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STUDIES ON THE RELATION OF *HETERODERA AVENAE* TO SUSCEPTIBLE AND RESISTANT WHEAT

by

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TABLE OF CONTENTS

	Page
SUMMARY	i
STATEMENT	iii
ACKNOWLEDGEMENTS	iv
SECTION 1: INTRODUCTION	1
1.1 Physiology of growth of wheat	2
1.2 Breeding and resistance	3
1.3 Aim of experimental work	7
SECTION 2: INTERACTION BETWEEN H. AVENAE AND ROOTS OF SUSCEPTIBLE PLANTS	8
2.1 Relationship between density of inoculum, penetration of larvae and growth of roots	8
2.1.1 Materials and Methods	
2.1.1.1 General	9
2.1.1.2 Experimental	10
2.1.2 Results	11
2.1.3 Discussion	12
2.2 Effect of three media on penetration of larvae and growth of roots	13
2.2.1 Materials and Methods	13
2.2.2 Results	14
2.2.3 Discussion	15
2.3 Effect of density of inoculum on penetration and establishment of larvae and growth of roots	16
2.3.1 Materials and methods	16
2.3.2 Results	18
2.3.3 Discussion	22
2.4 General discussion	24
SECTION 3: INTERACTION BETWEEN H. AVENAE AND WHOLE PLANTS	28
3.1 Effect of inoculation, cultivar and root growth on development of adult nematodes	29
3.1.1 Materials and Methods	
3.1.1.1 General	29
3.1.1.2 Experimental	32
3.1.2 Results	34
3.1.3 Discussion	37
3.2 Effect of density of inoculum and time of inoculation on growth of Halberd and development of nematodes	39
3.2.1 Materials and Methods	40
3.2.2 Results	42
3.2.3 Discussion	52
3.3 Conclusions	56
SECTION 4: EFFECT OF H. AVENAE ON GROWTH OF WHOLE PLANTS OF SUSCEPTIBLE AND RESISTANT CULTIVARS OF WHEAT AND BARLEY	58
4.1 Materials and Methods	
4.1.1 Growth room study	59
4.1.2 Field study	60

Page

4.2 Results
4.2.1 Growth room study 62
4.2.2 Field study 68
4.3 Discussion 71

SECTION 5: MECHANISM OF RESISTANCE TO *H. AVENAE* IN WHEAT 76

5.1 Materials and Methods 76
5.2 Results 84
5.3 Discussion 95

SECTION 6: EXAMINATION OF *H. AVENAE* FROM DIFFERENT SITES FOR BIOTYPES 102

6.1 Materials and Methods 102
6.2 Results 104
6.3 Discussion 109

SECTION 7: INHERITANCE OF RESISTANCE TO *H. AVENAE* IN WHEAT AND BARLEY 112

7.1 Materials and Methods 112
7.2 Results 114
7.3 Discussion 116

SECTION 8: GENERAL DISCUSSION 119

BIBLIOGRAPHY 122

SUMMARY

The relation of *H. avenae* to resistant and susceptible cultivars of wheat and barley, the mechanism of resistance in wheat, inheritance of resistance in wheat and barley, and the possibility of biotypes of the nematode in South Australia were studied.

Because of variability in published results, methods of studying the relation of the nematode to either a single root tip or the entire root system of plant were developed in which the environment, plant growth and density of larvae in the inocula were controlled. These methods were used throughout this study, but one field trial was done to relate these results with the field situation.

Similar numbers of larvae invaded, established and developed in susceptible and resistant cultivars of wheat at each density of inoculum when larvae were inoculated onto a single root tip. Each of these three aspects had a linear phase of increase in numbers of larvae and this was followed by a plateau phase as the density increased, but only the number of developing larvae decreased as the density of inocula continued to increase. The differences between numbers of larvae invading, establishing and developing increased with density of inoculation until the plateau phases were reached and rates of linear increase were progressively reduced from invasion to establishment to development of larvae. Numbers of females showed similar phases to those of developing larvae, but although the number of females was initially half the total number of larvae developing, this relation was not maintained. The ratio of males to females was unity at the low density of inocula and then increased with increases in the density of inoculation. Initially there was a similar number of females on resistant and susceptible cultivars, but as density of inocula increased, fewer females were on AUS 90248 than

Halberd and on AUS 10894 than AUS 90248.

Field and growth room studies showed that invasion of wheat and barley seedlings affected plant growth and reduced both the numbers of fertile spikelets and grains per inflorescence. This effect of the nematode on growth and yield of cereals was probably related to nutrient and moisture supply. The resistant wheat (cv. AUS 10894) was usually more tolerant than Halberd and the expression or absence of this tolerance was associated with the mechanism of resistance. There was a suggestion that the resistance induced into the early roots of AUS 10894 following invasion was transferred to the later developing roots.

Resistance was confirmed for five barley cultivars. This and the resistance in wheat was usually associated with partial dominance of a single gene with the possibility of polygenes or modifier genes also being associated. The same major gene occurred in the two resistant cultivars of wheat (cvs. AUS 10894 & AUS 90248), but at least two and possibly four different genes were present in the barley cultivars.

The two resistant wheat cultivars were resistant against H. avenae at 27 different sites within South Australia and the reaction of an 'International' range of cultivars against 4 different sites suggested only one biotype. Because the few females on resistant wheat were probably a result of the mechanism of resistance, no resistance-breaking nematodes were found or were likely within nematode populations in South Australia.

STATEMENT

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 and belief, contains no material previously published or written by another person, except where due reference is made in the text.

P.C. O'BRIEN

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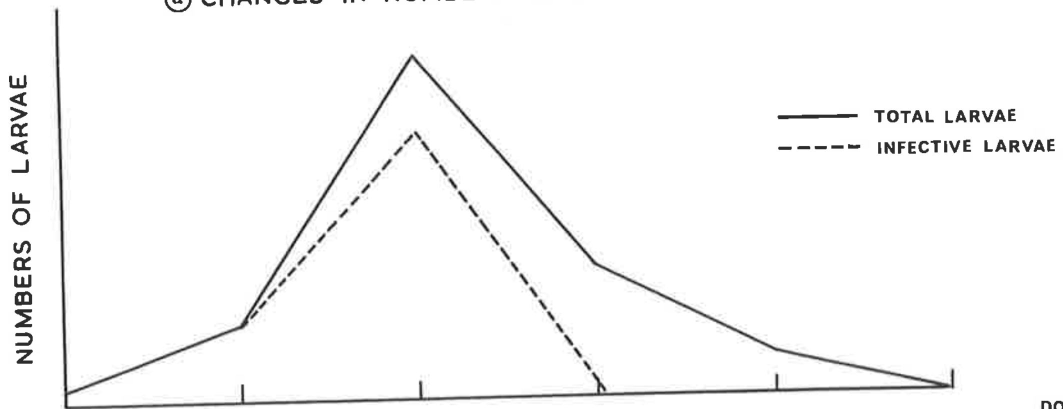
1. INTRODUCTION

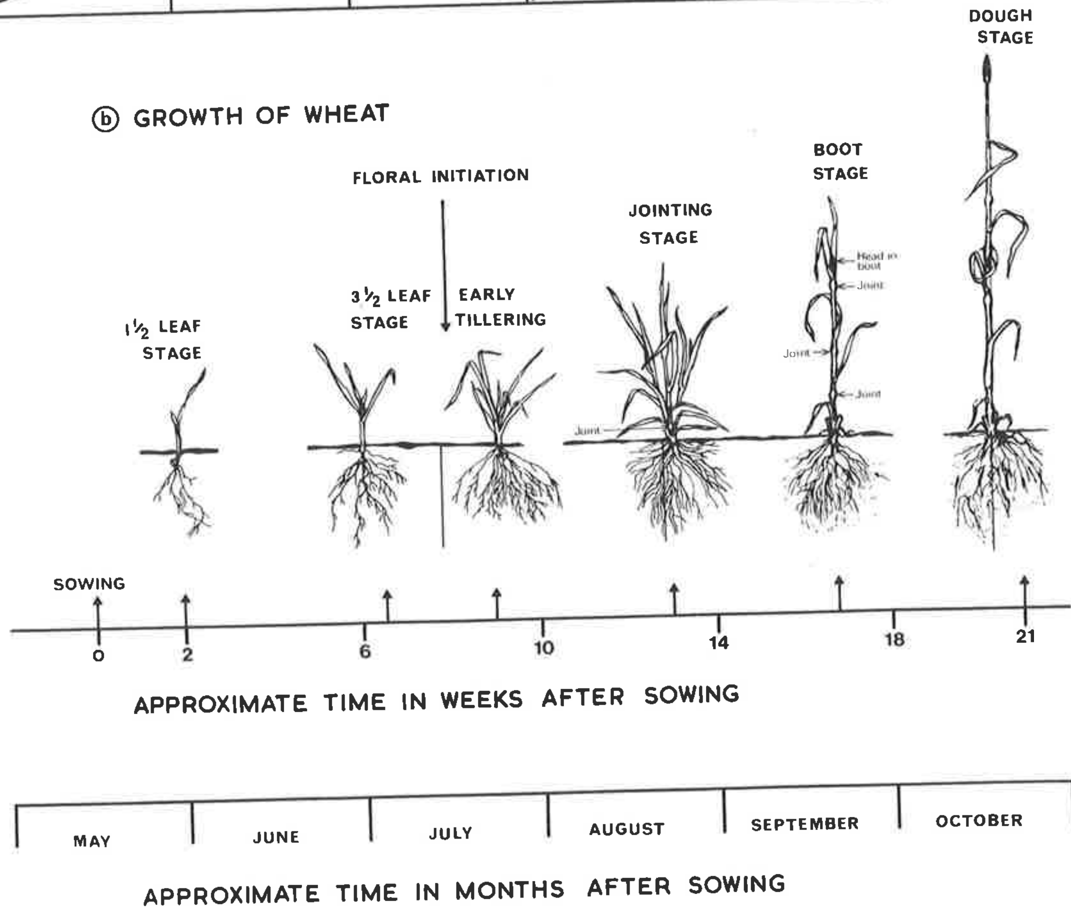
Cereal root eelworm (Heterodera avenae, Wollenweber 1924, (Filipjev, 1934)) was first reported in South Australia by Davidson (1930) and descriptive accounts of the effects of this organism on cereal growth within Australia (Hickinbotham, 1930; Millikan, 1938; Banyer, 1966; Parkin & Goss, 1968; Meagher, 1968) have indicated that it is of economic importance. However, a synoptic study on the growth of wheat (Triticum aestivum cv. Halberd) in South Australia by Stynes (1975) suggests that the nematode affects the early, or seedling, growth of the plant and because the plant could compensate for this effect during later growth, other environmental factors are more important in determining the final crop yield.

The major cereal growing areas within South Australia are bound roughly by mean winter (May to October) rainfall totals between 150 and 350 mm. and summer rainfall is relatively insignificant, i.e., bound roughly by a mean annual rainfall between 200 and 550 mm. Slow maturing cultivars are susceptible to the periods of high evaporative demand which normally occur late in the season, therefore, the genetic adaptation by Australian wheats has been through early maturity and insensitivity to both vernalisation and photoperiod (Donald & Puckridge, 1975). Highest yields of wheat are obtained when sowing is between mid-May and mid-June (Donald & Puckridge, 1975); this results in floral initiation occurring during July (Symes, 1974; Nix, 1975) and anthesis occurring during early October when the optimal combination of water availability, temperature and light regimes occur (Nix, 1975). These stages of wheat growth during the season are shown in relation to time of sowing in Fig. 1b.

Seasonal fluctuations in the total numbers of larvae of H. avenae, modified from Meagher (1970), are also shown (Fig. 1a) and these clearly

Figure 1. Changes in total (modified from Meagher, 1970) and infective larvae of *H. avenae* and in growth of wheat predicted for an average season in South Australia.


ⓐ CHANGES IN NUMBERS OF LARVAE IN SOIL
NUMBERS OF LARVAE
TOTAL LARVAE
INFECTIVE LARVAE


ⓑ GROWTH OF WHEAT
FLORAL INITIATION
1½ LEAF STAGE
3½ LEAF STAGE
EARLY TILLERING
JOINTING STAGE
BOOT STAGE
DOUGH STAGE
SOWING
0
2
6
10
14
18
21
APPROXIMATE TIME IN WEEKS AFTER SOWING
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
APPROXIMATE TIME IN MONTHS AFTER SOWING

indicate that wheat grown in infested soil can be infested until the end of July or early August. Absence of larvae after this time is apparently due to an induced dormancy in the unhatched larvae during July (Banyer & Fisher, 1971 a). Therefore, wheat growth can be affected by the nematode until after floral initiation, and because the final grain yield of wheat is a reflection of the accumulation of the effects of crop growth from germination to anthesis, as well as from anthesis to maturity (Nix, 1975), *H. avenae* is likely to affect the final yield of wheat.

1.3 Physiology of growth of wheat

During vegetative growth, leaf primoidea are initiated and develop at the apical meristem until floral initiation occurs and final leaf number is determined (Bonnett, 1966). Although only four leaves may be visible at floral initiation (Single, 1964), further growth of the leaf primoidea already initiated continues and usually 8 or 9 leaves are developed on the main tiller (Symes, 1974). Nutrient reserves in the endosperm of the seed normally affect only the growth of the first 4 leaves and, because the duration of growth before the exponential phase of each leaf is progressively increased, the appearance of successive leaves is delayed (Williams, 1960). The largest leaf is often the one elongating at the time of floral initiation (Borrill, 1959). The importance of nutrient supply for leaf growth is demonstrated by a reduction in leaf area with reduced availability of nutrients (Langer, 1966) and an increase in number of leaves, without delaying the time of floral initiation, with increased nitrogen supply (Single, 1964).

Between floral initiation and anthesis, maximum grain number is determined through development of spike-bearing tillers, spikelet number per spike and florets per spikelet. The maximum number of spikelets is dictated by genotype and the actual number is the result of the interaction of genotype and environment (Rawson, 1970), and because spikelet differentiation

seems to be completed when the apex has a "double ridge" appearance (Single, 1964; Lucas, 1971), spikelet differentiation in the field is probably completed by the end of August. This is also a period of low temperature and poor light intensity (Nix, 1975), and these factors with leaf area index, nutrient supply and plant competition (Single, 1964; Davidson, 1965; Friend, 1965; Aitken, 1966; Puckridge & Donald, 1967; Puckridge, 1968) are important factors in determining spikelet number. Therefore, it is also likely that plant diseases will also reduce spikelet number by affecting some of these factors.

A reduction in spikelet number may be compensated within a plant by an increased number of florets in the spikelets (Bonnett, 1966). However, the number of fertile florets or grains per spikelet are also affected by the same factors which affect spikelet number (Single, 1964; Davidson, 1965; Puckridge & Donald, 1967; Puckridge, 1968; Frankel, 1976). Other environmental factors, especially frost and moisture stress, can also affect the number of grains per spikelet with an effect on grain set at anthesis (Nix, 1975). Accumulation of photosynthate in the grain is almost entirely from photosynthesis in the flag leaf, peduncle and spike (Evans & Rawson, 1970), and therefore, an earlier effect of either environment or disease on plant growth could affect grain weight as well as numbers of grain per plant.

Similar patterns of development are described for wheat and barley (Williams, 1966) and the yield accumulation process seems to operate similarly for barley and wheat, as the number of leaves and duration of the preanthesis period are similar when both are grown under field conditions in South Australia (Gardener, 1974).

1.2 Breeding and Resistance

Resistance in plants to most nematodes has been identified and the inheritance of this resistance is similar to the inheritance of any

other type of resistance and other qualities in plants (Hare, 1965). Cultivars of wheat (Triticum spp.; O'Brien & Fisher, 1974), barley (Hordeum spp.; Hayes & Cotten, 1970) and oat (Avena spp.; Cotten & Hayes, 1972) with resistance to H. avenae have been identified and some of these cultivars are resistant to the nematode in Australia (Millikan, 1938; Brown, R., 1969; Brown & Meagher, 1970; O'Brien & Fisher, 1974; Ellis & Brown, 1976). Inheritance of this resistance includes both the dominant and recessive expression of the genes, and although a single gene is usually involved, two, three or several genes within the host have also been identified (Anderson, 1961; Cotten & Hayes, 1969; Cotten & Hayes, 1972; Cook, 1974). Therefore, the genetic material is available within each of the three cereal genera to introduce resistance into commercial cultivars.

Because of the possibility of new strains, races or biotypes of a pathogen developing and breaking down the resistance to the disease which is introduced into a new cultivar, polygenic rather than monogenic resistance is preferable (Van der Plank, 1968). However, polygenic resistance is not always available and the assessment of this expression of resistance is difficult during a breeding programme. The nature and habitat of most of the plant nematodes is restricted and physiological races that break resistance would spread slowly (Hare, 1965). Therefore, the rotation of resistant and susceptible crops as recommended by Jones et al. (1967) could delay or even prevent the selection of a new biotype.

Several biotypes of H. avenae have been identified within Europe (Andersen, 1959; Cotten, 1963; Kort et al., 1964; Neubert, 1966) and often two or more biotypes are present within one population of the nematode. If two or more biotypes within a nematode population is the normal situation, the few females on roots of the resistant cultivars in South Australia (O'Brien & Fisher, 1974) may represent a different biotype and the

resistance in cultivars will be lost because the resistance breaking biotypes are already distributed throughout the infested areas. However, only one biotype is suggested for Australia (Brown, R., 1969) and this biotype differs from those in Europe. This aspect needs further attention as a different biotype in South Australia is possible (O'Brien & Fisher, 1974) and resistance breaking biotypes may occur within Victorian populations of the nematode (Ellis & Brown, 1976).

Genetics of virulence in H. avenae has been studied (Anderson, 1965). However, these studies are difficult and the probability of selecting for virulence or avirulence in the nematode is more easily assessed by either measuring the reaction of the same resistant cultivar to successive generations of the nematode or understanding the mechanism of resistance within the host. Because one life cycle of H. avenae is likely each year (Wallace, 1965), the mechanism of resistance in wheat needs to be examined to assess the likely stability of the resistance.

The expression of resistance (Rohde, 1972) and biochemical mechanisms of resistance (Giebel, 1974) in plants to nematodes have been recently reviewed. Little is known of the response of plants resistant to H. avenae infection, but resistance in barley cultivars does not affect larval penetration (Cotten, 1967; 1970 a), female development is retarded and the number of nematodes within the roots decrease (Cotten, 1970a). In resistant wheat, penetration and the number of sexually mature males did not seem affected, but the number of sexually mature females is reduced (Brown, J., 1974). Therefore, the sex ratio of males to females is higher on the resistant than the susceptible cultivars of wheat. Variable sex ratios also occur on susceptible hosts to Heterodera spp. (Ellenby, 1954; Kampfe & Kerstan, 1964; Trudgill, 1967) with different densities of nematodes invading plants and different environments for plant growth. Sex reversal may occur, but most Heterodera spp. (including H. avenae) are heterosexual

and show sexual dimorphism (Shepherd, 1965) and genetical sex determination of larvae is more likely in these bisexual species (Triantaphyllou, 1973). Therefore, variable sex ratios reflect the effects of environment, nematode density and host resistance on the establishment, development and maturation of the female larvae in the nematode population.

