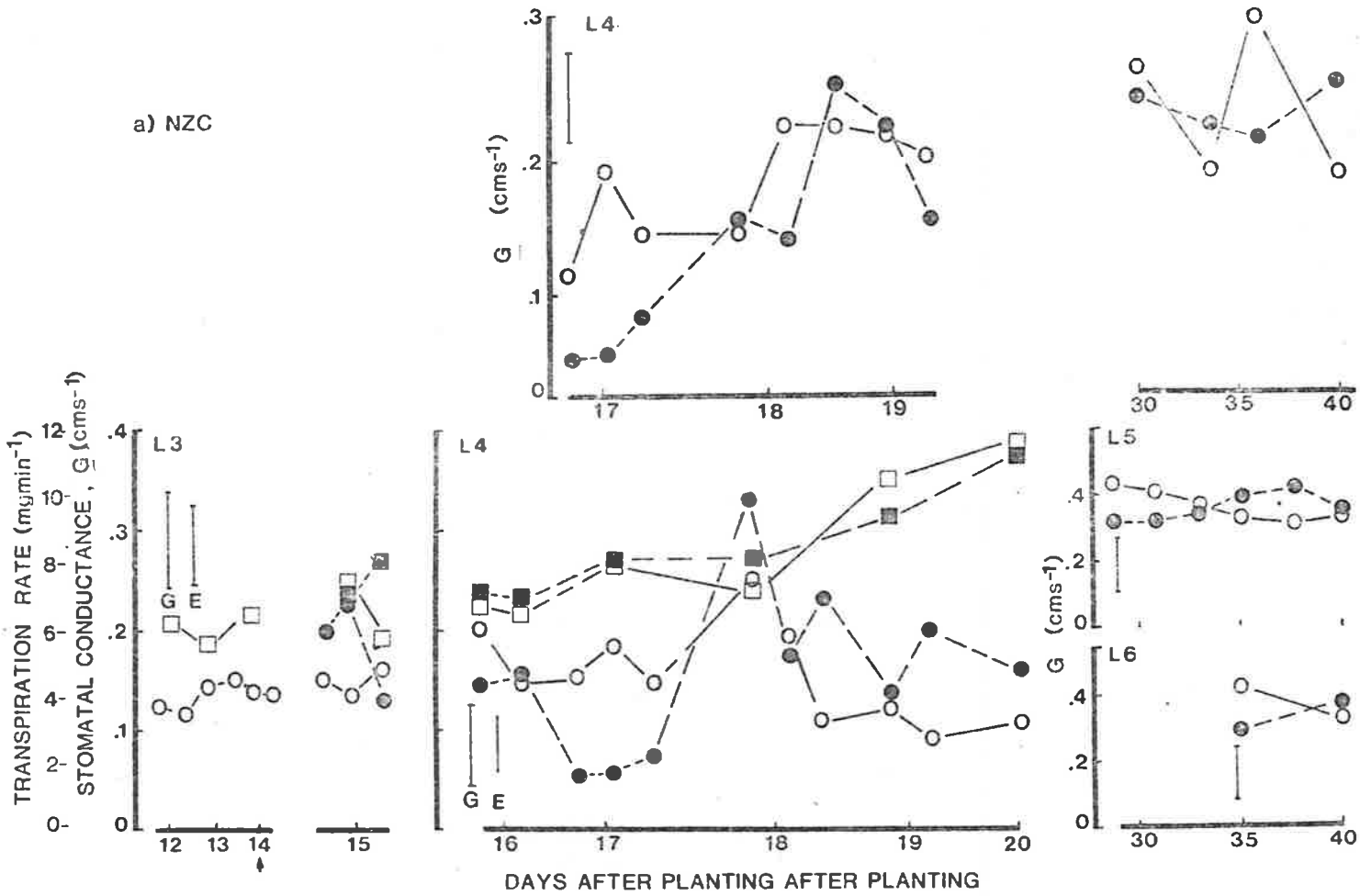
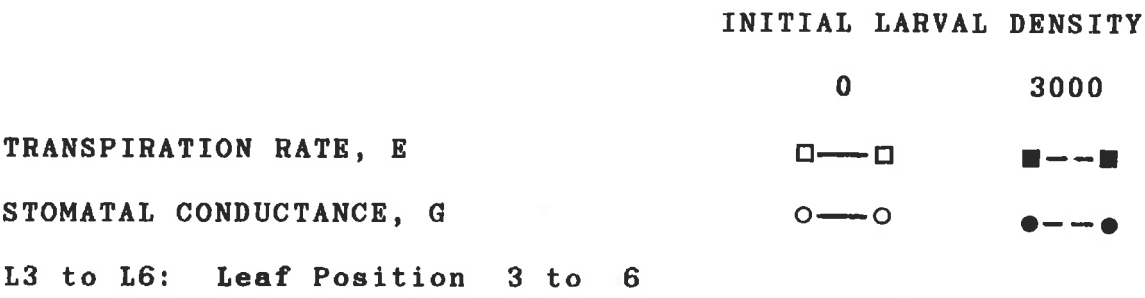

a) NZC
L4
L3
L4
L5
L6
G (cms-1)
TRANSPIRATION RATE (mgmin-1)
STOMATAL CONDUCTANCE, G (cms-1)
G E
DAYS AFTER PLANTING AFTER PLANTING

FIG 4.3.1 b) The effect of Heterodera avenae infestation on stomatal conductance (G) and transpiration rate (E) of b) Sual 0 - 36 days after inoculation (12 - 40 days after planting)



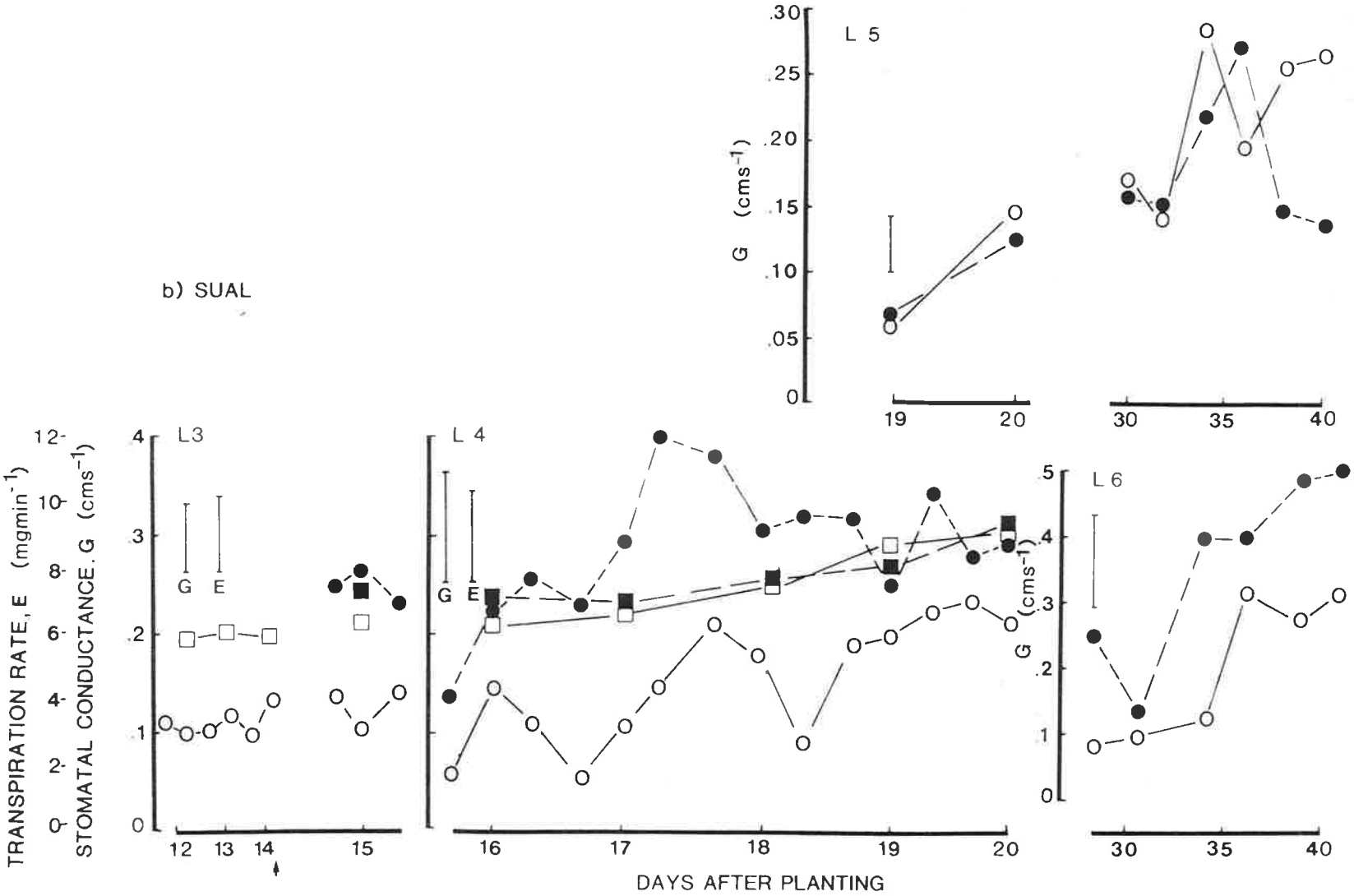
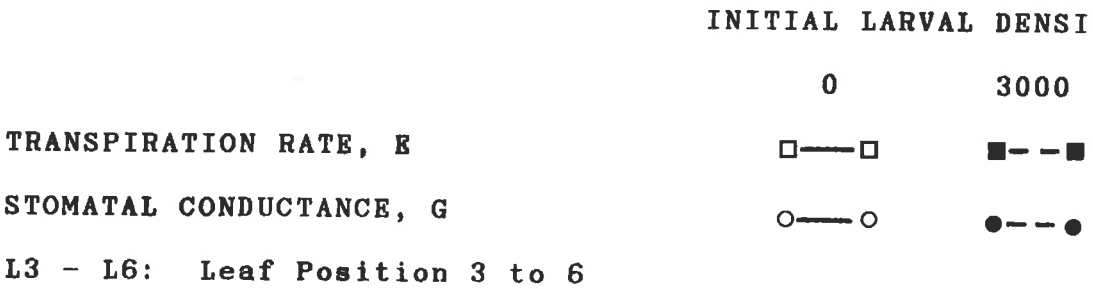

b) SUAL
L 5
L3
L 4
L 6
G E
TRANSPIRATION RATE, E (mgmin⁻¹)
STOMATAL CONDUCTANCE, G (cms⁻¹)
G (cms⁻¹)
DAYS AFTER PLANTING

FIG 4.3.1 c) The effect of *Heterodera avenae* infestation on stomatal conductance (G) and transpiration rate (E) of c) Swan 0 - 36 days after inoculation (12 - 40 days after planting)



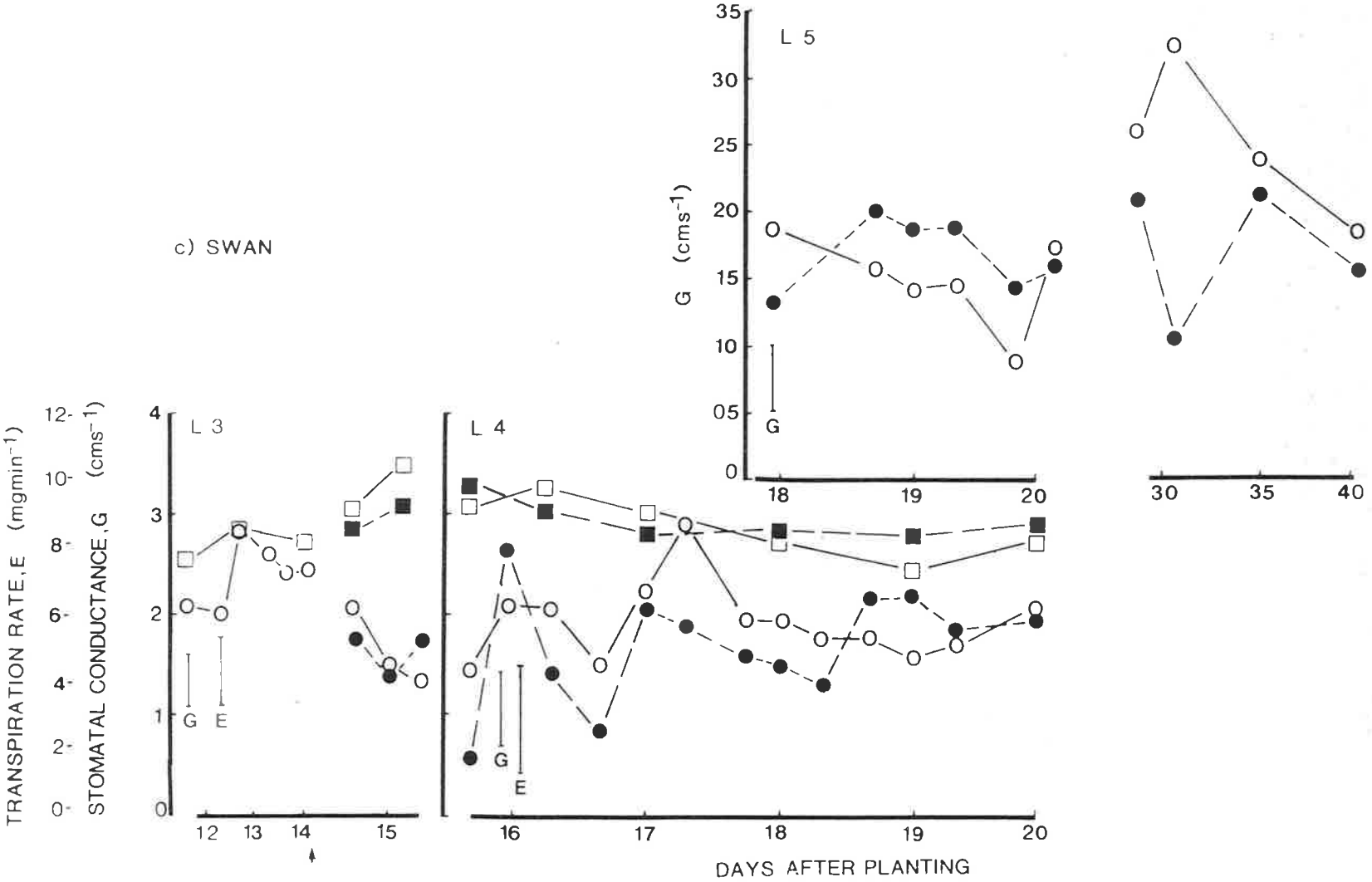
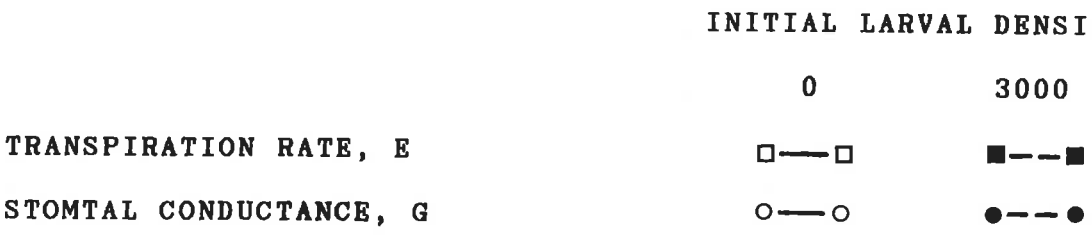

c) SWAN
TRANSPIRATION RATE, E (mgmin⁻¹)
STOMATAL CONDUCTANCE, G (cms⁻¹)
L 3
L 4
L 5
G
E
DAYS AFTER PLANTING

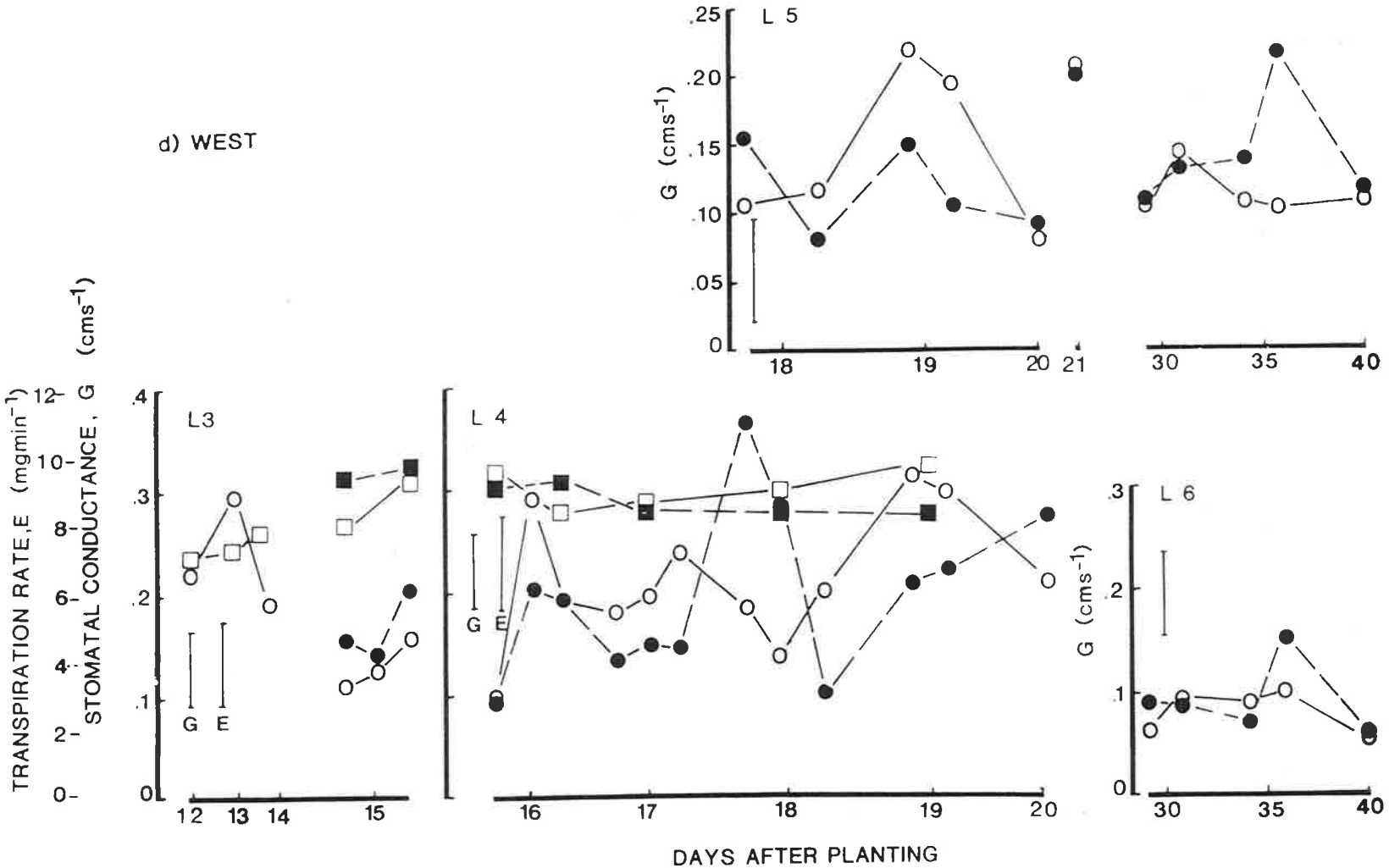
FIG 4.3.1 d The effect of Heterodera avenae infestation on stomatal conductance (G) and transpiration rate (E) of d) West 0 - 36 days after inoculation (12 - 40 days after planting)



L3 to L6: Leaf Position 3 to 6

↑ - indicates date of larval application

I LSD (P = 0.05)


d) WEST
L 5
L3
L 4
L 6
G (cms-1)
TRANSPIRATION RATE,E (mgmin-1)
STOMATAL CONDUCTANCE, G (cms-1)
G
E
DAYS AFTER PLANTING

All cultivars were found to have substantial larval populations on their roots (Table 4.3.1). No significant differences between cultivars were observed.

Root length was not significantly reduced by nematode infestation though roots of infested plants tended to be shorter.

Table 4.3.1: Effect of Heterodera avenae Inoculation[a] Fourteen Days After Sowing on the Total Root Length and Larvae Number 10 Days Later

CULTIVAR	NO. LARVAE	ROOT LENGTH (% OF UNINOCULATED CONTROL)
NZC	572 ± 46	98.4
SUAL	639 ± 68	92.9
SWAN	519 ± 32	89.6
WEST	596 ± 74	95.1

a - Initial Density = 3000 2nd stage larvae.

± - Standard error of mean.

4.3.4 Discussion

Differential effects of nematode infestation on stomatal physiology between cultivars might be associated with tolerance of plants to infestation. Stomatal closure induced by nematode infestation could reduce net CO_2 assimilation and thereby reduce the rate of dry matter accumulation.

Intolerant cultivars might show greater sensitivity to this effect than tolerant ones. The present study showed, however, that infestation had an unexpected effect on leaf conductance.

Nematode infestation was found to temporarily enhance stomatal conductance of the intolerant cultivar, Sual, and to have no consistent influence on the remaining cultivars. Transpiration rate was unaffected by nematode invasion. The effect of infestation on the stomatal conductance of Sual found in this study confirmed the higher conductances observed in Sual in Chapter, 4.2. In view of the close relationship between stomatal conductance and leaf water potential (Ψ_L) the higher conductance values of infested Sual may have indicated that these plants had a higher plant water status than uninfested plants. Alternatively stomata of infested Sual may be less sensitive to incipient water deficit stress than uninfested plants. This could occur if infestation ~~increased~~ decreased the critical threshold Ψ_L at which stomata close so that at a given Ψ_L, leaf conductance would be greater than on uninfested plants. The net effect would be to reduce the water use efficiency of infested plants. This will be discussed in more detail in the following section.

On the basis of the results of this section intolerance could not be attributed to unavailability of CO_2 supply arising from nematode-induced stomatal closure. For the cultivars Swan and NZC, there is no reason to attribute differences in stomatal conductivity between infested and uninfested plants observed in the previous section to anything other than indirect effects of nematode invasion on root surface area. Further work is required before this can be said about Sual.

4.4 The Influence of *Heterodera avenae* Infestation on the Relationship Betwen Leaf Water Potential and Stomatal Conductance

4.4.1 Introduction

Transpiration rates of well-watered plants have been shown to be unaffected by the initial effects of nematode invasion (Chapter 4, 4.3). Nematode invasion was found to temporarily induce higher stomatal conductivity (G) in leaves of one less tolerant oat cultivar (Sual). Insensitivity of stomatal guard cells to plant water deficit could reduce overall water use efficiency (WUE) (Turner *et al*. 1978; Schulze and Hall 1982). Results of Chapter 4, 1.1 indicated that the WUE of Sual was most reduced by infestation, of the four cultivars examined. Insufficient data was collected from Chapter 4, 4.3 to attempt to generalize on the relationship between Ψ_L and stomatal conductance (G) or on how different cultivars or the presence of nematode infestation may influence this relationship.

The following study was undertaken to test the hypothesis that nematode infestation has no effect on the relationship between Ψ_L and G. Cultivar differences were also investigated.

4.4.2 Methods

Seeds of four oat cultivars, NZC, Sual, Swan and West were germinated and seedlings were planted into 5.0 x 7.0 cm pots containing John Innes sandy-loam soil with crushed gravel placed at the bottom to facilitate drainage. After nine days growth in a growth cabinet (conditions described Chapter 3, 2.3.1) plants were selected for uniformity from that population and received either 2000 or 4000 second stage larvae in water suspensions. Control plants were given water without nematodes. Ten ml distilled water was added every two days starting two days after nematode inoculation. Sixteen days after planting water was withheld from the plants for the remainder of the experiment.

Measurements of G (Chapter 3.2.10.5), Ψ_L (Chapter 3.2.10.1) and RWC (Chapter 3.2.10.3) were determined on plants over the course of the next six days, 30 to 120 minutes ~~before~~ after the start of each new light cycle. Measurements of G were replicated 6 times, Ψ_L measurements, 3 times, and RWC 2-3 times per cultivar within each density.

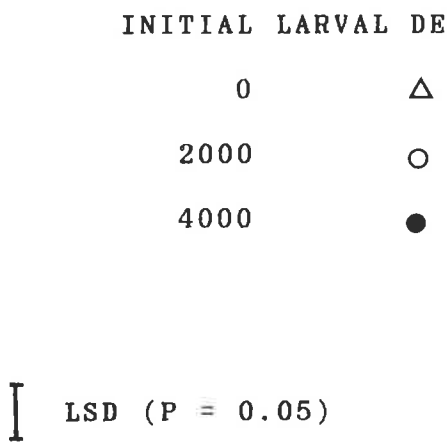
4.4.3 Results

Considerable variability in the data precluded significant relationships being found between G and Ψ_L. Regression analysis of Ψ_L on RWC (Figure 4.4.2) on the straight line segment of the relationship (Figure 4.4.1) in which G values were greater than 0 indicated that Ψ_L values closely depicted the actual water status of the plant, dismissing one possible source of error. Neither cultivar nor nematode effects were significant, although slopes for infested plants tended to be steeper than for controls.

4.4.4 Discussion

If soil water is not replenished, plants develop water deficits and leaf conductance decreases. Under conditions of artificially imposed drought of the type induced in this study, stomates close fairly abruptly at a critical threshold leaf water potential (Turner 1974; Ludlow 1980) although there is uncertainty as to whether this phenomenon can be always applied to field conditions (Schulze and Hall 1982). Nonetheless, differences in the water potential threshold for stomatal closure have been reported both between species (Sanchez-Diaz and Kramer 1971, Turner and Begg 1978) and varieties (Blum 1974, Henzell et al. 1975). There was therefore some reason to expect differences in the present study.

FIG 4.4.1 (a-d) The effect of infestation on the relation between stomatal conductance (G) and leaf water potential (Ψ_L) of the penultimate leaf (L_3 or L_4) of a) NZC, b) Sual c) Swan and d) West. (Water was with held 16 days after planting and measurements were taken during the next 6 days).



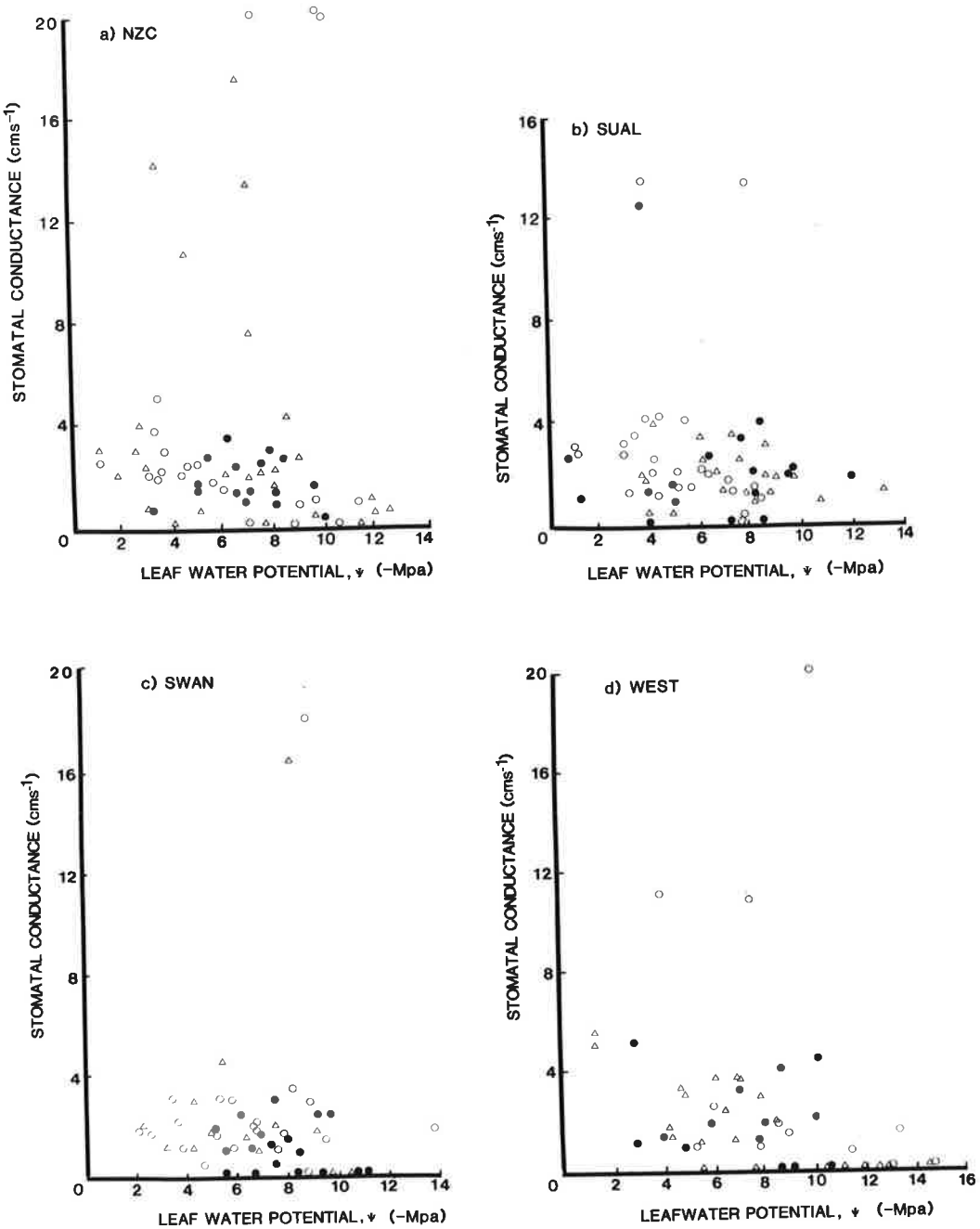
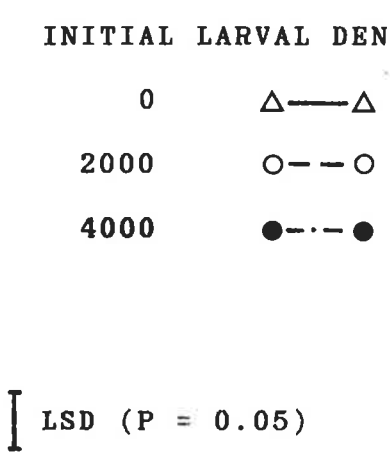
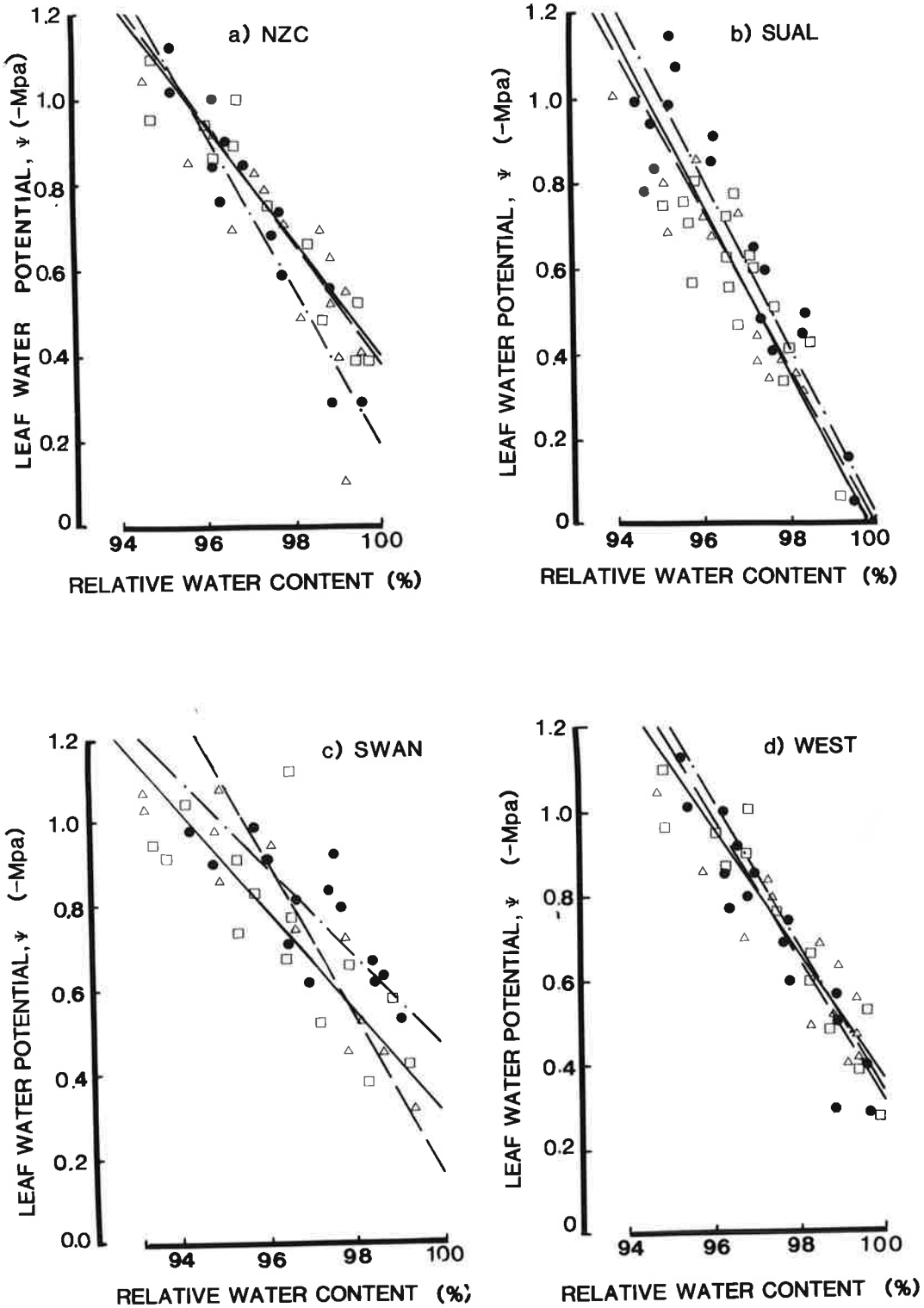

a) NZC
b) SUAL
c) SWAN
d) WEST
STOMATAL CONDUCTANCE (cms-1)
LEAF WATER POTENTIAL, ψ (-Mpa)
LEAFWATER POTENTIAL, ψ (-Mpa)