Resistance in cultivars of barley to H. avenae increase grain yield, not only by reducing the density of the nematode population (Cotten, 1970b; Williams, 1970), but also by having better tolerance to nematode infection than the susceptible cultivars (Cotten, 1967). Cultivars of oat are generally regarded as less tolerant to nematode invasion than cultivars of wheat and wheat is regarded as less tolerant than barley (Hesling, 1959; Stone, 1960). Evidence on the tolerance of cultivars of oat and wheat is lacking and the general relationship between the genera may not apply as there may be intra-species variation in tolerance within each of the genera similar to that which occurs with resistance (Millikan, 1938; Brown & Meagher, 1970). Cook (1974) suggests the difference in tolerance between barley and oats is related to the host response to invasion. This aspect needs further investigation as tolerance associated with resistance would increase the probability of selecting resistant plants when plants are selected for increasing yields during the later stages of a breeding programme. Another possibility of assisting selection of resistant plants is the identification of marker genes i.e., a character genetically linked with resistance which is easily recognised, and some success has been achieved with marker genes for resistance to H. avenae in barley (Andersen & Andersen, 1973).

During the identification of resistant cultivars, the selecting of resistant progency in the early stages of breeding for resistance and the assessing of progeny in tests on the inheritance of resistance, it is essential to use uniform methods of assessment to ensure that genetic resistance, and not some other factor influencing development of female

nematodes, is measured (O'Brien & Fisher, 1974). The method of assessment should also allow large numbers of plants to be tested at the one time (McKenna et al., 1963). Different methods have been used (Shepherd, 1958; Andersen, 1961; Andersen, 1963; McKenna et al., 1963; Brown, J., 1974) but wide variations in host reaction still occur. Therefore, further information on the relationship between the nematode and the host plant during assessment of the reaction of the host to infection is required to eliminate variation in the reaction of the host plants.

1.3 Aim of Experimental Work

The primary aim of the experimental work presented here was to examine the resistance in two cultivars of wheat, AUS 10894 and AUS 90248, and to evaluate the effectiveness of this resistance as a means of controlling *H. avenae* in South Australia. Cultivars of barley were included in some experiments to permit a comparison of the two genera as most of the information available is on resistance in barley. Experiments are described which indicate the effect of *H. avenae* on cereal growth, the mechanisms of resistance in wheat, the possibility of different biotypes in South Australia and the inheritance of resistance.

In order to obtain this information, two methods were developed to examine the relation between the nematode and the host. One method was developed to examine this relationship on individual roots of the host and the other was developed to examine the relationship on the whole plant. The reaction of plants to nematode infection was tested by the latter method and these tests were done before the optimal densities of nematode and times of inoculation were determined.

2. INTERACTION BETWEEN HETERODERA AVENAE AND ROOTS OF SUSCEPTIBLE PLANTS

A cereal plant is susceptible to *H. avenae* if it supports the production of large numbers of prolific females (Shepherd, 1959) and resistant if no, or very few, females are produced (Andersen, 1961).

When cereal cultivars were tested for their reaction to this nematode, variations occurred in the number of females which developed within a cultivar and between different tests for the same cultivar (O'Brien & Fisher, 1974). This variation was attributed to one or more of the following: genetic variation in the host or nematode, variations in methods of assessment, or the use of different densities of the nematode.

In a monoxenic culture method (Brown, J., 1974), a single cyst of *H. avenae* was used to infect a susceptible wheat (cv. Olympic) and a susceptible barley (cv. Weeah). Again, the number of females produced on each cultivar was variable and this appeared to relate to the variations in the number of larvae hatched from the cysts. Wide fluctuations noted in both the total number of larvae hatched and rate of hatch of these larvae from individual cysts were considered a normal feature within populations of *H. avenae* in South Australia (Davies, pers. comm.). Most assessments of plants' reactions to *H. avenae* have used cysts as the source of inoculum, and much of the variation recorded in the reaction of plants during these assessments may be due to consequent differences in density of inoculum.

The development of a method of application of second-stage larvae of *H. avenae* to seminal roots of cereals and the effects of this on the penetration, establishment and development of nematodes and growth of seminal roots are examined and discussed in this section.

2.1 Relationship between density of inoculum, penetration of larvae and growth of roots

A turbidimetric method of estimating the number of nematode larvae in a suspension has been described by Blake (1958) in which a 0.5% solution

of carboxymethyl cellulose (c.m.c) was used to retain larvae in suspension. Therefore, provided c.m.c. has no effects on either the nematode or plant, it would minimise variation in the number of larvae between inocula from the same suspension of nematodes, and so the value of c.m.c. as a medium for inoculation of larvae of *H. avenae* and the method of application of the inoculum was examined.

2.1.1 Materials and Methods

2.1.1.1 General

Mature cysts of *H. avenae* were collected with associated roots of barley (cv. Clipper) in March, 1972, from a field near Bow Hill in South Australia and stored at 20°C. When nematodes were required, the cysts were transferred to a temperature of 10°C for approximately 8 weeks as an increase in rate of hatching of larvae from cysts was expected following a low temperature treatment (Banyer & Fisher, 1971b). Cysts were then separated from roots by washing, collected with associated organic material in a 0.25 mm. sieve and then placed, with the organic material, onto bolting silk in large trays (Southey, 1970). Each day, the trays were moved from 10°C to 15°C, or back, and larvae were collected, counted and stored in shallow water at 5°C until required. Davies & Fisher (1976) reported no loss of infectivity of larvae for up to six weeks under these conditions of storing.

Different densities of inoculum were prepared by dilution of the highest density. Larvae in excess of the total number required were placed in a volume of water calculated to give a density of double the highest density required. This was mixed with an equal volume of a 2% solution of c.m.c. to give the highest density needed in a 1% solution of c.m.c. This density of larvae was checked by sub-sampling single drops with a pasteur pipette and counting the number of larvae per drop in a modified Doncaster dish (Southey, 1970). Other densities were prepared by dilution with a 1%

solution of c.m.c.

Seeds of Halberd, a susceptible (O'Brien, 1972) and the most widely grown wheat in South Australia, were pregerminated. Fifty seeds were placed on filter paper in a petri dish of 95 mm. diameter with 7 ml. of water and incubated at 20°C in the dark. Germinated seeds were selected following the emergence of the radicle and three seeds were placed on 1.75% distilled water agar in each 95 mm. petri dish. Incubation continued at 20°C in the dark until each seedling had grown the first three seminal roots (Fig. 2 a), when each tip of these roots was inoculated with a single drop of inoculum of the same density with a pasteur pipette. Inoculations of the seedlings within a petri dish were independent of one another.

Penetration of larvae following inoculation was the number in a root after a duration and temperature of incubation determined by experimental design. Seedlings were removed from agar, washed and stained in lactophenol cotton blue (Southey, 1970). The stained roots were treated with acid (Davies, 1974), squashed between two glass slides and the number of larvae counted.

The position of root tips was marked on the base of dishes at inoculation and after incubation, and the increase in length of root measured in mm. was recorded as root extension. Extension was measured on the first seminal root and alternately for the second and third roots to give equal replications between the two types of root.

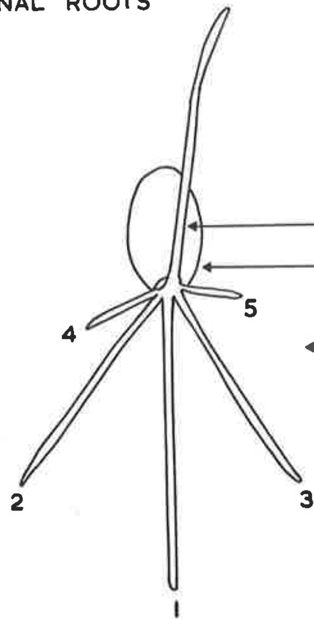
2.1.1.2 Experimental

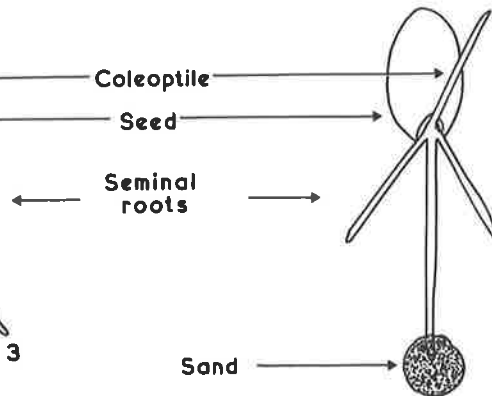
Initially, to measure the effect of density of inoculation on both the penetration of larvae and growth of roots, inocula were added directly onto roots. Six densities of 5(±1), 11(±1), 49(±4), 93(±3), 224(±30) and 360(±23) larvae (±S.E.) were used and the seedlings were incubated at 15°C for 48 hours in the dark following inoculation.

Figure 2. Schematic diagrams showing the arrangement of seminal roots on a seedling of wheat, sand over the root tip before inoculation with larvae of *H. avenae* and both the normal and abnormal growth of a root growing on agar.

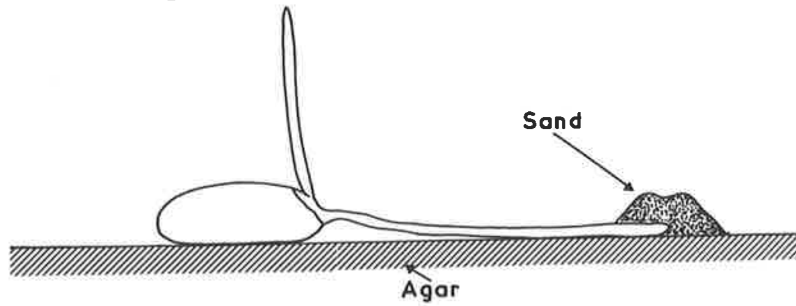
Ⓐ SEMINAL ROOTS

Ⓑ INOCULATION WITH SAND (TOP VIEW)

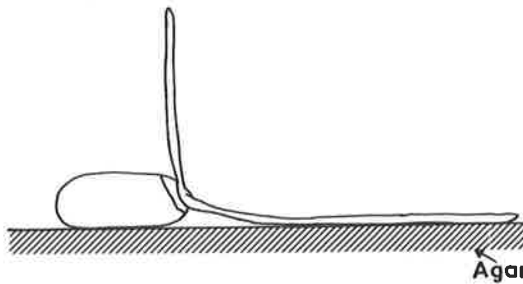




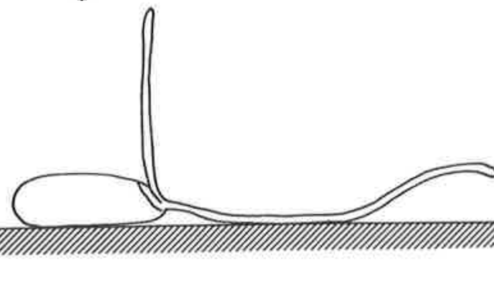
Ⓒ INOCULATION WITH SAND (SIDE VIEW)



Ⓓ NORMAL ROOT GROWTH



Ⓔ ABNORMAL ROOT GROWTH



Penetration of larvae and extension of the first seminal root were measured on all seedlings, but only root extension for one of the other two seminal roots of the seedling was measured. Six replicates (seedlings) of each density of inoculum were completely randomised and analyses of variance were done on the raw data.

Direct inoculations of larvae onto roots caused some roots to grow abnormally (Fig. 2 e), therefore a similar experiment was done to compare two methods of inoculation. Six densities of 6($\pm$1), 13($\pm$1), 66($\pm$2), 113($\pm$6), 219($\pm$11), and 399($\pm$17) larvae ($\pm$S.E.) were used and inoculations were directly onto roots and onto particles of sand of 150 - 250 µm. diameter covering the root tips (Fig. 2 b, c). After inoculation, incubation was at room temperature in the dark for 36 hours. Penetration of larvae, extension of roots and experimental design were as described above. Correlation coefficients and regression analyses between the number of larvae penetrating and length of extension of root were performed on both the raw and transformed data ($\log_{10}$) and analyses of variance were also done on the raw data.

2.1.2 Results

Growth of most roots was either along the surface of agar (Fig. 2 d) or down into the agar, except that some roots inoculated without a covering of sand grew abnormally by growing upwards from the agar surface (Fig. 2 e). Only a small percentage of roots grew abnormally at the two lowest densities of inoculum, but about 50% of the roots grew abnormally at the next three densities (Table 1). This abnormal growth seemed to reduce the number of larvae penetrating but insufficient replication prevented analysis of these results.

Penetration of larvae into roots increased with density of inoculum in both experiments and this increase was not proportional to the increase in density of inoculation (Tables 1 & 2). This relationship was

TABLE 1.

EFFECT OF DIFFERENT DENSITIES OF *H. AVENAE* OF PENETRATION OF LARVAE AND GROWTH OF SEMINAL ROOTS OF WHEAT (cv. HALBERD) DURING 48 HOURS AT 15°C.

Inoculum density	5	11	49	93	224	360
Penetration (Nos. of larvae)	1	2	8	12	19	24
		L.S.D. (P = 0.05) = 3				
Root extension (mm.)						
root 1	42	36	17	12	8	5
root 2 or 3	36	32	17	13	9	5
		L.S.D. (P = 0.05) = 7				
% of abnormal roots	5	0	68	58	50	0

TABLE 2.

EFFECT OF APPLYING DIFFERENT DENSITIES OF *H. AVENAE* DIRECTLY ONTO ROOTS OR ONTO SAND COVERING ROOTS ON PENETRATION OF LARVAE AND GROWTH OF SEMINAL ROOTS OF WHEAT (cv. HALBERD) DURING 36 HOURS AT ROOM TEMPERATURE.

Inoculum density	6	13	66	113	219	399
Nos. of larvae penetrated						
Root 1 (Direct)*	2	4	9	14	24	28
Root 2 (")	1	3	8	12	20	32
Root 1 (Sand)	3	7	20	28	47	75
Root 2 (")	3	7	17	26	46	58
		L.S.D. (P = 0.05) = 9				
Root extension (mm.)						
Root 1 (Direct)	60	58	39	25	16	11
Root 2 (")	61	54	32	25	17	9
Root 1 (Sand)	41	37	11	9	6	6
Root 2 (")	45	32	17	9	6	5
		L.S.D. (P = 0.05) = 9				

( )* Method of inoculation onto roots.

similar for both methods of inoculation, but more larvae penetrated when roots were covered with sand before inoculation (Table 2). Except with the highest density (399) of inoculum onto roots covered with sand, no difference occurred between the two types of seminal root (Table 2).

Root extension was repressed as the density of inoculum increased, but the relationship was not linear (Tables 1 & 2). No differences occurred between the two types of seminal root inoculated by both the same method and the same density of larvae. However, root extension was more severely restricted when roots were covered with sand before inoculation (Table 2).

The relationship of density of inoculum to penetration seemed to complement that of density of inoculum to root extension (Table 2). Regression analysis on $\log_{10}$ of the data showed a highly significant correlation between penetration of larvae and root extension (Fig. 3 a). A close relationship between the raw data of both parameters was also apparent in a scatter diagram (Fig. 3 b).

2.1.3 Discussion

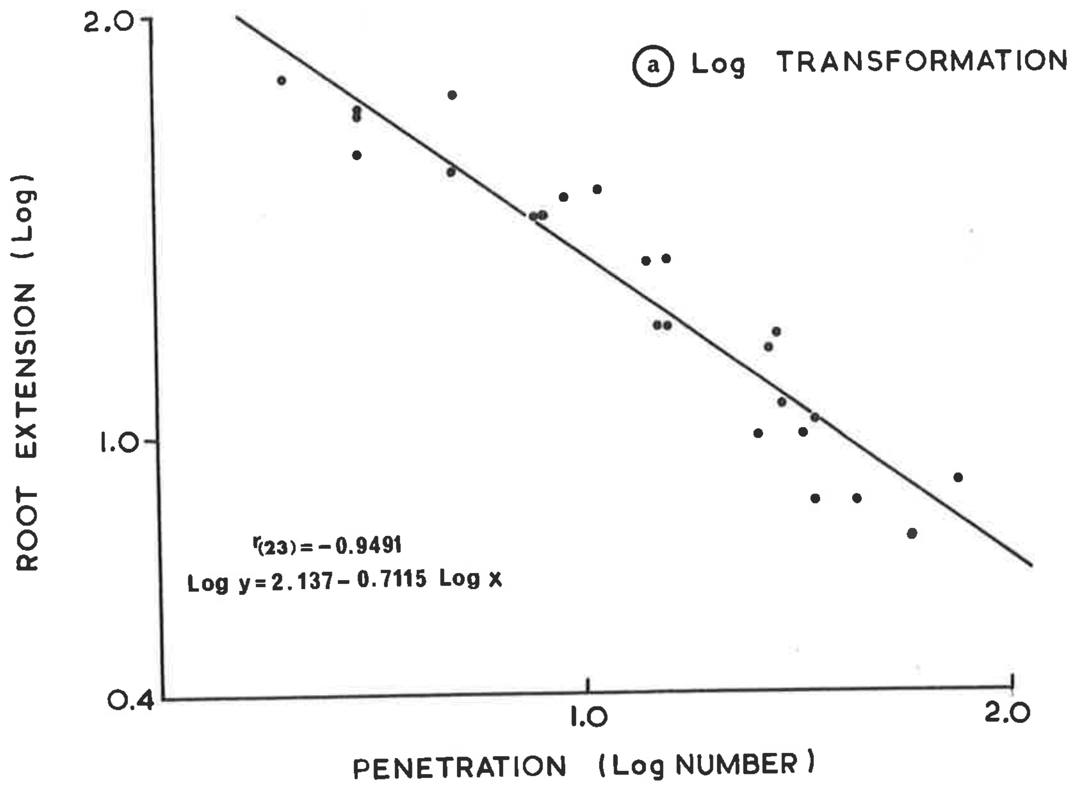
A horizontally placed root could be expected to show a positive geotropic response (Whittington, 1969) and roots which gave a negative geotropic response were described as "abnormal". Abnormal growth occurred in roots inoculated without sand covering the root tip and appeared to be an effect of nematodes because few roots grew abnormally at the lower densities of inocula. Therefore, root tips were covered with sand to avoid these effects on root growth following inoculation, and the sand particle sizes were selected from evidence of Wallace (1963) which permitted optimal movement of larvae of *H. avenae*.

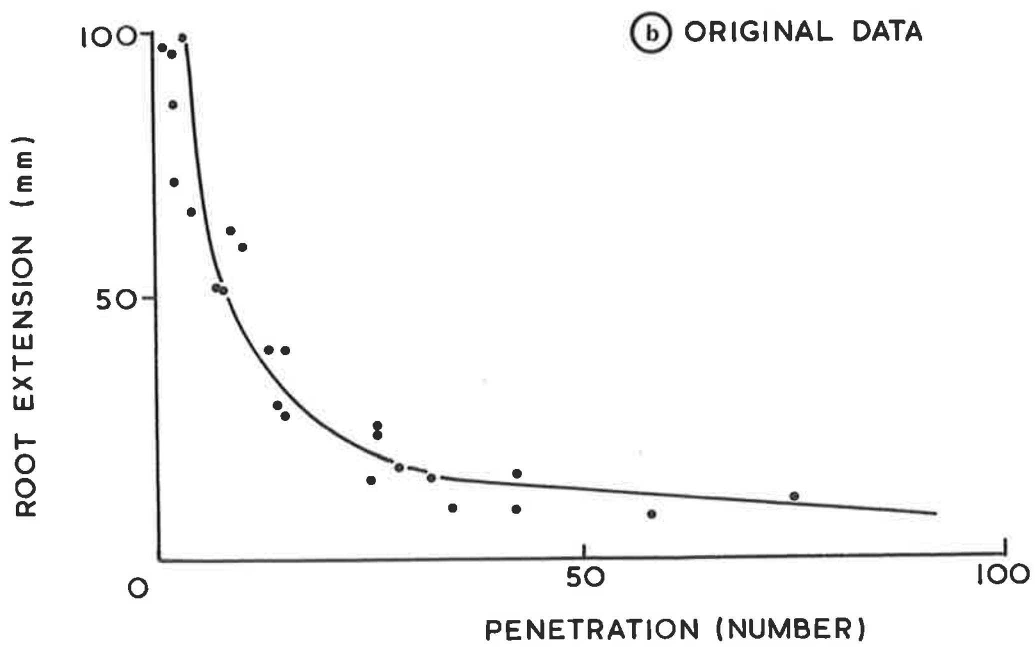
Increased penetration and reduced extension of roots resulted from using sand and this was probably due to several factors. Following

Figure 3. Scatter diagrams showing the relationship between the number of larvae penetrating a single root and the growth of the root expressed as root extension

(a) Log_{10} transformation of data with the line of best fit determined from the equation of the linear regression.

(b) Original data with the line of best fit determined by an antilog. transformation from the equation of the linear regression.


2.0
1.0
0.4
ROOT EXTENSION (Log)
a Log TRANSFORMATION
r(23) = -0.9491
Log y = 2.137 - 0.7115 Log x
1.0
2.0
PENETRATION (Log NUMBER)


100
50
0
ROOT EXTENSION (mm)
b ORIGINAL DATA
50
100
PENETRATION (NUMBER)

incubation, liquid with larvae was observed around tips of roots directly inoculated and this probably affected penetration (Sandstedt & Schuster, 1962). Sand would reduce the amount of liquid and assist movement of larvae, therefore less energy would be expended by the larvae and penetration could be increased (Wallace, 1969). Also, the increased area of root available to the larvae would result in less interference between larvae at the root surface and increase penetration (Davies & Fisher, 1976). Since various factors associated with root exudates may attract larvae (Lowenberg *et al.*, 1960; Bird, 1962; McClure & Viglierchio, 1966; Johnson & Viglierchio, 1969 a), greater attraction of larvae could occur with sand.

Although analyses showed no interaction between the two groups of seminal roots examined, more larvae penetrated the first seminal roots at the highest density of inoculum in the second experiment. This influenced the decision to use only the first seminal root in future experiments.

2.2 Effect of three media on penetration of larvae and growth of roots

The different growth responses of roots inoculated with *H. avenae* in a solution of c.m.c. were probably due to the nematode, but some doubt existed on the effect of c.m.c. Therefore, distilled water and distilled water agar were considered as possible alternatives and an evaluation of the three media was made by comparing similar densities of inoculum on covered and uncovered roots at two temperatures.

2.2.1 Materials and Methods

Larvae of *H. avenae* were collected from the same tray of matured cysts used in Section 2.1.

Inocula in solutions of water, 1% c.m.c. and 0.15% distilled water agar were prepared (Section 2.1.1.1) by mixing aliquots from the same mixture of larvae and water with an equal volume of distilled water, 2% c.m.c. or 0.3% distilled water agar. Similar densities of 94(±8), 85(±4) and

78 ($\pm$3) larvae ($\pm$S.E.) in water, 1% c.m.c. and 0.15% agar respectively were obtained.

Seeds of wheat (cv. Halberd) were pregerminated, placed on agar in petri dishes and then inoculated (Section 2.1.1.1). Only one root per seedling, the first seminal root, was inoculated and inoculation was either directly onto roots or onto sand covering the root. With each inoculum, a solution without nematodes was also tested.

Length of extension of all first seminal roots and penetration of larvae inoculated onto sand covering roots were measured (Section 2.1.1.1) following incubation of seedlings in the dark for 24 hours at either 15°C or 25°C.

There were six replications per treatment completely randomised within each temperature of incubation and analyses of variance were performed for each parameter measured.

2.2.2 Results

Although similar densities were used in each medium (Section 2.2.1), the variance of density from six replicates was 346.2 for water, 71.5 for c.m.c. and 40.7 for agar and there was greater variation within water than agar while c.m.c. was intermediate.

Penetration of larvae was unaffected by the solution used as the medium, but more larvae penetrated roots incubated at 15°C than 25°C (Table 3).

TABLE 3.

EFFECT OF THREE MEDIA AND TWO TEMPERATURES ON PENETRATION DURING 24 HOURS OF *H. AVENAE* INTO A SEMINAL ROOT OF WHEAT (cv. HALBERD) COVERED WITH SAND.

	Water	1% c.m.c.	0.15% Agar
25°C	11	12	14
15°C	19	23	20

L.S.D. ($P = 0.05$) = 6

TABLE 4.

EFFECT OF THREE MEDIA, TWO TEMPERATURES AND SAND ON GROWTH OF SEMINAL ROOTS OF WHEAT (cv. HALBERD) INOCULATED AND UNINOCULATED WITH H. AVENAE FOR 24 HOURS.

(a) Nos. of roots without sand with abnormal growth.

	Water		1% c.m.c.		0.15% Agar	
	15°C	25°C	15°C	25°C	15°C	25°C
+ larvae	6	5	6	5	4	5
- larvae	5	2	1	3	1	2

(b) Root extension (mm.)

	Water		1% c.m.c.		0.15% Agar	
	15°C	25°C	15°C	25°C	15°C	25°C
+ sand, + larvae	4	7	7	14	7	6
- sand, + larvae	8	10	10	24	5	9
- sand, - larvae	12	27	16	29	15	28
+ sand, - larvae	17	30	15	29	15	32

L.S.D. (P = 0.05) = 5

Abnormal growth (Section 2.1.2) was more frequent among roots with than without larvae and this frequency was similar at both temperatures (Table 4 a).

Extension of roots without nematodes was unaffected by sand or media, but was longer after incubation at 25°C (Table 4 b). Inoculation of roots with larvae in water or agar, directly or onto sand, reduced root extension similarly at both temperatures of incubation. The effect was also similar when c.m.c. was used as the medium and roots were incubated at 15°C, but at 25°C, a smaller effect on root extension occurred and this effect was least when inoculation was directly onto roots.

2.2.3 Discussion

Extension of roots incubated at 25°C varied when inocula of c.m.c. were used, but not when inocula of water or agar were used. Water and agar were probably absorbed by agar in petri dishes, while c.m.c. was not and

spread across the surface. Since increasing temperature decreases viscosity of liquids, the spread of c.m.c. would be greater at 25°C than 15°C and the associated nematodes would have further to move to reach the roots. Therefore, these nematodes would have less effect on root extension because either the total number penetrating was reduced or a delay in penetration occurred and roots grew for a longer period before being affected.

Abnormal growth of roots was associated with nematodes, but also occurred in some roots without nematodes and seemed to be more frequent when water was added and roots were incubated at 15°C. Increased elongation of cells in roots near the agar surface probably occurred where free water was present (Whittington, 1969).

However, less variation in numbers of larvae in inoculum with agar was the main reason for selecting 0.15% distilled water agar as the medium for inoculations in further experiments performed in petri dishes.

2.3 Effect of density of inoculum on penetration and establishment of larvae and on growth of roots

A suitable method of applying *H. avenae* to single roots has been developed and some information on the relationship between density and penetration of larvae was obtained. Further information on the effects of the density of inoculation and duration of incubation on both the penetration and establishment of larvae, and on root growth was sought before investigating the differences between susceptible and resistant cultivars.

This section describes a series of experiments designed to acquire this information.

2.3.1 Materials and Methods

Four experiments are described in this section and materials and methods, unless otherwise stated, were either the same as, or modifications

of, those described in Section 2.1.1.1. Larvae of H. avenae were collected from the same tray of cysts used for Section 2.1, but the different densities of larvae were prepared in a 0.15% solution of distilled water agar.

To examine the effect of duration of incubation on the number of larvae penetrating, the first seminal root of Halberd was inoculated, either directly or onto sand covering the root tip, with 24(±2), 142(±9) or 256(±17) larvae (±S.E.). The number of larvae in these roots was counted after 12, 24, 36, 48 or 72 hours incubation at 20°C. All treatments were randomised and there were six replicates at each time of sampling. Analyses of variance were calculated on the raw data.

The number of larvae which became either established or immobile in roots following penetration was obtained by using a Seinhorst mistifier to extract the motile larvae from the roots. The first seminal root of Halberd was covered with sand, inoculated with 15(±1) or 140(±4) larvae and incubated at 15°C for ½, 1, 2, 4 or 6 days. The number of larvae in these roots was counted both immediately after incubation and again following 24 hours in the mistifier. All treatments were randomised, there were eight replicates and analyses of variance were conducted.

To gain further information on the effect of both the density of inoculation and duration of incubation on the number of larvae either established or immobile in roots, the previous experiment was repeated with the following modifications. Three densities of inocula were used, 22(±1), 106(±11) or 191(±10) larvae, and larvae were counted after 1, 2, 4 or 6 days of incubation.

To compare the relationship between penetration of larvae and growth of roots on susceptible wheat (cv. Halberd) and barley (cv. Clipper), the first seminal root was covered with sand and inoculated with 0, 28(±3), 65(±5), 155(±3) or 332(±11) larvae and incubated at 20°C for 48 hours. Two

seedlings, one of each cultivar, were placed in a petri dish for inoculation with the same inoculum. Extension of the one inoculated root (including that of the control treatment without larvae) was measured between 24 and 48 hours of incubation and the length of all other roots of a seedling measured after 48 hours incubation was combined to give a total length in mm. Length of each root of the seedlings without larvae was measured after 24 and 48 hours of incubation. The number of larvae in each of the roots of a seedling was measured following 48 hours of incubation and the number in roots other than the inoculated root was combined for each seedling to give the total number of larvae in uninoculated roots. Analyses of variance were conducted on the raw data of five replications.

Extension of the uninoculated roots of seedlings inoculated with larvae was estimated by subtraction of their final length from the mean length of the equivalent roots on the control seedlings after 24 hours incubation. Individual uninoculated roots with the same number of larvae in them were grouped together and both the average extension and percentage growth relative to that of the control were calculated. Estimated extension of individual roots on the same seedling were combined to give total extension per seedling, and from this, the average extension per seedling was calculated for seedlings inoculated with the same density of larvae. Then the percentage growth of roots of seedlings relative to that of the control was calculated. This data was not statistically analysed.

2.3.2. Results

The number of larvae penetrating at each density of inoculation showed a similar trend over the period of incubation (Fig. 4 a, b, c), but fewer larvae penetrated when inoculation was directly onto roots than onto sand and this difference increased as the density of inoculum increased.

When roots covered with sand were inoculated, the number of larvae penetrating roots increased with increased density of inoculum and

Figure 4. Effect of density of H. avenae on number of larvae in roots inoculated directly or onto sand covering roots at three different durations of incubation at 20°C.

(a), (b), (c).

●——● Inoculation onto sand

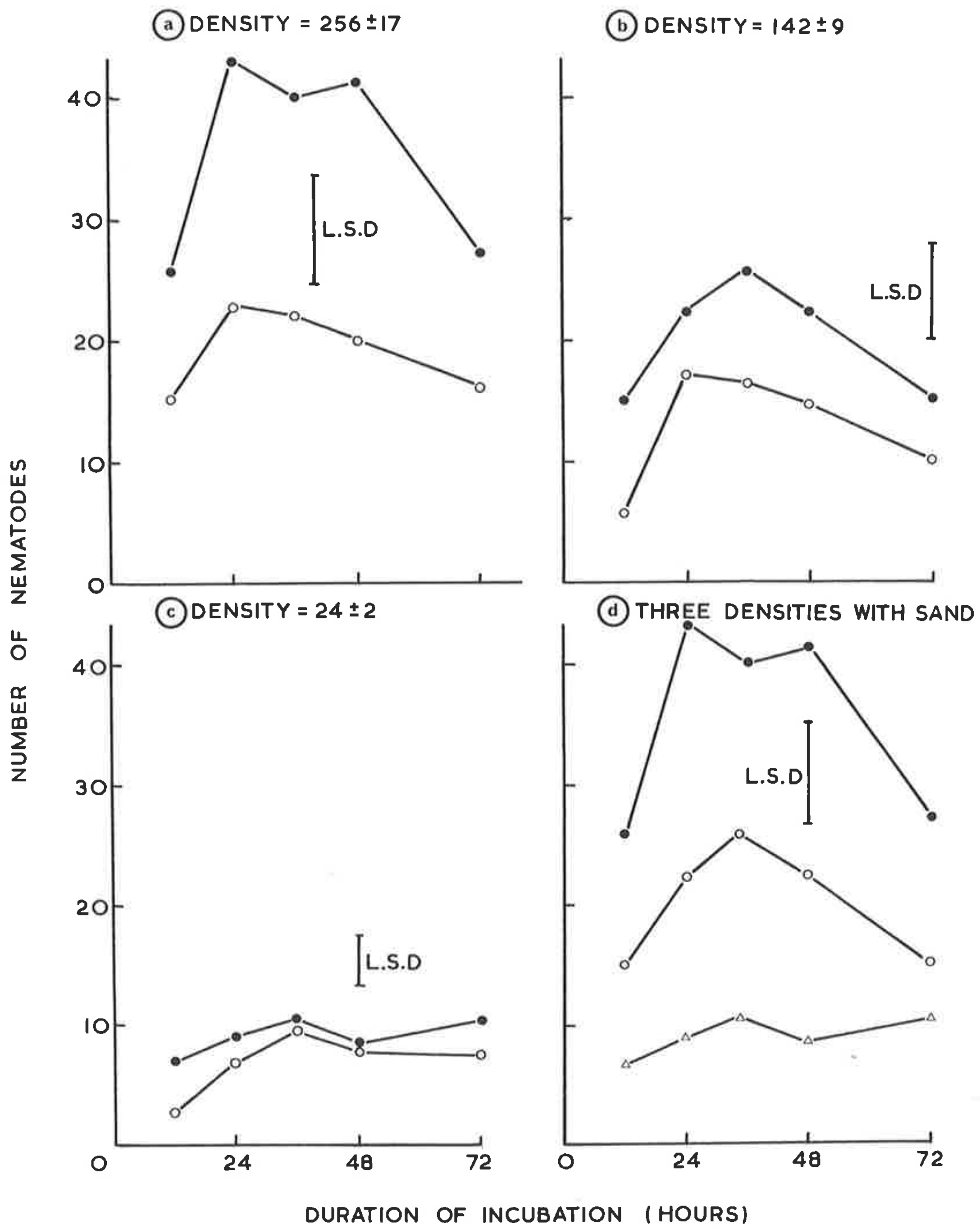
○——○ Inoculation directly onto roots.

(d) ●——● Inoculum density = 256 larvae

○——○ Inoculum density = 142 larvae

△——△ Inoculum density = 24 larvae.

L.S.D. (P = 0.05)



a DENSITY = 256 ± 17
b DENSITY = 142 ± 9
c DENSITY = 24 ± 2
d THREE DENSITIES WITH SAND
L.S.D
NUMBER OF NEMATODES
0
10
20
30
40
24
48
72
DURATION OF INCUBATION (HOURS)

varied with duration of incubation (Fig. 4 d). At the two highest densities of inoculation, three phases of nematode activity were apparent during 72 hours of incubation. An initial increase in the number of larvae in roots, a period of fairly constant numbers of larvae and finally a decrease in numbers of larvae in roots. The duration of incubation required for the maximum number to penetrate decreased from 36 to 24 hours as density of inoculum increased. At the lowest density of inoculation, only the first two phases were apparent as the number of larvae in roots increased until 36 hours of incubation when they remained fairly constant.

In examining further the decrease in the number of larvae in roots with duration of incubation, density of inoculation affected the time needed for maximum numbers of larvae to penetrate (Table 5). At the high

TABLE 5.

EFFECT OF TWO DENSITIES OF H. AVENAE AND DURATION OF INCUBATION AT 15°C ON NUMBERS OF LARVAE IN WHEAT (CV. HALBERD) BEFORE AND AFTER 24 HOURS IN A SEINHORST MISTIFIER.

Duration of incubation (days)	½	1	2	4	6
Inoculum of 140 larvae					
Before mistifier	15	13	15	14	11
After mistifier	6	8	12	11	10
Inoculum of 15 larvae					
Before mistifier	5	7	7	8	6
After mistifier	3	4	4	5	5

L.S.D. (P = 0.05) = 2.8

density, ½ a day was sufficient but at the low density, numbers tended to increase up to 4 days after inoculation. At the high density of inoculation, the number of larvae in roots remained the same up till 4 days of incubation and then declined, but no decline in numbers occurred at the low density. The number of larvae remaining in roots after 24 hours in a Seinhorst

mistifier were markedly fewer than the number penetrating from the high density of inoculum after ½ a day of incubation, but this difference gradually became less the longer the incubation period and finally disappeared after 6 days of incubation. At the low density, statistical differences occurred between the number remaining and penetrating after 2 and 4 days of incubation and the number of larvae remaining in roots showed a tendency to increase during 4 days of incubation.