FIG 4.4.2 The effect of infestation on the relation between leaf water potential (Ψ_L) and relative water content (RWC) of the penultimate leaf (L_3 or L_4) of a) NZC, b) Sual, c) Swan and d) West. (Water was withheld 16 days after planting and measurements were taken during the next 6 days)



REGRESSION EQUATIONS:

a)	NZC	Y	= -14.41 0.14x;	r = 0.88
		Y	= -14.23 0.13x;	r = 0.95
		Y	= -17.98+0.17x;	r = 0.95
b)	SUAL	Y	= -15.67+0.14x;	r = 0.91
		Y	= -15.43+0.15x;	r = 0.90
		Y	= -16.76+0.17x;	r = 0.92
c)	SWAN	Y	= -8.69+0.08x;	r = 0.91
		Y	= -12.14+0.12x;	r = 0.73
		Y	= -8.51+0.091x;	r = 0.82
d)	WEST	Y	= -12.74+0.12x;	r = 0.82
		Y	= -15.09+0.15x;	r = 0.94
		Y	= 17.06+0.174;	r = 0.95


a) NZC
b) SUAL
c) SWAN
d) WEST
LEAF WATER POTENTIAL, ψ (-Mpa)
RELATIVE WATER CONTENT (%)
0
0.2
0.4
0.6
0.8
1.0
1.2
94
96
98
100

Given the substantial evidence supporting a close relationship between Ψ_L and G indicated above, the very haphazard relationship between Ψ_L and G observed in this study may have been indicative of artefactual error inherent in the experimental method. However no single element of the experimental procedure could have so critically affected the results. More replicates would have undoubtedly reduced effects due to error. Pot size was considered sufficiently large to support the relatively small water requirements of a 15 to 20 day old seedling. Water was applied at a rate sufficient to permit water to leak from the bottom of the pots. Soil texture was loose enough to prevent anaerobiosis. Measurements at pre-dawn would have reflected the least water stressed condition. Yet relatively low stomatal conductances coupled with relatively few Ψ_L values less than -2 MPa were indicative of plants suffering mild drought stress. Poor correlations between Ψ_L and G have been reported in other studies (Turner et al. 1985, Gollan et al. 1985).

In spite of the unexpectedly poor relationship between Ψ_L and G a couple of useful conclusions could be drawn from the results. Infestation appeared to have little influence on the dispersion of ~~conductivity~~ conductance values of all cultivars above and below the rough mean G value of 2.0 cms^{-1} except for NZC. Infestation of NZC reduced the percentage of G values greater than 2 from about 0.65 to 0.35%. In spite of this the cultivar NZC has been shown in previous studies to be little affected in root and shoot growth by infestation at the nematode densities used in this study, so this effect is

likely to have had little affect on CO_2 assimilation. From a different viewpoint, NZC may be more tolerant as a result of a better ability to conserve water when infested, compared to the other cultivars. Inefficient water use has been related to intolerance in work on potato cultivars (Evans 1982a).

Sual has been shown to be strongly affected by infestation yet no differences were evident in effects of infestation on G. On the basis of these observations it would appear that nematode effects on stomatal aperture do not influence the growth response of oats to Heterodera avenae. This conclusion can only be provisional in view of the acknowledgement of the possibility of an unknown error source that induced mild drought stress on well-watered plants.

4.5 Effect of Heterodera avenae Infestation on Root Hydraulic Conductivity

4.5.1 Introduction

Evidence collected from previous studies in this section have suggested both direct (Section 4.4) and indirect (Section 4.3) effects of infestation on stomatal control. There is some evidence to equate these effects with tolerance

The rate of plant water loss is a function of both above and below ground resistances (Cowan and Milthorpe 1968). Under conditions in which water is in unlimited supply the amount of water reaching the shoot for a given pressure gradient (Δp) depends upon the resistances in the pathway along which water moves from the root surface to the leaves above. If the sum of the root resistances or its inverse, the hydraulic conductance (C) was a static entity then only total root surface area could influence the rate of flow (Jv) of water into the shoot at a given root to shoot Δp. However, it has been repeatedly shown that Jv and Δp are not linearly related but rather that C increases as Δp increases (Mees and Weatherly 1957, Newman 1976, Michel 1977).

The potential variability of root hydraulic conductance may be relevant to the relative tolerance cultivars to infestation by Heterodera avenae. Infested plants have been observed to maintain Ψ_L values similar to uninfested plants without a corresponding reduction in stomatal conductance,

and inspite of a reduction in root surface area (section 4.1.4.3). This could occur if the permeability to water of the infested root system was higher than that of uninfested plants. The extent to which this adjustment in hydraulic conductance might occur may vary between cultivars and as such could account for differences in growth response to infestation.

Authors studying the relative magnitude of resistance to water flow in root systems have varied Δp by regulating the solute potential of the root medium (Mees and Weatherly 1957; Newman 1973; Michel 1977), varying the transpirational rate by manipulation of air humidity, temperature and wind speed (Hailey et al. 1973; Lawlor and Lake 1977) or by increasing the hydrostatic pressure around the root system (Jensen et al. 1961, Duniway 1976; Ramos and Kaufmann 1979). In the following set of studies in which the effects of water stress on Jv, C and K, the hydraulic conductivity per unit root surface area of infested plants was examined using all three methods of varying Δp.

Methods and results of each study will be presented in sequence and will be discussed under a single heading at the end.

4.5.2 Methods

4.5.2.1 Manipulation of Δp using Polyethylene Glycol (PEG)

Four oat cultivars, NZC, Sual, Swan and West were examined in this study. Seeds were pregerminated as described (Chapter 3.2.1) and seedlings were individually sown in specially prepared 3.0 (diameter) x 10 cm plastic bags containing John Innes soil. Pebbles (2-4 mm) placed at the base of the plastic bags prevented soil from washing out through slits made in the plastic to allow drainage. Seedlings were grown in a growth cabinet.

Heterodera avenae larvae were applied after three and six days at a density of 2000 larvae each day. Controls remained nematode free. Twelve days after planting soil was removed from the roots by cutting the plastic bags and immersing the bag with the plant into water, and gently agitating until the soil fell away. Plants uniform in both root and shoot size were selected from each of the two inoculation treatments and placed into blackened test tubes (25 x 250 mm) containing nutrient solution. The composition of the nutrient solution was as follows (mM): KNO_3, 2.0; $Ca(NO_3)_2$, 2.0; $MgSO_4$, 0.9; KH_2PO_4 0.25; one half strength Hoagland's solution micro nutrients and NaEDTA-Fe (9.8 mg NaFEDTa and 13.0 mg $Fe(NO_3)_2$ per cm^3 stock solution. Plants were supported in solution with foam blocks. Air filtered through cotton wool was continuously provided from a

centrifugal pump forcing air into a manifold and distributed to individuala tubes via slender plastic tubing (1.0 mm ID). After approximately 24 hours, nutrient solutions were replaced with solutions of identical composition but varying in polyethylene glycol 4000 (PEG) concentrations having solution osmotic potentials of 0, -0.2, -0.4 and -0.6 MPa. There was a total of 8 treatments, inoculated and controls each being subjected to the range of PEG concentrations. Over the following 3 days, solutions were replaced daily with fresh solution of the same PEG concentration. Transpirational water loss was measured 1 and 3 days after initiation of the PEG treatments. Daily transpiration rates were the mean of 4 readings taken at 4-hourly intervals over a 12 hour period starting 2 hours after the lights were turned on. Transpiration was measured gravimetrically to an accuracy of 1 mg. Corrections for water loss from the tube through the foam were made from measurements of tubes containing no plants. Leaf length measurements were taken after the final water loss was recorded on each of the three days. Two hours after the lights were switched off, the penultimate leaf was excised and placed into a Spanner psychrometer thermocouple chamber for measurement of leaf water potential (Ψ_L). The root system was retained for measurement of root length. Nematode infestation was assessed at the start and end of the trial. Measurements of Ψ_L were replicated 4 times. Root measurements were replicated 6 times. The experiment was arranged in a randomized complete block design.

Hydraulic conductance (C), the reciprocal of hydraulic resistance (R) was calculated according to the equation (Ramos and Kaufman 1979):

$$R = \frac{L \times \Delta\Psi_p}{Jv}$$

where L is root length (m), $\Delta\Psi_p$ is the water potential gradient between the root and leaf, and Jv is volume flow equivalent to water flux through the whole plant measured on a unit leaf area basis ($m^3\ m^2 s^{-1}$), R represents the hydraulic resistance per unit meter of root. To obtain the total resistance R_T, R is divided by the total length of the root system (Ramos and Kaufmann 1979).

Root conductivity (K) differs from C in that K is defined for a specific area of root system (Passioura 1984). K ($m^2\ s^{-1}\ MPa^{-1}$) is therefore described by the equation

$$K = C/A$$

where A is the total surface area (m^2) of the root system.

Values of Ψ_L were obtainable directly from psychrometry and $\Delta\Psi_p$ was regulated by the PEG solution concentration. A basic stock nutrient solution had a Ψs = -0.047 MPa. Jv was measured as transpiration rate (E) expressed on a unit leaf area basis with units $mg\ m^{-2}\ s^{-1}$ which can be reduced to ms^{-1}.

Root system area was determined by recording lengths of roots categorized on the basis of two root diameters: 0.025 and 0.05 cm. The narrower roots were mainly first and higher order laterals, and the thicker roots were the main axes of seminal and nodal roots. In general, uninfested root systems were composed of about 15% thick roots while infested root systems consisted of between 25 to 50% thick roots. Area was calculated using the equation $A = \Pi dL$, where d is root diameter and L is total root length.

4.5.2.2 Manipulation of Δp by Varying Relative Humidity (RH)

A pressure potential gradient (Δ_P) was manipulated by regulating growth chamber relative humidity (R.H.) inducing increased vapor pressure deficit (VPD) at low R.H.

Seedlings were grown as described in the previous study. Larvae were applied three days after planting at densities of 1000 and 4000 per plant. After 16 days plants were exposed to either 90, 60 or 30% R.H. A humidifier was used to circulate moist cool air through a plexiglass chamber housed within a growth cabinet. Relative humidity was regulated by a humidostat positioned inside the chamber. The wet/dry bulb method was used to verify humidities. R.H. fluctuated by about 7% at the highest (90%) R.H., and about 11% at 60% R.H.

To prevent moisture build up within the chamber at the lowest RH, air passed through a silica gel filter and was pumped through the chamber. In addition the door of the chamber was left slightly ajar to reduce moisture build up. Variability in R.H. was approximately 4% at 30% R.H. A constant gentle flow of air was maintained across the canopy using a fan positioned within the chamber. The chamber was equipped with both an air entry and exit port.

Transpirational water loss was measured as described in the previous section 18 and 24 days after sowing. Between measurement dates sufficient distilled water was added daily until excess water drained from the base. Leaf water potential was recorded as in the previous study using either psychrometric methods or a Scholander pressure chamber. Comparable results were obtained using either technique within an accuracy of $\pm$0.014 MPa. Leaf lengths and root lengths as well as nematode larvae number were obtained from plants on the day of measurement.

Total hydraulic root conductance (C) and root conductivity (K) were calculated from equations previously described (section 4.5.2.1). It was assumed that the pressure gradient between the leaf and root system was equal to Ψ_L minus the Ψ_s of the xylem (-0.023 MPa) since the soil was kept saturated.

4.5.2.3 Manipulation of Δp by Varying Hydrostatic Pressure

Hydraulic root conductance was determined by methods described by Ramos and Kaufmann (1979) following principles developed by Mees and Weatherly (1957).

Plants were grown in growth cabinet as described previously in 2.7 x 26 cm tubes containing John Innes soil. Heterodera avenae larvae were applied three days after planting at a density of 4000 larvae per plant. Measurements were carried out on plants between 15 and 20 days after planting. At 800 hours (2 hours into the light cycle) the tubes were placed in a container of water to saturate the soil for about 5 minutes. They were then returned to the growth cabinet for 3 hours to allow drainage. One hour before measurement the plants were taken to a constant temperature room (25°C) and soil was gently washed from the roots. The stem was excised from the root about 2 cm from the base and the root system was submerged in a vessel containing aerated 1/2 strength Hoagland's solution. The vessel was placed inside a pressure chamber with the cut end of the stem protruding about 0.75 cm from the chamber. Once a tight seal was obtained the air pressure was gradually increased to a final pressure of either 0.25 or 0.5 MPa. Exudate collected during the first 6 minutes was discarded.

Volumes of exudate over three successive 3 minute intervals were measured by inverting a small glass vial 0.9 (I.D) x 3.0 cm containing a strip of filter paper over the stump and covering this with a larger vial to minimize evaporative loss. Rates of exudation were found to be constant for at least one hour at either pressure. After measurements at the first pressure, chamber pressure was released and the root system was aerated for 3 minutes. The same procedure was then repeated at the alternate pressure. After the final measurement the length of the root system was measured.

Hydraulic root conductivity was obtained from measured values of J_v Δp and root length using the equations in section 4.5.2.1.

4.5.3 Results

4.5.3.1 PEG Study - Infestation, Leaf Area, Root Length

There were significantly ($P = 0.05$) fewer larvae on NZC on day 17 than on day 13, but the other cultivars had similar larval numbers on both days (Table 4.5.1). The number of larvae on roots of NZC and Swan was significantly ($P = 0.05$) fewer at -0.04 MPa and -0.2 and -0.4 MPa, respectively, than on day 0. All other differences were not significant.

Table 4.5.1: The Effect of Nutrient Solution Osmotic Potential (Ψs) on Larval Population in Roots of Four Oat Cultivars 13 and 17 Days After Planting

NO. OF LARVAE IN ROOTS (X 10^{-2})

CULT.		Ψs (MPa)				MEAN
		-0.04	-0.2	-0.4	-0.6	
NZC	13	8.4	8.1	9.6	8.9	8.8
	17	7.5	6.9	9.1	7.2	7.7
MEAN		8.0	7.5	9.4	8.1	8.3
SUAL	13	3.8	3.5	3.4	2.8	3.4
	17	4.6	4.2	4.0	4.1	4.3
MEAN		4.2	3.9	3.7	3.9	3.9
SWAN	13	7.9	8.1	7.9	7.7	7.9
	17	7.1	6.9	7.1	7.3	7.1
MEAN		7.5	7.5	7.5	7.5	7.5
WEST	13	8.2	6.2	6.1	9.3	7.5
	17	8.3	7.3	6.9	8.4	7.7
MEAN		8.3	6.8	6.5	8.9	7.6
MEAN	Day 13	7.1	6.5	6.8	7.2	6.9
(DAY X SP)	Day 17	6.9	6.3	6.7	7.0	6.5
MEAN (Ψs)		7.0	6.4	6.8	7.1	

ANALYSIS	LSD (P = 0.05,*; P ≤ 0.01 **)	
CULTIVAR (CV)	0.6**	
DAY	NS	
SOLUTE POT. (SP)	0.6*	
CV X DAYS	NS	NS
CV X SP	1.0*	
DAY X SP	NS	NS
CV X DAY X SP	NS	

Leaf area was not significantly affected by solute potential (Ψs) (Table 4.5.2). Infestation had no influence on leaf area of NZC, Swan and West but significantly reduced that of Sual after 20 days growth.

Root length was not significantly affected by solute potential. Infestation reduced root length of all cultivars except NZC (Table 4.5.2). Swan had a longer root system than the other cultivars at both nematode densities.

Table 4.5.2: The Effect of Larval Density on Mean Leaf Area And Root Length of Four Oat Cultivars 20 Days After Planting

	LEAF AREA (cm^2)			
DENSITY OF LARVAE	CULTIVAR NZC	SUAL	SWAN	WEST
0	31.2	23.6	27.5	25.3
4000	27.7	19.4*	30.0	24.2
	ROOT LENGTH (cm)			
0	536	501	772	487
4000	443	329*	630*	316*

* significantly different from uninfested control plants ($P = 0.05$)

Transpiration Rate vs Root Length

Because transpiration rate (E) and transpiration rate per unit root length (E_R) responded similarly to changes in Ψs and to infestation, only E_R data will be presented. The transpiration rates per unit root length (E_R) for day 1 and 3 of each treatment are shown in Table 4.5.3. The E_R of uninfested NZC, Swan and West declined as Ψs declined, NZC and Swan significantly ($P = 0.05$) on both measurement days where the E_R of Sual was unaffected by decreased Ψs. Infestation influenced E_R differently 1 and 3 days after PEG treatment initiation. E_R of NZC again declined with decreasing Ψs while that of Sual Swan and West either tended to decline (Swan, West) or remained unaffected (Sual) by a decline in Ψs.

ANALYSIS

SOURCE OF VARIATION	DAY 1	DAY 3
CULTIVAR (CV)	4.1**	3.6**
OSMOTIC POTENTIAL (OP)	4.7**	4.5**
DENSITY	3.8**	3.2**
CV X OP	8.1*	7.9*
CV X DENSITY	NS	7.1*
OP X DENSITY	NS	NS
CV X OP X DENSITY	12.2*	NS

Table 4.5.3: Transpiration Rate Per Unit Root Length (E_R) of Inoculated and Uninoculated Oat Cultivars 1 and 3 Days After Commencement of PEG Treatment (14 and 16 Days After Planting)

E_R ~~(m s^{-1} PER ROOT LENGTH)~~ [kg m^{-2} (Leaf area) m^{-1} (Root) s^{-1}] X 10^9

CV	SOLUTE POTENTIAL (-MPa)	DAYS AFTER TREATMENT INITIATION				
		1		3		
		0	4000	O	4000	MEAN
NZC	0.0	27	58	27	31	29
	0.2	24	50	10	32	21
	0.4	20	38	18	23	21
	0.6	11	22	13	20	17
MEAN				17	27	22
SUAL	0.0	33	26	39	21	30
	0.2	32	37	39	23	31
	0.4	28	35	47	18	33
	0.6	36	16	47	20	34
MEAN				53	21	32
SWAN	0.0	26	25	19	12	16
	0.2	19	23	20	12	16
	0.4	18	19	16	9	14
	0.6	13	15	14	9	7
MEAN				17	11	13
WEST	0.0	23	25	30	27	29
	0.2	18	42	26	24	25
	0.4	14	34	26	14	20
	0.6	13	31	20	19	20
MEAN				26	21	24
MEAN (OP X DENSITY)				29	23	24
				24	23	24
				27	16	22
				24	17	20
MEAN (DENSITY)				26	20	23

$\Delta\rho$ vs Ψ_s

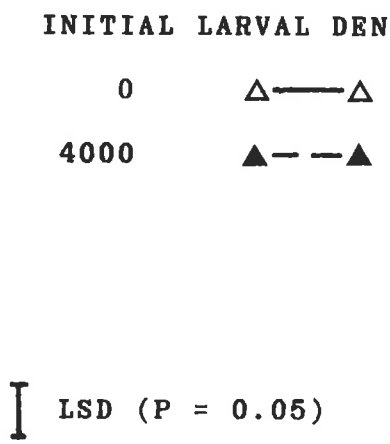
The effects of the nutrient solution solute potential (Ψ_s) on $\Delta\rho$ ($\Psi_L - \Psi_s$) of each cultivar were significantly different (Figure 4.5.1).

Since Ψ_L is the only variable component in $\Delta\rho$ at each solution Ψ_s, an increase in $\Delta\rho$ with decreasing Ψ_s (NZC, day 1 and 3, West day 3) indicated a decline in Ψ_L that was larger than the corresponding decline in Ψ_s. A constant $\Delta\rho$ (West day 1) reflected a decline in Ψ_L equal to the corresponding decline in Ψ_s, and smaller values of $\Delta\rho$ with declining Ψ_s (Sual and Swan) indicated either that Ψ_L increased as Ψ_s was reduced.

Total Hydraulic Root Conductance (C) and Hydraulic Conductivity K

Values of C represent conductance of the entire root system whereas K provides an indication of the permeability to water of the major barrier to radial water flow in the roots, possibly the endodermal membrane, on a unit surface area basis (Newman 1973; Ramos and Kaufmann 1979; Passioura 1984). C and K responded very similarly to changes in $_s$ and infestation (Fig. 4.5.2). C and K values of NZC and West declined with decreasing Ψ_s whereas those of Sual and Swan increased. Differences in C and K values between infested and control plants of Sual, Swan and West increased with time and solution Ψ_s. With the exception of NZC control plants

FIG 4.5.1 The effect of infestation and the nutrient solution solute potential (Ψ_S) on Δ_p ($\Psi_L - \Psi_S$) of NZC, Sual, Swan and West 1 and 3 days after commencement of PEG treatment (15 and 17 days after planting)



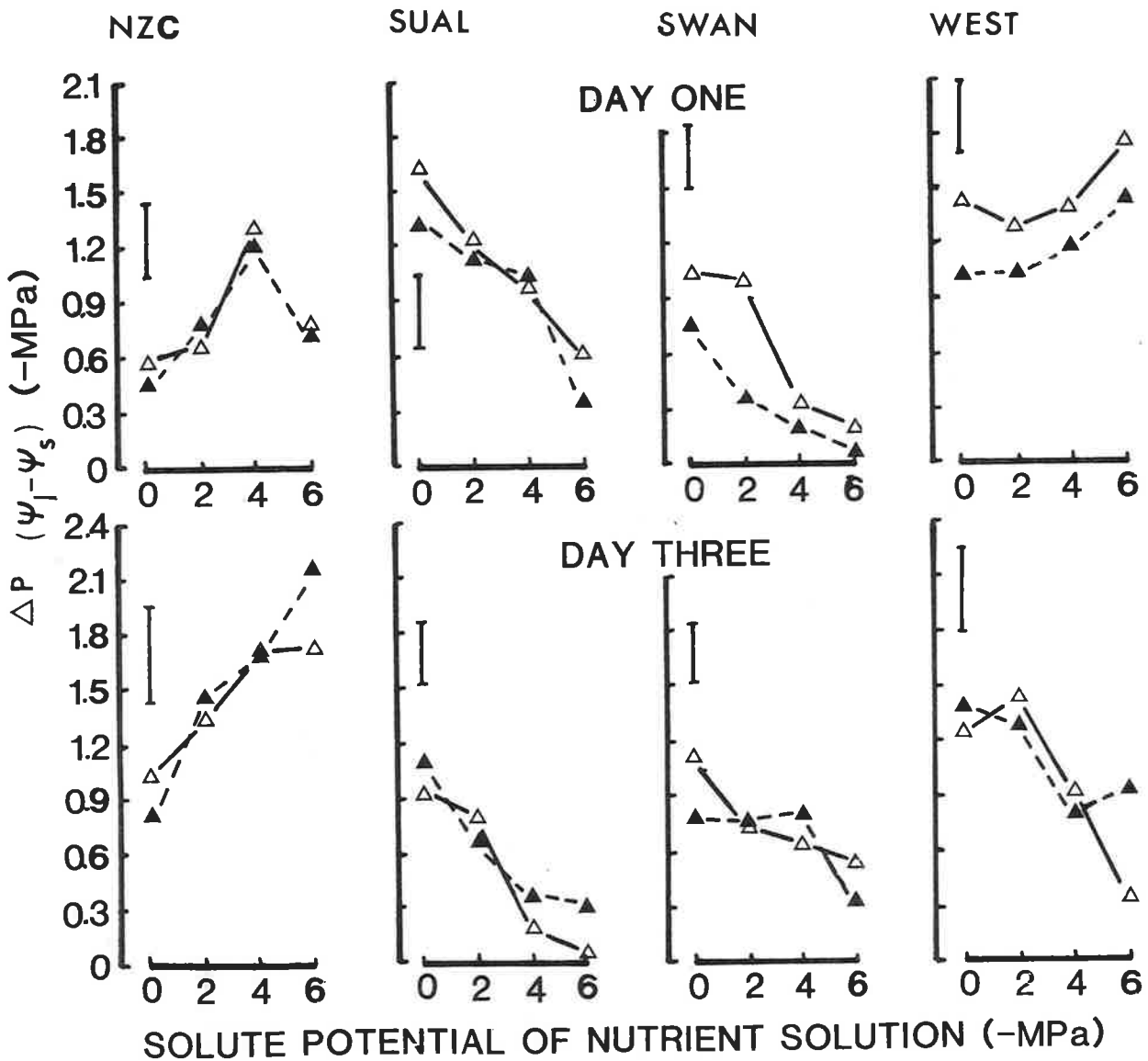
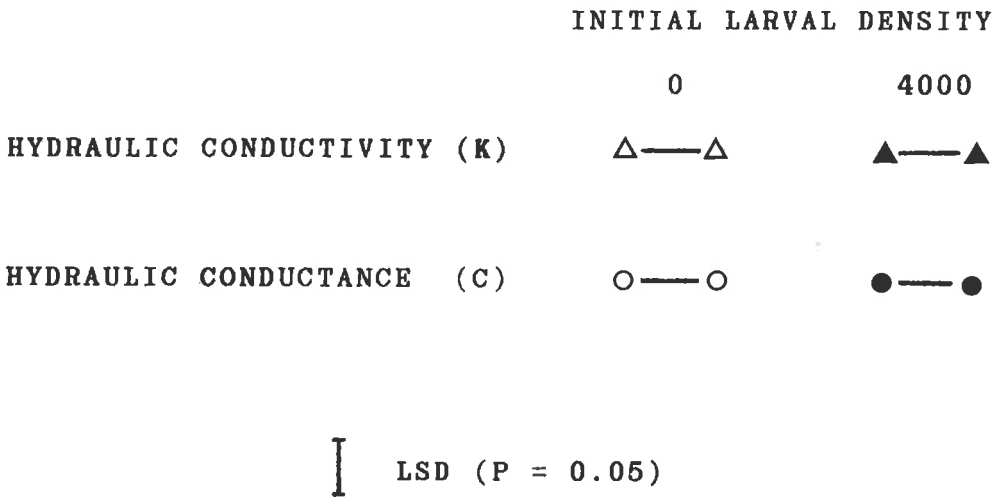
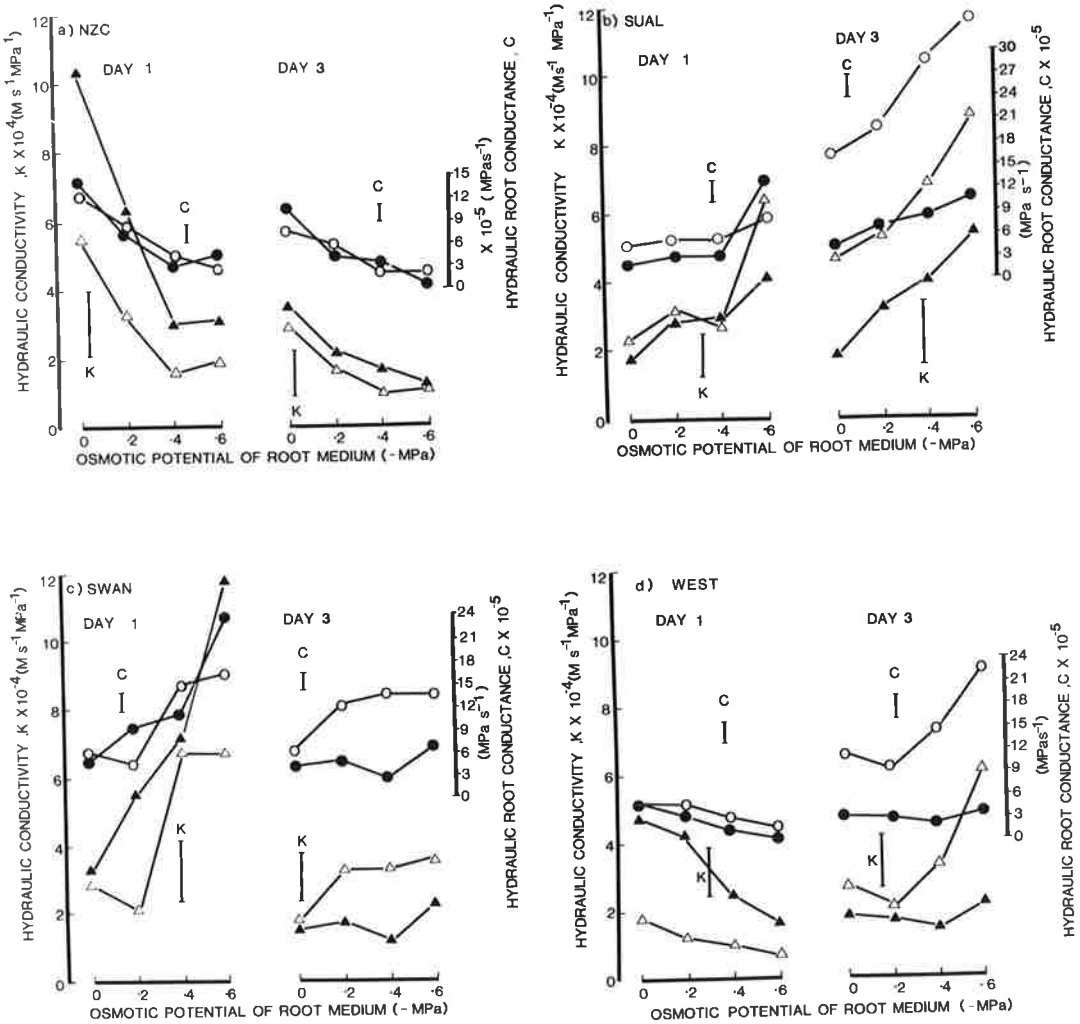

NZC
SUAL
SWAN
WEST
DAY ONE
DAY THREE
ΔP (ψ_i-ψ_s) (-MPa)
SOLUTE POTENTIAL OF NUTRIENT SOLUTION (-MPa)
0
0.3
0.6
0.9
1.2
1.5
1.8
2.1
2.4
0 2 4 6

FIG 4.5.2 a-d The effect of infestation on the hydraulic root conductance (C) and hydraulic root conductivity (K) of a) NZC, b) Sual c) Swan and d) West 1 and 3 days after commencement of PEG treatment (15 and 17 days after planting)




a) NZC
DAY 1
DAY 3
HYDRAULIC CONDUCTIVITY ,K X 10⁻⁴(M s⁻¹ MPa⁻¹)
HYDRAULIC ROOT CONDUCTANCE ,C X 10⁻⁵ (MPas⁻¹)
OSMOTIC POTENTIAL OF ROOT MEDIUM (-MPa)
b) SUAL
DAY 1
DAY 3
HYDRAULIC CONDUCTIVITY K X10⁻⁴(Ms⁻¹ MPa⁻¹)
HYDRAULIC ROOT CONDUCTANCE ,C X 10⁻⁵ (MPa s⁻¹)
OSMOTIC POTENTIAL OF ROOT MEDIUM (-MPa)
c) SWAN
DAY 1
DAY 3
HYDRAULIC CONDUCTIVITY ,K X10⁻⁴(M s⁻¹ MPa⁻¹)
HYDRAULIC ROOT CONDUCTANCE ,C X 10⁻⁵ (MPa s⁻¹)
OSMOTIC POTENTIAL OF ROOT MEDIUM (-MPa)
d) WEST
DAY 1
DAY 3
HYDRAULIC CONDUCTIVITY ,K X 10⁻⁴(M s⁻¹MPa⁻¹)
HYDRAULIC ROOT CONDUCTANCE ,C X 10⁻⁵ (MPas⁻¹)
OSMOTIC POTENTIAL OF ROOT MEDIUM (-MPa)

responded to declining Ψ_s with increased conductance whereas infested plants either did not change (Swan and West) or increased only slightly (Sual).