From a similar experiment in which three densities were compared, the maximum number penetrated roots inoculated with the highest densities within 1 day of incubation (Fig. 5 a, b) and at the lowest density, the number increased during 4 days of incubation (Fig. 5 c). With all densities of inoculation, no statistical decrease occurred between the number of larvae in roots with further incubation after the maximum number was obtained. The number of larvae remaining in roots after 24 hours in a Seinhorst mistifier remained constant at the two highest densities and was not significantly different from the number penetrated at the higher density (Fig. 5 a) but fewer remained at the lower density (Fig. 5 b). At the lowest density, the only statistical difference between the number remaining and penetrating occurred after 4 days of incubation and the number of larvae remaining in roots gradually increased during the 6 days of incubation (Fig. 5 c). Also, the number of larvae remaining in roots increased with each increase in density of inoculation (Fig. 5 d).

A comparison of growth of control seedlings (i.e. without nematodes) of Halberd and Clipper showed individual roots of Halberd extended further over 24 hours than roots of Clipper (Table 6), but Clipper developed more roots than Halberd (Table 7). Also, the roots of Halberd were separated, i.e. the distance between roots 2 and 3, 20 mm. away from the seed was approximately 30 mm., while those of barley were closer together, i.e. a distance of approximately 15 mm. between the two outer roots at the same

Figure 5. Effect of density of *H. avenae* and duration of incubation at 15°C on number of larvae in wheat (cv. Halberd) before and after 24 hours in a Seinhorst mistifier.

(a), (b), (c).

●——● Before mistifier

○——○ After mistifier

(d) ●——● Inoculum density = 191 larvae

○——○ Inoculum density = 106 larvae

△——△ Inoculum density = 22 larvae

L.S.D. ($P = 0.05$) are given where statistical differences occurred. No statistical difference occurred with inoculum density = 191 larvae.

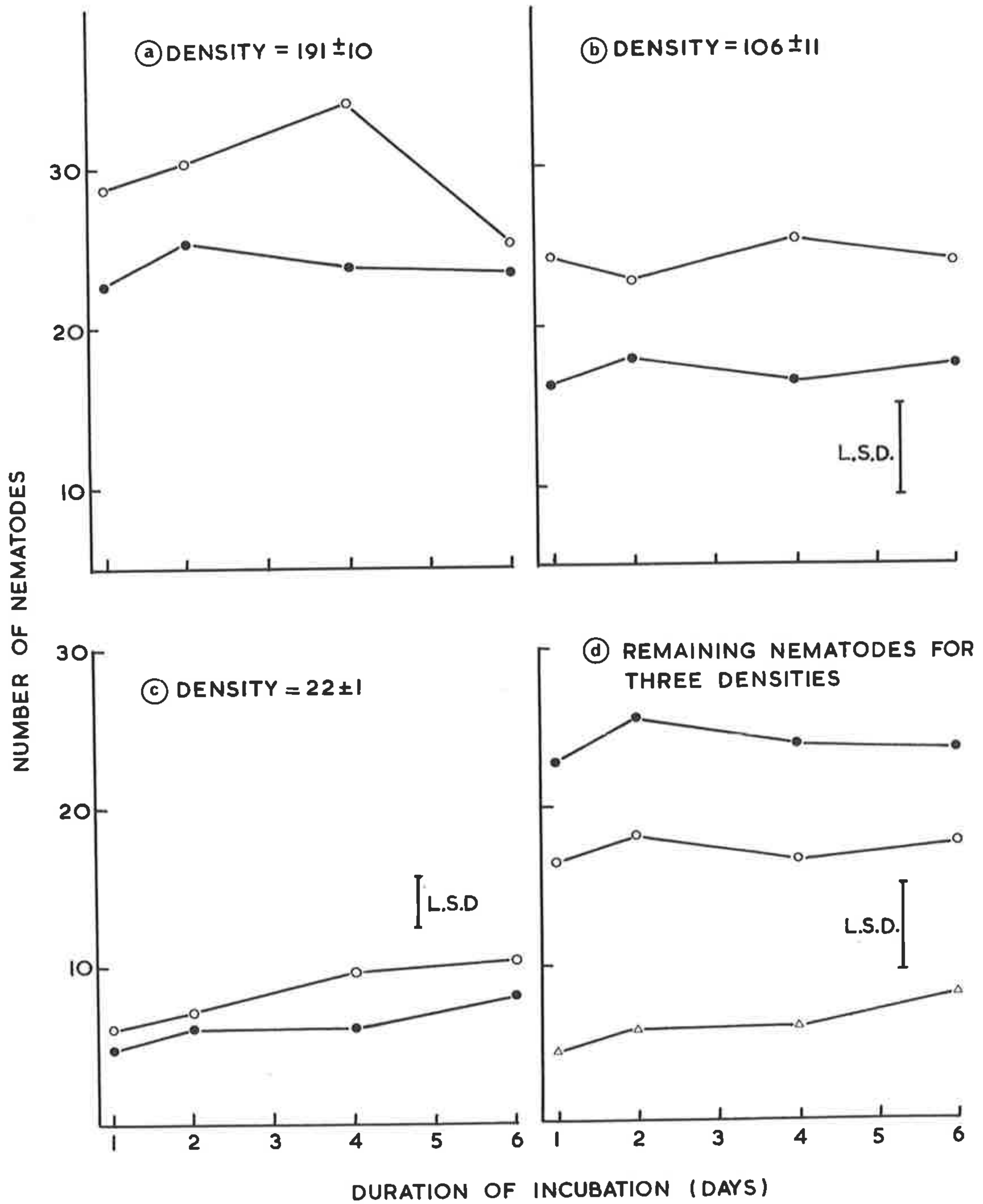

ⓐ DENSITY = 191 ± 10
ⓑ DENSITY = 106 ± 11
ⓒ DENSITY = 22 ± 1
ⓓ REMAINING NEMATODES FOR THREE DENSITIES
L.S.D.
L.S.D
L.S.D.
30
20
10
30
20
10
1
2
3
4
5
6
1
2
3
4
5
6
NUMBER OF NEMATODES
DURATION OF INCUBATION (DAYS)

TABLE 6.

EFFECT OF DENSITY OF H. AVENAE ON NUMBERS OF LARVAE PENETRATING AND EXTENSION OF INOCULATED ROOTS OF SUSCEPTIBLE WHEAT AND BARLEY.

Nos. of larvae in inoculum	0	28	65	155	332
Nos. penetrated - wheat		6	10	18	35
- barley		5	9	18	39

L.S.D. (P = 0.05) = 7

Root extension (mm.)					
wheat	39	7	3	2	1
barley	22	7	3	2	1

L.S.D. (P = 0.05) = 4

TABLE 7.

EFFECT OF DENSITY OF H. AVENAE INOCULATED ONTO ONE ROOT OF SUSCEPTIBLE WHEAT AND BARLEY ON VARIOUS PARAMETERS OF THE UNINOCULATED ROOTS ON A SEEDLING.

Nos. of larvae in inoculum	0	28	65	155	332
Nos. of roots - wheat	2.2	2.4	2.4	2.2	2.4
- barley	4.4	4.6	4.4	4.2	3.8

L.S.D. (P = 0.05) = 0.6

Total length (mm.) - wheat	102	80	104	64	61
- barley	206	189	168	164	137

L.S.D. (P = 0.05) = 22

Larvae penetrated (nos.)					
- wheat		4	2	8	
- barley		4	9	15	

L.S.D. (P = 0.05) = 5

Ave. nos. penetrated/root					
- wheat		1.5	0.8	3.5	
- barley		1.0	2.1	3.5	

L.S.D. (P = 0.05) = 1.3

position. Following inoculation with different densities of inoculum, extension of and the number of larvae penetrating roots were similar at each density for both species and as density increased, the number of larvae penetrating increased and extension of roots decreased (Table 6).

The total number of larvae in uninoculated roots showed a general increase with increasing density of inoculum and this was more obvious with Clipper than Halberd (Table 7). However, the average number of larvae penetrating each of these uninoculated roots was similar in both species at each density, but more penetrated these individual roots with 155 larvae as the inoculum. A comparison of the total length of uninoculated roots with density of inoculum suggested the total length was less affected in Clipper than Halberd, and this was also suggested when estimations of percentage growth relative to the control were compared with numbers of larvae penetrating roots for whole plants (Fig. 6 a) and individual roots (Fig. 6 b).

2.3.3 Discussion

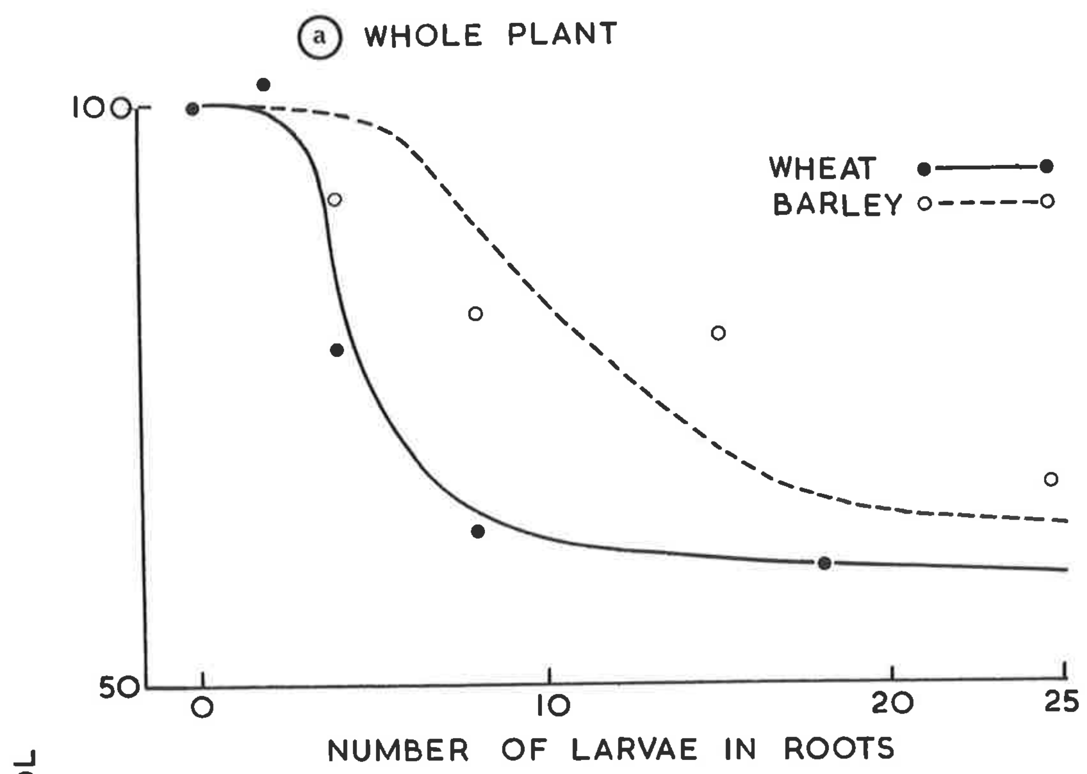
The effects of duration of incubation of high densities of inoculum on the number of larvae within roots before and after 24 hours in a Seinhorst mistifier were inconsistent between experiments and on one occasion this number of larvae was similar throughout the duration of incubation. Although all larvae used in the inoculum were stored for less than 6 weeks, they had been stored for different periods. Thus, although the invasion of roots by the larvae was normal, they probably had different amounts of stored energy and some became immobile more quickly than others. Therefore, only fresh larvae should be used in inocula, especially in experiments on the establishment and development of larvae.

The effects of different densities of *H. avenae* on susceptible wheat (cv. Halberd) and barley (cv. Clipper) were examined because barley is

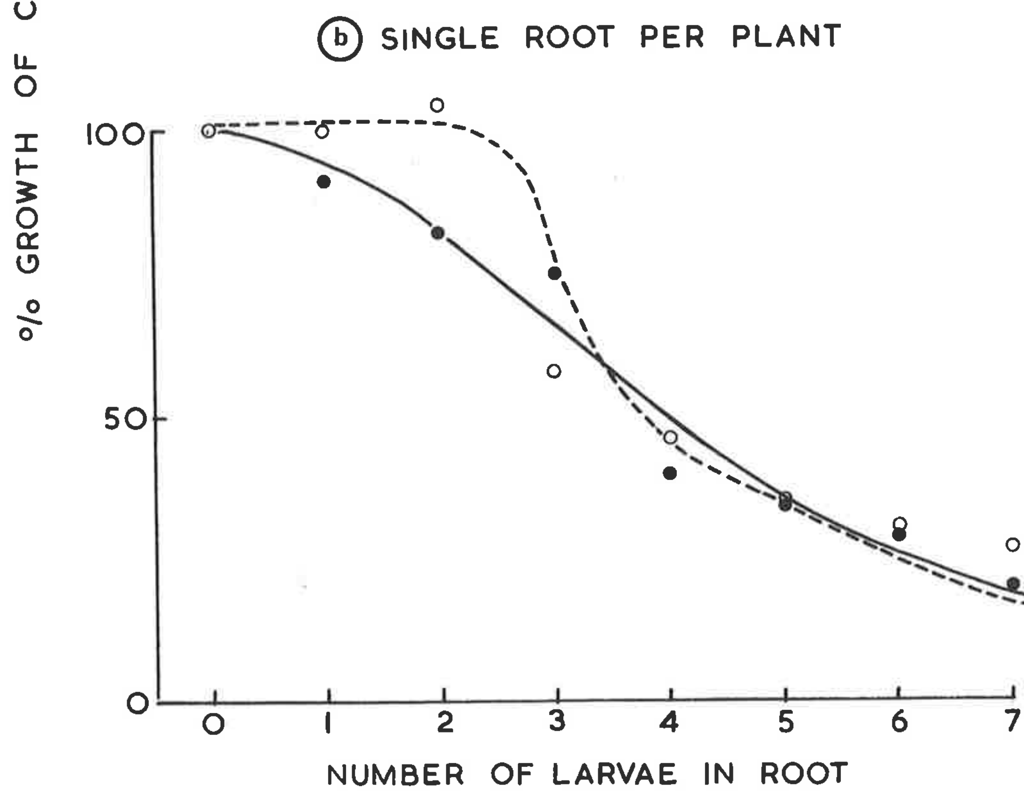
Figure 6. Comparison of the effect of larvae of *H. avenae* within roots on the growth of roots of susceptible wheat and barley.

(a) Total extension of uninoculated roots per seedling (mm.).

(b) Extension of single uninoculated roots (mm.).



% GROWTH OF CONTROL



usually regarded as being more tolerant than wheat. Without nematodes, individual roots of wheat grew faster than those of barley, but the number of seminal roots was higher and the total growth of roots per seedling was greater with barley. With nematodes, growth of the individual inoculated roots and the number of larvae penetrating these roots were similar on both species at each density of inoculation. Therefore, individual roots of barley were not markedly more tolerant than wheat at the densities of inoculum used in this experiment. These densities were selected from Fig. 3 b as being those associated with the rapid changes in root extension of Halberd and even the lowest density of 15 larvae was probably much higher than that normally experienced by individual root tips in the field.

An indication of the effects on root growth of lower densities of larvae and densities nearer to those normally in the field were obtained by examining the uninoculated roots on seedlings in which one root was inoculated. Extension of individual roots of wheat was apparently reduced with each additional nematode penetrating, but barley was not affected until three larvae had penetrated. Because barley produces more seminal roots faster than wheat following germination, more larvae would be needed with barley to give a similar number at each root tip and give a similar number penetrating individual roots. Therefore, given similar densities of larvae for both wheat and barley, fewer larvae would penetrate individual roots of barley and these roots would maintain better growth than wheat. With the lower densities of larvae normally in the field during the early growth of seedlings, growth of roots of barley may be only slightly affected while those of wheat are restricted, and this may explain the tolerance of barley to _H. avenae_. This aspect needs confirmation, but this was not possible within the time available for these studies.

2.4 General discussion

A suitable method of inoculating individual roots of seedlings in a petri dish was developed with sand (150-250 µm.) being placed over the tip of the root. Freshly hatched larvae were prepared in a 0.15% distilled water agar at appropriate densities and a single drop, from a pasteur pipette, of this inoculum was placed onto the sand. Using this method, an understanding of penetration and establishment of *H. avenae* in individual roots of susceptible hosts was obtained.

Changes in the number of *H. avenae* penetrating roots of susceptible wheat and barley with increasing densities of inoculation were similar to those which occurred with *Ditylenchus dipsaci* on oats (Blake, 1962) and *Meloidogyne incognita* (McClure & Viglierchio, 1966). The number of larvae in roots increased linearly as the density of inoculum increased until a constant number was reached and maintained at the highest densities and the percentage of the inoculum penetrating roots decreased at the highest densities with each increase in density. As the density of inoculum increased, competition between larvae on the root surface for invasion sites and/or interference increased (Davies & Fisher, 1976) and although larvae may share sites of entry into roots (Peacock, 1959), the severe inhibition of root growth caused by a small number of larvae limited the space available for nematodes within roots.

Invasion of roots by different densities of nematodes during incubation showed three phases of nematode activity and a fourth occurred if the period prior to entry of the first larva into a root (lag phase) was included. This first, or lag phase was the time needed for larvae to move to and begin widespread exploration of roots prior to penetration (Doncaster & Seymour, 1973) and would be dependent upon the density of inoculum and the distance travelling by larvae to reach the root.

The second, or exponential phase was the period of incubation

when the number of larvae penetrating roots increased linearly with time and the rate of increase in the number penetrating increased with increased density of inoculum. During this phase, larvae entered roots, moved within roots seeking a suitable site to establish and some larvae established and began development, while others either continued to move within roots or became quiescent. Entry of larvae was likely to be random and the first larvae entering the roots were likely to establish themselves with little competition with other larvae within roots, particularly at the low and moderate densities of larvae in inoculum. Because competition and/or interference occurs between larvae, even when a small number of larvae was on the root surface (Davies & Fisher, 1976), this competition and/or interference has occurred throughout this phase and the linear increase in the number of larvae within roots has continued until larvae within the root affected penetration.

In the third, or plateau phase, the number of larvae within roots remained constant and although this number of larvae increased with density of inoculum, the increase was not proportional to the increase in density of inoculum. Larvae within the root had established or became quiescent or remained motile within the root. Doncaster & Seymour (1973) observed larvae of *H. cruciferae* in these stages within roots and also noted that quiescent larvae could eventually regain motility to leave roots. At low densities of inoculum, almost all larvae established and began development, but as density increased there were three separate effects of competition between larvae likely to influence the number of larvae in roots during this plateau phase. The first effect would be due to the interference between larvae on the root surface resulting in some larvae leaving the root surface; more larvae would leave as density increased because of increased interference. Therefore, the number of larvae in roots

would be associated with a lower number of larvae on the root surface than was in the inoculum and this may be a proportional relationship. Secondly, as numbers of larvae within a root increased, the increased interference between larvae within the root would cause some of the larvae to move out of the root and these larvae would interfere with the other larvae entering the root. Therefore, a dynamic equilibrium would exist between larvae entering and leaving the root. Finally, because root growth was restricted by larvae, the space available to larvae within roots would be occupied and there would be no space for any more larvae to penetrate. One of these three effects would be the dominant effect at different densities of inoculation, with the first effect occurring at lower densities than the second which would occur at lower densities than the third effect.