4.5.3.2 Relative Humidity Study - Infestation, Leaf Area, Root Length

Cultivars differed in the total number of larvae per root system only at the highest density (Fig. 4.5.3a). NZC had significantly more larvae than Sual and West. Total larvae number did not differ between days. The number of females did not differ between cultivars but increased from day 18 to 24 at the highest density (Fig 4.5.3b). Humidity had no effect on larval number.

Leaf area per plant was influenced by all independent variables except humidity (Fig. 4.5.4). The density x day interaction was not significant.

The intermediate density did not significantly reduce leaf area of any of the cultivars tested. The highest inoculation rate reduced leaf area of all cultivars except NZC on both sample dates.

Root growth was strongly inhibited by infestation, but was not affected by humidity (Fig 4.5.5). Cultivar x density effects were only significant on day 24. Infestation reduced total root length of Sual, West and Swan at inoculation densities of 1000 and 4000 larvae but of NZC at

FIG 4.5.3 a) The relation between initial inoculum density and the total number of larvae on four oat cultivars 7 days after commencement of relative humidity treatment (each value is the mean of 6 replicates)

b) The relation between initial inoculum density and the mean number of male and female larvae (averaged over four oat cultivars) 1 and 7 days after commencement of relative humidity treatment (each value is the mean of 24 replicates)

LSD (P = 0.05)

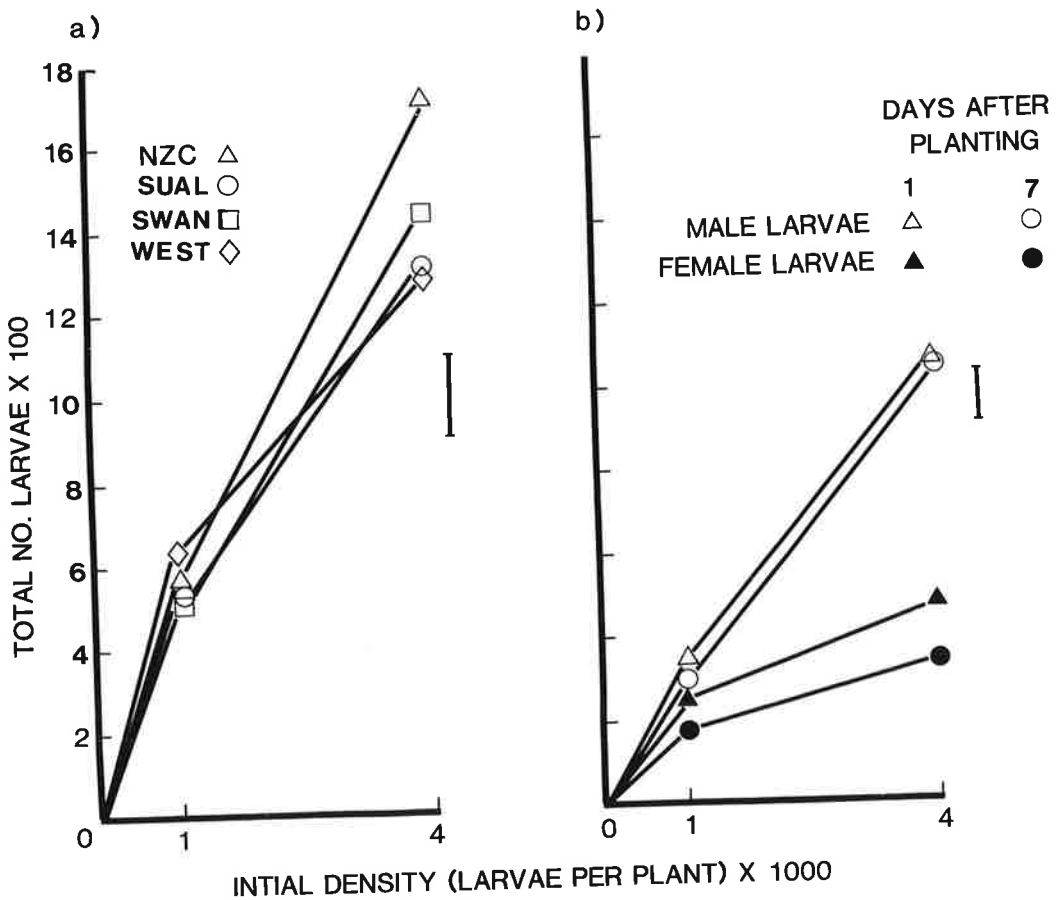

a)
b)
18
16
14
12
10
8
6
4
2
0
1
4
NZC
SUAL
SWAN
WEST
DAYS AFTER PLANTING
1
7
MALE LARVAE
FEMALE LARVAE
TOTAL NO. LARVAE X 100
INTIAL DENSITY (LARVAE PER PLANT) X 1000

FIG 4.5.4 The effect of Heterodera avenae infestation on total leaf area of 4 oat cultivars 1 and 7 days after commencement of relative humidity treatment (18 and 24 days after planting)

I LSD (P = 0.05)

FIG 4.5.5 Effect of Heterodera avenae infestation on total root length of four oat cultivars 7 days after commencement of relative humidity treatment (24 day after planting)

Figs. 4.5.4 and 4.5.5 have common legends:

CULTIVAR

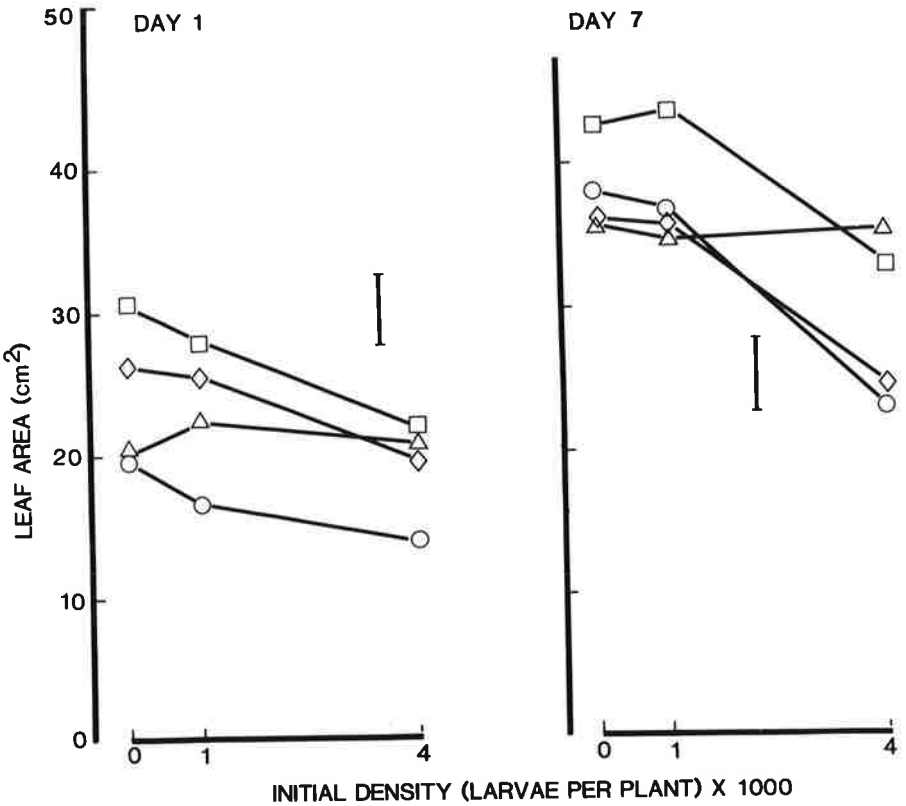
NZC △——△

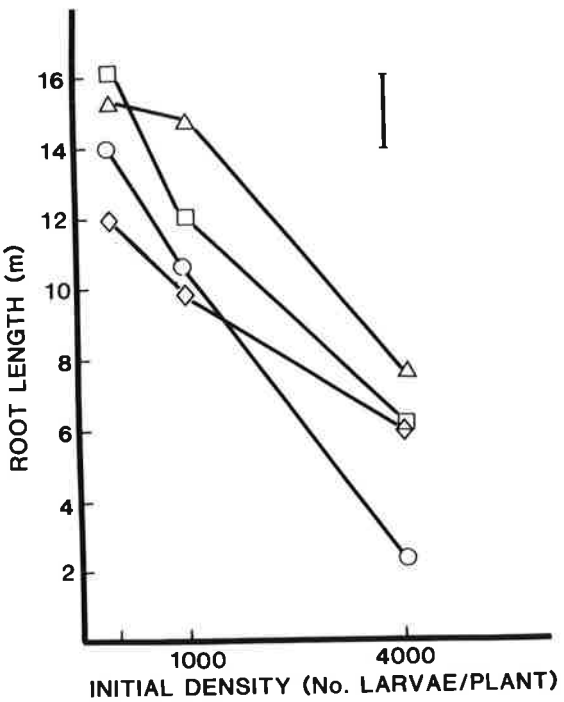
SUAL ○——○

SWAN □——□

WEST ◇——◇

I LSD (P = 0.05)


DAY 1
DAY 7
LEAF AREA (cm2)
50
40
30
20
10
0
0
1
4
INITIAL DENSITY (LARVAE PER PLANT) X 1000


ROOT LENGTH (m)
16
14
12
10
8
6
4
2
1000
4000
INITIAL DENSITY (No. LARVAE/PLANT)

only 4000 larvae. Root length of Sual did not differ from that of Swan and West when inoculated with 1000 larvae but was significantly shorter than these 2 cultivars at the highest inoculum density.

Transpiration Rate vs Root Length

Transpiration rate (E) was stable over a wide range of root lengths. As a result transpiration rate per unit root length (E_r) on day 7 values were less than day 1 values (Table 4.5.4). Heavily infested plants transpired more water per unit root length and this held for all cultivars at all relative humidites (RH) on day 1. However, on day 7, only Sual and Swan had significantly ($P = 0.01$) higher transpiration rates at the highest inoculation density compared to uninfested plants. All cultivars grown at 30 and 60% humidity levels transpired more water per unit root length than those grown at high RH on day 1. This was only true for Swan on day 7, all other cultivars showing significant increases in transpiration rate only at 30% RH. The interaction between density and RH was significant on both day 1 and 7. On day 1 and 7 plants inoculated with 4000 larvae had E_R values more than twice those of uninoculated plants at the same RH.

ANALYSIS
SOURCE OF VARIATION LSD (P = 0.05,*; P < 0.01,**)

	DAY 1	DAY 7
Cultivar	1.2*	1.1*
Relative Humidity (RH)	1.1**	1.0**
Density	1.2**	1.0**
CV x RH	NS	1.8*
CV x Density	NS	1.8*
RH x Density	2.0*	1.9*
CV x RH x Density	NS	NS

Table 4.5.4: Transpiration Rate Per Unit Root Length (E_R) of Inoculated and Uninoculated Oat Cultivars

CV	RELATIVE HUMIDITY %	E_R (10^9) (Kg m^{-2} (Leaf Area) m^{-1} (root) s^{-1}) DAYS AFTER COMMENCEMENT OF HUMIDITY TREATMENT 1 — INOCULATION DENSITY (NEMATODE NO.) 0	1000	4000	MEAN		7 — 1000	4000	MEAN	
NZC	90	1.8	1.6	3.4	2.3		0.6	0.6	1.0	0.7
	60	4.9	4.6	7.5	5.7		1.5	1.6	2.8	2.0
	30	8.1	8.8	12.8	9.9		2.9	3.4	4.8	3.7
MEAN		5.0	5.0	7.9	6.0		1.7	1.9	2.9	2.2
SUAL	90	0.7	2.2	5.9	2.9		0.7	0.8	2.8	1.4
	60	4.5	5.1	9.8	6.5		1.8	2.3	9.4	4.5
	30	10.0	13.1	22.1	15.1		2.7	3.4	12.9	6.3
MEAN		5.1	6.8	12.6	8.2		1.7	1.9	8.4	4.0
SWAN	90	1.2	1.5	4.6	2.4		0.5	0.6	1.3	0.8
	60	2.8	3.9	10.2	5.6		1.3	1.6	2.8	1.9
	30	5.2	8.3	16.3	9.9		2.6	2.9	6.8	4.1
MEAN		3.1	4.6	10.4	6.0		1.5	1.7	3.6	2.3
WEST	90	1.0	1.7	2.3	1.7		0.7	0.8	1.1	0.9
	60	2.9	3.4	7.5	4.6		2.4	1.6	2.4	2.1
	30	6.5	7.5	18.7	10.9		3.9	4.7	8.0	5.5
MEAN		3.5	4.2	9.5	5.7		2.3	2.4	3.8	2.8
MEAN		1.2	1.8	4.1	2.3		0.6	0.7	1.6	1.0
(RH X DENSITY)		3.8	4.3	8.8	5.6		1.8	1.8	4.4	2.7
		7.5	9.4	17.5	11.5		3.0	3.6	8.1	4.9
MEAN (DENSITY)		4.2	5.2	10.1	6.5		1.8	2.0	4.7	

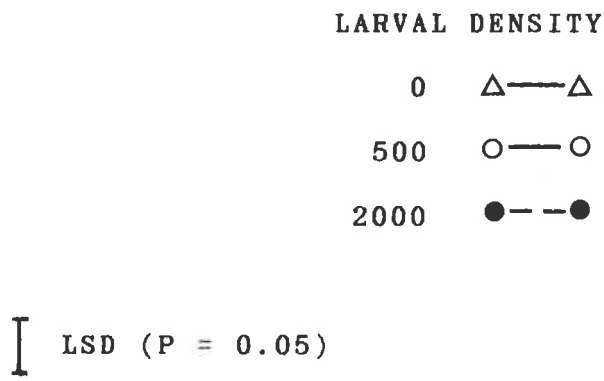
Leaf Water Potential (Ψ_L) vs Transpiration Rate (E)

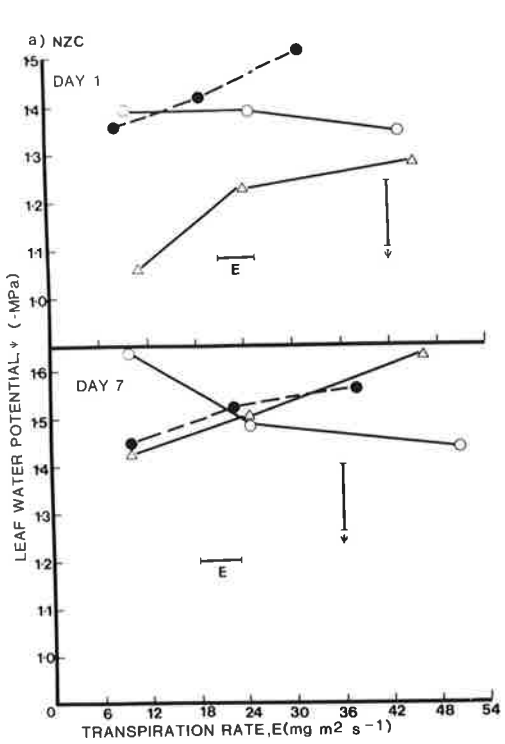
Because the soil in this experiment was well watered the soil water potential was assumed to be 0. Leaf water potential (Ψ_L) therefore equalled Δp. Since Δp is the driving force behind water flow through the plant an increase in Δp, as reflected in a reduction in Ψ_L, should increase the transpirational rate (E) of affected plants. However inspite of signifcantly lower Ψ_L values on infested NZC, Swan and West at 90% RH on day 1 compared to uninfested plants, transpiration rates of these plants did not differ from uninfested control plants (Fig 4.5.6). On day 7 neither E nor Ψ_L of infested plants differed from uninfested controls. At 60% RH, while infested plants tended to have lower transpiration rates, differences were still not significant. Again the Ψ_L of NZC and West at the highest inoculum density was significantly lower than that of uninfested plants at this RH on day 1. At 30% RH, inoculation of plants with 4000 larvae significantly reduced E values of NZC and Sual on day 1 and of NZC on day 7, corresponding to similar declines in Ψ_L of NZC on day 1, but on Sual or NZC on day 7. In addition inspite of small, though insignificant, increase in E of heavily inoculated West on day 7, the Ψ_L of these plants was significantly lower than uninfested plants.

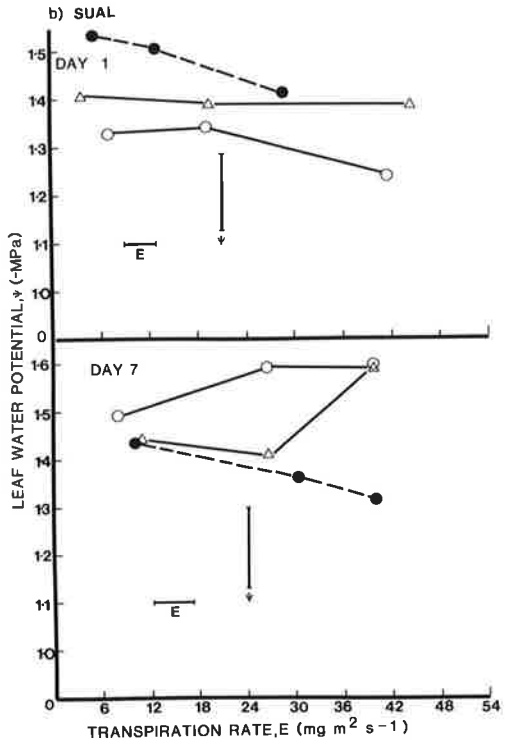
Hydraulic Conductivity (K)

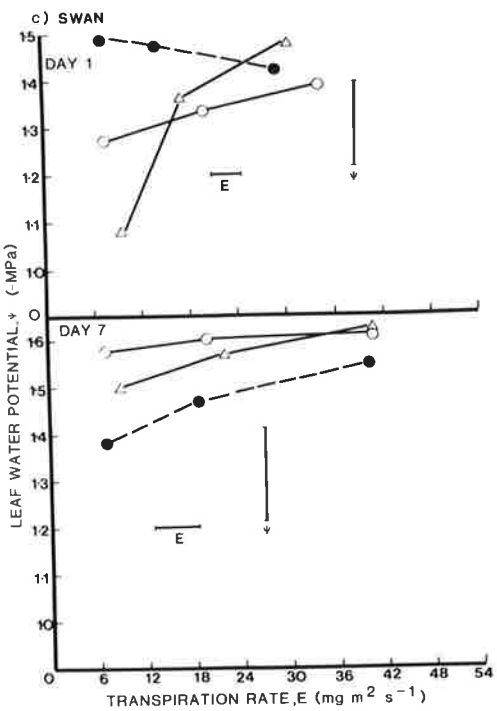
Because values of C and K showed similar patterns of response in section 4.5.3.1, C values were not calculated for this experiment.

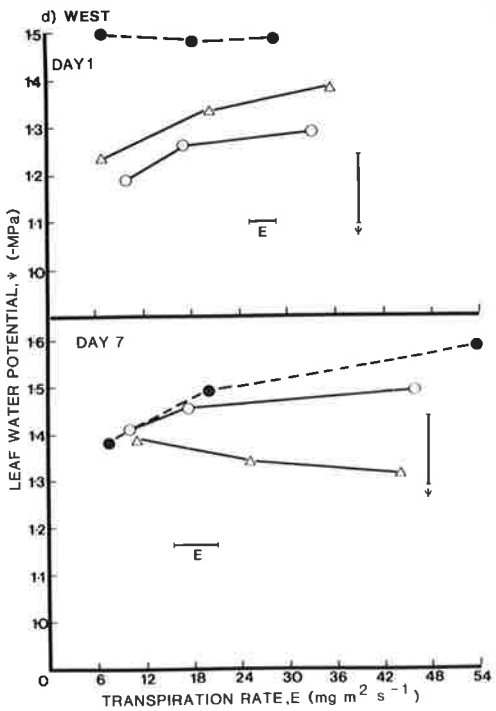
FIG 4.5.6 (a-d) The effect of Heterodera avenae infestation on the relationship between leaf water potential and transpiration rate of a) NZC b) Sual, c) Swan and d) West 1 and 7 days after commencement of relative humidity treatmer (18 and 24 days after planting)




a) NZC
DAY 1
DAY 7
E
LEAF WATER POTENTIAL, ψ (-MPa)
TRANSPIRATION RATE, E (mg m2 s-1)


b) SUAL
DAY 1
DAY 7
E
LEAF WATER POTENTIAL, ψ (-MPa)
TRANSPIRATION RATE, E (mg m2 s-1)


c) SWAN
DAY 1
DAY 7
E
LEAF WATER POTENTIAL, ψ (-MPa)
TRANSPIRATION RATE, E (mg m2 s-1)

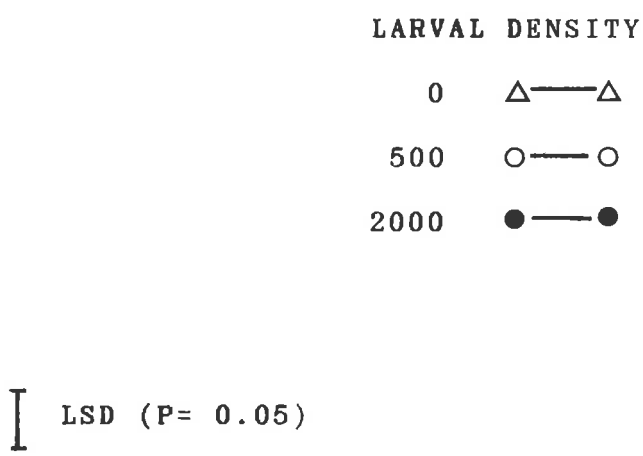

d) WEST
DAY1
DAY 7
E
LEAF WATER POTENTIAL, ψ (-MPa)
TRANSPIRATION RATE, E (mg m2 s-1)

The hydraulic conductivity increased significantly with decreasing relative humidity (RH) (increasing vapor pressure deficit) (Fig 4.5.7). This was significant only on day 1 on control and lightly infested plants. At high RH, infestation at the highest density had no effect on K. Under conditions of low RH heavily infested Sual and Swan had significantly higher values of K than lightly infested and control plants. hydraulic Conductivities of NZC and West did not differ from controls.

4.5.3.3 Pressure Chamber Study

Because inoculum densities in this trial were the same as in the first 2 studies in this series it was assumed that larval numbers were similar to values reported earlier. Measurements were conducted between 15 and 20 days after sowing during which time root lengths more than doubled in some cases. In preliminary calculations classifying C values within cultivars on the basis of root system size had no effect on the calculated values and therefore results from a range of root system surface areas were pooled. The total root surface area per plant was significantly reduced by infestation on all plants, the greatest decline occurring on Sual in which area was reduced to 26% of uninfested plants, compared to 54, 41 and 60% for NZC, Swan and West respectively (Table 4.5.5).

FIG 4.5.7 (a-d) The effect of Heterodera avenae infestation on the hydraulic root conductivity of a) NZC b) Sual, c) Swan and d) West 1 and 7 days after commencement of relative humidity treat (18 and 24 days after planting)



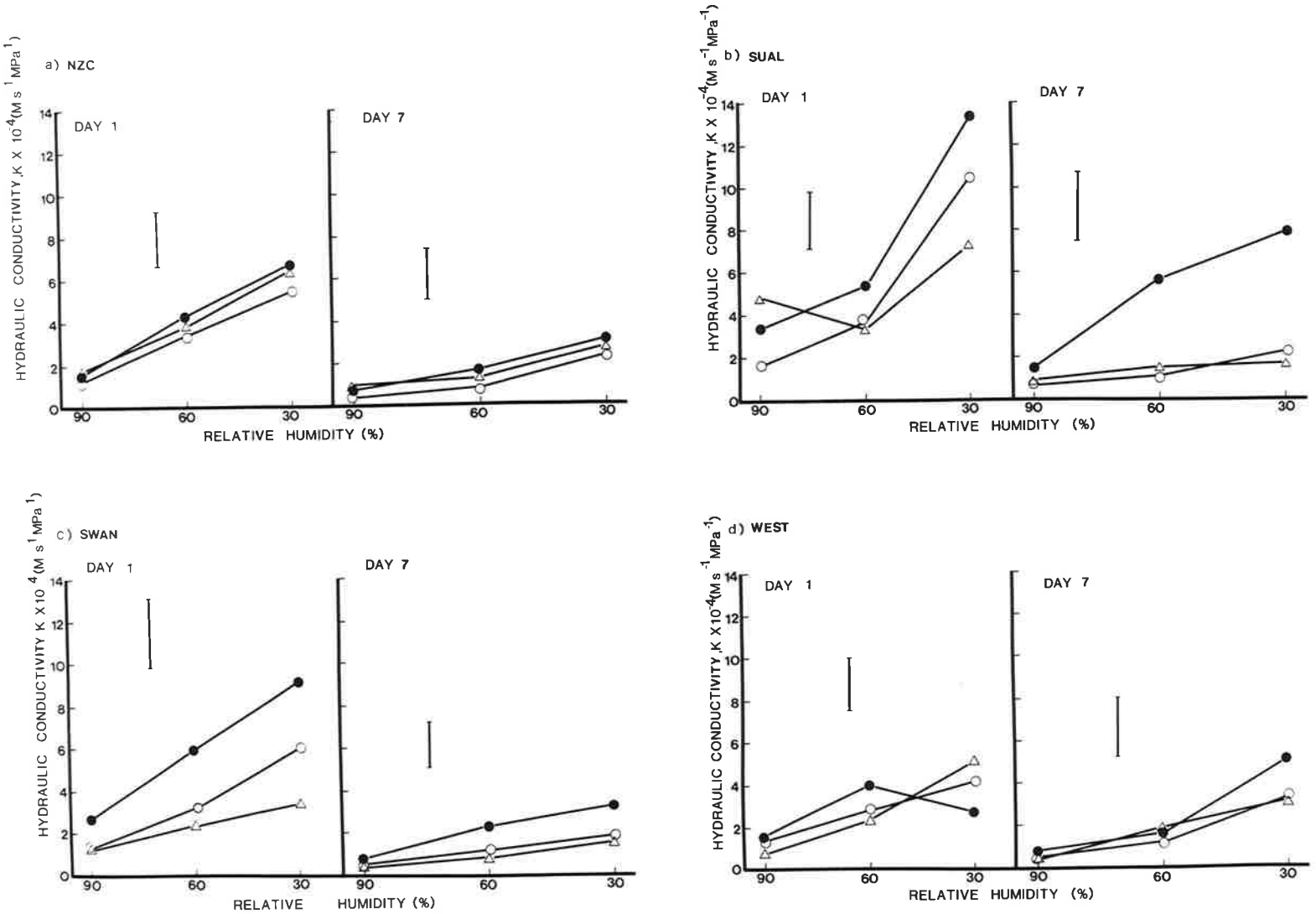

a) NZC
b) SUAL
c) SWAN
d) WEST
DAY 1
DAY 7
HYDRAULIC CONDUCTIVITY, K X 10⁻⁴ (M s⁻¹ MPa⁻¹)
RELATIVE HUMIDITY (%)
90
60
30
0
2
4
6
8
10
12
14

Table 4.5.5 The Effect of *Heterodera avenae* Infestation and Hydrostatic Pressure (Δp) on Root Length, Flow Rate (Jv) Hydraulic Conductance (C) of Four Oat Cultivars. (values obtained between 16 to 20 days after planting)

CULT.	DENSITY	ΔP	ROOT LENGTH (M)	RATE OF FLOW (Jv) (Ms^{-1})	HYDRAULIC CONDUCTANCE, C ($MPa\ ms^{-1}$)
NZC	0	0.25	915	16.2	7.1
		0.50	915	33.8	7.4
	4000	0.25	494	18.3	14.9
		0.50	494	38.1	14.3
SUAL	0	0.25	726	10.1	5.6
		0.50	726	22.1	6.1
	4000	0.25	189	4.1	8.7
		0.50	189	9.7	10.3
SWAN	0	0.25	937	13.9	5.9
		0.50	937	27.6	7.8
	4000	0.25	384	7.2	7.5
		0.50	384	15.2	7.9
WEST	0	0.25	702	8.9	5.1
		0.50	702	16.8	4.8
	4000	0.25	427	7.9	7.3
		0.50	427	14.5	6.8

ANALYSIS	LSD (P = 0.05)		
Significant interactions	Root Length	Jv	C
CV X Density	0.82	–	–
CV X Density X ΔP	–	6.2	2.9

The rate of flow (Jv) of exudate from the cut stump of infested Sual was not increased at the higher hydrostatic pressure (0.5 MPa) flow rates and all other infested and uninfested cultivars were significantly enhanced by the increase in pressure. Infestation reduced the flow rate of Sual and Swan at root pressures of 0.25 and 0.5 MPa, respectively. The flow rate of NZC and West were unaffected by infestation.