With high densities of inoculum, there was usually a phase when the number of larvae within roots decreased to a number similar to the number of larvae established. A loss of ability of larvae to invade the root as body reserves of larvae were depleted seemed unlikely because some larvae within the root retained the ability to move throughout the duration of incubation and these larvae have not been observed to feed during this time (Davies & Fisher, 1976). However, larvae within the root may have extended the duration of their activity by conserving body reserves during periods of quiescence. It seems more likely that larvae eventually moved away from the damaged and unfavourable environment of the root, while larvae unable to establish within the root have moved out, either randomly or in response to changes in the root, in search of other sites to establish themselves.

Establishment of larvae in a root would show three phases of activity, a lag phase, exponential phase and a plateau phase. Apart from the effect of an increase in duration of the lag phase while larvae move within the root to sites of establishment, these phases are likely to be

related to the equivalent phases of invasion by nematodes and density of inoculation seemed to have a similar effect on invasion and establishment of larvae. This and other aspects of establishment and development of larvae are discussed further in a later section.

3. INTERACTION BETWEEN HETERODERA AVENAE AND WHOLE PLANTS

A consistent, repeatable method for measuring the genetic reaction of up to 2,000 plants of cereals to H. avenae in a single test was requested by plant breeders, but assessment based on the number of females has been difficult as the number of females on genetically homozygous genotypes vary (Cotton & Hayes, 1969). Therefore, the genetic reaction of cereals to nematodes was masked by variations related to changes in the environment.

The testing of host reactions to cyst-forming species of nematodes is usually performed either outdoors in the natural environment or in a glasshouse and usually cysts are used as inoculum. Density of nematodes in inocula has been a major source of variation (O'Brien & Fisher, 1974) and using hatched larvae as inoculum would seem preferable to using cysts as hatching from cysts can be unpredictable. When hatched larvae of H. schachtii were used (Shepherd, 1958), variations in host reactions were similar to using cysts as inoculum, but larvae were added in large numbers at only one stage of plant growth, therefore careful management during inoculation of larvae could reduce these variations. Other environmental factors affecting plant growth could be more easily controlled within a growth room and, although this would limit space available for testing plants, small tubes could be used as growth containers to increase numbers of plants tested within this space (Anderson, 1963; McKenna et al., 1963; Brown, J., 1974).

Cultivars of wheat and barley were grown in small tubes under controlled environmental conditions and were inoculated with second-stage larvae of H. avenae. The development of a method to study interactions between nematodes and plants is described and these interactions are discussed in this section.

3.1 Effect of inoculation, cultivar and root growth on development of adult nematodes

Laboratory studies showed hatching of larvae of *H. avenae* was sigmoidal and that the rate of hatching was dependent upon the environment (Banyer & Fisher, 1971 a) and this seemed similar to hatching in the field (Meagher, 1970) so that plants were affected by increasing densities of larvae during growth. Greater numbers of larvae penetrated roots after several inoculations with low densities of larvae than one inoculation with a high density of larvae (Fisher, pers. comm.). Therefore, inoculation of plants at several times with an increase in the number of larvae from one inoculation to the next would seem preferable to the single inoculation used by Shepherd (1958). The effects of different inoculations and plant management on the number of female nematodes developed on different cereals are described.

3.1.1 Materials and Methods

3.1.1.1 General

Cereal cultivars were grown in open-ended tubes, 13 cm. long and 2.5 cm. internal diameter, cut from rigid P.V.C.* conduit which contained washed river sand (Waikerie sand) with a wide range of particle sizes (Table 8).

TABLE 8.

COMPOSITION OF WAIKERIE SAND CALCULATED BY SIEVING SAND THROUGH SIEVES OF DIFFERENT MESH SIZE

Mesh size (µm.)	>1,200	>1,000	>700	>500	>250	>150	>100	<100
Proportion collected	.23	.13	.12	.05	.16	.24	.06	.01

Tubes were prepared for planting by placing them upright on a

* Poly vinyl chloride

firm and even base, adding a small quantity of moist sand, ramming this sand vigorously to compact it to give a firm base which prevented the loss of the extra sand to be added and to allow free drainage of solutions. Further sand was then added until the tubes were full and this sand was then lightly compacted. This was repeated until the level of sand was 1 cm. below the top of the tubes, then 5 ml. of Hoagland solution (Hoagland & Snyder, 1933), containing iron chelate (Jacobson, 1951) in the place of Iron Tartrate, was added with an automatic pipetting machine. The level of the sand usually subsided after adding nutrient solution and therefore more sand was added to maintain the original level. These prepared tubes were then stored on trays, 85 cm. x 40 cm., until the plants were ready for planting.

Seeds were pregerminated (Section 2.1.1.1) and viable seeds were selected as early as possible. One seed per tube was sown approximately 50 mm. deep into the sand and a further 5 ml. of nutrient solution was added, either alone or with larvae, to the sand surface. Trays of tubes sown with seeds were transferred to a growth room until emergence of the coleoptiles occurred and the final selection of plants could be made. Selected plants were arranged in accordance with experimental design and returned to the growth room. The growth room was set to give a constant environment of 20°C and 10 hours of continuous fluorescent light of uniform intensity in each 24 hour period.

When the sand in most tubes seemed to be dry during the early growth of plants (approximately once per week) 10 ml. of either water or nutrient solution were added to the surface of the sand. Water and nutrient solution were added on alternate occasions with an automatic pipetting machine unless the addition coincided with an inoculation, then the inoculum was added using a hand pipetting machine. Maintenance of plants continued in this manner until 5 days after the final inoculation when only water was used;

it was added to the trays and absorbed through the base of tubes. Water was added in this manner when plants began to show signs of wilting.

Inocula of larvae were prepared with either water or nutrient solution in a manner similar to that described in Section 2.1.1.1, except that the required density of larvae were in 5 ml. of solution.

Plants were examined regularly for pests and diseases, but only green aphid and powdery mildew occurred on infrequent occasions. Plants were sprayed with "rogor"* to control green aphid and dusted with sulphur to control powdery mildew.

The number of female nematodes were counted a minimum of 35 days after the final inoculation. This allowed sufficient time for development of the nematode to the adult stage under the conditions used for growth of the plants (O'Brien, 1972). Growth tubes were soaked in water, plants and sand were removed from the tubes, placed on a 1.68 mm. sieve, washed with a fast jet of water and females, sand and associated organic material collected in a 0.152 mm. sieve. The collected material was then mixed with water, agitated vigorously and decanted into a 0.152 mm. sieve. Sand was retained as sediment and females were collected in the sieve. The numbers of females were counted in a large counting dish under 10 x magnification.

Root weight of plants was measured as wet root weight. Growth tubes were soaked in water and both the plant roots and associated sand were removed from the tubes. Sand was removed from roots by agitation in water and by washing roots in a 1.68 mm. sieve with a moderate jet of water. Roots were cut from the plant, rolled into a ball, blotted dry with water absorbent tissues to remove excess water and then weighed.

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* 40% W/V dimethoate (0,0 - dimethyl S - (N - dimethyl S - (-methyl carbamoylmethyl) phosphorodithioate.

3.1.1.2 Experimental

The effects of density, time and number of inoculations on the number of females on susceptible (cv. Halberd) and resistant (cv. AUS 10894) wheat were compared. Waikerie sand with a particle size of less than 500 µm. diameter was used as the medium for plant growth because these particles were expected to aid nematode movement (Wallace, 1963). A single inoculation with 200, 600 or 1,500 larvae was applied to Halberd on days 0, 7 or 14 after sowing and to AUS 10894 14 days after sowing. Inoculations of 200 larvae at sowing followed by 400 or 1,300 larvae at 7 days or 400 or 1,300 larvae at 14 days after sowing were given to both cultivars. Inoculation of 200 larvae on days 0, 7 and 14 after sowing was also given to both cultivars. A randomised block design containing five replicates was used and the number of females was compared after an analysis of variance.

The numbersof females developed on one susceptible and six resistant cultivars of barley were assessed following inoculation of seedlings with 500 larvae at days 0, 7 and 14 after sowing. Seedlings were grown and inoculated in tubes with Waikerie sand in a randomised block design with 7 replicates.

The barley cultivars used were as follows; Clipper as the susceptible standard because it is the most widely grown cultivar in South Australia, Morocco (WI* 2398, CI 3902) was reported resistant in Australia (Brown & Meagher, 1970) and seed was obtained from the Victorian Wheat Research Institute at Horsham, and Nile (WI 1470, CI 3582), Athinais (WI 938), Orge Martin 839 (WI 858), CI 8147 (WI 1944) and WI 2231 which were rated resistant in an unpublished field test in South Australia (Sparrow, pers. comm.). Results were compared by analysis of variance.

The number of females was also assessed on a susceptible

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* WI - Waite Agricultural Research Institute accession number.

(cv. Halberd) and two other (cvs. AUS* 10894 & AUS 90248) wheats, which were resistant in South Australia (O'Brien & Fisher, 1974), by growing and inoculating seedlings in tubes of Waikerie sand with 300 larvae at sowing, 500 larvae 5 days and 800 larvae 15 days after sowing. Six replicates of the three cultivars were completely randomised and an analysis of variance was done on results.

Following a successful test of the reaction of barley cultivars to H. avenae earlier in this section, an attempt to test the reaction of parents, F1, F2 and backcrosses of progeny from crosses of resistant and susceptible barley was made. A maximum of 355 growth tubes were placed on a tray to test a maximum number of plants in the minimum space and at this density the growth tubes were self-supporting. After germination and sowing test plants in tubes (Section 3.1.1.1), plants were scattered at random throughout the tray before being inoculated with 500 larvae at sowing, 7 and 14 days after sowing. During the 75 days of growth of all plants, general management (Section 3.1.1.1) of these densely packed plants was very difficult, therefore, the 24 plants of susceptible barley (cv. Clipper) scattered throughout the tray were examined after this time to determine the effects of management on the number of females in relation to plant growth. Wet weight of root and the number of females on roots were measured and a regression analysis was performed on raw data.

A relationship between the number of females and root weight was only expected when management of plants affected plant growth and such a relationship was obtained when 355 tubes were grown on a tray (Fig. 7 a). Therefore, 156 plants were grown in tubes arranged in 13 rows of 12 across a tray, and except for one row, rows were paired and each pair of rows was separated by a single spacing of the rigid net of wire used to support the

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* AUS - Accession number of the Australian Wheat Collection at Tamworth, N.S.W.

growth tubes, and this was similar to that shown in Plate 1. Progeny of crosses of barley selected, germination, inoculation and general management were similar to when 355 plants were grown on a tray. But plants were only grown for 45 days before wet weight of roots and the number of females on the 24 plants of susceptible barley (cv. Clipper) were measured and a regression analysis was performed on the raw data.

3.1.2 Results

Waikerie sand with particle sizes less than 500 μm. in diameter was an unsuitable medium for plant growth as seminal roots of both cultivars of wheat were thick, stunted and developed few lateral roots because of compaction of the sand. Poor development of roots was also reflected by the small number of females on the roots of Halberd inoculated at one time (Table 9).

Halberd inoculated with 200, 600 or 1,500 larvae 7 days after sowing seemed to produce fewer females than similar inoculations at or 14 days after sowing (Table 9). This was probably the result of using larvae stored for a longer period for inoculations 7 days after sowing than at 0 or 14 days after sowing. Increased number of females occurred on Halberd when inoculations of 200 larvae at sowing and 400 larvae at 7 or 14 days after sowing were used, compared with one inoculation of 600 larvae. However, the number of females was similar on Halberd when inoculations of 200 larvae at sowing and 1,300 larvae at 7 or 14 days after sowing were compared with the one inoculation of 1,500 larvae. Most females developed when Halberd was inoculated with 200 larvae at sowing, 7 and 14 days after sowing. Few females developed on resistant wheat (cv. AUS 10894) and the method of inoculating did not affect numbers of females.

When a complete mixture of the particle sizes in Waikerie sand was used to grow plants, seminal roots of wheat and barley grew normally.

Plate 1. Arrangement of growth tubes within trays for growth of plants within an environmentally controlled growth room.

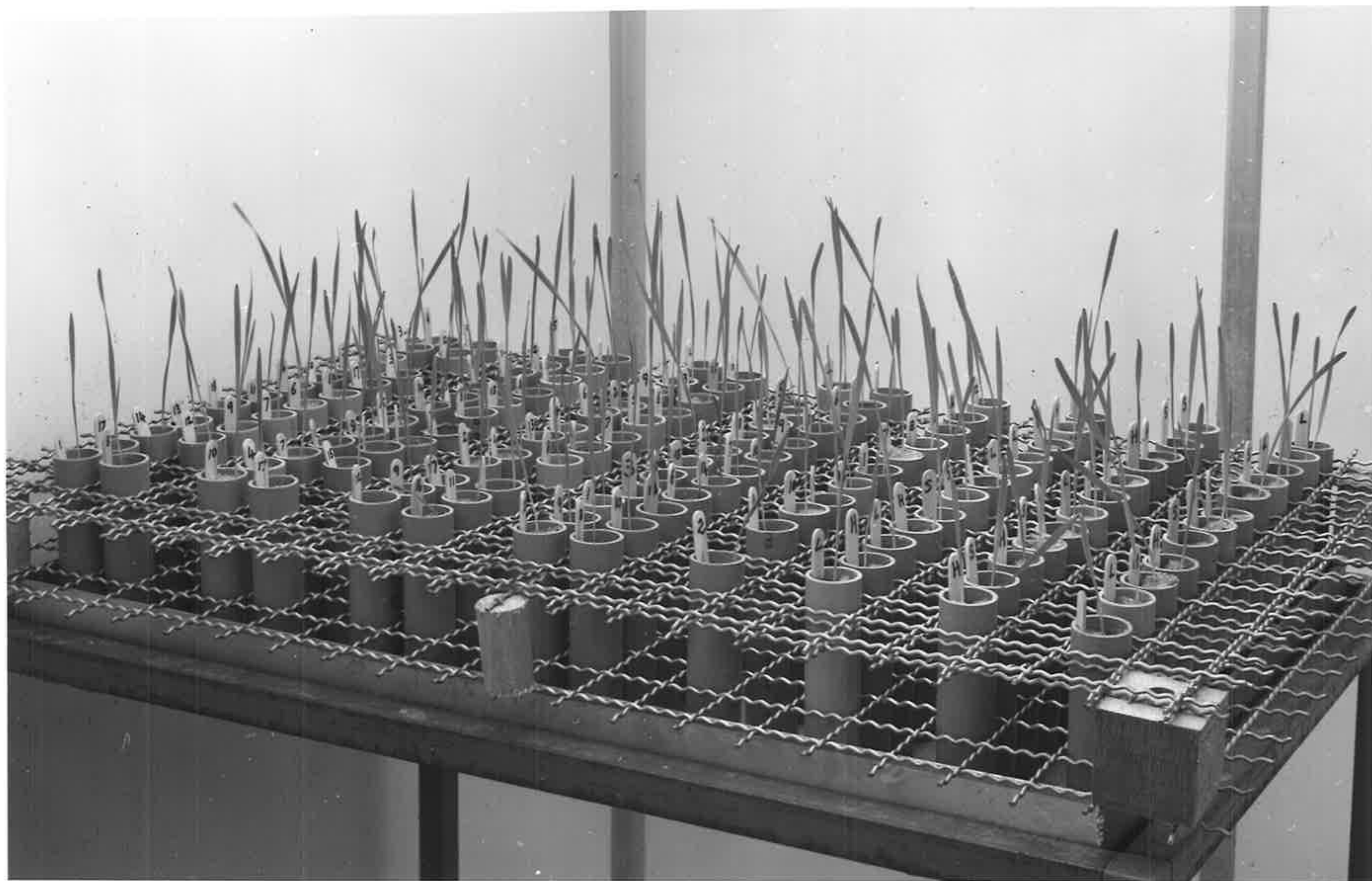


TABLE 9.

EFFECT OF DENSITY, TIME AND NUMBER OF INOCULATIONS OF H. AVENAE ON THE NUMBER OF FEMALES ON SUSCEPTIBLE (CV. HALBERD) AND RESISTANT (CV. AUS 10894) WHEAT.

Inoculation Nos. of larvae in inoculum on: Day 0	Day 7	Day 14	Halberd	AUS 10894
200	0	0	5	-
0	200	0	2	-
0	0	200	4	1
600	0	0	6	-
0	600	0	4	-
0	0	600	4	0
1,500	0	0	7	-
0	1,500	0	3	-
0	0	1,500	9	1
200	400	0	14	1
200	0	400	10	1
200	1,300	0	8	1
200	0	1,300	10	2
200	200	200	24	2

L.S.D. (P = 0.05) = 5

Roots grew the depth of tubes and lateral roots permeated the sand within tubes.

The number of females on cultivars of barley and wheat was used to assess the reaction to infection by H. avenae. With the barley cultivars, Clipper was very susceptible with an average of 60 females per plant and the other cultivars were more resistant (Table 10). Although WI 2231 was more resistant than Clipper and averaged 21 females per plant, it was more susceptible than the other 5 cultivars which averaged 7 or less females per plant. Morocco had the best resistance of the barley cultivars tested as there were no females on the roots of this cultivar. With the wheat cultivars,

TABLE 10.

EFFECT OF CULTIVARS OF BARLEY ON THE NUMBER OF FEMALES OF H. AVENAE ON ROOTS

	Clipper	Morocco	Nile	Athinais	Orge-Martin	WI2231	CI8147
Females	60	0	5	4	3	21	7
± S.E.	6	0	1	1	1	2	1

L.S.D. (P = 0.05) = 7

Halberd was susceptible with an average of 35 females per plant and the two other cultivars, AUS 10894 and AUS 90248 were resistant with an average of 5 or less females per plant (Table 11).

TABLE 11.

EFFECT OF CULTIVARS OF WHEAT ON THE NUMBER OF FEMALES OF H. AVENAE ON ROOTS

	Halberd	AUS 10894	AUS 90248
Nos. of Females (± S.E.)	35 ± 3	2 ± 1	5 ± 1

L.S.D. (P = 0.05) = 6

Management of the 355 barley plants crowded together on a tray was very difficult. Watering plants with an automatic pipetting machine was difficult and time consuming due to the top growth of plants. Adding water to trays was easier, but water was distributed unevenly to the plants and those plants growing in the centre of the tray were often under moisture stress. Powdery mildew was difficult to contain and either other organisms or physiological disorders caused a blackening at the base of stems and poor root growth in many plants. When 156 plants were grown on a tray, uniform management of plants was easier and plant growth appeared to be normal.