Root hydraulic conductances (C) of NZC and Sual were significantly increased by infestation whereas infestation had no effect on the K values of the other 2 cultivars. The K value of infested NZC was almost three times that of uninfested plants, averaged over the two root pressures, whereas that of Sual was only just significant. Values of C did not differ significantly between the two root pressures.

4.5.4 Discussion

In using three aproaches to study the hydraulic properties of infested root systems the experiments provided an opportunity to look at nematode effects on net shoot plus root resistance to water movement as well as on root resistances alone. The results demonstrated clear differences between cultivars in their hydraulic properties and depending upon the conditions of the experiment, in their pattern of response.

In the first study in which PEG was used as an osmoticum root conductance of two cultivars Sual and Swan declined or remained constant depending upon the measurement date with while those of NZC and West increased with increasing Ψ_s.

Reports in the literature of varietal differences in root conductances are rare (Syvertsen 1982) but studies describing interspecies differences in both magnitude of root permeability as well as the pattern of response to variable Δ P are common (Hailey et al. 1973; Newman 1976) and lend at least some credence to the observation of varietal differences noted in the present work.

The second study in which relative humidity was varied to induce different rates of water flow gave results in general agreement with a number of studies using similar methods which reported constant Ψ_L over a wide range of transpiration rates. These findings are widely interpreted as indicating that resistance to water flow through plants varies with rate of flow (Tinklin and Weatherly 1966; Lawlor and Milford 1975). In the final study, while K values of all cultivars rose with increasing hydrostatic pressure, there were again significant differences between cultivars, most important of which was a significant increase in root conductivity of infested roots of NZC.

Differences in rates of water flow E_R between studies were compatible with differences in conditions under which measurements were taken. Flow rates of PEG treated plants were more than double those of soil tested plants, reflecting a component of resistance to water flow in the soil. Nonetheless K values for both methods were within similar

ranges. It is also significant to note that the magnitude of K did not differ substantially between cultivars and that major differences were observed only in their responses to infestation.

Measurements of hydraulic conductivity using PEG and pressure chamber techniques indicated that infestation increased the permeability to water of NZC as Jv increased but had the opposite or no effect on the remaining cultivars. On the other hand results of the humidity experiment showed that, while the root permeability of all cultivars increased with increasing Jv, that of NZC and West were least responsive. In addition infestation had no effect on K of NZC and West but enhanced K of Sual and Swan.

Several explanations are possible to account for both the observed differences between cultivars within each study and between studies. The concurrence of the PEG and pressure chamber data suggests some error either in methodology or assumption in the humidity study. One possible source of error is the assumption that the soil water potential at the root surface was 0. With large overlap in root absorption zones likely in the confined root containers used in this study the probability may be high that the actual root/soil interface soil water potential was considerably less than zero for a large part of the root system, particularly for large rooted control plants. If so, predicted Δp values would be over estimated and actual conductances would be

underestimated. The PEG and the pressure chamber studies are not susceptible to this criticism. However, since water movement toward the root is propelled by water potential gradient, it is likely that on average the more rapidly water was depleted the faster was its movement to the root. Therefore the argument proposed above may not be relevant.

A second potential weakness in the interpretation of the humidity experiment is that it does not take into account possible differences in guard cell sensitivity to vapour pressure deficit. Large vapor pressure deficits can induce stomatal closure (Sheriff 1979). In a previous study (section 4.4), infestation was observed to increase stomatal conductance of Sual. If this is one manifestation of a generally reduced sensitivity to low Ψ_L or low RH, then the apparently higher root conductances of infested Sual may be a reflection of higher leaf conductances. In effect, whole plant measurements of the type carried out in this study may reflect the resilience of the root system in responding to increased water loss from the tops. If the results of the PEG and pressure chamber studies are accepted, then the higher K of infested Sual at low RH may be evidence for a signal sent from the shoot to the root causing increased root permeability, an effect that would not be observable in excised plants or where demand for water was low.

A further possible explanation for the differences in cultivar response noted between methodologies relates to soil water availability in the R.H. study. Reduced hydraulic contact with the soil may occur as a result of root shrinkage (Huck et al. 1970), particularly at high Jv.

Polysacchacharides, collectively called mucigel, exuded near the root tip may improve root contact with the soil (Greaves and Darbyshire 1972, Foster 1981). In the present study, it was found that infested roots of the cultivar Swan were consistently more stubbornly coated with soil particles than were other cultivars. It is possible that the higher conductivities recorded for Swan were an indication of increased hydraulic contact with the soil and not of a higher interval root conductivity.

Several hypotheses may be put forward to account for variability between cultivars in the effects of infestation on root permeability. Treatment of root systems with hot water, O_2 free air or metabolic inhibitors (Ginzburg and Ginzburg 1970, Stoker and Weatherly 1971, Parsons and Kramer 1974) has led to the conclusion that the pathway of water movement through the root cortex and up to the endodermis into the xylem is symplastic. On the other hand a body of evidence associating reduced hydraulic conductance with endodermal suberization (Clarkson and Robards 1975) has led others to suggest that the pathway of water movement through the root cortex is apoplastic, that is, along the outside cell walls and that the endodermis is the major barrier to entry of water (Newman 1976b, Weatherly 1982).

Despite this controversy over the origin of root resistance itself, three tentative hypotheses will be tendered to account for the variability between cultivars in the effect of infestation on root permeability. If infestation leads to endodermal disruption, and if the endodermis constitutes a barrier to water entry, it is easy to see how infestation could induce a rise in conductance of the root system. Differences between cultivars could derive from differences in either susceptibility to endodermal penetration or to endodermal repair (say by further suberization). Alternatively if cortical cell protoplasts and associated plasmodesmata constitute the major obstacle to passage of water, and if syncitial formation following nematode invasion reduces the number of cells separating the rhizosphere from the periderm and xylem trachea, the net effect would be an elevated conductance following infestation. Again intra-varietal differences could be associated with effects of infestation on cortical thickness, syncitial size, or number and efficiency of plasmodesmata.

The possibility that changes in root permeability following infestation have a hormonal basis cannot be ruled out either. Work described earlier (Section 3) showed that nematode invaded roots had elevated levels of ABA. ABA has been reported to stimulate water flow and increase root hydraulic conductance (Tal and Imber 1971, Glinka 1977 1980). However, given an absence of cultivar variation in nematode induced changes in ABA levels in the study reported in section 3 the likelihood that the relationship betwen K and infestation is hormonally mediated may be small.

If results of the PEG and the pressure bomb studies are accepted as reliable, the findings may in part account for the higher level of tolerance of NZC and possibly also West to Heterodera avenae compared to Sual and Swan. Higher conductance to water flow would be an asset under well watered conditions. Section 4.2 showed that because of a smaller effect of infestation on root growth NZC was able to penetrate the water table earlier than the remaining cultivars.

Interestingly the transpiration rate (E_R) of Sual was generally less responsive (i.e. did not decline) to increasing solution PEG concentrations than the other cultivars, particularly NZC. It was already suggested that stomatal operation of Sual may be less sensitive to low (30%) R.H., and to soil water deficit stress (Chapter 4, Section 4.3), though the results of Chapter 4, Section 4.4 failed to confirm this. If it is assumed that E_R largely reflects stomatal aperture, and that changes in root hydraulic conductance are consequent to changes in plant water status arising from the sensitivity of stomata to the environment, then the stable E_R values under conditions of reduced water availability observed in Sual are suggestive of an insensitivity of stomata to water stress compensated for by increased root hydraulic conductance. Given the sensitivity of the root system of Sual to stunting by nematode infestation such a compensatory mechanism would be ineffective under conditions of soil water deficiency.

The association between nematode tolerance and drought resistance has been observed before: e.g. Fatemy *et al*. (1985) reported that infested nematode tolerant potato plants used water more efficiently than intolerant plants because stomata were more sensitive to apparent changes in plant water status. The findings of the present study provide an explanation for the lower water use efficiency of infested Sual observed in Chapter 4, Section 1.1.

To conclude, the higher root conductance of infested NZC particularly under high shoot-root pressure potential gradients, coupled with vigorous root growth may contribute significantly to a high level of tolerance to *Heterodera avenae*.

SECTION 5: ASSIMILATE SUPPLY, CO_2 ASSIMILATION RATE AND *Heterodera avenae* INFESTATION

5.1 Effect of CO_2 Supply on Early Root Growth of Infested Oat Cultivars

5.1.1 Introduction

Previous studies in this thesis have shown that minimal disruption of root growth following nematode invasion may be a characteristic of tolerant cultivars (Chapter 4, section 2). An investigation into the potential mediatory role of 2 plant growth substances, ABA and Ethylene, failed to detect any strong cultivar variation that could account for the more vigorous root growth of tolerant compared to intolerant cultivars (Section 3). It was observed however that the differential root response to infestation could be neutralized by removal of the shoot (Section 3.1) possibly implicating a requirement for a shoot-derived component necessary for expression of a characteristic associated with tolerance. One such component may be carbohydrate. Reduced concentration of carbohydrate in the root has been associated with a decline in the initiation and growth of root meristems (Davidson 1968, Brouwer 1977) as well as a reduced capacity for uptake and transport of minerals, especially nitrogen, to the shoot (Koster 1973).

The following study was carried out to find out if the root growth response to infestation could be manipulated by varying the supply of carbohydrate coming from the shoot.

5.1.2 Methods

Seedlings of four oat cultivars (NZC, Sual, Swan and West) were grown in 26 x 2.7 cm tubes containing John Innes soil capped at the bottom with Clipsal caps as previously outlined (Section 4.3.2). Plants were inoculated with Heterodera avenae larvae at larvae/plant densities of 500, 1400, 2800 and 4200 between 3 and 10 days after planting and maintained until ten days after planting in standard growth cabinet conditions (Chapter 3, 2.3.1). On the tenth day plants were selected for uniformity and transferred to growth chambers having reduced (150 μbar), ambient (310 μbar) or enriched (900 μbar) CO_2 partial pressure ($p(CO_2)$), other environmental variables being held constant at pre-CO_2 treatment levels (Chapter 3, 2.3.2). The CO_2 treatment was for 8 hours during the light cycle every day for 14 days following commencement of the treatment.

Destructive sampling was conducted 0, 7 and 14 days after initiation of the CO_2 treatment. Measurements included root and shoot mass, root length and larval population in the root. In addition, estimates of total root soluble carbohydrate were obtained on roots 14 days after commencement of CO_2 treatment (Chapter 3, 2.9.8) with final values being the mean of determinations on three plants per treatment. Root and shoot mass measurements were replicated 6 to 8 times. A randomized complete block design, where chamber position represented a block, was implemented by rotating positions of the chambers.

5.1.3 Results

Exposure to reduced or increased CO_2 partial pressure ($p(CO_2)$) had no consistent effects on total Heterodera avenae larval population in roots during treatment (Table 5.1).

Table 5.1: Effect of Density of Heterodera avenae on Total Nematode Number (Male and Female) 0, 7 and 14 Days After Commencement of CO_2 Treatment

		NEMATODE NUMBER						
CULTIVAR	DENSITY	DAYS AFTER COMMENCEMENT OF CO_2 TREATMENT						
		0	7			14		
		CO_2 PARTIAL PRESSURE, $P(CO_2)$ (μ bars)						
		310	50	310	900	50	310	900
NZC	1400	272	372	455	296	319	343	328
	4200	607	692*	1099	680*	827	780	719
SUAL	1400	296	286*	414	394	354	263	289
	4200	803	829	972	889	721	639	792
SWAN	1400	341	534	605	524	287*	482	476
	4200	737	946	1021	1023	762	786	891
MEAN			610	761	634	545	549	582

* Significantly different from ambient CO_2 treatment at the relevant density and date ($P = 0.05$).

Significant interactions: Cultivar (CV) X Density ($P = 0.05$)

Density X Days ($P = 0.05$)

Density X CO_2 ($P = 0.05$)

CV X Density X CO_2 ($P = 0.01$)

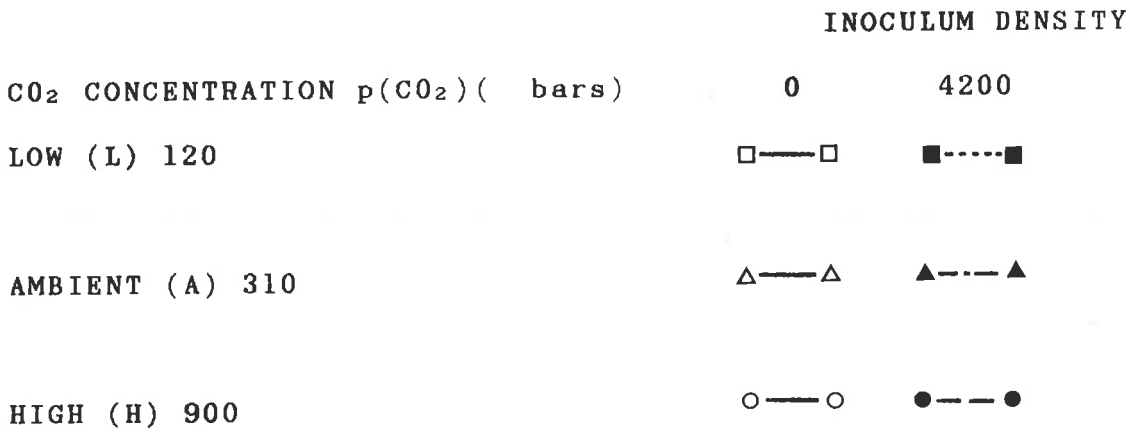
CV X Density X CO_2 X Day ($P = 0.01$)

A significant four-way interaction was a result of reduced larval numbers on CO_2 deprived NZC and Sual on day 17 and on Swan on day 24.

$P(CO_2)$ had a significant ($P = 0.01$) effect on shoot growth of all three cultivars on both day 14 and 24 (Fig. 5.1). Effects of CO_2 enrichment were significant by the fourteenth day of CO_2 treatment. CO_2 deficiency significantly slowed growth after 7 days of treatment. Exposure to enriched and ambient CO_2 resulted in a significant decline with time in relative growth rate (Rw) of NZC. Infestation had no effect on shoot growth of any cultivar.

Both $p(CO_2)$ and infestation strongly influenced root growth, with effects differing between cultivars (Table 5.2, Fig. 5.2). CO_2 supply had a similar effect on root extension of all three cultivars, reducing growth to between 30-40% and 40-50% that of ambient controls on week 1 and week 2 respectively. Effects were similar over all densities. Root growth of all cultivars was significantly ($P = 0.01$) increased at high CO_2 on week 1 of CO_2 treatment. A significant CO_2 x density x cultivar interaction was observed by the end of week 2. An enriched CO_2 supply had no effect on root extension of Sual and Swan at the highest inoculum density compared to ambient CO_2 grown plants, but significantly ($P = 0.001$) improved growth of NZC at the same density. Infestation had no effect on root length of low CO_2 treated roots on either day 7 or 14.

FIG 5.1 a-c The effect of Heterodera avenae infestation on shoot mass and relative shoot growth rate of a) NZC, b) Sual and c) Swan 7 and 14 days after exposure to low, ambient or high CO_2 concentrations (17 and 24 days after planting)



Infestation (4200 larvae) indicated in bar graph by shading.

I LSD (P = 0.05)

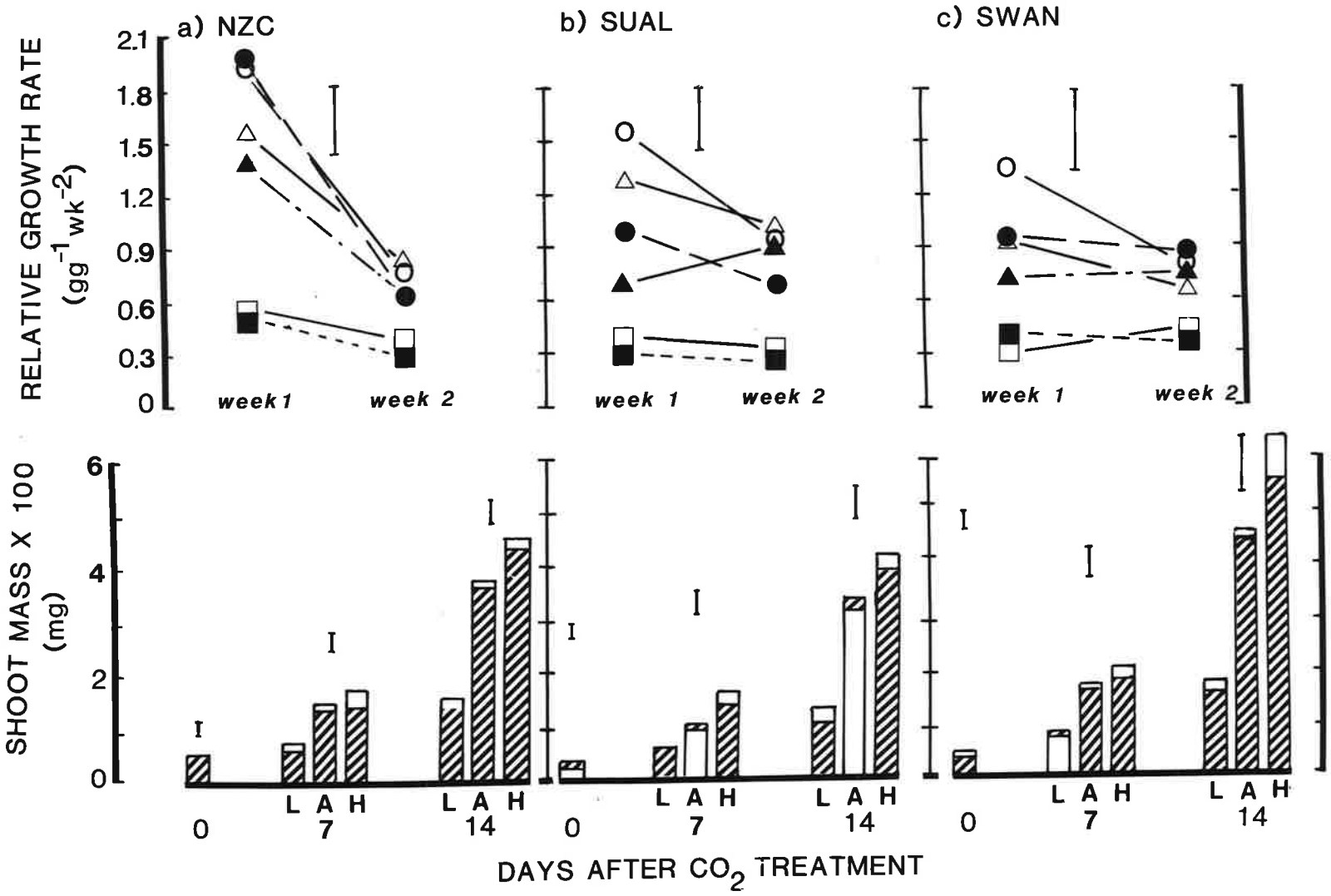
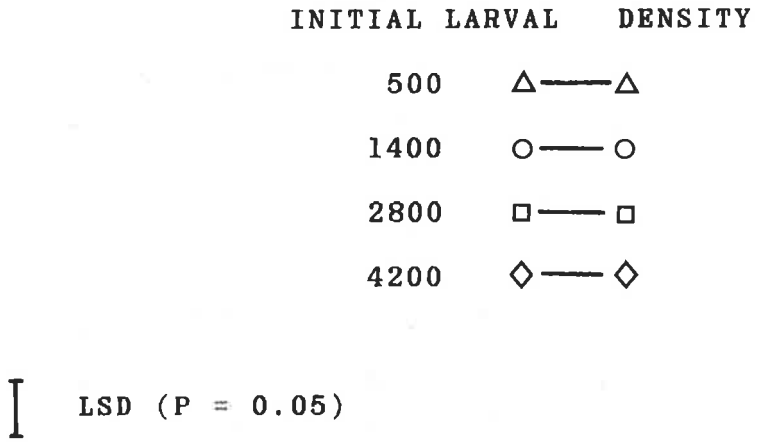

a) NZC
b) SUAL
c) SWAN
RELATIVE GROWTH RATE
(gg^{-1}wk^{-2})
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0
week 1
week 2
SHOOT MASS X 100
(mg)
6
4
2
0
L A H
0
7
14
DAYS AFTER CO_2 TREATMENT

FIG 5.2 The effect of infestation and CO_2 concentration on % decline in root length of a) NZC, b) Sual and c) Swan 7 and 14 days after commencement of exposure to 120, 310 or 900 bars CO_2 (17 and 24 days after planting)



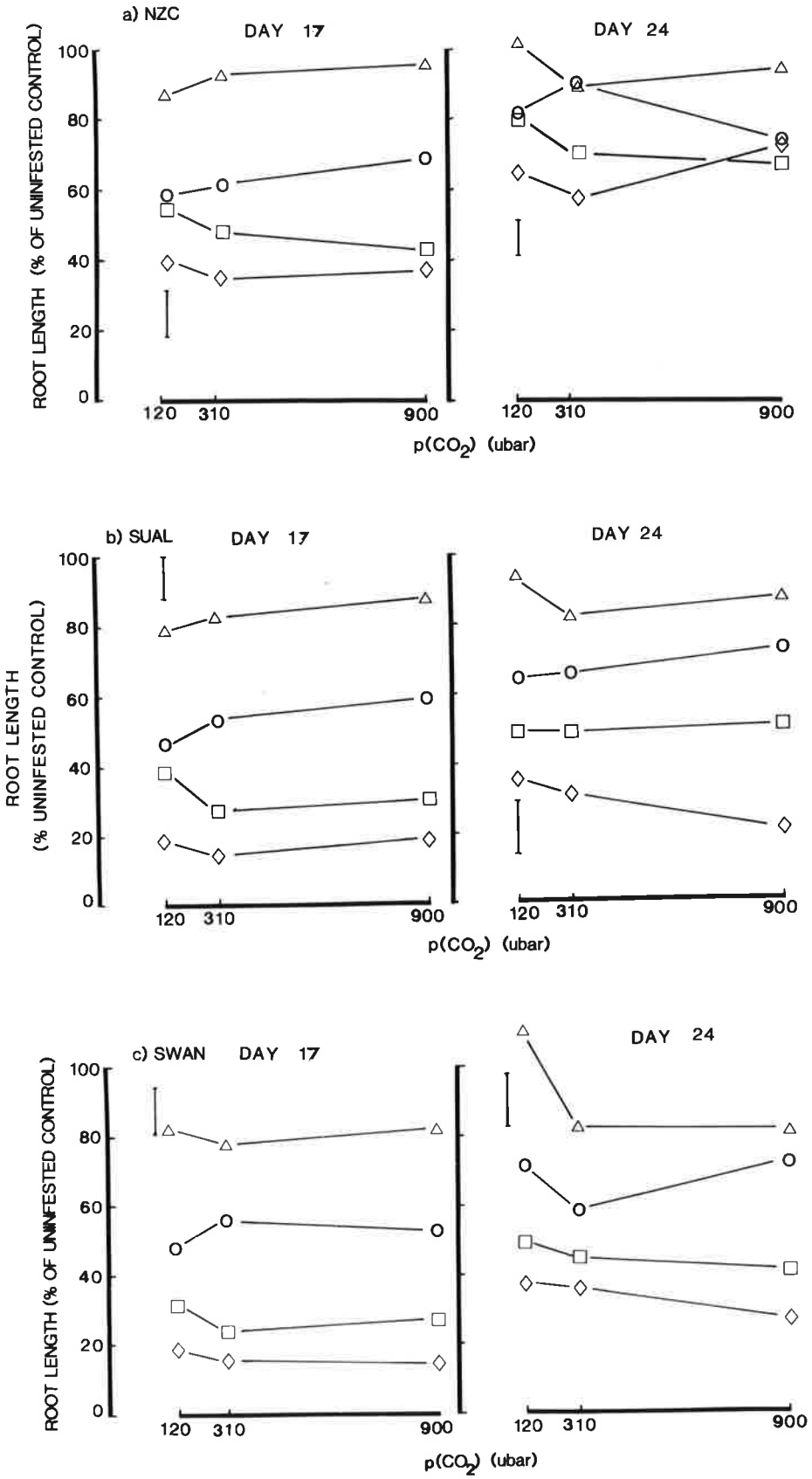

a) NZC
DAY 17
DAY 24
ROOT LENGTH (% OF UNINFESTED CONTROL)
100
80
60
40
20
0
120 310 900
120 310 900
p(CO2) (ubar)
b) SUAL
DAY 17
DAY 24
ROOT LENGTH
(% UNINFESTED CONTROL)
100
80
60
40
20
0
120 310 900
120 310 900
p(CO2) (ubar)
c) SWAN
DAY 17
DAY 24
ROOT LENGTH (% OF UNINFESTED CONTROL)
100
80
60
40
20
0
120 310 900
120 310 900
p(CO2) (ubar)

Table 5.2: Effect of Density of *Heterodera avenae* on Total Root Length of 3 Oat Cultivars over 2 Weeks of CO_2 Treatment, 10-24 Days After Planting

ROOT LENGTH (m)

DAYS AFTER START OF CO_2 TREATMENT

	0	7			14		
	PARTIAL PRESSURE P(CO_2) (μ bars)						
CULTIVAR/ DENSITY	310	50	310	900	50	310	900
NZC 0	0.92	0.94	3.48	6.69	2.56	5.59	9.38
500	0.83	1.24	3.23	6.42	2.62	4.91	8.80
1400	0.61	0.87	2.12	4.74	2.07	4.98	5.93
2800	0.52	0.58	1.70	2.74	1.83	3.94	5.48
4200	0.49	0.46	1.28	2.60	1.64	3.24	6.96
SUAL 0	0.69	0.78	2.45	3.78	1.80	4.85	6.05
500	0.62	0.68	2.02	3.31	1.70	3.97	5.27
1400	0.35	0.47	1.29	2.29	1.15	3.19	4.41
2800	0.21	0.30	0.67	1.17	0.89	2.40	3.08
4200	0.13	0.15	0.35	0.66	0.59	1.50	1.24
SWAN 0	1.31	1.22	4.84	7.03	2.50	6.12	7.86
500	1.18	1.03	3.79	5.79	2.75	4.98	6.40
1400	0.61	0.74	2.70	3.75	1.82	3.54	6.03
2800	0.19	0.46	1.14	1.89	1.24	2.78	3.26
4200	0.19	0.24	0.76	1.04	0.97	2.18	2.11

ANALYSIS LSD (P = 0.05,*;P 0.01,**)

SOURCE OF VARIATION	0	7	14
Cultivar (CV)	0.087*	0.371**	0.523**
Density	0.058**	0.329**	0.542**
CO_2	-	0.394**	0.580**
CV X Density	0.119*	0.796	NS
CV X CO_2	-	NS	1.241*
CO_2 X Density	-	0.838	1.298*
CV X Density X CO_2	-	NS	1.792*

TABLE OF MEANS FOR TABLE 5.2

CV X Density

Day 7

	0	500	1400	2800	4200	MEAN
NZC	3.70	3.63	2.58	1.67	1.44	2.62
SUAL	2.33	2.00	1.35	0.71	0.39	1.36
SWAN	4.36	3.54	2.40	1.16	0.68	2.43
MEAN (DENSITY)	3.46	3.06	2.11	1.04	0.83	

Day 14

NZC	5.84	5.44	4.33	3.75	3.95	4.66
SUAL	4.23	3.65	2.92	2.12	1.11	2.81
SWAN	5.49	4.71	3.80	2.43	1.75	3.64
MEAN (DENSITY)	5.19	4.60	3.68	2.77	2.27	

CV X CO_2

	DAY 7				DAY 14			
	50	310	900		50	310	900	
NZC	0.82	2.36	4.69	2.62	2.14	4.53	7.31	4.6
SUAL	0.48	1.36	2.28	1.36	1.23	3.19	4.01	2.8
SWAN	0.74	2.65	5.31	2.43	1.86	3.92	5.13	3.6
MEAN (CO_2)	0.68	2.12	4.08	1.74	3.88	5.48		

DENSITY X CO_2

Day 7

	0	500	1400	2800	4200
50	0.98	0.98	0.69	0.45	0.28
310	3.59	3.01	2.04	1.17	0.79
900	5.83	5.17	3.59	1.93	1.43

Day 14

50	2.29	2.36	1.68	1.32	1.07
310	5.52	4.62	3.90	3.04	2.30
900	7.76	6.82	5.46	3.94	3.44

Root mass was not as affected by infestation as root length (Table 5.3).

Table 5.3: The Effect of Infestation and (CO_2) on the Dry Root Mass of 3 Oat Cultivars 14 Days After Commencement of CO_2 Treatment

ROOT MASS PER PLANT (mg)

CO_2 PARTIAL PRESSURE p(CO_2) (ubars)

CULT.		150	310	900	MEAN
NZC	0	72	178	241	163
	500	61	172	207	146
	1400	66	160	189	138
	2800	78	169	253	166
	4200	69	220	218	169
MEAN		69.2	179	222	157
SUAL	0	18	91	155	88
	500	16	110	162	96
	1400	19	110	133	87
	2800	17	135	162	105
	4200	23	92	103	72
MEAN		18.6	108	143	89
SWAN	0	49	158	171	119
	500	22	141	219	127
	1400	25	151	207	127
	2800	25	132	143	100
	4200	30	146	144	106
MEAN		26.2	156	176.8	116

ANALYSIS

SOURCE OF VARIATION	LSD(P = 0.05)
CV X DENSITY X CO_2	36*

Infestation had no effect on root mass of any cultivar exposed to low or ambient $p(CO_2)$. At the highest inoculum density only Sual was reduced in root mass at high $p(CO_2)$. The root mass of all cultivars was significantly reduced at 150 $p(CO_2)$ at all inoculum densities. Exposure to increased $p(CO_2)$ increased root mass of NZC at densities of 0 and 2500 larvae, of Sual at 0 and 500 larvae, and of Swan at 500 and 1400 larvae per plant.

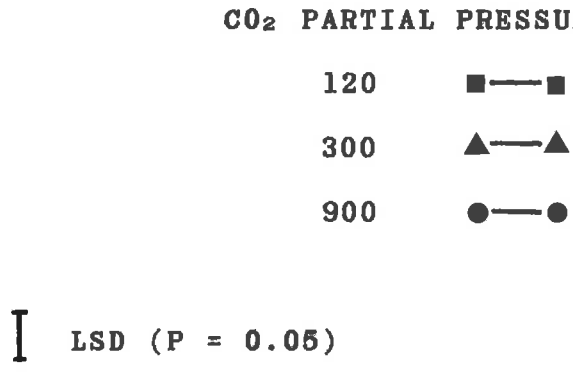
The effect of $p(CO_2)$ on the mean root total soluble carbohydrate concentration (TSC) is shown in Table 5.4. Infestation had no effect on root TSC, nor were CV or CO_2 interactions with inoculum density significant, roots of plants exposed to 150 ubars $p(CO_2)$ had significantly lower TSC content than on ambient CO_2 control plants. High CO_2 treatment significantly increased the TSC content of all cultivars, though only marginally in the case of Sual.