Regression analyses on the relationship between wet root weight and the number of females on roots of Clipper showed a second order

correlation when 355 plants were grown on a tray (Fig. 7 a). This relationship was represented by the algebraic equation, $Y = 9.4 + 8.4\ x - 0.9\ x^2$. The curve derived from the equation showed an initial increase in the number of females per plant as root weight increased. An optimum number of females per plant was reached and then the number began to decrease with further increases in the root weight of plants. No correlation occurred between the number of females per plant and root weight of Clipper when 156 plants were grown on a tray (Fig. 7 b).

3.1.3 Discussion

Variations in the number of female nematodes on genetically homozygous genotypes (Cotton & Hayes, 1969) made assessments of genetic reaction of different cultivars difficult. Cultivars of wheat and barley are normally self-fertilising and the individual cultivars are described as homozygous, but unless rigid selection of plants is maintained, minor genetical differences can occur which cause variations in the genetic influences within a cultivar. Therefore, growth responses to the environment and reactions to the nematodes by plants would vary within the same cultivar, but these are likely to be under a different genetic control and would vary independently of one another unless extreme environmental or nematode effects occur. This means no correlation should occur between the root weight and the number of females when both suitable environment and inoculations of nematode are used to assess the plant reaction.

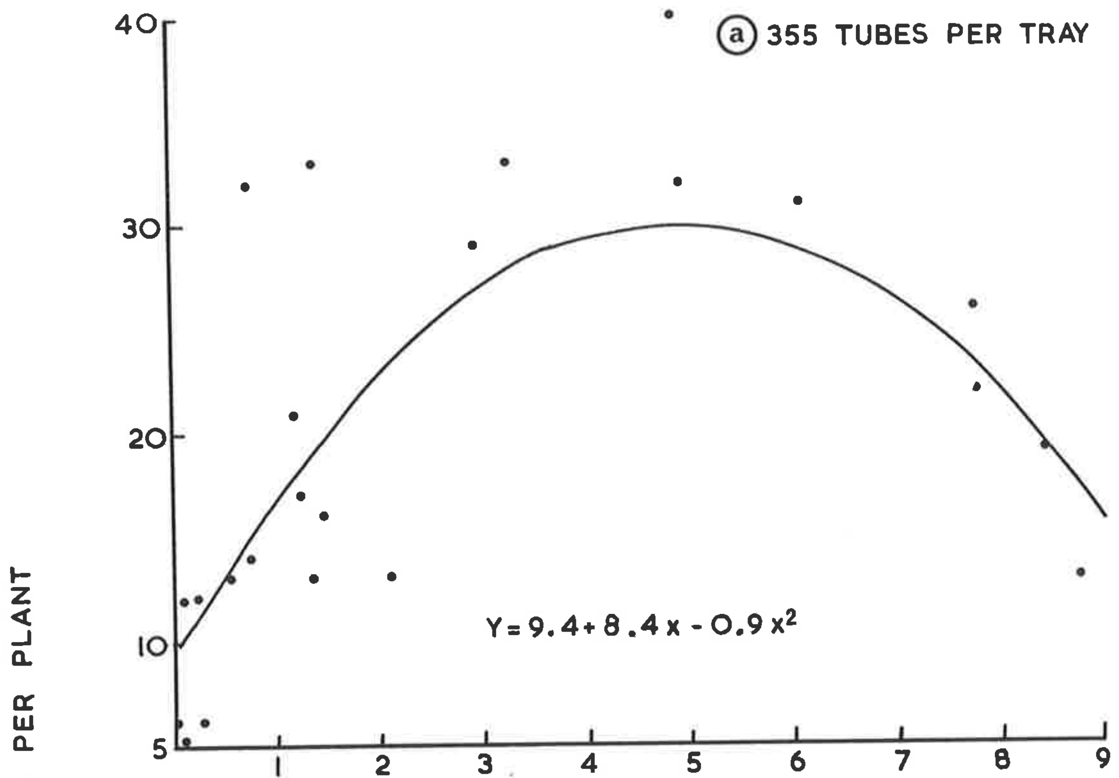
Composition of the sand used as a growth medium affected root growth and also affected the number of females on plants. Therefore, Waikerie sand was used in all later assessments to ensure good root growth and the maximum expression of the genetic reaction of plants to nematode infection.

A correlation occurred between root weight and number of females per plant when maximum numbers but not when fewer plants were grown on a

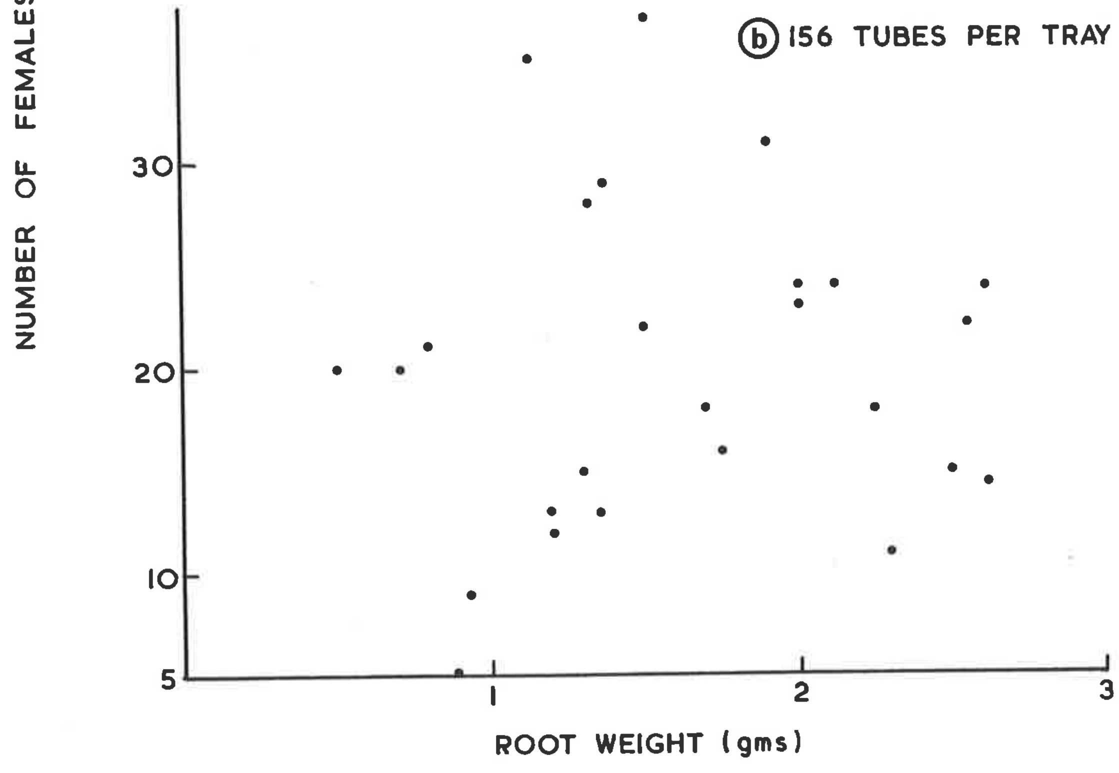
Figure 7. Scatter diagrams showing the relationship between the fresh weight of barley (cv. Clipper) roots and the number of females of *H. avenae* on the roots.

(a) High density of plants per tray (355 growth tubes).

(b) Low density of plants per tray (156 growth tubes).



40
30
20
10
5
(a) 355 TUBES PER TRAY
$Y = 9.4 + 8.4x - 0.9x^2$
1 2 3 4 5 6 7 8 9
NUMBER OF FEMALES PER PLANT



(b) 156 TUBES PER TRAY
30
20
10
5
1 2 3
ROOT WEIGHT (gms)

tray. This showed that difficulties in the management of large numbers of plants on a tray caused extreme differences in the environment between plants and prevented some plants from reacting to nematode infection independently of the environment. Therefore, spacing and careful management of plants are important during the assessment of plants to nematode infection.

Because root growth was affected by compaction of sand when Halberd was inoculated with different densities of larvae at different times after sowing, the effects of inoculations on the number of females were not clear. However, the number of females did not seem to increase with increased densities of inoculum or with delay in inoculations and this suggested competition between larvae as a result of density and/or poor root growth limited the number of females. Most females were produced on seedlings inoculated on three successive occasions with the lowest density of nematode. This suggested the low density allowed penetration, establishment and development of larvae into females, and allowed further growth of roots so that the increased root growth between inoculations permitted at least a similar number of females to develop with each additional inoculation. With normal root growth of wheat, a similar effect using higher densities as inoculum should further increase the number of females. Therefore, the reactions of susceptible and resistant wheats were compared by increasing the number of larvae in the initial and the two subsequent inoculations. As barley (cv. Clipper) developed more roots than wheat (cv. Halberd) during early growth (Section 2.3.2), an even higher initial density of inoculum was used to compare the reaction of barley cultivars to nematode infection.

In the two tests, one on the reactions of barley and the other on the reactions of wheat to H. avenae, a large difference in reaction occurred between the susceptible and resistant cultivars. Resistance in

AUS 10894 and AUS 90248 was confirmed for wheat and in Morocco, Nile, Athinais, Orge Martin 839 and CI 8147 for barley. The barley cultivar, Wl 2231, was rated as susceptible, but was more resistant than Clipper and would assist control of *H. avenae* if it were a commercial cultivar.

The number of females on Halberd increased following increases in the density of inoculations when Waikerie sand was used, but they did not increase in proportion to the increases in densities of inoculations. Also, the number of females was greater on Clipper than Halberd when both were grown under similar conditions. This, with other observations discussed in this section, showed the number of females on cereal roots was the end result of complex interactions between environment, nematodes and plant growth. By controlling the environment and gaining a better understanding of the interactions between nematodes and plants, both the density and time of inoculations could be determined to give the maximum number of females on cereals grown within a particular environment.

3.2 Effect of density of inoculum and time of inoculation on growth of Halberd and development of nematodes

Competition between larvae during penetration of roots and/or development within roots seemed the main causes of variation in the number of females on roots of cultivars inoculated with larvae of *H. avenae*. General management during growth of plants, spacing of plants on trays and environment affected competition between nematodes. But these factors were controlled in the later assessments and only caused minor variations in the number of female nematodes on roots. Density of inoculum and time of inoculation became the major causes of competition between larvae and variations in the number of female nematodes on roots. Further information was needed to select both the densities of inoculum and times of inoculations to avoid competition and to limit variation in the reaction of cultivars.

Effects of density of inoculum and time of inoculation on growth of wheat (cv. Halberd) and development of nematodes are described and discussed in this section.

3.2.1 Materials and Methods

Seedlings of Halberd were inoculated once with a low, medium or high density of larvae (Table 12) either at sowing, 7 or 14 days after

TABLE 12.

AVERAGE NUMBER OF LARVAE OF *H. AVENAE* INOCULATED ONTO WHEAT (CV. HALBERD) AT DIFFERENT TIMES FROM SOWING.

Days from sowing	Number of Larvae	Density
0	167	Low
0	325	Medium
0	436	High
7	145	Low
7	286	Medium
7	422	High
14	165	Low
14	395	Medium
14	594	High

L.S.D. (P = 0.05) = 41

sowing and were compared with uninoculated seedlings. Seedlings were to be sampled at three times up till 36 days after inoculation for information on the number of adult nematodes and root growth. Early growth of seedlings inoculated at sowing suggested top growth should also be examined. Sufficient plants were available from inoculations at sowing for an extra one sample at low and high densities and two samples at the medium density of inoculations. Therefore, additional plants were inoculated with all densities and the effects of *H. avenae* on plant growth were examined till 50 days after sowing for each time of inoculation and most densities of inoculation. The low and high densities at sowing were only examined till 36 days after sowing.

Destruction of seedlings was necessary at each sampling for the

measurement of root growth. Six replicates of seedlings were randomly organised onto two trays in a manner similar to that described for barley in Section 3.1.1.2 and shown in plate 1. Sampling was at random from the trays and seedlings were reorganised to maintain rows of 12 within the trays.

Length of the first four seminal roots was measured on each seedling sampled 7 and 14 days after sowing and an average length of these roots was calculated. Total length of nodal roots was measured on each seedling sampled until 36 days after sowing. The number of root tips per seedling were assessed until 21 days after sowing. Assessment of root tips was by a modification of the method described by Southey (1970) to assess the number of nematodes within roots. Sand was washed from the roots, the roots were stained in lactophenol cotton blue, macerated in an homogeniser and the number of tips in a 5 ml. aliquot of a 100 ml. suspension were counted. Wet weight of roots of each seedling sampled was obtained in the same manner described for barley in Section 3.1.1.2.

The number of expanded leaves, length of lamina of expanded leaves and seedling height were measured on the same seedlings selected for root growth. Lamina length (Williams & Rijven, 1965) was used to assess leaf growth. Seedling height was measured from the sand surface to the tip of the youngest emerged leaf. Breadth was measured at the widest part of lamina for the first four expanded leaves on seedlings sampled 28 days from sowing.

Adult nematodes were counted either 35 or 36 days after inoculation. Females were collected and counted as described in Section 3.1.1.1. Males were collected from sand as well as being washed off the surface of roots during the collection of females. A 0.53 mm. sieve was placed beneath the 1.68 mm. sieve used to collect the females washed from roots. The water used to soak the growth tubes was poured through a 1.68 mm. sieve and material in this sieve was rinsed thoroughly with water. Males were

collected in the 0.53 mm. sieve, a 10 ml. suspension was prepared from material collected in this sieve and the number of males was assessed from counts of 1 ml. aliquots of the suspension (Southey, 1970).

Seedlings were inoculated and maintained in tubes as described in Section 3.1.1.1 until 5 days after the final inoculations. Then seedlings were watered with 10 ml. from an automatic pipetting machine when indicated by either dry sand in tubes or signs of wilting of plants. This was done to maintain uniform conditions within the tubes and to prevent the loss of male nematodes. Other materials and methods used were as described in Section 3.1.1.1.

Analyses of variance were done on raw data for most results, but log (x+1) transformation were analysed for the length of nodal roots and the number of male nematodes.

3.2.2 Results

The number of larvae used as the different densities of inoculum was similar at sowing and 7 days after sowing (Table 12). But there were more larvae in the medium and high densities of inocula 14 days after sowing than in the equivalent densities of the earlier inocula.

Roots of uninoculated seedlings were observed to meander through the sand as they grew down within tubes and therefore were as long as the tube after 7 days (Table 13) although they had not emerged from the tube.

TABLE 13.

EFFECT OF DIFFERENT DENSITIES OF *H. AVENAE* AT TIME OF SOWING ON THE AVERAGE LENGTH (MM.) OF SEMINAL ROOTS OF WHEAT (CV. HALBERD) COMPARED WITH THE DEPTH OF GROWTH TUBES.

Days from Sowing	Nematode Density				Growth Tube
	0	Low	Medium	High	
7	128	-	-	-	
14	133	90	85	58	128

L.S.D. (P = 0.05) = 19

Tips of uninoculated roots were observed near the base of tubes after 7 days growth and at the base after 14 days growth. Roots of seedlings inoculated at sowing were shorter than those of uninoculated seedlings after 14 days growth and the shortest roots were on seedlings inoculated with the highest density of larvae.

A comparison of growth of uninoculated and inoculated roots is shown (Plate 2) for each sampling until 21 days after sowing. Roots inoculated at sowing had growth severely affected at each sampling, but the effect of density of inoculum was not noticeable after 21 days growth or roots. Inoculation of roots with high densities of larvae 7 days after sowing was the only other method of inoculation to have some visual effect on growth of roots (Plate 2c).

Inoculated and uninoculated seedlings had a similar number of root tips 7 days after inoculation, e.g., measurements 14 and 21 days after sowing for inoculations 7 and 14 days after sowing respectively (Table 14). But 14 days after inoculation, i.e., measurements 14 and 21 days after sowing for inoculations at sowing and 7 days after sowing respectively, the inoculated seedlings had developed fewer root tips than uninoculated seedlings. After 21 days growth, seedlings inoculated at sowing had fewer root tips than uninoculated seedlings and seedlings inoculated with the higher densities had fewer root tips than those inoculated with the low density.

Initiation of nodal roots was delayed with inoculation of seedlings at sowing and this delay was noticeable until 36 days after sowing (Table 15). When inoculation was later, i.e., 7 or more days after sowing, no differences between the length of nodal roots on inoculated and uninoculated plants could be shown.

The root weight of uninoculated seedlings 36 and 42 days after sowing were similar and showed an effect of moisture stress on root weight as a result of a malfunction in the pipetting machine between these two

Plate 2. Root growth of seedlings of wheat (cv. Halberd) inoculated and uninoculated with *H. avenae*.

(a) Growth 7 days following sowing.

1. Uninoculated
2. Low density at sowing.
3. Medium density at sowing.
4. High density at sowing.

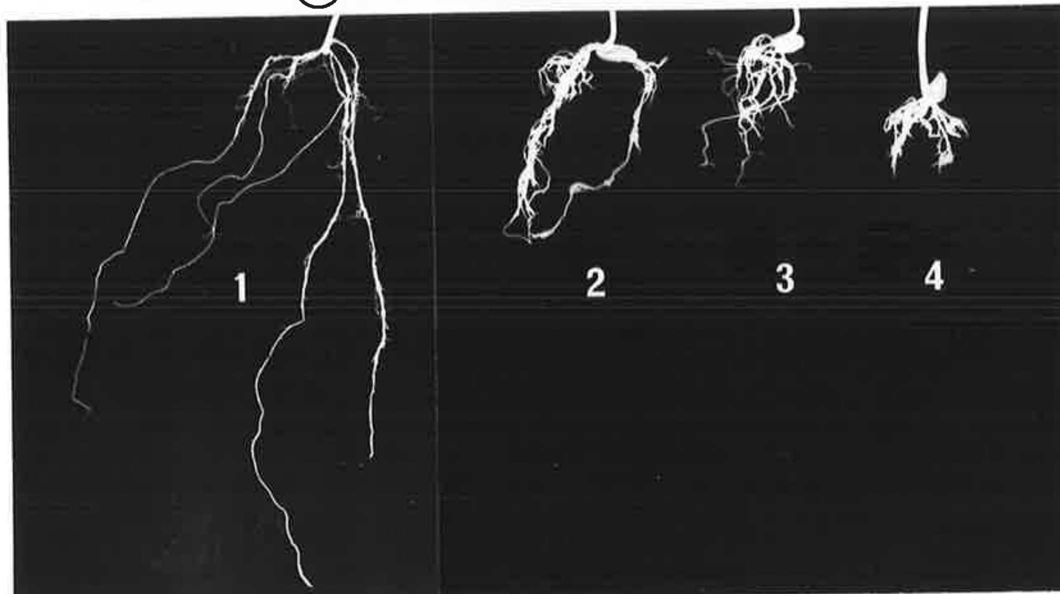
(b) Growth 14 days following sowing.