The relationship between root mass and the % total soluble carbohydrate (TSC) is presented in Fig. 5.3. Root mass was directly related to TSC. Differences between cultivars in slopes relating mass and TSC did not differ.

5.1.4 Discussion

Photosynthesis is limited in C_3 plants by low $p(CO_2)$ (Gaastra 1959) and its enhancement by increased CO_2 is understood in terms of competitive interactions between O_2 and CO_2 in the carboxylation of RuP_2 (Lorimer et al. 1977).

FIG 5.3 Relation between root mass and total soluble carbohydrates in a) NZC, b) Sual and c) Swan after 14 days exposure to 120, 310 and 900 μbars CO_2 (24 days after planting)

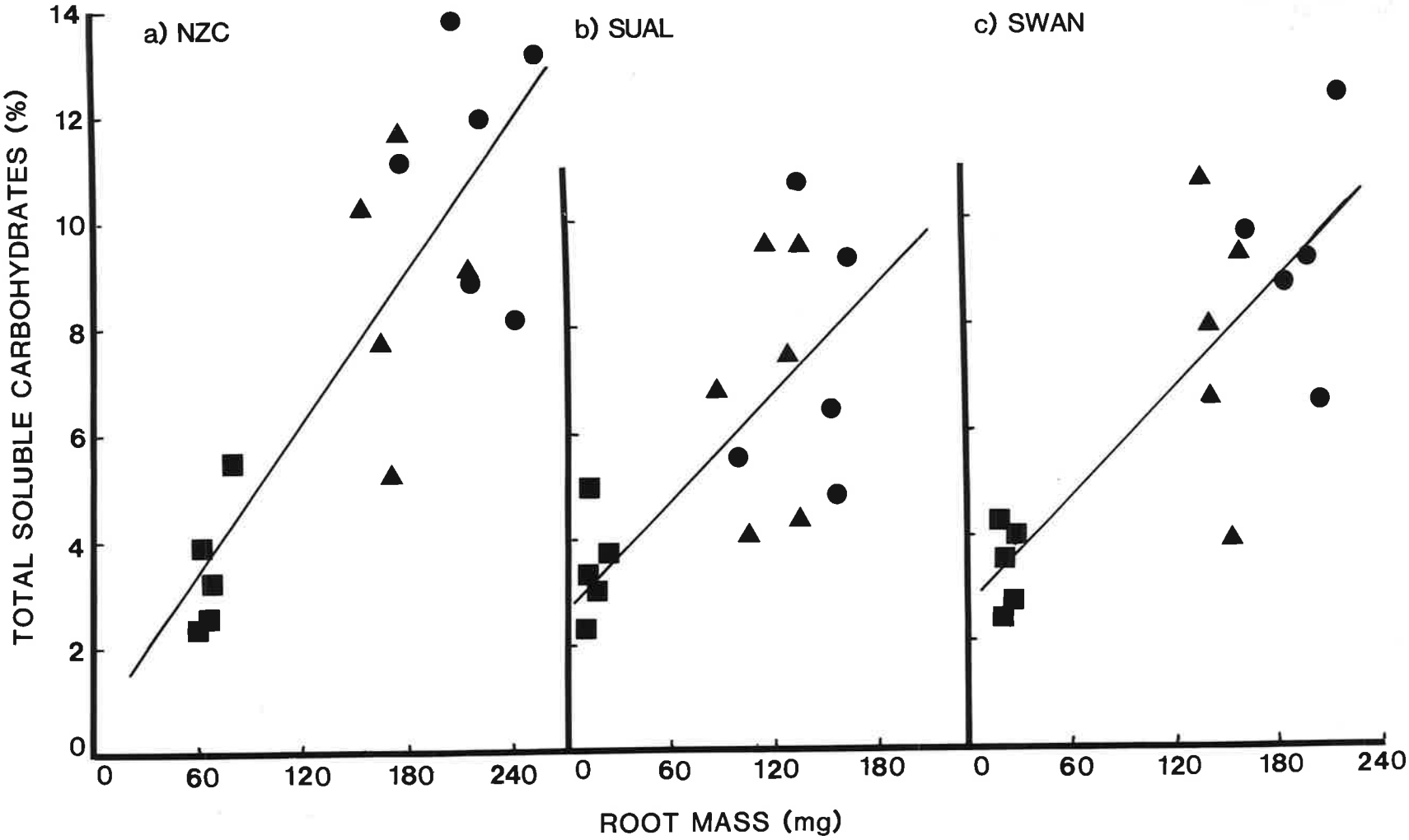


REGRESSION EQUATIONS:

a) NZC Y = 0.623+0.0461x r = 0.81

b) SUAL Y = 2.82+0.031x r = 0.79

c) SWAN Y = 2.82+0.033 r = 0.76


a) NZC
b) SUAL
c) SWAN
TOTAL SOLUBLE CARBOHYDRATES (%)
0
2
4
6
8
10
12
14
0
60
120
180
240
ROOT MASS (mg)

ANALYSIS	LSD (P = 0.05,*)
SOURCE OF VARIATION	
CULTIVAR (CV)	0.8*
CO_2	0.7*
DENSITY	NS
CV X CO_2	NS
CV X DENSITY	NS
CO_2 X DENSITY	1.6*
CV X DENSITY X CO_2	NS

Table 5.4: The Effect of Infestation and p(CO_2) on the Total Soluble Carbohydrate Concentration in Roots of 3 Oat Cultivars 14 Days After Commencement of CO_2 Treatment

CULT.	DENSITY	TOTAL SOLUBLE CARBOHYDRATE CONCENTRATION (% DRY ROOT MASS)			
		CO_2 PARTIAL PRESSURE, p(CO_2)(μbars)			
		150	310	900	MEAN
NAC	0	2.7	11.6	8.1	7.4
	500	3.98	5.1	13.6	7.5
	1400	2.60	10.0	11.1	7.9
	2800	5.4	7.60	12.8	8.6
	4200	2.30	8.9	8.6	6.6
MEAN		3.4	8.6	10.8	7.6
SUAL	0	4.8	6.9	6.7	6.1
	500	3.01	8.8	4.8	5.4
	1400	2.1	3.8	10.4	5.5
	2800	2.9	4.0	9.0	5.1
	4200	3.5	6.4	6.1	5.3
MEAN		3.3	6.0	7.2	5.5
SWAN	0	2.3	9.2	9.6	7.0
	500	2.1	7.6	12.1	7.3
	1400	4.2	3.7	9.2	5.7
	2800	3.4	10.6	6.2	6.7
	4200	4.0	6.4	8.6	6.3
MEAN		3.2	7.5	9.1	6.6
					MEAN (DENSITY)
		3.3	9.2	8.1	6.8
MEAN		3.0	7.2	9.9	6.7
(DENSITY X CO_2)		2.9	5.9	10.2	6.4
		3.9	7.3	9.2	6.8
		3.3	7.2	7.8	6.1
MEAN (CO_2)		3.3	7.6	9.4	

CO_2 enrichment has been shown to enhance relative growth rate (Rw) of monocots (Gifford et al. 1973, Gifford 1977) though the response has been found to vary with plant age and CO_2 concentration (MacDowell 1972, Neales and Nicholls 1978). The effects of $P(CO_2)$ on plant growth in the present study were in general agreement with these studies. The absence of a sustained effect of CO_2 enrichment on Rw found in this and other studies (Attwell 1971, Neales and Nicholls 1978, Kramer 1981) may have been due to a growth limiting deficiency acting elsewhere as well as a reduction in the intrinsic efficiency with which CO_2 is incorporated with time (Bjorkman et al. 1980, Kriedemann 1984).

While nematode infestation had no effect on growth of shoots at any level of CO_2 supply, root growth was strongly influenced. The effect of infestation on root length at densities of 1400 or greater were more pronounced at high $p(CO_2)$ than at ambient conditions. In contrast not only did CO_2 enrichment increase root growth of NZC more than that of Swan and Sual, but in addition, the length of the most heavily infested roots of NZC at high CO_2 was greater than that at the same density grown at ambient CO_2 concentrations. The close correlation between total soluble carbohydrates and total root mass observed in plants in this study supports the proposition that a faster rate of root extension of plants grown in an enriched CO_2 environment was a direct result of an improved assimilate supply.

However, the absence of an effect of infestation on root TSC indicated that the differences in infested root growth between cultivars was not because the roots that were most reduced by infestation had less carbohydrate in them.

One explanation for the independence of infested root growth of Sual and Swan on carbohydrate supply is that effects of localized cellular disruption due to nematode invasion prevailed over that of carbohydrate supply in influencing root extension. The fact that infestation did not significantly reduce the root mass of Swan at any density and that that of Sual was reduced significantly only at the highest density when plants were exposed to high $p(CO_2)$ indicated that the primary effect of infestation was on root extension, not on root mass.

Another possible explanation that might account for the independence of infested root growth on CO_2 supply and carbohydrate content is that infestation reduced the efficiency by which soluble carbohydrate was used for root growth. If carbohydrate utilization efficiency of NZC was unaffected by nematode infestation while that of Sual and Swan were, then when supplied with an equivalent amount of carbohydrate growth of infested NZC roots would surpass that of Sual and Swan.

As root growth encompasses the dimensions of both mass and length, and considering that root mass was less affected by infestation than root length, differences in carbohydrate

utilization efficiency might be more accurately described in terms of root length. The manner by which carbohydrate use efficiency might be influenced by infestation is open to speculation, but is unlikely to be due to loss of carbohydrates through, for example, nematode feeding or root exudation, since the TSC content of roots was unaffected by infestation.

To conclude, an enriched CO_2 environment increased the growth of heavily infested NZC but reduced root mass of Sual and had no effect on Swan relative to infested ambient Co_2 plants. Differences were not due to a reduced supply of carbohydrates to the root of the latter cultivars. Instead either less localized cell disruption in roots of NZC by nematode invasion or a higher carbohydrate utilization efficiency of infested NZC might explain the smaller effects of infestation on root growth of this cultivar than on Sual and Swan.

Section 5.2: The Influence of Infestation on CO_2 Assimilation Rate

5.2.1 Introduction

The cultivar NZC has consistently been found to develop a larger root system following nematode invasion than the other oat cultivars studied (eg. Section 2.3, Section 5.1). The previous experiment (Section 5.1) indicated that the supply of carbohydrate to the root was not an important factor limiting the length of infested roots of Sual and Swan, but rather that an inability to utilize carbohydrates for root extension may be important. If assimilate supply is not a significant factor in limiting growth of infested roots, then reports of reduced rates of photosynthesis on plants having nematode infested roots (eg. Melakberhan and Webster 1985) may be relevant to root growth only if the effects on CO_2 assimilation are large. An inability to utilize carbohydrates efficiently coupled with reduced capacity to supply carbohydrates could make the roots of Sual more subject to growth impairment by infestation. This experiment was carried out to determine the effects of infestation of Heterodera avenae on photosynthesis of Sual and NZC.

5.2.2 Methods

Seedlings of NZC and Sual were grown in 4.5 x 27 cm plastic tubes in a growth cabinet under conditions previously described (Section 4.3.2). Twelve days after planting nematodes were applied to one half the plants of each

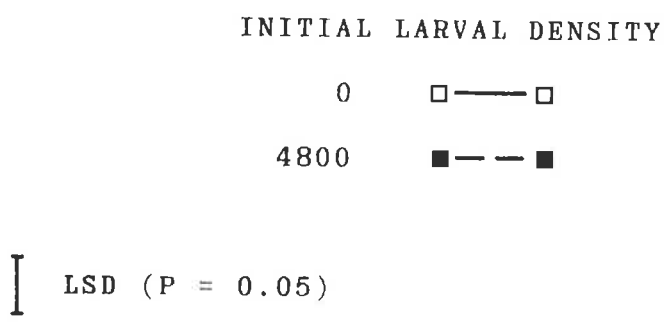
cultivar at a density of 4800 larvae per plant. Measurements of water vapour and CO_2 exchange were carried out on the fully expanded penultimate leaf 0, 5, 10, 20 and 40 days after inoculation (Chapter 3.2.11). On the evening prior to measurement plants were watered with one-quarter strength Hoaglands solution (Hoagland and Arnon 1950). Measurements were started usually at 900 hours the following day and continued until 1700 hours. The response of CO_2 assimilation rate (A) to the intercellular $p(CO_2)$ ($A;p_i$ curves) were measured on four plants per treatment.

Chlorophyll was determined on leaf discs from each leaf following gas exchange measurements (Chapter 3, 2.12).

5.2.2 Results

Infestation had no effect on the mean CO_2 uptake rate of either cultivar at ambient CO_2 partial pressure (Fig. 5.4). The maximal rate of photosynthesis was ~~at~~ high $p(CO_2)$ (CO_2 saturated rate of photosynthesis) was reduced by infestation within 10 days of nematode inoculation (Fig. 5.5). Both cultivars had virtually recovered from this effect within 40 days after treatment, NZC recovering sooner than Sual. Stomatal conductance of Sual was consistently lower than that of NZC both in the presence and absence of infestation (Fig. 5.6). As a result water use efficiency of NZC was lower than that of Sual (Fig. 5.7). Infestation resulted in significantly lower water use efficiency of Sual 10-20 days after infestation owing to the combined effect of somewhat higher conductances and lower assimilation rates of infested plants.

FIG 5.4 (a,b) The effect of infestation on mean CO_2 assimilation rate of a) NZC and b) Sual 0, 5, 10, 20 and 40 days after inoculation (12 - 52 days after planting)



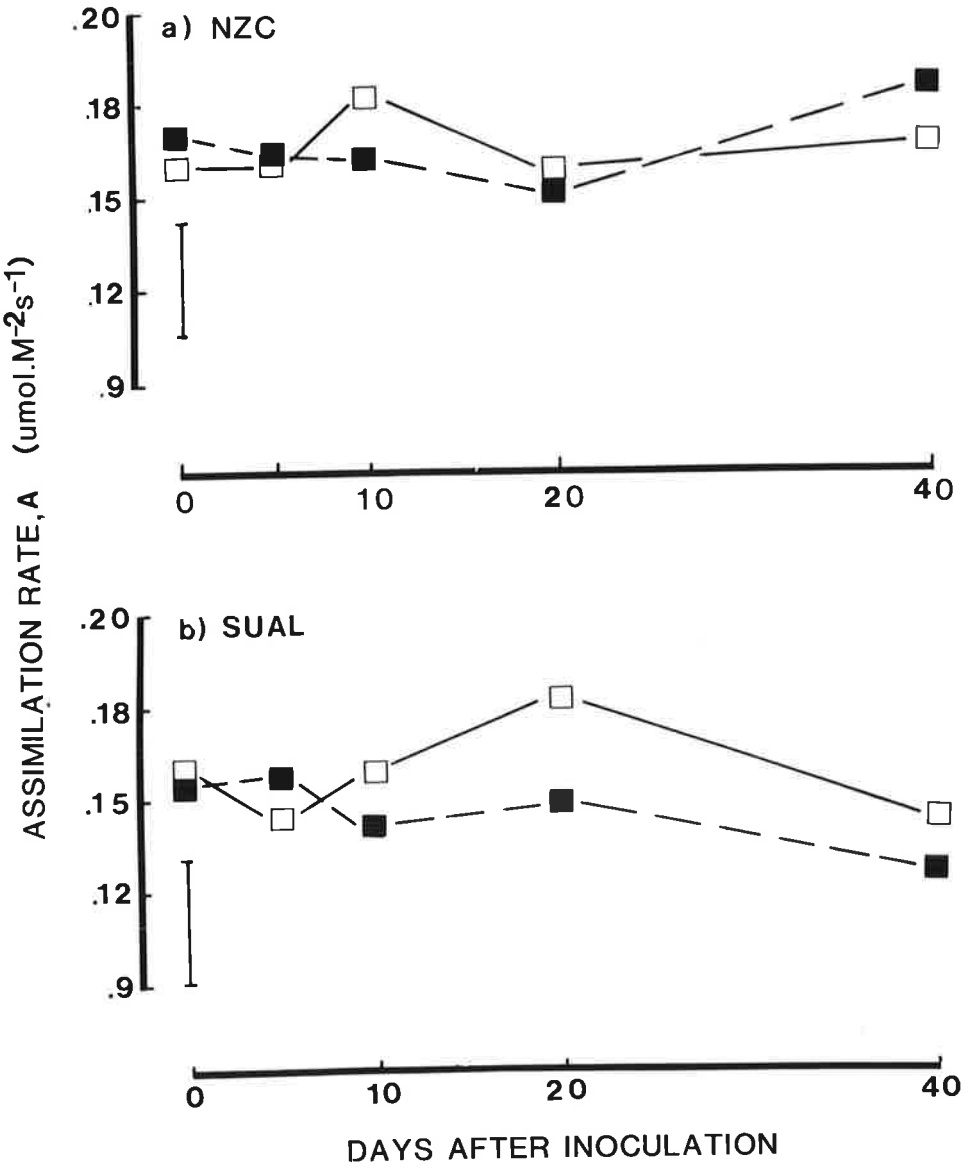
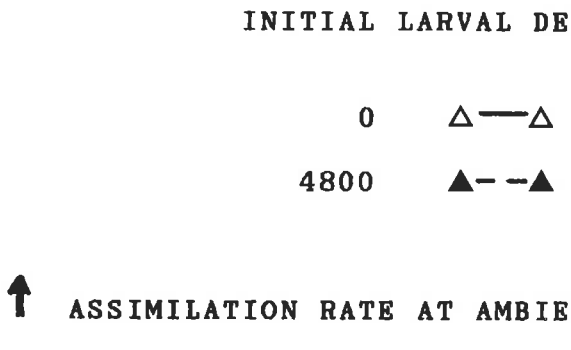
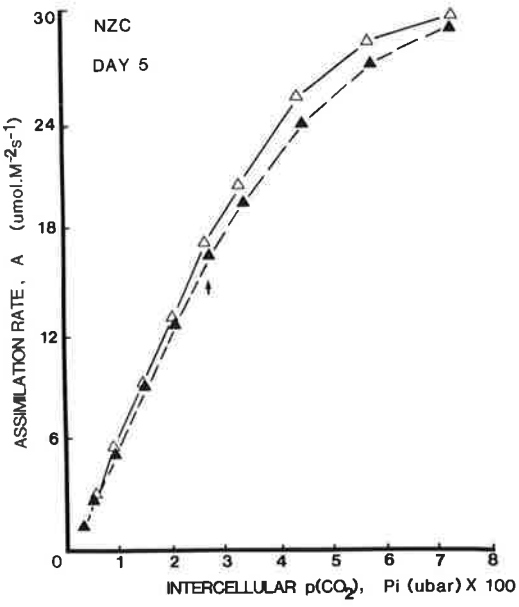

a) NZC
.20
.18
.15
.12
.9
0
10
20
40
b) SUAL
.20
.18
.15
.12
.9
0
10
20
40
ASSIMILATION RATE, A (umol.M-2s-1)
DAYS AFTER INOCULATION

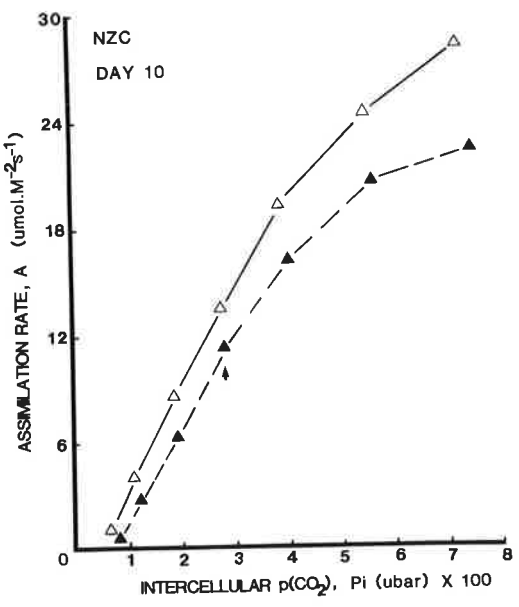
FIG 5.5 a,b The effect of Heterodera avenae infestation on the relation between intercellular CO_2 partial pressure and CO_2 assimilation rate of a) NZC and b) Sual 5, 10, 20 and 40 days after inoculation (17 - 52 days after planting)

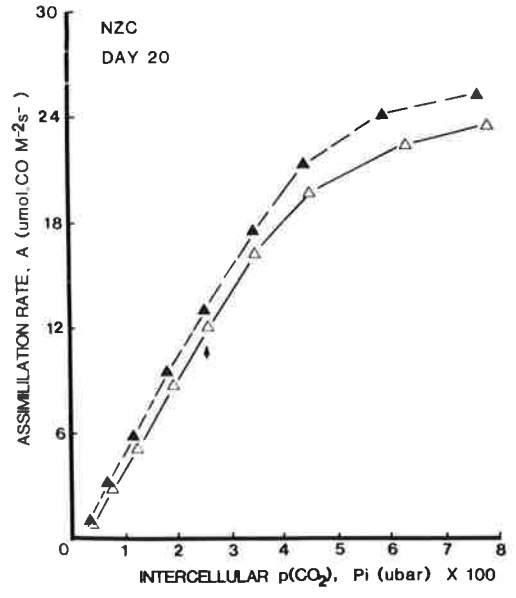
FIG. 5.5 a) NZC



a)


NZC
DAY 5
ASSIMILATION RATE, A (umol.M-2s-1)
INTERCELLULAR p(CO2), Pi (ubar) X 100


NZC
DAY 10
ASSIMILATION RATE, A (umol.M-2s-1)
INTERCELLULAR p(CO2), Pi (ubar) X 100


NZC
DAY 20
ASSIMILATION RATE, A (umol.CO M-2s-)
INTERCELLULAR p(CO2), Pi (ubar) X 100

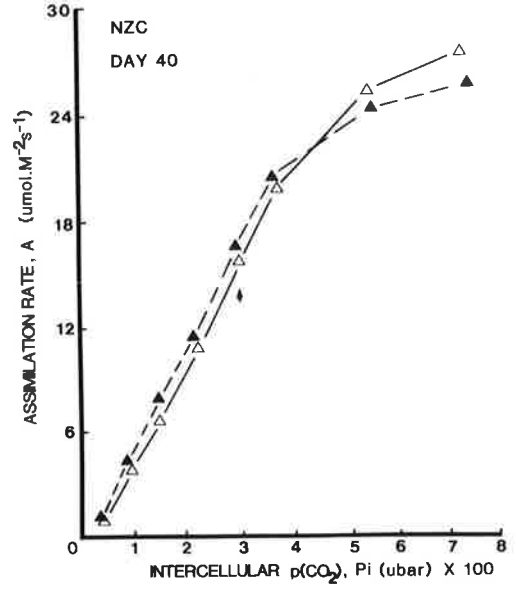
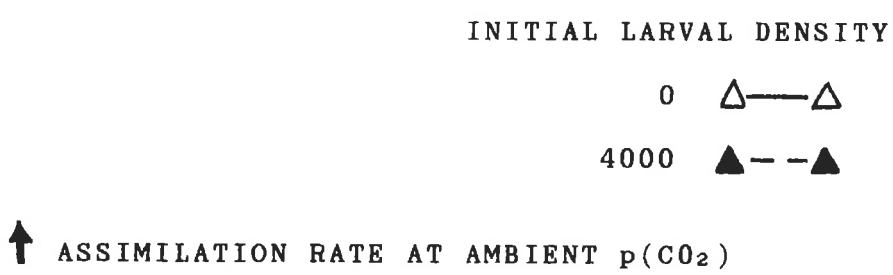

NZC
DAY 40
ASSIMILATION RATE, A (umol.M-2s-1)
INTERCELLULAR p(CO2), Pi (ubar) X 100

FIG 5.5 b) SUAL


INITIAL LARVAL DENSITY
0
4000
ASSIMILATION RATE AT AMBIENT p(CO2)

b)

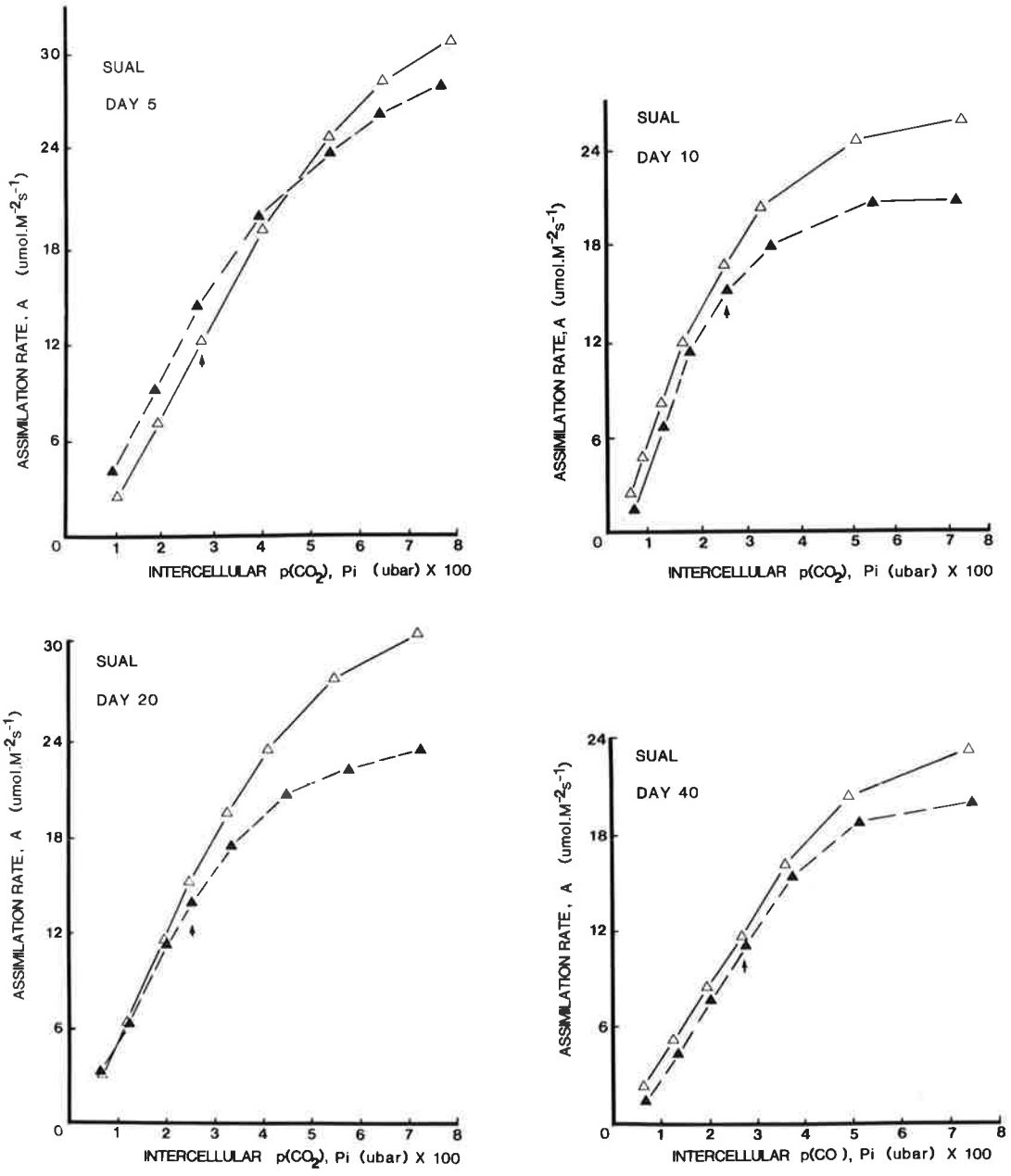

SUAL
DAY 5
ASSIMILATION RATE, A (umol.M-2s-1)
INTERCELLULAR p(CO2), Pi (ubar) X 100
SUAL
DAY 10
ASSIMILATION RATE, A (umol.M-2s-1)
INTERCELLULAR p(CO2), Pi (ubar) X 100
SUAL
DAY 20
ASSIMILATION RATE, A (umol.M-2s-1)
INTERCELLULAR p(CO2), Pi (ubar) X 100
SUAL
DAY 40
ASSIMILATION RATE, A (umol.M-2s-1)
INTERCELLULAR p(CO), Pi (ubar) X 100

FIG 5.6 The effect of infestation on the water use efficiency of NZC and Sual at ambient p(CO_2) (310 μbars) 2, 5, 10, 20 and 40 days after inoculation (12 - 52 days after planting)

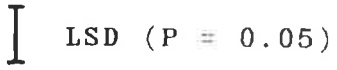
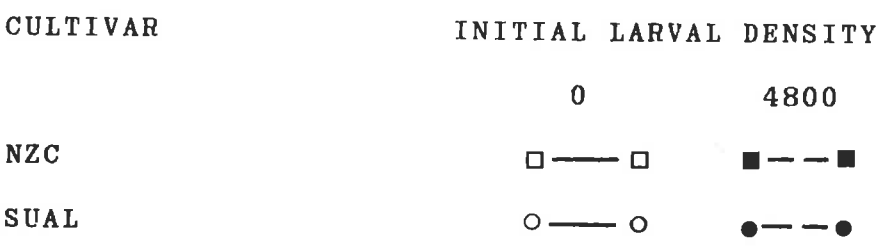
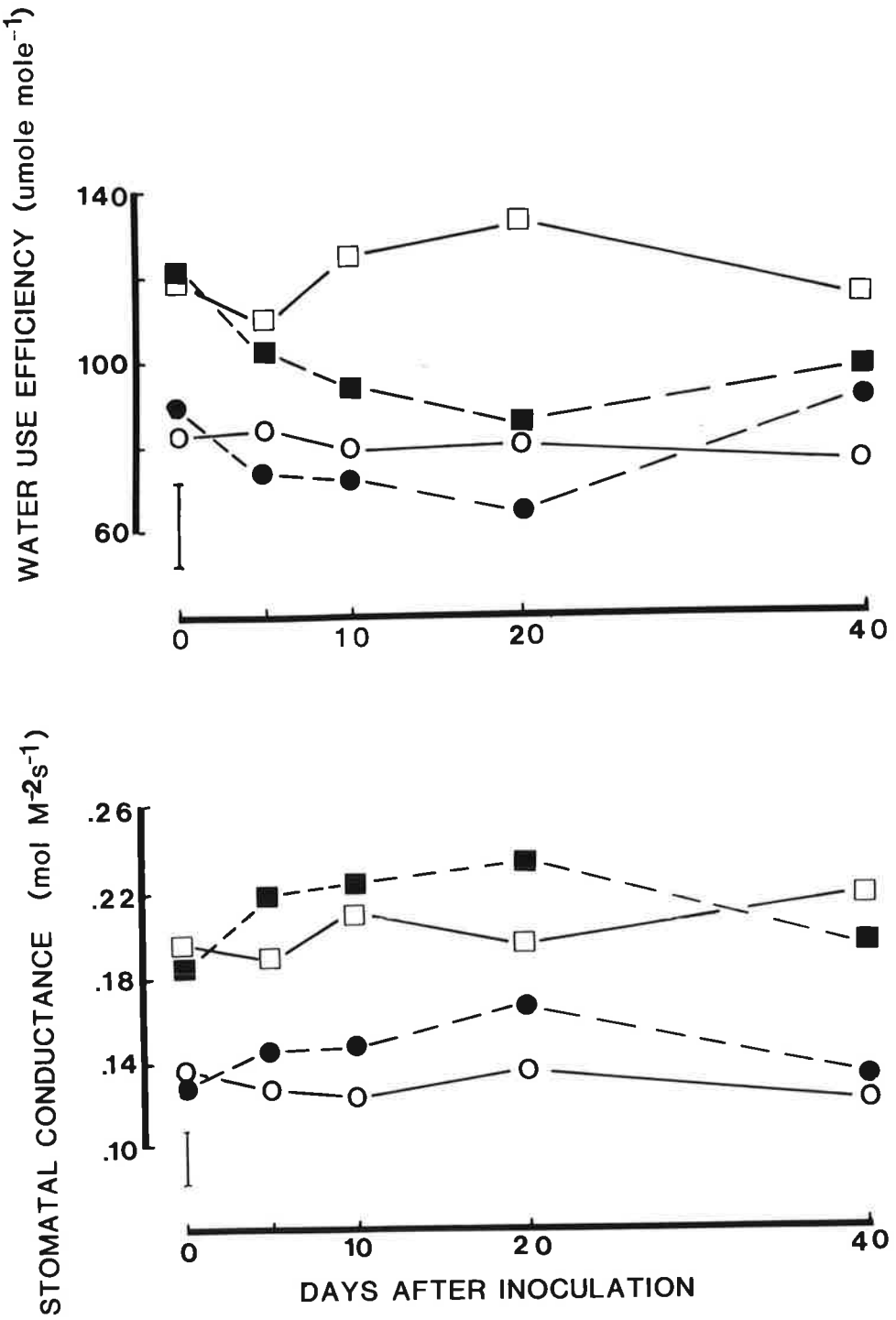


FIG 5.7 The effect of infestation on the stomatal conductance of NZC and Sual at ambient p(CO_2) (310 μbars) 2, 5, 10, 20 and 40 days after inoculation (12 - 52 days after planting)

NOTE: Fig. 5.6 and 5.7 have common legends:




WATER USE EFFICIENCY (umole mole^{-1})
140
100
60
0
10
20
40
STOMATAL CONDUCTANCE (mol M^{-2}s^{-1})
.26
.22
.18
.14
.10
0
10
20
40
DAYS AFTER INOCULATION

Chlorophyll contents of infested and control plants did not differ (data not shown).