1. Uninoculated or low, medium or high density 7 days following sowing.
2. Low density at sowing.
3. Medium density at sowing.
4. High density at sowing.

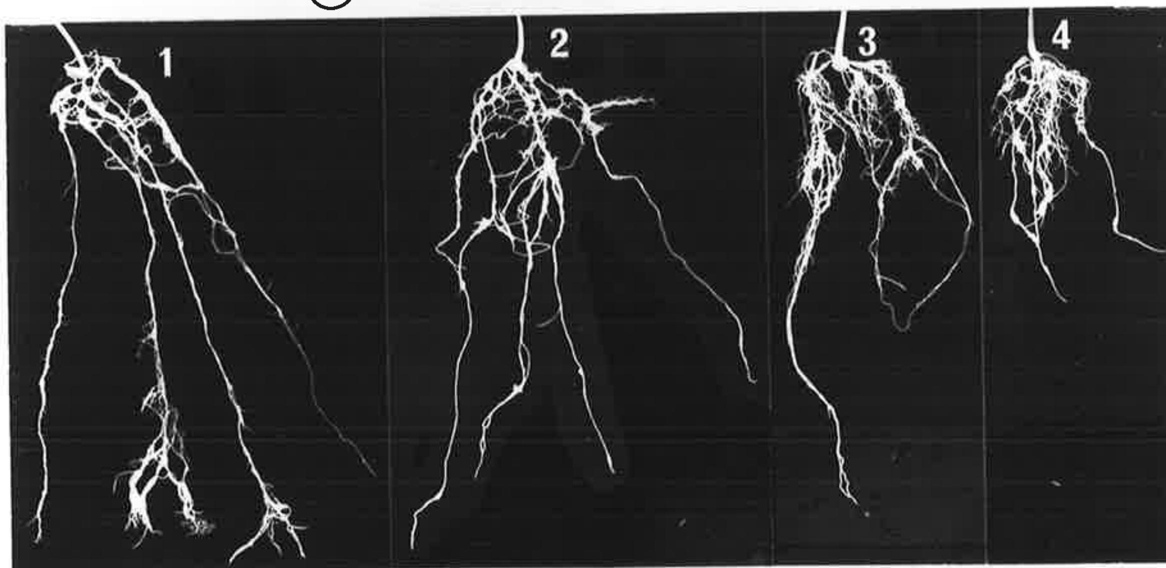
(c) Growth 21 days following sowing.

1. Uninoculated, low or medium density 7 days following sowing, low, medium or high density 14 days following sowing.
2. High density 7 days following sowing.
3. Low, medium or high density at sowing.

ⓐ 7 DAYS



ⓑ 14 DAYS



ⓒ 21 DAYS

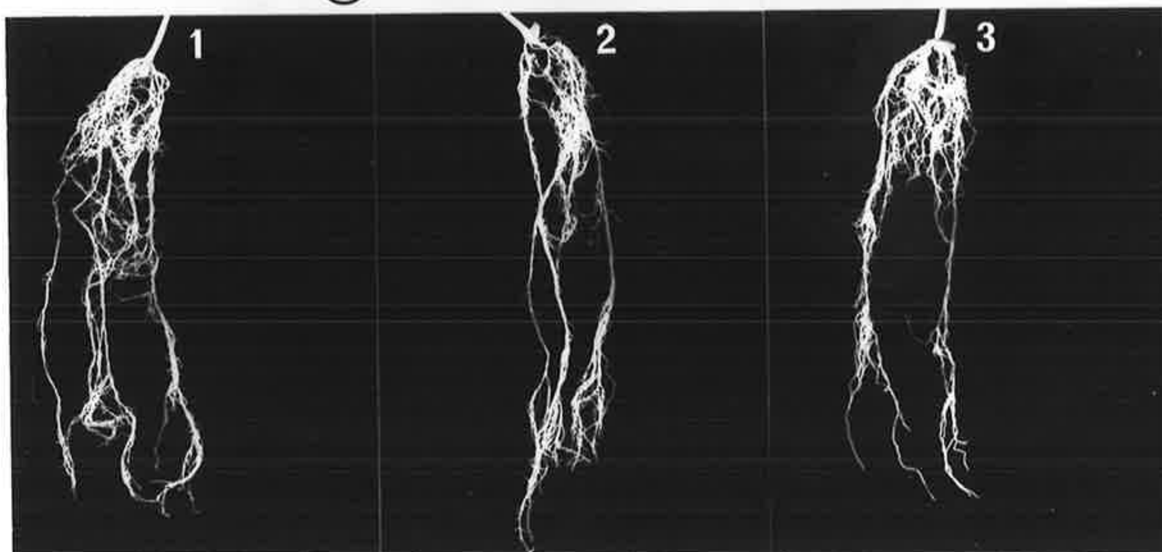


TABLE 14.

EFFECT OF DIFFERENT DENSITIES AND TIME OF INOCULATION OF OF H. AVENAE ON THE NUMBER OF ROOT TIPS PER PLANT OF WHEAT (CV. HALBERD).

Inoculation		Harvest (Days from Sowing)		
Density	Time (Days)	7	14	21
0		83	256	577
Low	0		221	442
Medium	0		196	271
High	0		178	276
Low	7		243	345
Medium	7		247	371
High	7		259	368
Low	14			514
Medium	14			608
High	14			615
L.S.D. (P = 0.05)		=	49	= 60

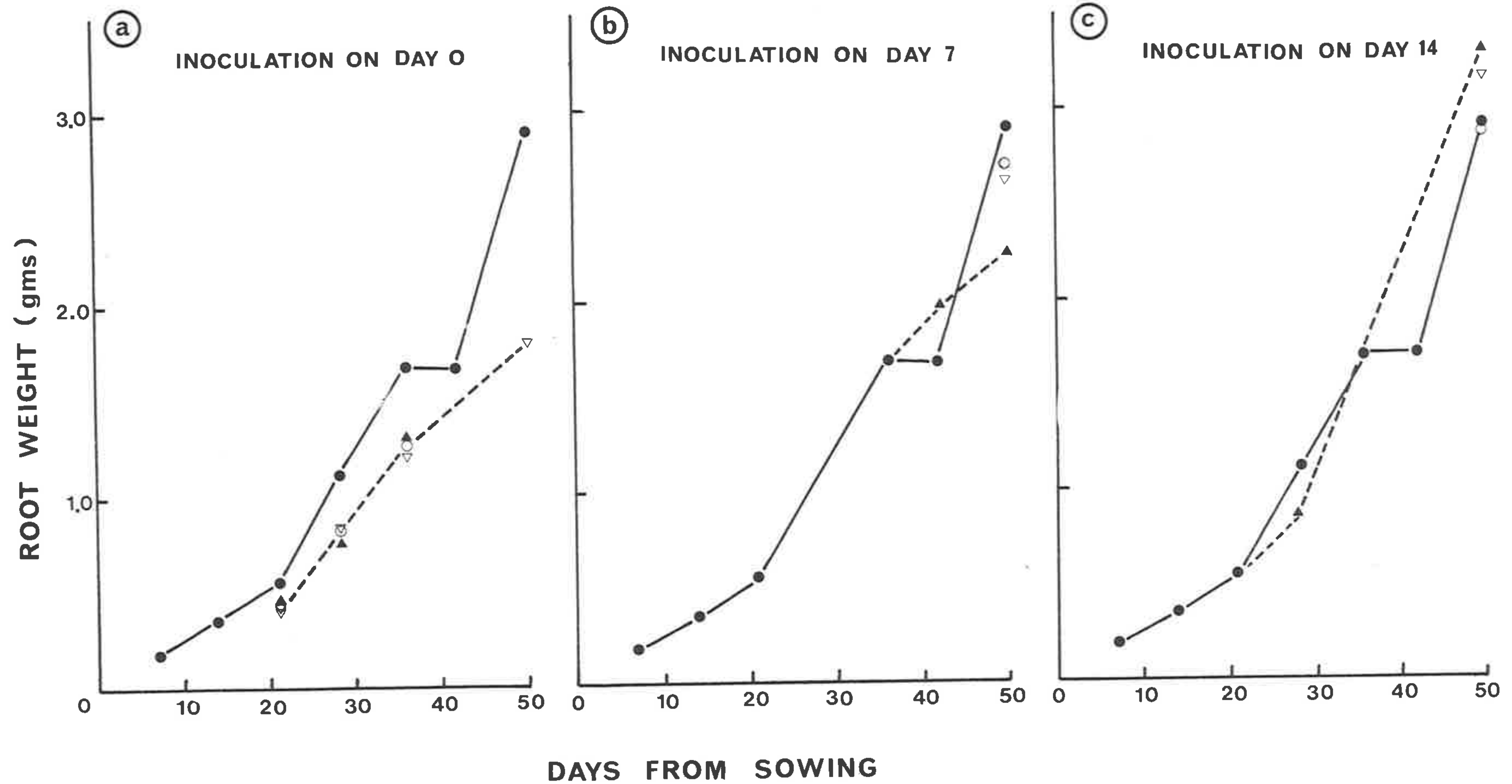
TABLE 15.

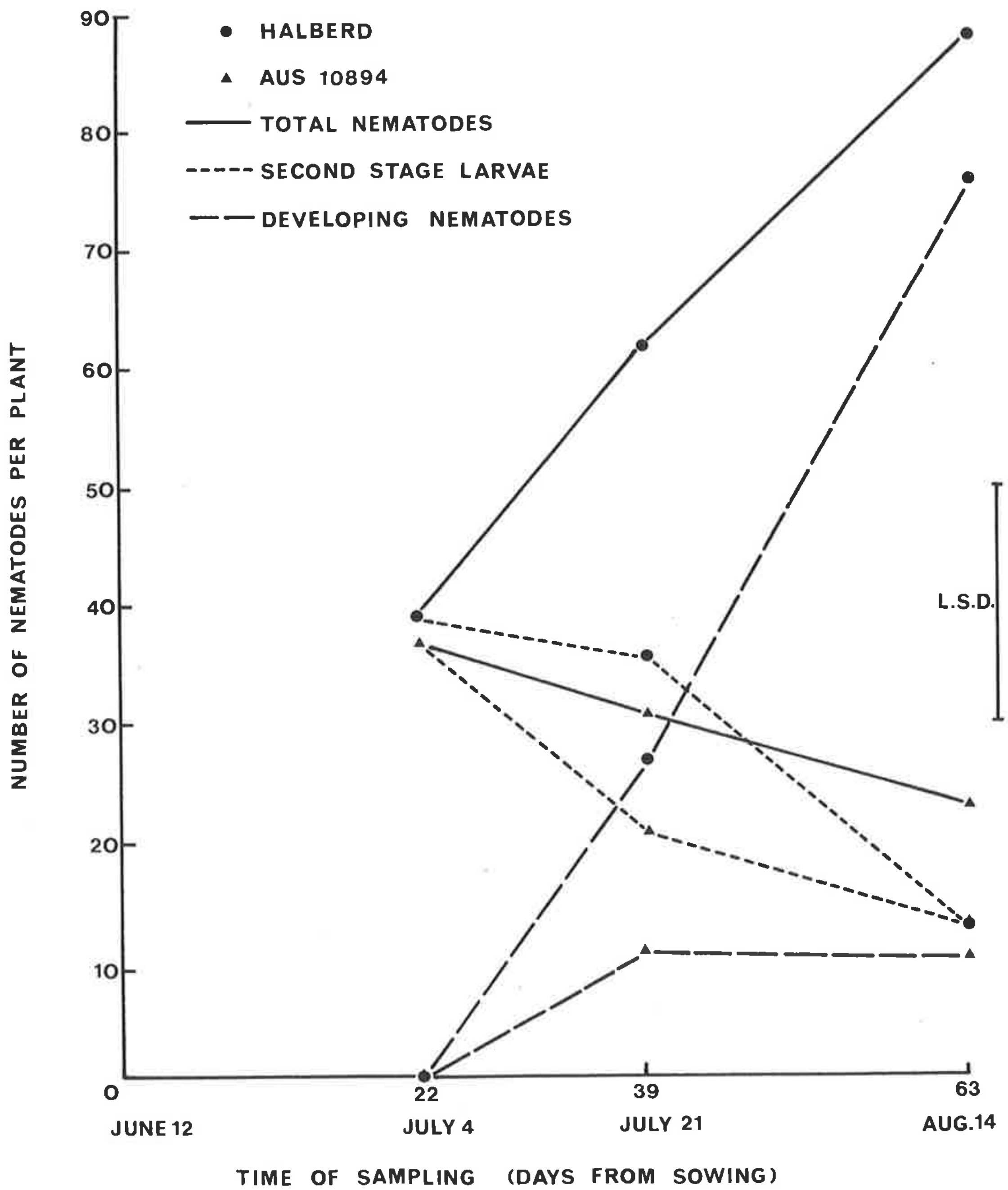
EFFECT OF DIFFERENT DENSITIES AND TIME OF INOCULATION OF *H. AVENAE* ON THE LENGTH OF NODAL ROOTS OF WHEAT (CV. HALBERD)

Inoculation		Harvest (Days from Sowing)		
Density	Time (Days)	21	28	36
0		1.25 (31)*	2.32 (214)	2.49 (311)
Low	0	0.76 (9)	1.74 (66)	2.57 (386)
Medium	0	0 (0)	1.55 (93)	2.50 (327)
High	0	0.15 (1)	1.83 (76)	2.57 (378)
Low	7	1.37 (41)	2.31 (218)	2.55 (357)
Medium	7	1.22 (27)	2.35 (235)	2.52 (336)
High	7	1.09 (21)	2.39 (256)	2.60 (402)
Low	14	1.31 (57)	2.43 (283)	
Medium	14	1.51 (47)	2.38 (241)	
High	14	1.54 (43)	2.34 (231)	
L.S.D. (P = 0.05)		= 0.39	= 0.36	N.S.

Results for each time of harvest is the mean of Log_{10} (x+1) transformation of six replicates.

( )* Mean of original data (mm.)


a
INOCULATION ON DAY 0
b
INOCULATION ON DAY 7
c
INOCULATION ON DAY 14
ROOT WEIGHT (gms)
3.0
2.0
1.0
0
10
20
30
40
50
DAYS FROM SOWING



HALBERD
AUS 10894
TOTAL NEMATODES
SECOND STAGE LARVAE
DEVELOPING NEMATODES
NUMBER OF NEMATODES PER PLANT
90
80
70
60
50
40
30
20
10
0
L.S.D.
22
39
63
JUNE 12
JULY 4
JULY 21
AUG.14
TIME OF SAMPLING (DAYS FROM SOWING)

Figure 8. Effect of different densities of *H. avenae* and time of inoculation on the growth of roots of Halberd wheat.

(a) Inoculation on the day of sowing

●—●	0 larvae - Treatment	0
○	167 larvae -	1
△	325 larvae -	2
▲	436 larvae -	3

(b) Inoculation 7 days after sowing

●—●	0 larvae - Treatment	0
○	145 larvae -	4
△	286 larvae -	5
▲	422 larvae	6

(c) Inoculation 14 days after sowing

●—●	0 larvae - Treatment	0
○	165 larvae -	7
△	395 larvae -	8
▲	594 larvae -	9

All data was analysed, and apart from the following exceptions, the points presented on the graphs differ significantly ($P < 0.05$).

EXCEPTIONS
(i) Treatment 0 at days 36 and 42.
(ii) Treatments 1, 2, 3 at each sample. N.B. Only treatment 2 was sampled on Day 50.
(iii) Treatment 9 and treatments 1, 2, 3 at day 28.
(iv) Treatments 6 and 5 on day 50.
(v) Treatments 0, 4 and 5 on day 50.
(vi) Treatments 8 and 9 on day 50.
(vii) Treatments 0, 7 and 8 on day 50.

times of sampling (Fig. 8). Because of this influence of moisture stress on uninoculated plants, differences between root weights 42 days after sowing will be ignored, with the exception that all inoculated plants did not show this effect of moisture stress.

Roots of seedlings inoculated at sowing with different densities had similar root weights and were always lighter than uninoculated roots (Fig. 8 a). With roots inoculated 7 days after sowing, only roots with the high density differed from uninoculated roots and were lighter 50 days after sowing (Fig. 8 b). After 50 days of growth, the root weight of seedlings inoculated 7 days after sowing showed a tendency to decrease as density of inoculum increased. Seedlings inoculated with low and medium densities 14 days after sowing had a similar root weight to that of uninoculated seedlings, but seedlings inoculated with the high density had lighter roots 28 days after sowing and heavier roots 50 days after sowing (Fig. 8 c). After 50 days growth, root weight of seedlings inoculated 14 days after sowing showed a tendency to increase as density of inoculum increased.

The number of adult nematodes on roots varied with the method of inoculation (Table 16). Density of inoculum had no effect on the number of females but delay in time of inoculation reduced the number of females, while the number of males increased as density increased and also increased with delay in the time of inoculation. These variations in the number of adult nematodes were reflected in male to female ratios. This ratio increased with increased density of inoculations at planting and 14 days after sowing but not with inoculations 7 days after sowing. Percentage of larvae which became adult seemed to increase with each delay in time of inoculation and decrease with increased densities of inoculations 7 and 14 days after sowing.

Length of lamina of expanded leaves of the first, second and

TABLE 16.

EFFECT OF DIFFERENT DENSITIES AND TIME OF INOCULATION OF H. AVENAE ON DEVELOPMENT OF ADULT NEMATODES ON WHEAT (CV. HALBERD).

Inoculation		No. of Females	No. of Males	M/F Ratio	Percent
Density	Time		(Log_{10} x)	(Log_{10} (x+1))	Adults
Low	0	14	1.07 (12)*	0.28 (0.9)*	16
Medium	0	14	1.38 (27)	0.45 (1.8)	13
High	0	10	1.69 (51)	0.81 (5.6)	14
Low	7	8	1.57 (38)	0.95 (13.3)	32
Medium	7	10	1.72 (56)	0.87 (7.8)	23
High	7	10	1.80 (66)	0.92 (7.9)	18
Low	14	4	1.72 (55)	1.25 (20.8)	36
Medium	14	4	1.91 (85)	1.39 (32.1)	23
High	14	4	2.15 (148)	1.58 (44.0)	26
L.S.D. ($P = 0.05$)		= 5	= 0.17	= 0.32	

( )* Mean of original data

third leaves on plants inoculated at sowing were shorter than equivalent leaves on uninoculated seedlings (Table 17). Inoculations 7 and 14 days

TABLE 17.