5.2.4 Discussion

Nematode infestation reduced the CO_2 saturated rate of photosynthesis but had no effect on the CO_2-limited rate of photosynthesis. Nematode effects were most pronounced on nematode intolerant plants recently inoculated.

Declines in rates of photosynthesis following invasion of tomato plants with Meloidogyne javanica (Loveys and Bird 1973, Wallace 1973, Melakberhan and Webster 1984) as well as on potato plants infested with Globodera rostochiensis (Franco 1980) have been reported. Because no measurement of stomatal conductance was obtained in those studies how much of the decline in photosynthesis was due to a nematode effect on stomatal closure is unknown.

In the present study the CO_2 assimilation rate (A) could be measured directly against the internal CO_2 partial pressure $P(CO_2)$. This facilitated discrimination between effects of stomatal and of mesophyll resistances on photosynthetic rates. However, as has been pointed out, (Sharkey 1985) a precise value cannot be assigned to the relative contribution of stomatal and mesophyll resistances to treatment effects on photosynthesis. At the inoculum density applied in this study infestation had no influence on CO_2 assimilation rate under ambient atmosphere CO_2

conditions. The CO_2-limiting, linear region of the A/Ci curve is considered to reflect the activity of ribulose-1,5-bisphosphate carboxylase (Farquhar and Von Caemmerer 1982). The lower CO_2 assimilation rates of inoculated plants under saturated CO_2 conditions indicated that infestation interfered either with the capacity of the leaves to regenerate ribulose-1,5-bisphosphate (Farquhar and Von Caemmerer 1982) or to utilize triose phosphate (Badger et al. 1984).

It is difficult to conceive how nematode infestation could affect RuBP regeneration since it is a process chiefly limited by the rate of photosynthetic electron transport, itself dependent upon light (Farquhar and Von Caemmerer 1982). It is easier to envisage how triose phosphate utilization (TPU) could limit photosynthesis of infested plants. TPU involves the conversion of triose phosphates, products of phosphorylation of 3-phospho glycerate, into starch and sucrose, with the resultant release of inorganic phosphate (Pi). If TPU proceeds at a rate less than one-third the rate of CO_2 fixation the Pi released will be insufficient to maintain photophosphorylation (Walker and Herold 1977) and CO_2 assimilation would decline. Other factors may also be involved (Sharkey and Badger 1984). In the case of the nematode infested plant, demand for assimilate, and therefore triose phosphates could be reduced as a consequence of the slowing of the rate of root extension arising from nematode infestation. If nematode feeding

imposed an insignificant carbon drain as reported by Wallace (1974) then an accumulation of chloroplast starch could inhibit CO_2 incorporation. Unfortunately no leaf starch analysis was performed in the present study. Such a scenario is in conflict with findings attributing reduced CO_2 assimilation rates of nematode infested plants (Loveys and Bird 1973, Wallace 1974) to an increased demand on the plant for carbon skeletons (Bird and Loveys 1975, McClure 1977).

The apparent discrepancy in results of the present study and that of others which reported photosynthesis of infested plants was reduced at ambient $P(CO_2)$ may have been reconciled if higher Heterodera avenae inoculum densities had been applied. However, previous studies (Section 1) have shown infestation to have significant effects on growth of the less tolerant cultivar Sual at the inoculum density used in this study. The evidence suggests that there is no reason to attribute affects on growth observed in Section 1 following inoculation of up to 4000 Heterodera avenae larvae to direct effects on photosynthesis. On the other hand infestation resulted in Sual having a greater water requirement per unit dry matter accumulation compared to NZC. This indicated that drought stress may have a stronger impact on CO_2 assimilation of Sual than NZC through the direct effects of water stress on photosynthesis. Both photosystem I and II are sensitive to water stress (Jones 1973, Govindjee et al. 1981, Bjorkman and Powles 1984).

In conclusion the difference between rates of extension of infested roots of NZC and Sual found in previous studies is not because CO_2 assimilation rate of NZC is less influenced by infestation than that of Sual.

SECTION 6: EFFECT OF PHOSPHORUS SUPPLY ON TOLERANCE OF OATS TO *Heterodera avenae*

6.1 EFFECTS OF INFESTATION ON GROWTH AND YIELD OF OAT PLANTS SUPPLIED WITH DIFFERENT AMOUNTS OF PHOSPHORUS

6.1.1 Introduction

Nematode tolerance has been associated with vigorous root growth (Evans *et al* 1977) and reduced sensitivity to root stunting induced by nematode infestation (Chapter 4, 3.2). Morphological factors which increase the absorptive surface area of a root system are of undisputed importance in facilitating acquisition of minerals, particularly phosphorus which is relatively immobile in the soil (Baldwin *et al*. 1972; Nye and Inker 1977; Schenk and Barber 1979) specific absorption rates and efficiency of mineral utilization are equally important (Loughmann 1966, Gerlof 1976, Nielsen 1979). The potential benefit of these latter properties to the ability of a plant to withstand nematode infestation has received little attention. Price *et al*. (1982) reported that infested plants absorbed phosphorus and potassium at a faster rate than uninfested plants but no cultivar comparisons were made. Evans *et al* (1977) found that the mineral concentrations of nematode infested potato cultivars could be related directly to their relative level of nematode tolerance. Yield increase has followed the supply of fertilizer to nematode infested potato (Evans *et al*. 1977) and wheat (Simon and Rovira 1985) grown in the field. It is therefore possible that adaptations rendering

a plant more tolerant to limited nutrient supply may also enhance nematode tolerance.

Phosphorus deficiency can seriously reduce crop yield (Bieleski and Ferguson 1983). The rate of release of P from a bound into a soluble form is slow and it is only slowly diffusible in solution (Reisenauer 1966, Lewis and Quirk 1967). Phosphorus supply could be an important factor limiting growth for nematode infested plants suffering root growth impairment. The ability to take up and/or use phosphorus efficiently would be advantageous in these circumstances.

Cultivars were compared for their tolerance to the presence of nematodes over a range of phosphorus concentrations to ascertain whether growth response to nematode infestation could be influenced by P nutrition.

6.1.2 Methods

Preliminary experiments were carried out to ascertain the suitability of the sand grain size with respect to root growth and inoculation efficiency. Results are presented in Appendix I. The results showed that if plants were grown in sand with particle dimensions of 250-750 µm, inoculum densities of 1000 or more larvae would significantly reduce the root length of Swan.

In the next study four oat cultivars, NZC, Sual, Swan and West, were prepared for planting as previously described (Chapter 3.2.1). Prior to sowing, approximately 2/3 of the endosperm was excised from each grain using a sterile scalpel, and seedlings were rinsed three times in deionized water. Seedlings were sown into 13.0 x 2.7cm tubes closed at one end with nylon mesh (50 μm) filled with acid-washed pre-sieved sand (250-750 μm) and stood upright in 15cm diameter plastic water-tight pots, eight tubes per pot. Nematodes were applied in aliquots of 1000 second stage larvae per plant between three and eight days after sowing, with a total density of 4000 larvae per plant. Larvae were prepared for inoculation in this study as outlined in Chapter 3.2.3 to minimize P added during inoculation. Uninoculated control plants received 3 mls of diluent used for nematode inoculum dilution, rendered nematode-free by passing once through a fritted glass buchner funnel. Nutrient solution was added to the bottom of the plastic pots and maintained at a level of about 3.0cm. The composition of the nutrient solution for the first 18 days was as follows (μM): $Ca(NO_3)_2$, 150; KNO_3, 500; KH_2PO_4, 1.0; $MgSO_4$, 150; K_2SO_4, 50 with 0.5 mg l^{-1} Fe as FeNaEDTA and micro nutrients. Solutions in pots were changed every two days. Pots were washed weekly to limit algal growth.

Eighteen days after sowing, plants were selected for uniformity in development and solutions were replaced with new solution identical in composition to the previous but differing in phosphorus concentration:

(1.0, 10.0 or 100.0 μM P in the form of KH_2PO_4). The low phosphorus (1 and 10 μM P) treatments received K_2SO_4 at concentrations sufficient to balance potassium levels between P treatments. Solutions were renewed every two days during early growth until harvest, a total of 72 days after sowing.

The experiment was carried out under standard growth cabinet conditions. Measurements of main-stem and tiller height during development, and final shoot mass, root length and mainstem and total grain yield were obtained. The experiment was designed as a randomized complete block, and an analysis of variance was performed on all variables measured.

6.1.3 Results

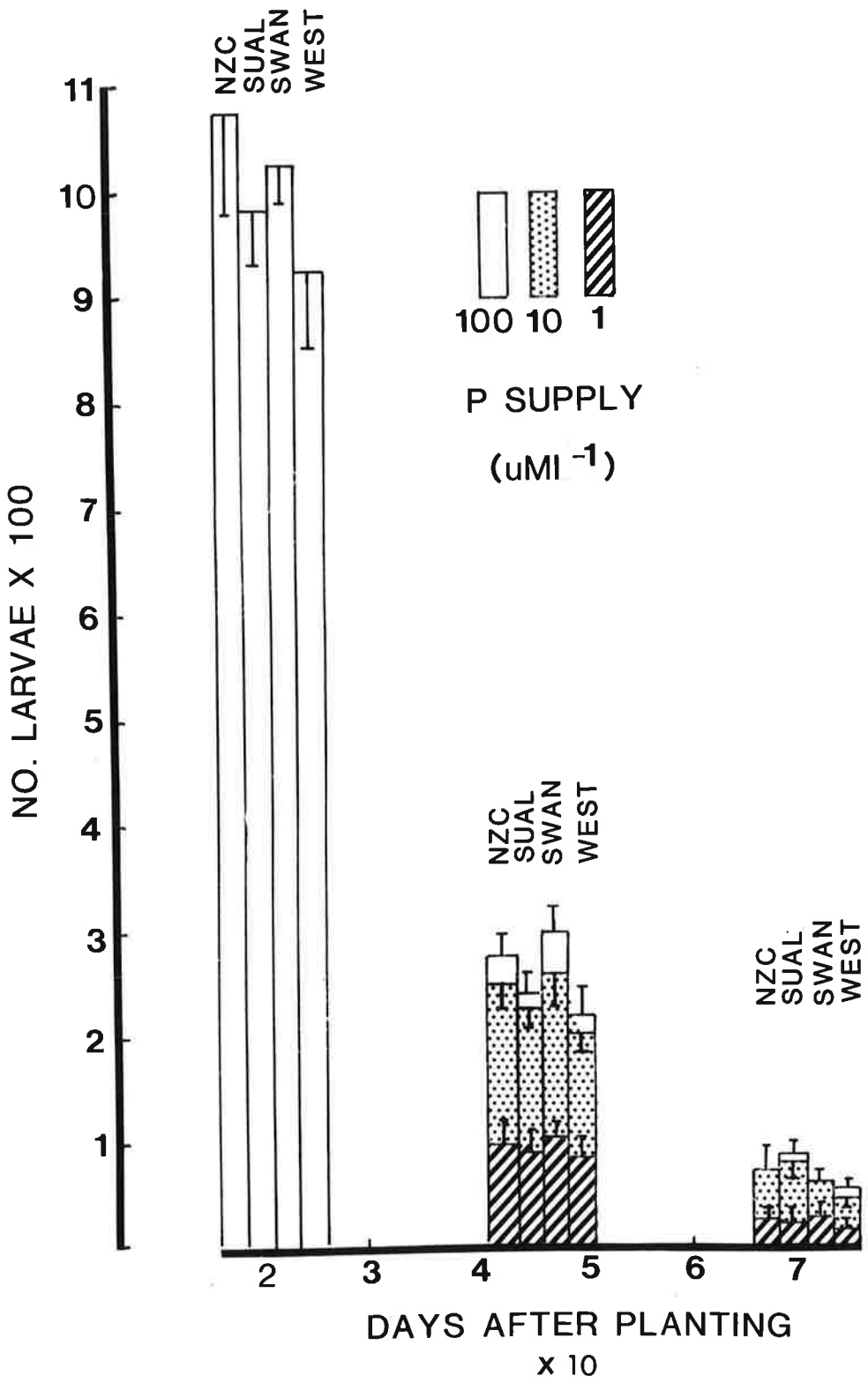
Nematode Infestation

Subsampling of the root nematode population was carried out on days 18, 45 and at harvest. P nutrition influenced nematode population only at the lowest P concentration (Fig. 6.1). By day 45 the number of females infesting the root systems had declined to about 37% of that of the higher P plants. Differences between cultivars were not significant.

Growth cabinet malfunction resulted in day/night temperatures rising from 20/14°C to 38/26°C and

FIG 6.1 The effect of initial *Heterodera avenae* density on the number of nematode larvae in roots of four oat cultivars 16, 45 and 72 days after planting

Standard Error of Mean


NZC
SUAL
SWAN
WEST
11
10
9
8
7
6
5
4
3
2
1
NO. LARVAE X 100
100 10 1
P SUPPLY
(uMl -1)
NZC
SUAL
SWAN
WEST
NZC
SUAL
SWAN
WEST
2
3
4
5
6
7
DAYS AFTER PLANTING
x 10

37/24°C on two consecutive weekends. NZC and Swan were 46 and 34 days old, respectively, and suffered severe wilting. Sual was in another growth cabinet, and West had almost fully matured, and therefore neither were affected. Results will be presented for only Sual and West.

Growth of Main Stem

The effect of phosphorus supply and nematode infestation on growth of the main shoot at maturity are shown in Table 6.1.

Table 6.1: Effect of P Supply and Infestation on Main Stem Height of Sual and West After 72 Days

CULTIVAR	DENSITY	STEM HEIGHT TO FLAG LEAF (cm) P SUPPLY (μM)			
		1	10	100	MEAN
SUAL	0	27	49	47	41
	4000	28	49	48	42
MEAN		28	49	48	42
WEST	0	24	33	34	30
	4000	23	35	33	30
MEAN		24	34	34	30

Significant interaction: LSD (P = 0.05)

Cultivar x Phosphorus 6.8

Cultivar differences in stem height were not expressed at the lowest P concentration, but at 10 and 100 μM Sual had longer culms than West.

Treatment effects on main stem grain mass are presented in Table 6.2. The grain mass of West was significantly greater than that of Sual with and without nematode infestation. Infestation had no effect on main stem grain mass of Sual and West at the lowest P concentration. Grain mass of Sual and West were significantly reduced (P = 0.05) at 10 and 100 μM P by infestation.

Table 6.2: The Effect of P Supply and Infestation on Main Stem Yield of Sual and West 72 Days after Planting

CULTIVAR	DENSITY	GRAIN MASS (mg)			
		P SUPPLY (μM)			
		1	10	100	MEAN
SUAL	0	49	125	187	121
	4000	46	91	148	95
MEAN		48	108	167	108
WEST	0	34	187	226	149
	4000	34	151	181	122
MEAN		34	169	203	136
MEAN		42	156	206	135
(P X DENSITY)		40	121	164	108
MEAN (P)		41	139	185	

ANALYSIS

Significant Effects	LSD (P = 0.05)
CV	16
Density X Phosphorus	27

The effects of infestation and P supply on the main stem grain number of Sual and West are presented in Table 6.3 The effects were very similar to those on grain mass. The mean grain number of Sual and West did not differ, indicating that the difference between Sual and West in grain mass was due in part to a smaller single grain mass of Sual.

Table 6.3: The Effect of P Supply and Nematode Infestation on Grain Number of Sual and West After 72 Days

CULTIVAR/ DENSITY	GRAIN NO. P SUPPLY (μM)			
	1	10	100	MEAN
SUAL				
0	2.6	5.2	7.2	5.1
4000	2.7	4.1	5.9	4.2
MEAN	2.7	4.7	6.6	4.7
WEST				
0	1.8	6.7	7.8	5.4
4000	1.9	5.4	6.4	4.6
MEAN	1.9	6.2	7.1	5.0
MEAN	2.2	5.9	7.5	5.3
(DENSITY X PHOS.)	2.3	4.8	6.2	4.4
	2.3	5.4	6.9	

ANALYSIS LSD (P = 0.05,*)

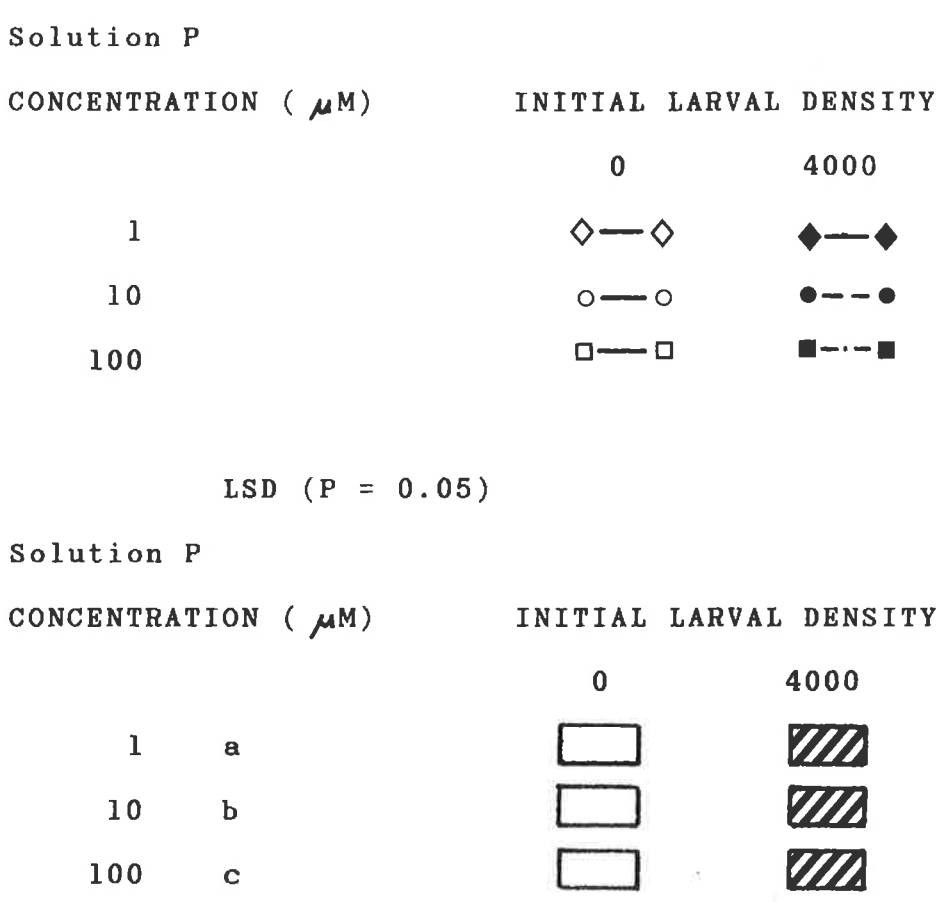
Significant Interactions:
DENSITY X PHOSPHORUS 0.7

Tiller Development

Fig 6.2 presents the effect of phosphorus supply on tiller number and mean total tiller length. Tiller number and total tiller height of Sual and West were significantly higher ($P = 0.001$) at a solution P concentration of 10 than at 1 μM P. A further increase in P supply to 100 μM P increased the tiller height of uninfested Sual after 72 days but had no effect on uninfested West. Similarly the number of tillers on uninfested Sual was significantly greater than on infested plants at both 10 and 100 μM P, whereas on West, tiller number was not influenced by infestation at either 10 or 100 μM. On the other hand, after 70 days growth, tiller number of uninfested Sual and West was enhanced by an increase in P supply from 10 to 100 μM but had no effect on infested plants.

The effects of P supply and infestation on final shoot mass of Sual and West are shown in Table 6.4. Statistical analysis followed a log e transormation of the data. The shoot mass of uninfested and infested Sual and West increased with an increase in P from 1 to 10 μM. The shoot mass of infested and uninfested Sual and West increased further with an increase in P from 10 to 100 μM.

FIG 6.2 a,b The effect of solution P concentration and Heterodera avenae infestation on tiller height and tiller number of a) Sual and b) West during 72 days growth



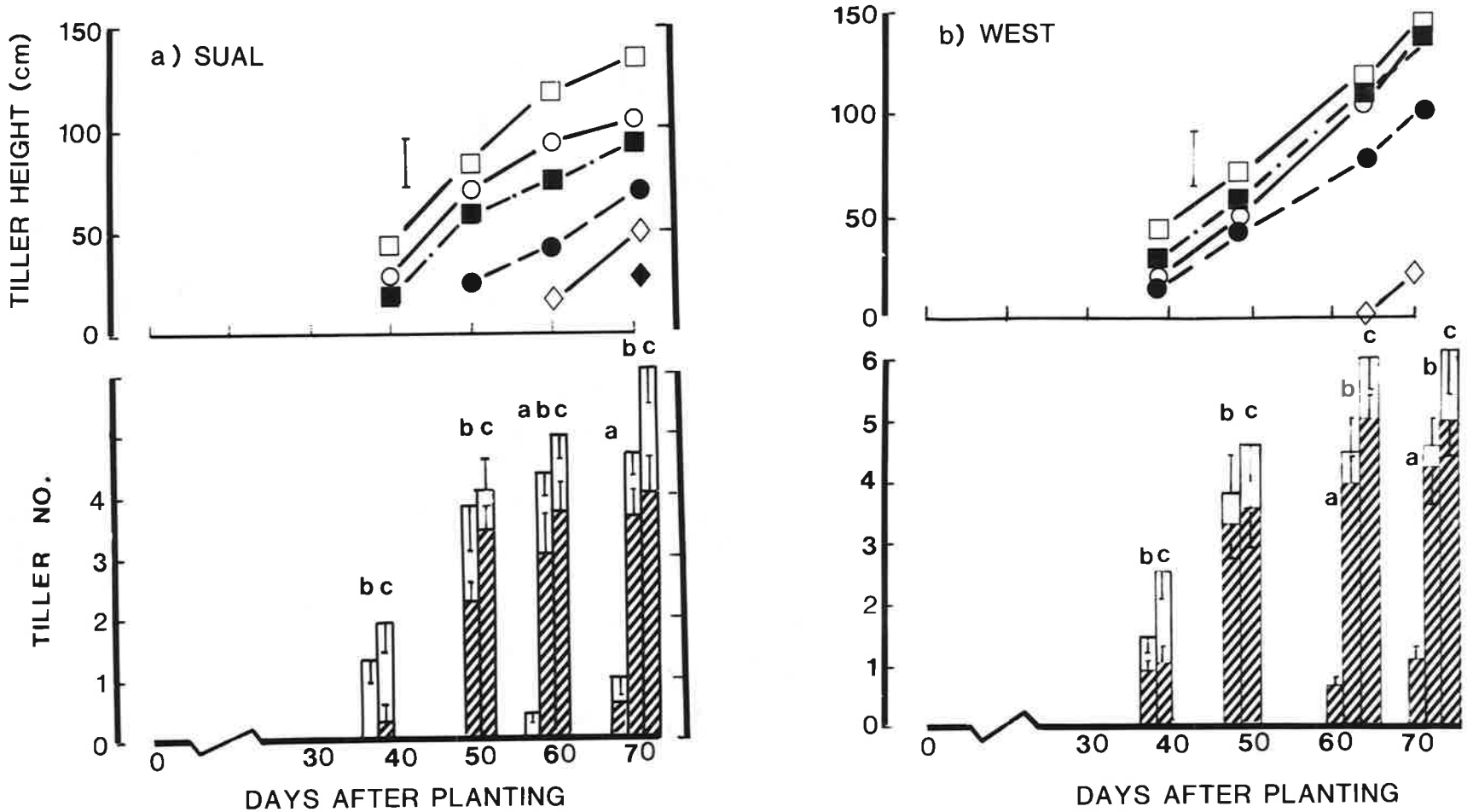

a) SUAL
b) WEST
TILLER HEIGHT (cm)
TILLER NO.
DAYS AFTER PLANTING
DAYS AFTER PLANTING
150
100
50
0
6
5
4
3
2
1
0
30
40
50
60
70
bc
abc
a
b
c

Table 6.4: The Effects of P Supply and Infestation on Shoot Mass of Sual and West 72 Days After Planting

SHOOT MASS g ($\log_e$ (100n) → (ln 100 n))

CULTIVAR/ DENSITY	P SUPPLY (μM) 1	10	100	MEAN
SUAL				
0	2.07	4.52	5.29	3.96
4000	1.94	4.14	4.60	3.56
MEAN	2.01	4.33	4.95	3.76
WEST				
0	3.13	5.68	6.04	4.95
4000	2.99	5.29	5.89	4.72
MEAN	3.21	5.48	5.97	4.88
MEANS	2.60	5.10	5.66	4.46
(DENSITY X P)	2.47	4.71	5.24	4.14
MEAN (P)	2.61	4.90	5.45	

ANALYSIS

Significant Interactions	LSD (P = 0.05)
CV X Density X P	0.32

Infestation of Sual reduced shoot mass of plants grown at 10 and 100 μM but had no effect at the lowest P level. The shoot mass of West was reduced by infestation only at 10 μM solution P.

Root length of West was considerably larger than that of Sual at the two higher P concentrations (Table 6.5)

The root length of infested and control Sual and West increased with an increase in P supply from 1 to 10 μM.

Table 6.5: The Effect of P Supply and Infestation on Root Length of Sual and West 72 Days After Planting

CULTIVAR/ DENSITY	TOTAL ROOT LENGTH (m) P SUPPLY			
	1	10	100	MEAN
SUAL				
0	0.45	1.13	1.10	1.34
4000	0.21	0.91	1.22	1.17
MEAN	0.33	1.02	1.16	1.42
WEST				
0	0.61	3.39	2.83	3.42
4000	0.29	3.75	1.94	2.99
MEAN	0.45	3.57	2.39	3.21
MEAN	0.53	2.26	1.97	2.38
(DENSITY X P)	0.25	2.33	1.58	2.08
	0.39	2.30	1.78	

ANALYSIS	(LSD P = 0.05)
Significant Interaction	
CV X Density X Phosphorus	0.37

A further increase in P supply brought no further change in infested or control root length of Sual. In contrast the root length of both infested and uninfested West declined significantly, infested roots declining the most, when P supply was increased from 10 to 100 μM. Infestation had no influence on root length of Sual grown at any P concentration. Infestation significantly reduced (P = 0.01) the root length of West grown in 100 μM P, but had no effect at 1 or 10 μM.

The effect of P supply and infestation on total grain mass of Sual and West are presented in Table 6.6.

Table 6.6: The Effect of Solution P Concentration and Nematode Infestation on Total Grain Mass of Sual and West After 72 Days

CULTIVAR/ DENSITY	GRAIN MASS (g) (ln 100n) ~~(log$_e$ (100n))~~ P SUPPLY (μM)			
	1	0	0	MEAN
SUAL				
0	0.69	4.41	4.82	3.31
4000	0.69	3.93	4.54	3.05
MEAN	0.69	4.17	4.68	3.18
WEST				
0	1.61	4.33	4.57	3.50
4000	1.39	3.96	4.17	3.17
MEAN	1.50	4.15	4.37	2.90
MEAN	1.15	4.37	4.69	3.18
(P X DENSITY)	1.04	3.95	4.35	3.11
MEAN (P)	1.09	4.16	4.52	

ANALYSIS	LSD (P = 0.05)
Significant Interactions	
Cultivar X Density	0.33
Cultivar X Phosphorus	0.35

Table 6.7: The Effect of Solution P Concentration and Nematode Infestation on Total Grain Number of Sual and West after 72 Days

CULTIVAR/ DENSITY	GRAIN NUMBER P SUPPLY (μ M)			
	1	10	100	
SUAL				
0	1.5	36.4	54.6	30.8
4000	1.4	21.5	34.6	19.2
MEAN	-	-	-	25.0
WEST				
0	1.8	34.3	50.6	28.9
4000	1.6	26.1	34.7	20.8
MEAN	-	-	-	24.9

ANALYSIS

Significant interactions	LSD (P = 0.05)
Cultivar X Density	8.97

The results are presented as transformed data (~~loge (100n)~~ ln 100n). Infestation significantly reduced (P = 0.05) total grain mass of Sual but had no effect on that of West. Grain mass of Sual was significantly increased from 1 to 10 and from 10 to 100 μM solution P, whereas the yield of West only increased from 1 to 10 μM solution P.

The effects of P supply and infestation on total grain number are presented in Table 6.7. Infestation significantly reduced grain number of Sual at both 10 and 100 μM solution P, but had no effect at the lowest P concentration. Infestation of West reduced grain number at only the 10 μM P concentration.

6.1.4 Discussion

In this experiment the effect of phosphorus supply on growth response of four oat cultivars to infestation was compared, though due to experimental mishap, only the results of two cultivars were presented. Optimum plant growth is obtained for a range of plant species with external P concentrations between 3 and 13 μM P (Asher and Edwards 1983) but, these values were derived from studies using continuous solution flow techniques.

The highest P concentration used in this experiment (100 μM) was well in excess of the optimum prescribed above, yet plant growth was significantly increased at this P concentration relative to 10 μM P. The rapid depletion of P around the roots of sand cultured plants in this experiment despite solution changes at short intervals likely resulted in the actual P concentration around the roots being less than that applied.

Infestation reduced the grain mass and grain number of the main-stem of both West and Sual at 10 and 100 μM solution phosphorus concentrations. In contrast infestation had no effect on total grain mass (main-stem plus tillers) of West at any P concentration. Total grain mass of Sual was reduced at both 10 and 100 μM P. This indicated firstly that tiller grain mass of infested West compensated for the smaller grain mass of the main stem, but did not in Sual.

Secondly it indicated that, in as much as P supply did not influence the response of either cultivar to nematode infestation, P supply and tolerance are unrelated, at least at the nematode density and P concentrations used in this study. The effect of the two lower P supplies on the response to infestation of Sual and Wes could be explained as follows: When P was limiting the effects of Heterodera avenae infestation were obscured. The effect of infestation on yield of the less tolerant cultivar, Sual became prominent

when the constraint on growth imposed by P limitation was removed. A higher level of tolerance precluded any decline in yield of West in response to infestation, at the density applied.

On the other hand, there appeared to be a possible indirect relation between P nutrition and nematode tolerance.

The total grain mass of uninfested and infested West did not increase from 10 to 100 μM P whereas that of Sual did, suggesting that the optimum P concentration for growth of Sual was higher than that of West. Considering that infestation had no effect on the total grain yield of West at any phosphorus solution concentration, whereas that of Sual was reduced significantly at 10 and 100 μM P. It is possible that the cultivar with the lower requirement for phosphorus West may also have a higher level of tolerance to Heterodera avenae.

If Sual had a higher optimum P concentration for growth than West and if a decline in root surface area reduced P absorption, then root growth impairment by nematode infestation during early development may have affected growth of Sual more than West.

Differences in the effect of phosphorus and nematode infestation on yield of Sual and West indicate that a closer examination of these two cultivars, with special regard to P absorption rates and P utilization efficiencies is warranted.

6.2 EFFECTS OF INFESTATION ON GROWTH AND PHOSPHORUS ABSORPTION OF OAT PLANTS SUPPLIED WITH DIFFERENT AMOUNTS OF PHOSPHORUS IN SOLUTION CULTURE

6.2.1 Introduction

In the previous experiment (section 6.1) the cultivar West was less responsive in grain yield to an increase in solution phosphorus (P) concentration above 10 μM than Sual and was also less affected by nematode infestation. These findings were indicative of a possible relationship between P nutrition and nematode tolerance, wherein plants unresponsive to increased external P supply may have higher tissue P concentrations at lower P supply conditions than more P responsive plants, thereby conferring a higher nematode tolerant status.

However the results of section 6.1 provided no firm evidence that plants with high internal P concentrations were more tolerant to nematode infestation since the plant tissue was not analysed for P.

The aim of this experiment was to obtain more information on the relationship between tissue P status and tolerance to Heterodera avenae. Because tissue P status is a function of the rate of P absorption and the rate of tissue growth (Williams 1948) the relationship between P uptake, P utilization efficiency and nematode tolerance was also examined.

6.2.2 Methods

Seedlings were germinated and two thirds of the endosperm excised as described in section 6.1.2. Instead of PVC tubes, medium guage plastic bags (10.0 x 4.0 cm when flat) with drainage slots cut at the base were used to contain the acid washed pre-sieved ($\leq$ 850 $\geq$ 250μm). Coarse acid washed sand ($\geq$ 2.0mm) was placed at the bottom of the plastic bags to prevent loss of sand through the drainage slots. Bags containing plants were supported upright in 10.0cm pots in groups of four, inside a growth cabinet maintained at standard conditions. Three to eight days after sowing, second stage larvae were applied in aliquots of 1000. Plants received either 0, or 4000 larvae. From the time of sowing until transfer to nutrient solution, plants received 25mls of complete low P nutrient solution every two days as described in section 6.1.2. This volume was sufficient to permit drainage through the bottom of the plastic bags.

Twelve days after sowing, plants were selected for uniformity then carefully removed from the bags, rinsed under deionized water to remove loosely clinging sand and transferred to 1 litre black plastic bottles containing a low P nutrient solution identical to that which they received previously. Solutions were aerated and stirred vigorously with a continuous supply of activated charcoal/cotton wool filtered air delivered through pasteur pipettes fitted to

the bottles. Plants were arranged in groups of five per bottle. They were supported by a 2.0 x 6.0 cm rectangle of 1.0 cm thick foam wrapped around the base of the stem and inserted into the mouth of the bottle. Each bottle represented a single replicate within a treatment. Following equilibration in solution for 40 hours to minimize possible effects of transplanting, plants were grown in solutions with phosphorus concentrations of 1, 10 or 100 μM with the remaining nutrient components being held at the pre-treatment level. Solutions were changed daily. Plants were sampled 16 days after sowing in consecutive four day intervals until 36 days after sowing. Treatments consisted of four cultivars, two inoculum levels, and three phosphorus concentrations. Each treatment was replicated three times. Treatments were randomly arranged within the growth cabinet initially but were rotated daily within the cabinet to minimize positional effects. Plants started to tiller by about the 20th day. Tiller buds were removed each time solutions were changed.

At each of 6 sampling dates, roots were rinsed under running deionized water to remove residual culture solution for about 1 minute. Shoots and roots were separated, placed in airtight glass bottles and refrigerated for a maximum of four hours at 2°C. Root lengths of individual root

systems were measured by laying the whole root system on a 15.0 cm diameter glass petri plate laid atop a one-half inch grid. 10 mls of deionized water wetted the surface of the plate to facilitate manipulation of the roots. After root intersections were counted, requiring about 1 minute, the root was returned to the glass vial capped, and refrigerated. The water was rinsed from the petri dish and replaced with fresh deionized water. Root and shoot tissues were then snap frozen in liquid N_2 and freeze dried. Dry weights of emerged leaves, stems and roots were obtained.

Shoot and root components were ground and duplicate subsamples were used to determine tissue phosphorus concentrations as described in Chapter 3.2.8.

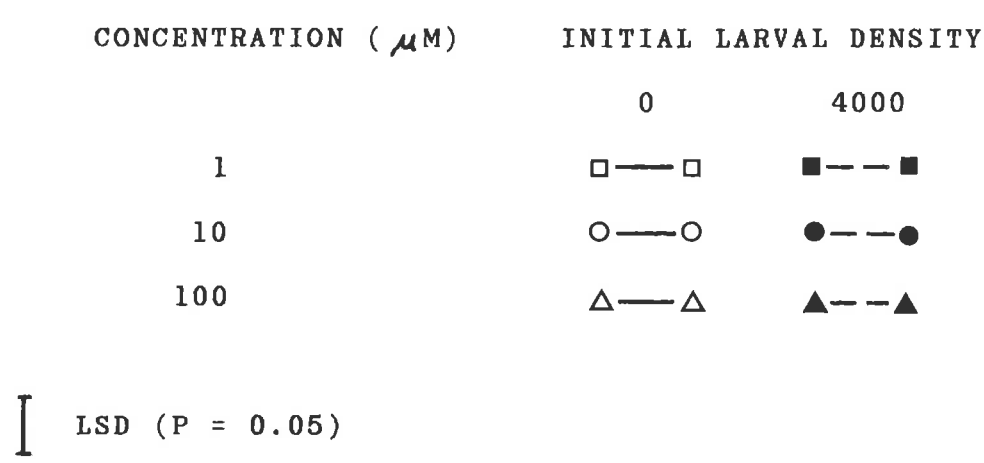
Treatments were arranged in a randomized completely block design. Analysis of variance was performed on all variables.

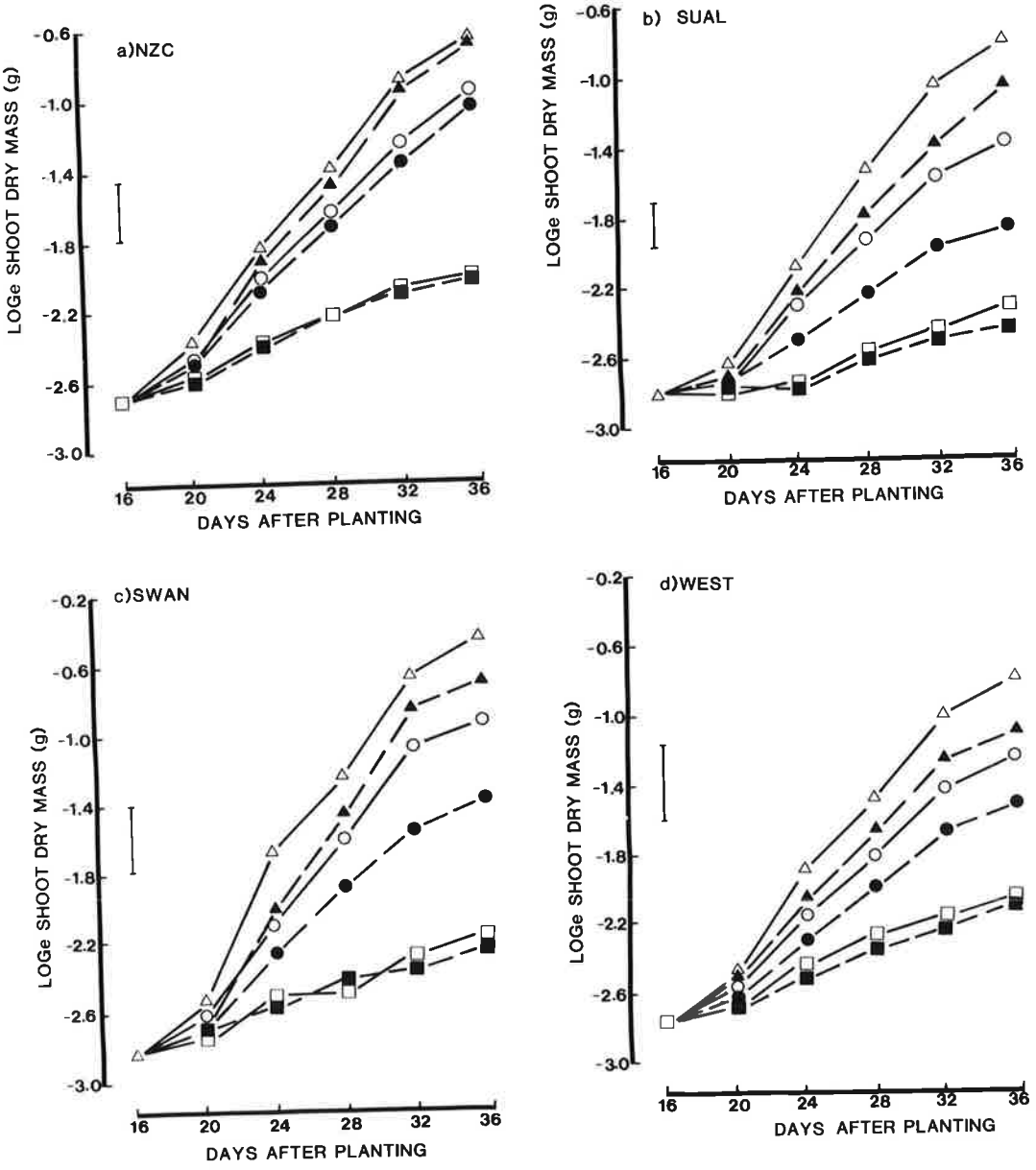
6.2.3 Results

Relative Growth Rate (R_W)

Data for shoot growth over the treatment interval (16 to 36 days after sowing) is presented in Fig. 6.3. Following an initial lag in growth between days 16 and 20 shoot growth was logarithmic up until day 32 when growth rate started to decline, particularly in those plants grown in P solutions of 10 and 100 μM.

FIG 6.3 (a-d) The effect of Heterodera avenae infestation and solution P concentration on Loge shoot mass of a) NZC, b) Sual, c) Swan and d) West 1 to 20 days after commencement of P supply treatment (16 to 36 days after planting)




a)NZC
b) SUAL
c)SWAN
d)WEST
LOGe SHOOT DRY MASS (g)
DAYS AFTER PLANTING
-0.2
-0.6
-1.0
-1.4
-1.8
-2.2
-2.6
-3.0
16
20
24
28
32
36

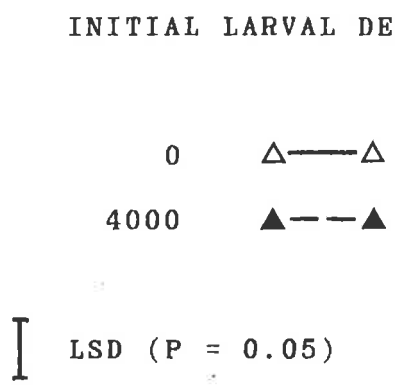
Mean relative growth rates (Rw) were calculated for each cultivar from the slopes of the linear regressions of the data in Fig. 6.3. Data are presented in Fig. 6.4. Each increment in solution phosphate concentration increased the Rw of all cultivars from a base level of about 0.4 gg^{-1} at 1. μM phosphorus. Infestation depressed Rw of all cultivars except NZC. Increasing the solution P concentration to 100 μM eliminated the effect of nematode infestation on Swan, reduced their effect on Sual, and had no effect on West and NZC.

Final Shoot Mass, Root Mass and Root Surface Area (Tables 6.8, 6.9, 6.10)

After 20 days of P treatment (36 days after planting, all cultivars showed significant ($P \leq 0.01$) increases in shoot mass, root mass and total root surface area (A) with an increase in solution P from 1 to 10 μM, irrespective of infestation status. An increase in solution P from 10 to 100 μM significantly enhanced shoot mass of all uninfested and infested cultivars (Table 6.8) and the root mass and root surface area of all infested and uninfested cultivars except West (Tables 6.9 and 6.10 respectively). In the latter case there was no increase root mass or A of infested West, while that of uninfested West increased.

Infestation had no effect on the shoot mass, root mass or A of any cultivar grown in 1 μM solution P. At 10 μM solution P, while infestation significantly reduced ($P = 0.01$) the shoot mass of Sual, Swan and West, infestation only

FIG 6.4 (a-d) The effect of Heterodera avenae infestation and solution P concentration on the mean relative growth rate of a) NZC, b) Sual, c) Swan and d) West during 20 days of P supply treatment



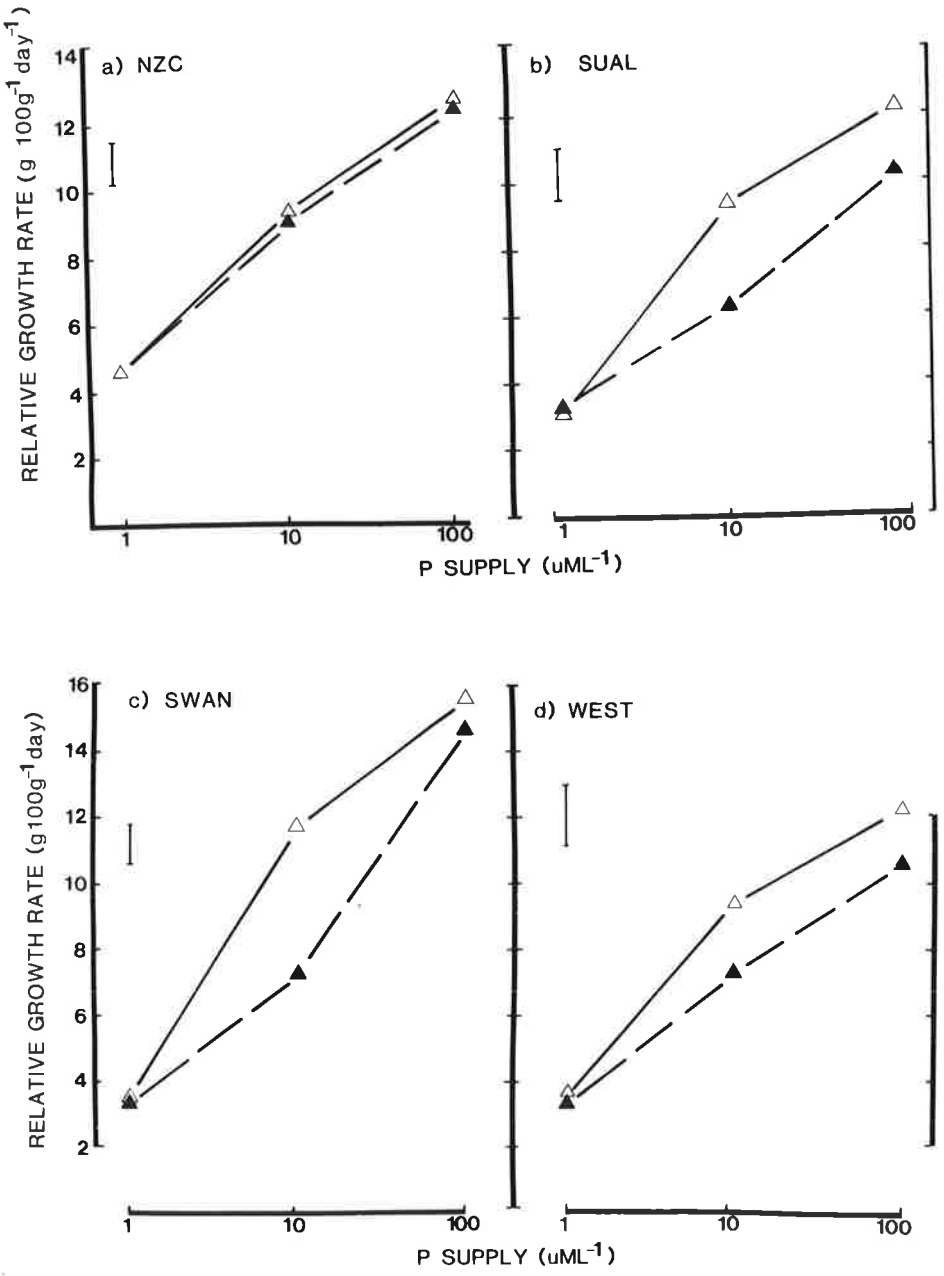

a) NZC
b) SUAL
c) SWAN
d) WEST
RELATIVE GROWTH RATE (g 100g^{-1} day^{-1})
RELATIVE GROWTH RATE (g100g^{-1}day)
P SUPPLY (uML^{-1})
P SUPPLY (uML^{-1})
2
4
6
8
10
12
14
16
1
10
100

Table 6.8: The Effects of Infestation and P Supply on the Dry Shoot Mass of 4 Oat Cultivars 36 Days After Planting

		SHOOT DRY MASS (g)		
CULTIVAR	DENSITY	PHOSPHORUS SOLUTION CONCENTRATION (μM)		
		1	10	100
NZC	0	0.145	0.390	0.538
	4000	0.137	0.381	0.521
SUAL	0	0.091	0.247	0.440
	4000	0.084	0.153	0.352
SWAN	0	0.111	0.385	0.622
	4000	0.106	0.252	0.494
WEST	0	0.124	0.288	0.449
	4000	0.121	0.219	0.324

ANALYSIS	LSD (P = 0.05)
Significant interaction	
CV X Density X P	0.066

Table 6.9: The Effect of Infestation and P Supply on the Dry Root Mass of 4 Oat Cultivars 36 Days After Planting

		ROOT DRY MASS (g)		
CULTIVAR	DENSITY	PHOSPHORUS SOLUTION CONCENTRATION (μM)		
		1	10	100
NZC	0	0.097	0.209	0.302
	4000	0.096	0.189	0.276
SUAL	0	0.069	0.161	0.243
	4000	0.061	0.109	0.162
SWAN	0	0.079	0.234	0.321
	4000	0.068	0.122	0.250
WEST	0	0.088	0.179	0.229
	4000	0.089	0.138	0.171

ANALYSIS	LSD (P = 0.05)
Significant interactions	
CV X Density X Phosphorus	0.052

While cultivars did not differ in root mass at the lowest P concentration the infested root mass of both Sual and Swan was significantly lower than that of NZC at 10 μM. At 100 μM the root mass of Swan no longer differed from that of NZC but the root masses of Sual and West were significantly smaller than NZC's in the presence and absence of infestation.

Table 6.10: Effect of Infestation and P Supply on Total Root Surface Area of 4 Oat Cultivars After 36 Days

CULTIVAR	DENSITY	TOTAL ROOT SURFACE AREA (cm^2) SOLUTION P CONCENTRATION		
		1	10	100
NZC	0	39.7	86.6	129.4
	4000	38.1	75.7	109.4
SUAL	0	32.3	74.9	106.3
	4000	27.8	47.1	71.2
SWAN	0	39.4	116.2	141.5
	4000	38.9	66.3	103.7
WEST	0	37.1	72.8	96.1
	4000	32.4	59.4	61.1

ANALYSIS	LSD (P = 0.05)
Significant interaction	
CV X Density X P	14

reduced the root mass of Swan and the root surface area of Sual and Swan. When plants were grown in 100 μM solution P, the shoot mass, root mass and root area were all reduced by infestation. Infestation reduced only the root surface area of NZC.

Phosphorus Concentrations of Shoot and Root 36 Days after Planting

As with plant growth variables, root and shoot P concentrations were significantly enhanced with an increase in solution P from 1 to 10 μM (Table 6.11). A further increase in solution P from 10 to 100 μM increased the root P concentrations of all cultivars irrespective of infestation status. At 100 μM only uninfested NZC did not show a significant increase in shoot P concentration.

Infestation had no effect on the shoot P concentration of Sual, Swan and West at 10 and 100 μM solution P. Infestation increased the ~~solution~~ shoot P concentration of NZC at 100 μM (Table 6.11). The root P concentration of infested Swan and NZC did not differ from that of uninfested control plants at either 10 or 100 μM. Infestation increased the root P concentration of Sual at both 10 and 100 μM P, but reduced that of West at 10 μM, with no effect on West at 100 μM solution P.

Table 6.11: Effect of P Supply and Infestation on Root and Shoot P Concentrations of 4 Oat Cultivars 36 Days After Planting

	TISSUE P CONCENTRATION (% DRY MASS)					
CULTIVAR/ DENSITY	ROOT			SHOOT		
	P SUPPLY (μM)					
NZC	1	10	100	1	10	100
0	0.081	0.301	0.468	0.081	0.425	0.491
4000	0.097	0.315	0.501	0.076	0.368	0.650
SUAL						
0	0.081	0.295	0.536	0.033	0.154	0.503
4000	0.075	0.206	0.629	0.040	0.161	0.481
SWAN						
0	0.100	0.252	0.396	0.048	0.171	0.331
4000	0.113	0.240	0.582	0.037	0.162	0.349
WEST						
0	0.087	0.279	0.429	0.058	0.232	0.581
4000	0.092	0.370	0.478	0.052	0.288	0.545

ANALYSIS LSD ($P=0.05$)

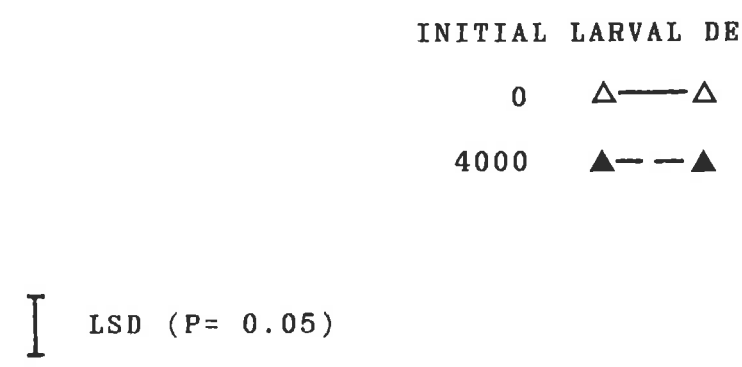
Significant interaction	Roots	Shoots
CV X Density X P	0.059	0.068

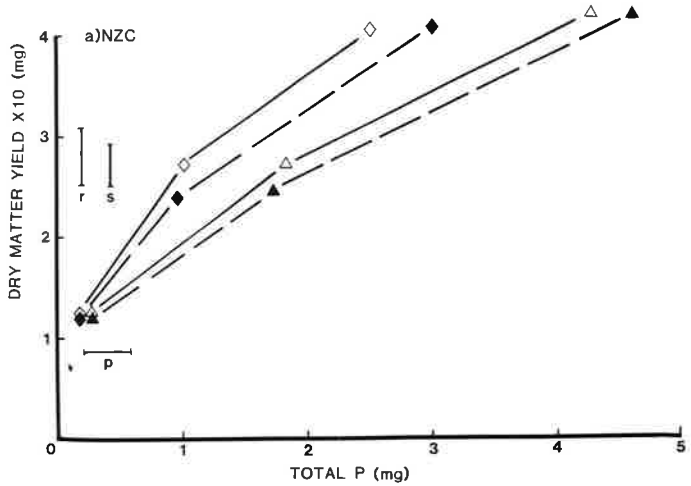
Tissue P vs Shoot Dry Matter Yield

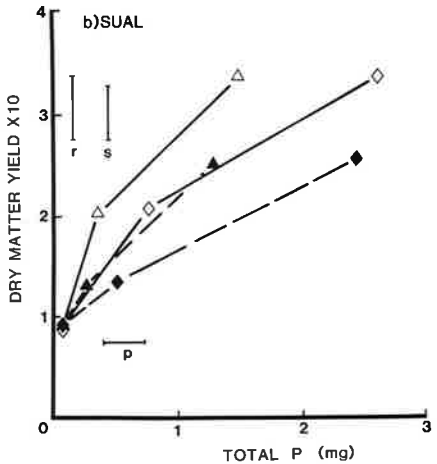
The relationship between shoot P concentration and shoot dry matter yield (day 32) is presented in Figure 6.5. Swan produced the greatest shoot dry matter at all tissue P concentrations, followed by Sual, NZC and West. The shoot P concentrations of infested and control Sual and Swan did not differ despite significantly lower shoot masses of infested plants, whereas shoot concentrations of infested NZC were significantly increased by infestation but shoot mass was unchanged.

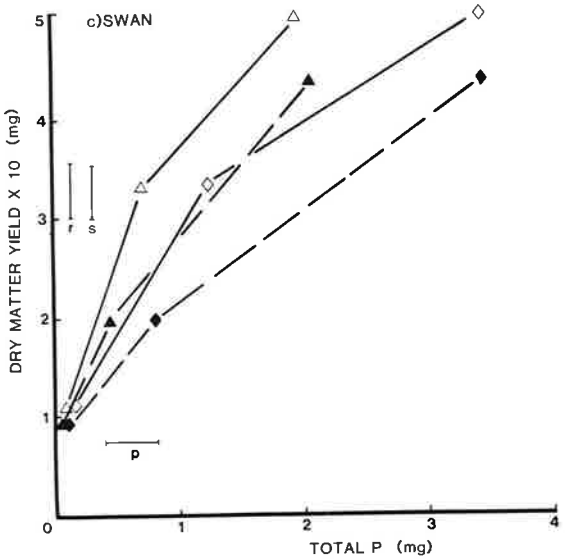
In Figure 6.6, values for shoot and root dry matter yield per total P uptake on day 32 of the experiment are presented. Increases in total P uptake were reflected in greater yields of all cultivars. If the relationship between total P uptake and dry mass is used as a criterion of efficiency of utilization of P (Blair and Cordero 1978) then because NZC took up the most P but produced no more mass than the other 3 cultivars, it could be classified as least efficient in utilizing P, followed by West, Sual and Swan, ranked in increasing order of efficiency. Infestation reduced the P utilization efficiency of Sual, Swan and West, though this effect was marginal, and had no effect on NZC.

FIG 6.5 (a-d) The effect of Heterodera avenae infestation on the relation between shoot P concentration and shoot dry matter yield of a) NZC, b) Swan and d) West 16 days after commencement of P supply treatment (32 days after planting).




a)NZC
DRY MATTER YIELD X10 (mg)
TOTAL P (mg)
r
s
p


b)SUAL
DRY MATTER YIELD X10
TOTAL P (mg)
r
s
p


c)SWAN
DRY MATTER YIELD X 10 (mg)
TOTAL P (mg)
r
s
p

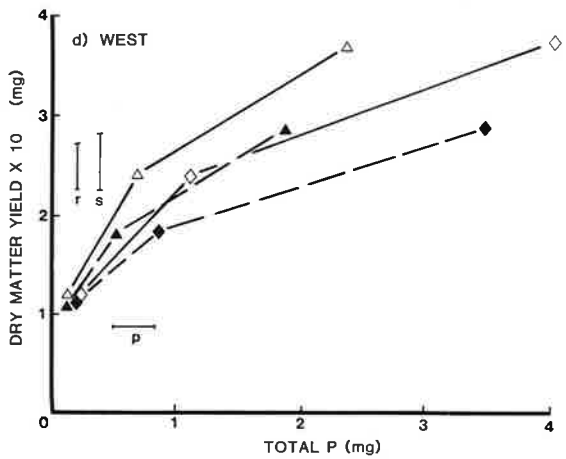
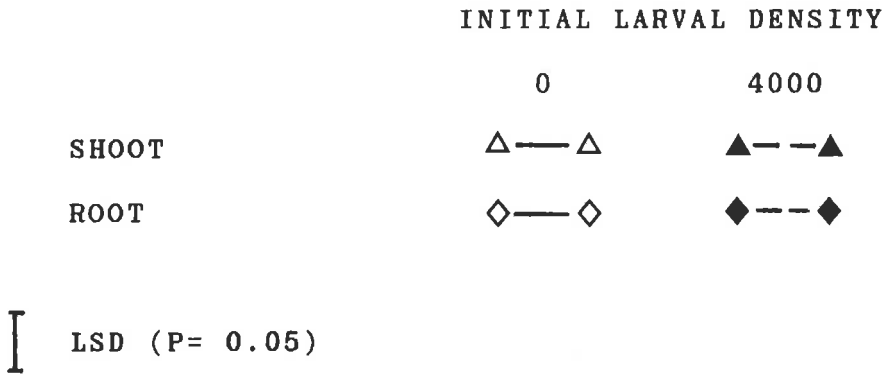
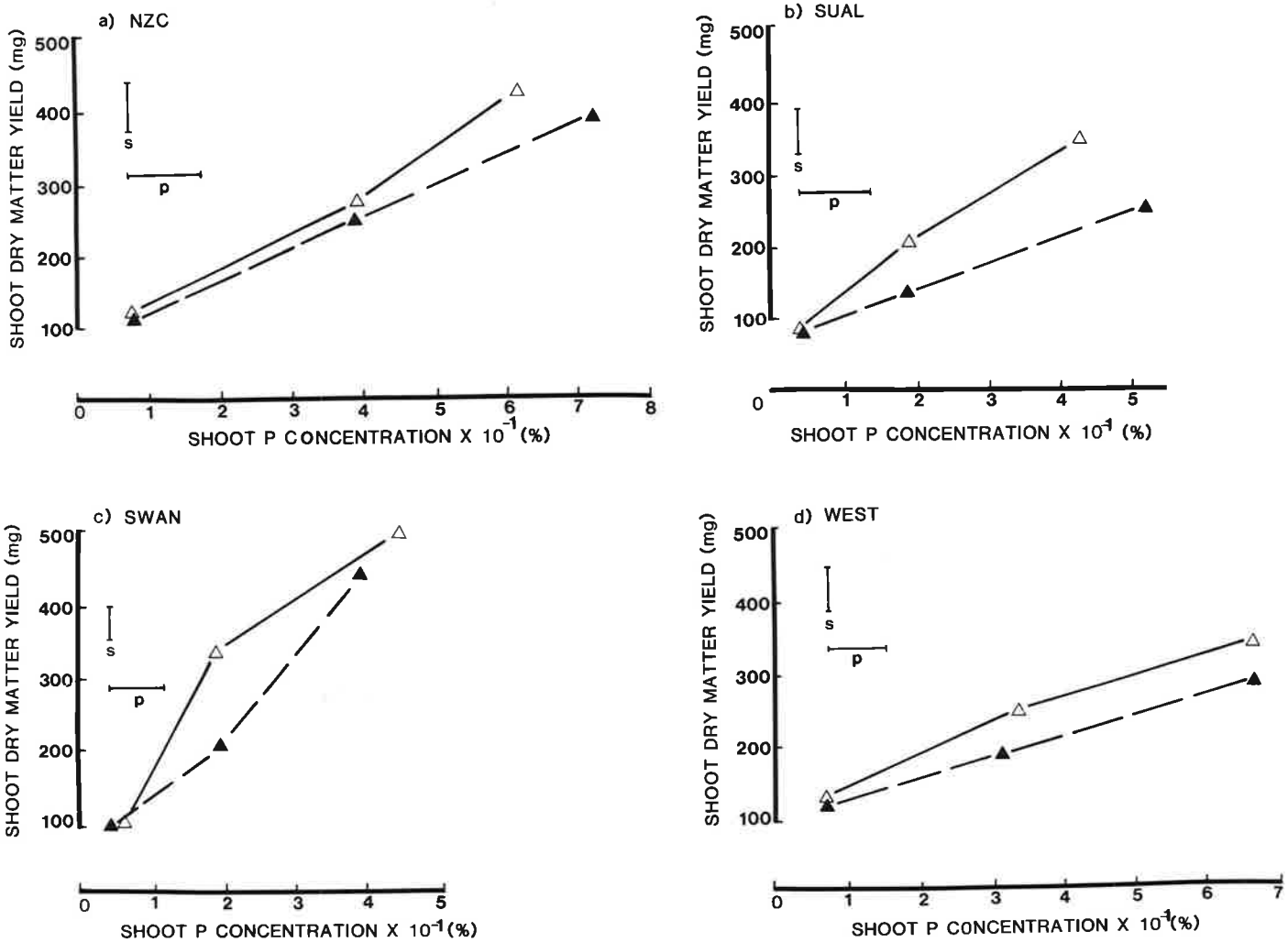

d) WEST
DRY MATTER YIELD X 10 (mg)
TOTAL P (mg)
r
s
p

FIG 6.6 (a-d) The effect of Heterodera avenae infestation on the relation between P uptake and shoot and root dry matter yield of a) NZC, b) Sual, c) Swan and d) West 16 days after commencement P treatment (32 days after planting)




a) NZC
b) SUAL
c) SWAN
d) WEST
SHOOT DRY MATTER YIELD (mg)
100
200
300
400
500
s
p
0
1
2
3
4
5
6
7
8
SHOOT P CONCENTRATION X 10^{-1} (%)

Rates of Phosphorus Absorption

The rate of phosphorus absorbed per unit root mass was derived from the equation (Williams 1948)

$$Ip = \frac{Q_2 - Q_1}{t_2 - t_1} \times \frac{L_N \; W_{R1} \; L_N \; W_{R2}}{W_{R1} - W_{R2}} \qquad \text{EQ. 6.1}$$

Where Q is the quantity of P absorbed, W the root mass, and t, the time. Over short time intervals ($t_2 - t_1 = 4$ days) Q and R may be assumed to be linearly related. Data was transformed (Loge (X 10 original values)) before performing statistical analysis. Transformed data are presented in Table 6.12.

Infestation had no significant effect on the rate of P uptake (Ip) of any cultivar at any date and at any P level. All cultivars had increased Ip with increasing external P concentration, with no differences observed in this effect at any of the three measurement intervals.

Ip of NZC at 10 μM was greater than that of Swan and Sual between days 20 to 24, and greater than that of Sual throughout the experiment at 10 μM. At 100 μM, Ip of Swan was less than that of the other cultivars between day 2-24, but afterwards Ip of all cultivars did not differ.

Table 6.12: Effect of P Supply, Infestation and Date of Measurement on the Rate of P Absorption of 4 Oat Cultivars (Analysis of Loge(X10) Transformed Data)

RATE OF P ABSORPTION, Ip (mg P g^{-1} Root Day^{-1})

CV.	DENS.	INTERVAL (DAYS) 20 - 24		24 - 28		28 - 32	
		0	+	0	+	0	+
NZC	1	1.39	1.09	4.69	4.72	5.2	5.44
	10	7.06	7.10	6.76	6.70	6.48	6.57
	100	7.66	7.66	7.27	7.31	7.57	7.88
SUAL	1	1.61	1.61	4.08	4.11	3.17	3.29
	10	6.32	6.78	6.28	6.51	6.11	6.18
	100	7.62	7.79	7.39	7.66	7.14	7.49
SWAN	1	3.64	3.71	4.30	4.41	2.40	2.48
	10	6.44	6.71	6.68	6.75	6.55	6.70
	100	7.21	7.29	7.53	7.61	7.25	7.45
WEST	1	1.95	1.95	4.25	4.43	4.68	4.38
	10	6.62	6.89	6.26	6.67	6.27	6.19
	100	7.59	7.88	7.17	7.49	7.74	7.66

Initial Density:

0,+; 0 and 4000 larvae per plant

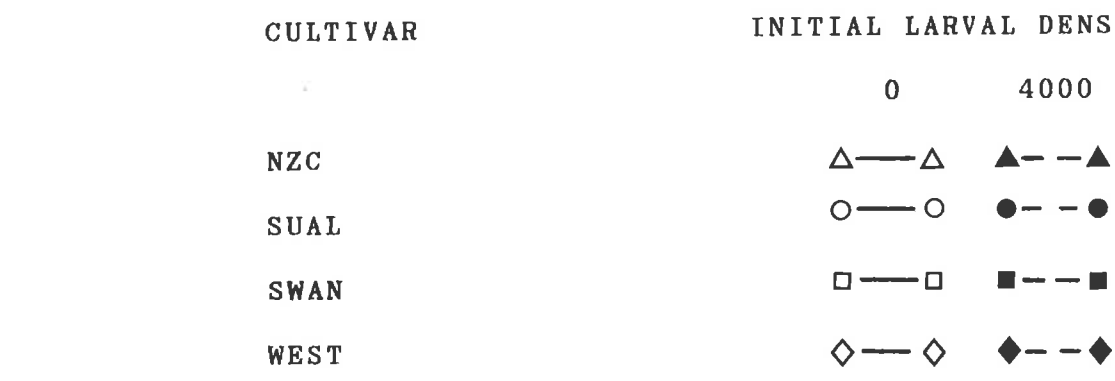
Rate of Phosphorus Flux

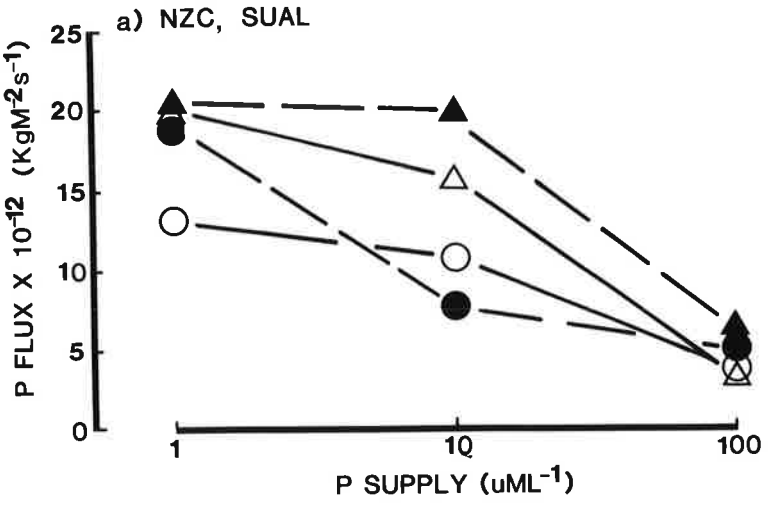
Nutrient uptake has been related to the surface area of the root system and the external concentration. Flux of phosphorus into the root was calculated using the equation (Christie and Moorby 1975):

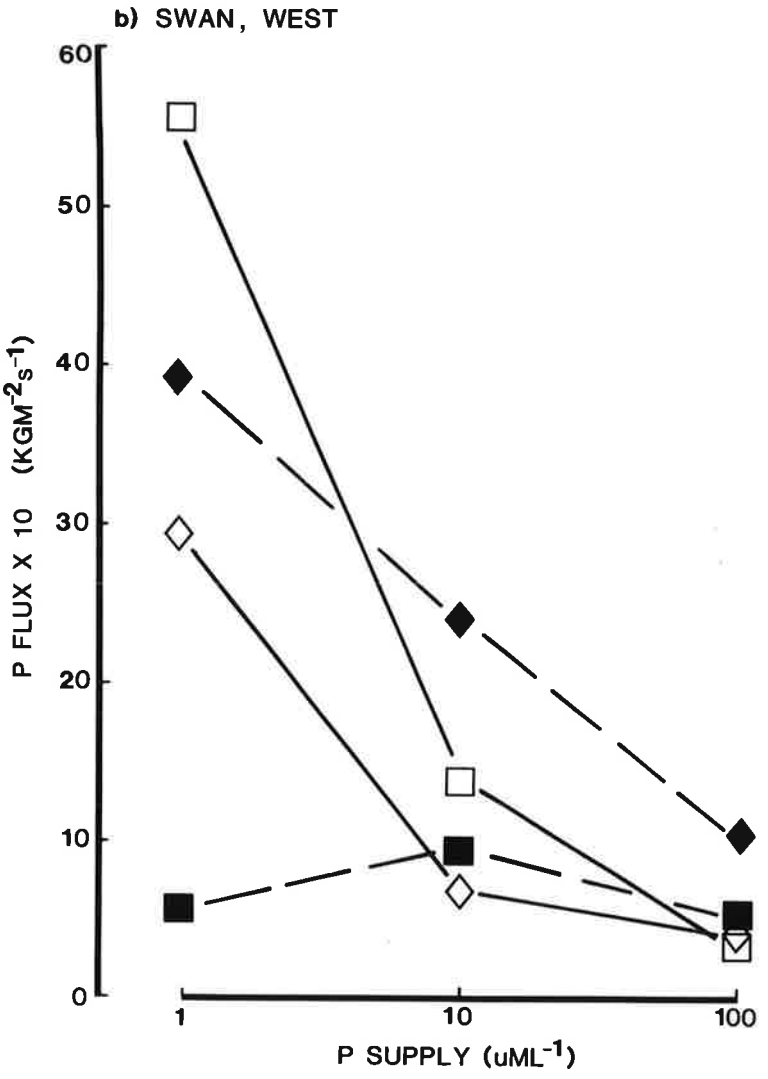
$$F = \alpha Cr \qquad \text{Eq. 6.2}$$

where F is the flux of phosphorus ions across the root surface (Kg M^{-2} sec^{-1}), Cr the concentration of ions at the root surface (Kg m^{-3}) and the mean root uptake coefficient, a value expressing the proportionality of flux to concentration (m sec^{-1}). The proportionaility coefficient α was derived from determinations of root surface area, A (m^2), relative growth rate of the plant (mg g^{-1} day^{-1}), and mean plant phosphorus concentration (mg g^{-1}). (See Appendix II for derivation of α value). Mean phosphate flux was calculated for the interval between days 20 and 32, an interval during which there was least data variability. Results are presented in Fig. 6.7. Results over the entire experimental period are presented in the Appendix (Table A1). Statistical analysis of the results was considered inappropriate because of the highly derived nature of the final values. In general, P flux decreased as external P concentration increased. Flux of P into the uninfested roots of Swan and West greatly exceeded that of NZC and Sual at the lowest external P concentration but were comparable at 10 and 100 μM external P.

FIG 6.7 a,b The effect of Heterodera avenae infestation and P supply on the mean P flux across the root surface of a) NZC and Sual and, b) Swan and West 4 to 16 days after commencement of P treatment (20 to 32 days after planting)




a) NZC, SUAL
P FLUX X 10^{-12} ($KgM^{-2}s^{-1}$)
25
20
15
10
5
0
1
10
100
P SUPPLY (uML^{-1})


b) SWAN, WEST
P FLUX X 10 ($KGM^{-2}s^{-1}$)
60
50
40
30
20
10
0
1
10
100
P SUPPLY (uML^{-1})

Infestation had the strongest influence on phosphate flux into the root at 1 and 10 μM solution P concentrations. The most noticable exception was Swan which had a consistently low phosphate flux at all P levels. Rate of phosphate flux of infested roots exceeded that of controls at all P concentrations for NZC and West.

6.2.4 Discussion

The relationship between phosphorus nutritional status and tolerance to Heterodera avenae was examined in this experiment. The four cultivars used in the study displayed differences in root and shoot growth in response to nematode infestation only at the two higher P concentrations. At the lowest P solution concentration the effects of P deficiency on shoot and root growth prevailed over that of nematode infestation, a finding also reported in section 6.1 This was not entirely expected since the solution culture method of supplying P in this study probably supplied more P at a given solution concentration than the sand culture method used in section 6.1. The significantly lower P concentrations in the roots and shoots of plants grown in 1.0 μM P compared to that of plants grown in the two higher solution P concentrations indicated that low tissue P status adequately accounted for slower growth of the former plants.

When the solution P concentration was increased the shoot mass, root mass, root length and tissue P concentration of all cultivars increased considerably but the effect of nematode infestation was not the same on all cultivars. Infestation had a larger effect on shoot and root variables of Sual, Swan and West than on NZC. The shoot mass of the three former cultivars was significantly reduced by nematode infestation, at 10 and 100 μM P while shoot P concentration was not affected by infestation. The maintenance of a constant shoot P concentration in spite of a significant decline in shoot mass in infested plants was either due to more phosphorus being held within the roots and ~~and~~ thereby inhibiting transportation to the shoot in infested plants, or because less phosphorus was absorbed by the root system of infested plants.

With Sual and Swan, root surface area was significantly reduced in infested plants. Infestation had no effect on the root P concentration of Swan and reduced that of Sual at 10 μM. Infestation also had no effect on P absorption rate of any cultivar nor did it reduce the phosphorus flux on these cultivars, supporting findings of Price et al. (1982).

The observations outlined above strongly indicated therefore, that the decline in P uptake that must have occurred in infested Sual and Swan was attributable to a reduction in root surface area.

On the other hand the root surface area of West at 10μM was not reduced by infestation, but the root P concentration increased. This increase may have accounted for the decline in shoot growth of infested West as well as the maintenance of a constant shoot P concentration in infested and uninfested West at 10 μM P.

Identical shoot P concentrations of infested and uninfested NZC at 10 μM solution P can be understood in terms of the absence of any affect of infestation on root surface area and shoot mass.

At the highest external P concentration, there was a significant increase in root P concentration of Sual and Swan, along with a decline in total root surface area. The sequestration of P within infested roots of Sual and Swan at this solution P concentration may partly explain the difference between these two cultivars and NZC. In the case of NZC root P concentration did not increase but shoot concentration in infested plants did, even though root surface area was slightly reduced. A decline in total P uptake in infested West at 100μM P likely arose from reduced root area, since there was no change in root P concentration.

Although a decline in root length of infested plants could be largely held responsible for the reduced shoot mass and the corresponding decline in total P uptake of Sual, Swan and West it cannot be concluded from this that phosphorus

deficiency was the reason for the poorer growth of infested plants. In no case did the P concentration of infested plants decline below that of uninfested plants. Increasing the external P concentration from 10 to 100 did not significantly alter the effect of infestation on shoot mass of NZC, Sual and West and at least with respect to these cultivars, nematode tolerance and phosphorus nutrition do not seem to be related. In the case of Swan, a higher phosphorus status in infested Swan at 100 compared to 10 μM solution P resulted in the relative growth rate of this cultivar increasing to a level similar to that of uninfested plants. The possibility that P deficiency was at least partly responsible for infested Swan's weaker growth cannot be ruled out.

Studies on factors relating to plant performance under conditions where phosphorus is limiting have shown that the ability to tolerate phosphorus deficiency (low-P tolerant) is often correlated with an unresponsiveness to increased P (Clarkson 1967, White 1972). Low-P tolerant plants also have higher tissue P concentrations and require more phosphorus to produce the same amount of dry matter than low-P intolerant plants (Christie and Moorby 1975, Chaplin et al. 1982). Such plants are therefore less efficient in utilizing internal P and also less responsive to increases in external P supply.

It was suggested at the outset that by virtue of their slower growth and higher internal P concentrations, such plants would be more nematode tolerant than low-P tolerant plants if phosphorus nutrition was related to nematode tolerance. However, the results of this experiment do not support this latter proviso.

There was nonetheless an indication that P utilization efficiency was associated with nematode tolerance of NZC and Swan. Shoot mass of uninfested NZC and Swan were equal at 10 μM even though the shoot P concentration of NZC was significantly greater than that of Swan, indicating that Swan may have been more efficient than NZC in utilizing P. At the same solution P concentration infested Swan had a smaller root mass than NZC. This was because infestation may have had no effect on the rate of mineral upake, including P, of NZC, since root surface area was unaffected whereas the root surface area of Swan was considerably reduced, with consequent effects on mineral uptake. When the solution P concentration was increased, P absorption rates of both cultivars increased similarly but there was less shoot growth, derived from this increase in tissue P concentration, in NZC than in Swan. Uninfested shoot mass of Swan was larger than that of NZC, and shoot mass of infested NZC and Swan were equal, even though the shoot P concentration of NZC exceeded that of Swan. It could be concluded that two factors may have differentiated the response of NZC and Swan to Heterodera avenae infestation;

the response of root growth to infestation and the efficiency with which P was utilized.

An increase in P supply may have had no effect on the tolerance of Sual to Heterodera avenae because the nematode density may have been too high to allow expression of P effects. This cultivar was found to be more efficient than NZC in utilizing P, and the relative growth rate of infested Sual increased at the highest P concentration. However a higher P utilization efficiency than NZC may not have been sufficient to compensate for a drastically reduced root surface area accompanying root infestation.

In the case of West it could be argued that at a lower P solution concentration the effects of infestation may have been more severe. Equally probable however is that infestation had effects on plant growth independent of P nutrition, the severity of which would not have altered at lower solution P concentrations. The experiments reported here do not allow a logical choice between these two possibilities.

While the treatment response of Sual in section 6.1 and 6.2 were the same, West was found to be less tolerant to infestation in section 6.2 than 6.1, at both 10 and 100μM. This may simply have been a reflection of differences in sampling times, so that with further growth West may have been unaffected by infestation in section 6.2.

Alternatively, while it was assumed that the growth cabinet malfunction had no significant effect on the already nearly matured West, effects may have been sufficient to confound the differences between infested and uninfested plants.

In conclusion there is evidence from this experiment that phosphorus limitation was a factor contributing to reduced growth of Heterodera avenae of infested plants. P deficiency of infested plants arose from a reduced root surface area and not from a decline in rate of P absorption. P deficiency was partly alleviated in at least one cultivar by increasing the solution P concentration from 10 to 100 μM. Tolerance to Heterodera avenae infestation was greatest on the cultivar least efficient in utilizing P, and tolerance was increased the most on the cultivar with the highest P utilization efficiency. Other factors, which may include the supply of other minerals, are also important since the tolerance level of two cultivars was unaffected by P nutrition.

CHAPTER 5: GENERAL DISCUSSION

CHAPTER 5: GENERAL DISCUSSION

Tolerance has been defined as the ability of plants to endure disease without severe loss in yield (Caldwell *et al.* 1958). Such a definition does not include mechanisms that result in the host's escape or exclusion of the pathogen. Yet it is difficult to conceive of any mechanism ultimately conferring disease tolerance that does not act through exclusion or escape. Whether the avoidance occurs at the macro level, through mechanisms that deter development of the parasite on the host or at the microscopic level, wherein, e.g. substances released by the pathogen alter the metabolism of an intolerant, but not a tolerant host, the underlying mechanism is one of escape, not tolerance in the strict sense. In the latter case failure to respond to the parasite could be viewed as having arisen from an incompatibility between the host and pathogen, enabling the plant to escape, not tolerate the invader.

In the present study cultivars varied in their yield response to *Heterodera avenae*. Nematode infested NZC was consistently less reduced in yield than Sual and Swan, with West showing a response somewhere in between. Using the term loosely, tolerance to *Heterodera avenae* therefore varied between cultivars, NZC was the most tolerant, Sual and Swan, the least.

In trying to track down what made NZC a more nematode tolerant cultivar than the others, some progress was made in determining the critical stage of the infestation process at which tolerance (or avoidance) of the parasite occurred.

Tolerance did not occur at the stage of nematode penetration or establishment, or at least the differences in larval numbers establishing within the roots did not appear to vary sufficiently to account for differences in growth response to infestation between cultivar.

Tolerance also did not appear to derive from inherently larger root systems, since cultivars with comparably sized root systems (NZC and Swan) differed considerably in their response to infestation.

Tolerance to Heterodera avenae in part derives from compensatory uninfested lateral and nodal root growth, though under field conditions the efficacy of this form of avoidance is strongly dependent upon a synchrony of larval hatch and seed germination, which is an uncommon occurrence in the cereal belt of south-east Australia. Differences in cultivar response to infestation could largely be accounted for by a greater insensitivity of the roots of NZC to growth impairment caused by nematode invasion compared to that of the other three cultivars (Chapter 4.2). The mechanism that conferred the insensitivity to root stunting brought on by infestation was itself an avoidance mechanism if considered in the light of the opening paragraphs of this chapter.

It has been recognised for quite a while that root growth may be an important contributing factor to nematode tolerance (e.g. Seinhorst 1961, Howard 1965). As far as the author is aware no reports have been published relating the more vigorous root growth of tolerant plants (Evans et al. 1977) to greater insensitivity to root stunting caused by infestation. However, attempts to elucidate the mechanism conferring increased root insensitivity to infestation were unsuccessful. There was no association between sensitivity of root growth to infestation and levels of or sensitivity to, growth regulators in the root (Chapter 4.3).

Considering the dependence of all metabolic processes on water and minerals supplied by the root, and that root growth is restricted by nematode infestation it is not surprising that edaphic and climatic factors have been found to influence the response of different plant species to nematode infestation (e.g. Wallace 1971) nor should it be surprising to find that plant processes which compensate for water and mineral deficit arising from nematode-induced root stunting have been associated with higher levels of nematode tolerance (e.g. Evans 1982b, Fatemy et al. 1985, this thesis). In all of the above studies, infested tolerant cultivars had a greater water use efficiency than infested intolerant cultivars. The first two studies referred to found a relation between stomatal sensitivity to water deficit and nematode tolerance, though the present study found no convincing evidence for this. While there is no disputing

that differences in stomatal regulation between cultivars was at the heart of the cultivar differences in water use efficiency it is not clear whether they arose from distinct shoot or root characteristics. The present investigation indicated that root factors, i.e., root extension (Chapter 4.4.2) and hydraulic conductivity (Chapter 4.4.5) differed more distinctly between tolerant and intolerant cultivars than shoot factors (i.e. stomatal sensitivity to water deficit (Chapters 4.4.3 and 4.4.4). However the two sets of factors are so interrelated that it is difficult to separate their individual contribution to tolerance. For example the infested roots of NZC were more permeable to water than those of the other cultivars when shoots were excised from the roots, yet under water limiting conditions the transpiration rate of infested NZC declined further than that of intolerant Sual, presumably indicating greater sensitivity of stomata to water deficit. On the other hand water permeability of infested, excised roots of Sual was also higher than uninfested roots, but this was not checked by stomatal sensitivity to water deficit. To the extent that nematode tolerance is related to maintenance of turgor, such tolerance derives from both conservatism and opportunism, whereas intolerance is a consequence of liberal and inopportunistic water use. These findings indicate that even within the context of drought resistance-related properties of nematode tolerant plants, several factors are involved which when acting singly may even disadvantage the infested plant.

While failing to convincingly demonstrate that phosphorus nutrition could influence the level of tolerance to Heterodera avenae, the phosphorus study indicated how important unimpeded root extension was in determining mineral nutrition. It also showed that unimpeded root growth was not a consequence of faster rates of P uptake or an ability to utilize P more efficiently. To the contrary, whereas nematode tolerance was associated with efficient water use, it was the less tolerant cultivars which used P more efficiently. These findings again support the earlier observation that the final outcome of the interaction between host and pathogen that is observed as tolerance is the consequence of interrelating tolerance conferring characteristics which in isolation may be of no significance.

It is important that cause and effect of nematode tolerance be disentangled. The physiological traits associated with tolerance may be the cause or the consequence of other more obscure, or perhaps a more generalized attribute. To distinguish the two possibilities is important, since the factors leading to expression of a trait associated with tolerance in one cultivar may not necessarily result in tolerance in another if they are not causally related. If root growth inhibition was only one manifestation of a generalized effect of infestation on, say, photosynthesis, then the tolerant cultivar would derive its tolerance from an insensitivity of its photosystems to

infestation, and not to unimpaired root growth. While the CO_2 studies described in this thesis indicated that assimilate supply was not the cause of root stunting, other, yet undefined causes, may be. The CO_2 studies also demonstrated the difficulties associated with an over simplification of events occurring during nematode infestation. While reduced rates of CO_2 assimilation may derive from mineral deficiency arising from stunted roots on infested plants it is equally likely that a decline in photosynthesis occurred as a consequence of feedback inhibition, an oversuply of reduced sugars inhibiting further CO_2 incorporation.

As work proceeds towards a better understanding of the physiology of nematode tolerance, it is possible that the single factor, unimpaired root extension, may be at the heart of tolerance, independent of all other traits. More likely it will be found that tolerance derives its character from a diversity of factors and it is how these factors interrelate that will determine the level of tolerance of a nematode infested plant.

CHAPTER 6: LITERATURE CITED

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APPENDICES

APPENDIX I

EFFECT OF SAND GRAIN SIZE ON INOCULATION EFFICIENCY AND ROOT GROWTH

Particle size of the rooting medium is inversely related to rate of root extension (Wiersum 1957). Optimum pore size for nematode mobility is in the range of 150 to 250 M diameter (Wallace 1963). The aim of this study was to assess the affect on root growth and inoculation efficiency (number of nematode larvae applied/number of larvae invading roots) of a sand particle size ranging from 250 to 750 M in diameter.

Methods and Materials

Seedlings of the cultivar Swan were sown after the usual pregermination treatment into 13 x 2.5 cm plastic tubes closed at one end with nylon mesh (50 M) and filled to within 2.0 cm of the top with acid-washed presieved sand ranging in diameter from 250 to 750 M. An identical set of tubes was filled with John Innes soil used in previous experiments into which seedlings of Swan were also sown. After three days, when coleoptiles had fully emerged, 20 plants were uniformly selected from each planting medium type and inculated with second stage Heterodera avenae larvae at a rate of 500, 1000, 2000 and 4000 per tube. Over a subssequent 12 day growth period plants in sand culture were grown in a one quarter strength Hoaglands solution (Hoagland and Arnon 1950) roots being fed by upward capillary movement of nutrient solution as described in section .

Soil grown plants wre grown on plastic lined trays and had access to distilled water free standing at the bottom of the tray.

The experiment was conducted in the glasshouse in which prevailing temperatures fluctuated between 16 and 22°C night/day. Daylength was 13 hours.

Fifteen days after sowing the oat plants were assessed for root length and total nematode larvae number on the roots, using methods previously described (Chapter 3, 2.5.2).

Results and Discussion

The effect on root length and nematode infestion of two rooting medium types is presented in Fig. A.1. Roots grown in sand culture had shorter root systems after 15 days growth than those grown in conventional soil. Mean inoculation efficiency over the 3 initial densities was greatly reduced on plants grown in sand (6.8%) compared to soil grown plants (16.4%). The specific root length decline (%) due to nematode infestation was 5.6 per 100 nematodes for plants grown in soil compared to 7.7 per 100 nematodes for sand grown plants, at the highest level of inoculation. Therefore at this level of inoculation sand culture using sand grain dimensions between 250 and 750 M diameter provides a satisfactory growth medium for testing nematode affects on plant growth.

APPENDIX II

DERIVATION OF PROPORTIONALITY COEFFICIENT α RELATING ION FLUX INTO THE ROOT AND EXTERNAL CONCENTRATION

Ion flux F ($Kgm^{-2}\ sec^{-1}$) into the root is related to external ion concentration at the root surface Cr (Kgm^{-3}) by the equation -

$$F = \alpha Cr$$

where α is the mean root uptake coefficient expressing proportionality of flux to solution concentration.

Nutrient uptake (Z) is proportional to the root surface area (Cm^2) and external ion concentration and related by the equation -

$$\frac{dZ}{dt} = 2 \quad rL \cdot \alpha \cdot Cr$$

where r and L are root radius (cm) and length (cm), respectively. Uptake also depends on plant growth rate according to the equation -

$$\frac{dZ}{dt} = \frac{d(xW)}{dt} = x \frac{dW}{dt} + W\frac{dX}{dt}$$

where x is the mean ion concentration in the plant ($g\ g^{-1}$) and W is the total plant dry mass (g) (Nye and Tinker, 1969).

Combining the two equations -

$$a = \frac{W}{2} \cdot \frac{x}{Cr} \cdot \left(\frac{1}{W}\frac{dW}{dt} + \frac{1}{x}\frac{dx}{dt}\right)$$

APPENDIX

Table A1: Effect of Solution Phosphate Concentration and Nematode Infestation on Phosphorus Flux (Kg m^{-2} s^{-1}) Across the Root Surface Between 16 and 32 Days After Sowing

		PHOSPHORUS FLUX (Kg m^{-2} s^{-1}) x 10^{-12}							
		DAYS AFTER SOWING							
CULTIVAR	PHOS. CONC. (M)	16-20		20-24		24-28		28-32	
		0	+	0	+	0	+	0	+
NZC	1	13.2	17.6	26.9	18.8	14.4	18.4	14.9	23.1
	10	20.7	4.9	18.3	14.1	12.9	22.2	18.3	22.1
	100	3.9	5.2	2.3	3.9	4.1	6.5	4.2	8.8
SUAL	1	6.7	8.4	14.2	28.5	13.2	13.6	12.6	13.2
	10	8.7	12.5	12.5	14.9	9.1	4.2	11.1	4.0
	100	2.8	3.4	4.2	5.7	2.9	3.9	3.3	4.6
SWAN	1	14.0	27.2	49.7	5.0	58.1	4.6	60.9	7.1
	10	1.1	18.2	11.6	5.0	14.4	11.5	15.1	12.9
	100	2.3	3.6	3.4	4.9	2.7	4.8	2.9	5.4
WEST	1	13.6	12.3	19.4	45.7	32.4	28.6	37.3	41.6
	10	1.6	11.9	13.5	40.7	4.3	27.8	3.9	4.4
	100	3.7	6.2	2.7	22.6	4.9	4.7	5.5	5.6

a - uninfested control; b - infested (initial density = 4000 nematodes)