EFFECT OF DIFFERENT DENSITIES AND TIME OF INOCULATION OF *H. AVENAE* ON THE LENGTH (MM.) OF EXPANDED LEAVES OF WHEAT (CV. HALBERD).

Inoculation		Leaf Number (order of development)					
Density	Time	1	2	3	4	5	6
0		98	198	288	277	295	275
Low	0	66	150	240	271		
Medium	0	64	148	240	272	304	273
High	0	60	138	243	275		
Low	7	94	195	272	268	303	277
Medium	7	91	188	275	259	296	274
High	7	89	184	266	268	303	287
Low	14	90	191	281	263	285	270
Medium	14	92	194	285	265	273	268
High	14	93	189	275	264	276	268
L.S.D. (P = 0.05)		= 9		= 17	= 20	N.S.	N.S.

after sowing seemed to affect length of the third and fourth leaves, but only the third leaf on seedlings with the high inoculation 7 days after sowing was shorter than the equivalent leaf on uninoculated plants. Breadth of lamina of expanded leaves was also affected by inoculations (Table 18). Second, third and fourth leaves of seedlings inoculated at

TABLE 18.

EFFECT OF DIFFERENT DENSITIES AND TIME OF INOCULATION OF *H. AVENAE* ON THE BREADTH (MM.) OF LAMINA OF EXPANDED LEAVES OF WHEAT (CV. HALBERD).

Inoculation		Leaf Number (order of development)			
Density	Time	1	2	3	4
0		3.8	4.0	5.0	6.5
Low	0	3.7	3.2	4.2	6.0
Medium	0	4.0	3.7	3.7	5.5
High	0	4.0	3.3	4.0	5.8
Low	7	4.0	3.8	4.3	6.2
Medium	7	4.0	3.5	4.3	6.3
High	7	4.0	3.7	4.2	6.2
Low	14	4.0	3.8	4.2	6.3
Medium	14	4.2	4.2	4.5	6.5
High	14	4.2	3.8	4.0	6.2

L.S.D. ($P = 0.05$) = 0.5

sowing and the third leaf of seedlings inoculated 7 and 14 days after sowing were narrower than equivalent leaves on uninoculated seedlings.

Length of the newest expanding leaf was included in the measurements of the height of seedlings. Because the length of expanding leaves was variable (Table 19), total length of laminae of green, expanded leaves was added to height and used as an index of stem growth of seedlings. Seedlings inoculated 7 and 14 days after sowing grew similarly to uninoculated seedlings and the variations, at 42 and 50 days after sowing, with inoculations 7 days after sowing (Fig. 9 b) were an

TABLE 19.

EFFECT OF A HIGH DENSITY OF H. AVENAE INOCULATED ONTO WHEAT (CV. HALBERD) AT TIME OF SOWING ON VARIATION IN PLANT HEIGHT AND NUMBER OF EXPANDED LEAVES AT TWO HARVESTS.

Harvest (Days from Sowing)	Plant Number	Height (mm.) Density 0	Height (mm.) Density High	Expanded Leaves (Nos.) Density 0	Expanded Leaves (Nos.) Density High
	1	312	95	2	3
	2	95	115	3	3
	3	105	162	3	3
21	4	115	192	3	3
	5	120	230	3	3
	6	120	297	3	3
	Mean	145	182	2.8	3.0
	S.E.	34	31	0.2	0
	1	288	118	3	4
	2	312	134	3	4
	3	102	147	4	4
28	4	110	155	4	4
	5	135	157	4	4
	6	140	271	4	4
	Mean	181	164	3.7	4.0
	S.E.	38	22	0.2	0

Figure 9. Effect of different densities of *H. avenae* and time of inoculation on growth of the stem of wheat (cv. Halberd) expressed as height plus the total length of lamina (m m.)

(a) Inoculation on day of sowing.

●——● 0 larvae - Treatment 0

o 167 larvae - Treatment 1

Δ 325 larvae - Treatment 2

▲ 436 larvae - Treatment 3

N.B. Means of all samples plotted.

(b) Inoculation 7 days after sowing.

●——● 0 larvae - Treatment 0

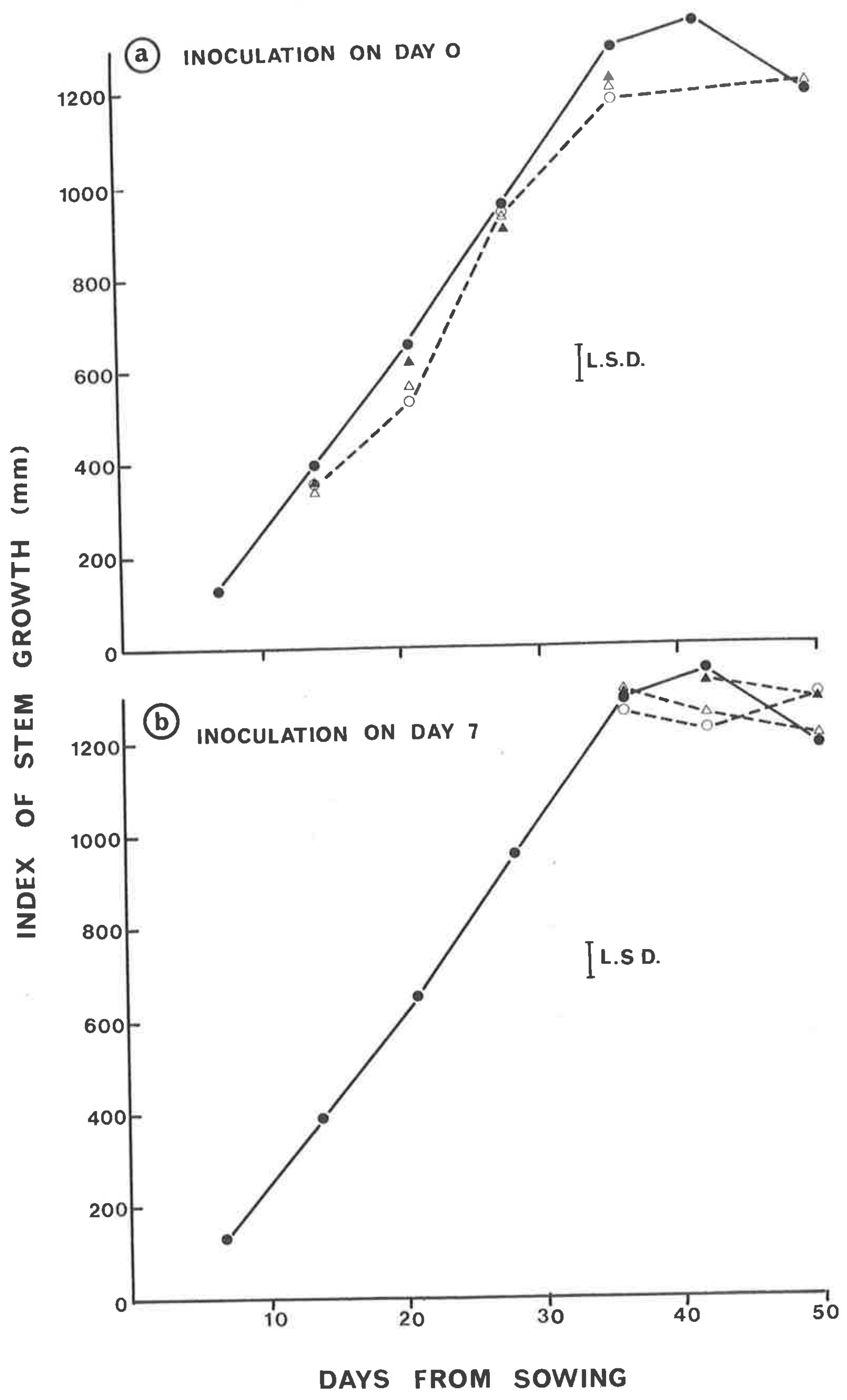
o 145 larvae - Treatment 4

Δ 286 larvae - Treatment 5

▲ 422 larvae - Treatment 6

N.B. No significant differences in means until day 42. Means of samples from Day 36 are plotted.

L.S.D. (P = 0.05)



a
INOCULATION ON DAY O
L.S.D.
b
INOCULATION ON DAY 7
L.S D.
INDEX OF STEM GROWTH (mm)
DAYS FROM SOWING
0
200
400
600
800
1000
1200
10
20
30
40
50

effect of either earlier or later senescence of leaves on one or two of the seedlings sampled. Stem growth of seedlings inoculated at sowing was similar at most samplings (Fig. 9 a), but 21 days after sowing, more growth occurred in seedlings inoculated with the high than the low density. However, seedlings inoculated with the high density at sowing also grew similarly to uninoculated seedlings, while seedlings inoculated with the low density grew similarly 14 and 28 days after sowing and grew less 21 and 36 days after sowing. Seedlings with the medium density of inoculation at planting were intermediate to the low and high densities.

Seedlings inoculated with the high density of larvae at planting were compared with uninoculated seedlings 21 and 28 days after sowing for differences in both the height and number of expanded leaves (Table 19). No differences occurred when height and the number of leaves were considered separately, but inocculated seedlings were either taller when seedlings with the same number of leaves were compared or shorter with one extra leaf on the seedling. These differences indicated the inoculated seedlings had grown more than the uninoculated seedlings.

An index for stage of growth of seedlings at each sampling was obtained from the height and the number of expanded leaves. A logarithmic transformation was done on height to minimise variation and when multiplied by the number of expanded leaves, gave an index of the stage of top growth of seedlings (Table 20). No differences occurred in this index between uninoculated and seedlings inoculated 7 and 14 days after sowing. But when uninoculated seedlings were compared with seedlings inoculated at sowing, the index was greater with the high density 21 days after sowing and greater with all inoculations 28 days after sowing. A bigger index was also obtained with the high than the low density of inoculation at planting 21 days after sowing.

TABLE 20.

EFFECT OF DIFFERENT DENSITIES AND TIME OF INOCULATION OF *H. AVENAE* ON DEVELOPMENT OF THE STEM OF WHEAT (CV. HALBERD) REPRESENTED AS NUMBER OF EXPANDED LEAVES X LOG_{10} HEIGHT.

Inoculation		Harvest (Days from Sowing)					
Density	Time	14	21	28	36	42	50
0		4.0	5.9	8.1	11.0	12.4	15.2
Low	0	4.4	5.4	8.9	10.9		
Medium	0	4.3	6.0	9.0	11.5		15.5
High	0	4.3	6.7	8.8	11.3		
Low	7	4.1	5.8	8.4	10.6	12.6	15.0
Medium	7	4.1	6.0	8.3	10.9	12.4	15.2
High	7	4.0	6.1	8.2	11.0	12.7	15.4
Low	14		5.5	8.5			15.5
Medium	14		5.9	8.5			15.1
High	14		6.1	8.7			15.2
L.S.D. (P = 0.05)		N.S.	= 0.67	= 0.54	= 0.65	N.S.	N.S.

3.2.3 Discussion

Increasing the age of seedlings before inoculation from 0 to 7 to 14 days changed the number of root tips available for invasion by nematodes from 5 to 83 to 256, but the percentage of larvae becoming adult increased only up to 7 days. This was best demonstrated by the low density of inoculum in which the number of females decreased and the

number of males increased as inoculation was delayed and suggested the number of females was inversely related to the number of root tips.

Three main seminal roots had emerged from the seed embryo at sowing and by 7 days there were five with their tips near the base of the growth tube. Thereafter, no increase in the number of main roots occurred, but they had branched (first order branching) within 7 days and by 14 days, second order branching had begun. This development of seminal roots was normal (Troughton, 1962) and increases in root tips from sowing to 7 days to 14 days after sowing were in tips of first and second order branches from the limited number of main seminal roots. In addition, these roots differ in diameter with the main roots widest (0.32 mm.), then first order branches (0.09 mm.) and finally the second order branches (0.03 mm.) were the narrowest (Troughton, 1962).

Thus, females probably developed only on the main roots and as these approached the bottom of tubes, fewer larvae were able to find them, penetrate and establish. Whereas, males could have developed in lateral roots as the number of males increased as the number of root tips increased with delays in inoculation. Similar observations with *H. rostochiensis* (Ellenby, 1954; Trudgill, 1967) have suggested an inability of larvae to develop suitable syncytia for female development within narrow lateral roots (Ross & Trudgill, 1969).

Den Ouden (1960) and Trudgill (1967) implied sex in *H. rostochiensis* was determined environmentally and that most larvae develop into females following inoculation of roots with single larvae. Approximately 30% of the total number of larvae they inoculated onto roots developed into females and the males may have developed but escaped detection. Davies and Fisher (1976) have shown 80% of larvae of *H. avenae* were able to penetrate roots. If no competition occurred between larvae at invasion sites of lateral roots and only males developed within lateral

roots, 40% of the inoculum developing into males would favour sex being genetically predetermined in second stage larvae (Triantaphyllou and Hirschman, 1964; Kerstan, 1969; Triantaphyllou, 1973). This almost was reached when low numbers of larvae of H. avenae were inoculated onto seedlings 14 days after sowing and probably did occur as some males would not have been recovered.

With either competition between larvae on main roots or development of male nematodes within lateral roots, the following sequence of events probably occurred. Genetically predetermined male and female second stage larvae penetrated roots randomly (Ross & Trudgill, 1969; Section 2.3.3) rather than selectively (Davide & Triantaphyllou, 1967). Following invasion, random establishment of larvae occurred until there was competition between larvae within the main roots, then the size of syncytia required for development of female larvae was restricted and both the main and lateral roots had a similar effect on the establishment and development of female larvae. Syncytia may be established and because of the restrictions on their development, they may either remain small, restrict larval development and prevent formation of adult females or degenerate and then the female larvae would die (Endo, 1965). It would also be possible for female larvae to remain motile and either leave roots because they were unable to establish (Davies & Fisher, 1976) or die in roots when energy reserves were depleted (Section 2.3.3). Therefore, changes in sex ratios were a result of differential development of larvae due to either female larvae leaving roots or selective death of female larvae within roots.

Growth of seminal roots was affected by nematodes and the greatest effect was with inoculations at sowing when both the root weight and number of root tips were reduced, but differences between densities of inocula were minor. Minor differences in root growth and a similar number

of females between densities of inocula at planting has suggested similar competition between larvae at the roots for each density and not the increased competition with increased density which was expected. Therefore, an optimum density of inoculum at planting for tests to assess the reaction of plants to nematodes was not determined. Fewer larvae than the number used for the low density would be optimal and would avoid unnecessary competition while allowing the maximum number of females.

Inoculations at sowing also had the greatest effect on top growth of plants. Because three leaf primordia are preformed in the embryo of the seed and early growth is dependent upon the endosperm (Wellington, 1966), the reduced growth of these leaves, especially of the first leaf, following inoculation at sowing has suggested a direct effect of the establishing nematodes on the top growth of plants. This needs confirmation as water and nutrient uptake by roots would also be affected by nematodes. As a result of nematodes affecting leaf growth, both the total leaf area and stem available for photosynthesis were reduced and further development of the plants would be affected. However, an index of stem growth (Fig. 9) showed that plants compensated for the effects of inoculations at sowing by an increased rate of growth.

With each delay in inoculation from sowing to 7 and then 14 days after sowing, the effect of nematodes on plant growth was reduced. This was due to a dilution of nematodes at each root tip as the number of tips increased and/or decreasing availability of the main seminal roots to nematodes as the plants aged, thereby reducing the effects of nematodes on both the growth and function of roots. A further indication of the decreasing influence of nematodes on roots was shown by the different responses of inoculated plants to moisture stress with delays in inoculation and increases in density of inoculum.

Although nematodes may benefit plants under some abnormal

environmental conditions, nematodes normally restrict both the growth and function of seminal roots, and delay development of the nodal roots during the period of infection. Thus, moisture and nutrient uptake by roots was affected and this was also reflected in the top growth of plants. Therefore, the restricted growth of roots during penetration and establishment of nematodes rather than development of nematodes after establishment was the major cause of the effect of *H. avenae* on growth of wheat.

3.3 Conclusions

The medium of plant growth, number of plants on a tray, control of pests and diseases, moisture, number and age of larvae in the inoculum, number of inoculations and age of plants at inoculation have affected both the growth of plants and number of female nematodes on roots. These effects emphasised the need for the standardisation of environmental conditions and method of inoculation when studying interactions between *H. avenae* and plants, especially when only the females are counted.

Comparisons of the reactions between a susceptible and resistant barley, and between a susceptible and resistant wheat gave satisfactory separations of the reactions of different cultivars to *H. avenae* and the variation in the number of females on a cultivar was relatively small. However, when the effects of different densities of inoculation onto seedlings at sowing were compared, the effects of nematode competition on nematode development and root growth of plants were re-emphasised, suggesting the number of larvae used in the previous tests of plants were too high and that only 50 to 100 larvae should be used in the initial inoculum. However, more information is required, especially on the importance of the nematode density to the possible induction of resistance in resistant cultivars.

Growth of wheat plants were affected by inoculations with nematodes at planting. The rate of top growth seemed faster, development of nodal roots was delayed and the lamina growth of early leaves was reduced when the inoculated were compared with uninoculated seedlings. These effects suggested plants infected by nematodes during early growth could have the growth, development and yield of mature plants affected. This aspect needed confirmation and has been examined by comparing the growth and development of a resistant and susceptible cultivar of wheat and of barley with and without inoculations of nematodes.

4. EFFECT OF H. AVENAE ON GROWTH OF WHOLE PLANTS OF SUSCEPTIBLE AND RESISTANT CULTIVARS OF WHEAT AND BARLEY

Reduced plant growth and grain yields due to infections with H. avenae were reported for susceptible cereals during the first investigations in South Australia (Hickinbotham, 1930). This has since been supported by increased grain yields in cereals following the reduction of nematode populations when different plant hosts were grown (Mathison, 1966; Meagher & Brown, 1974) and nematicides were used (Brown et al., 1970; Brown, R. 1973).

In Europe, comparable densities of H. avenae (Duggan, 1961; Gair et al., 1969) to those in Australia do not seem as severe in reducing yields of cereals and although this may be due in part to the cereal cultivars grown, environmental factors, e.g., soil type and both the nutrient and moisture supply, also affect the host response to nematode infection (Jones et al., 1965; Wallace, 1970). Severe crop losses in the field may be through the interaction between the nematode population and the environmental stresses to which the plant is subjected (Wallace, 1970). However, Stynes (1975) concluded from a synoptic study of wheat grown in South Australia during 1972 that although H. avenae affected the growth of wheat early in the season, this effect was not related to the final yield of grain as increased plant growth later in the season compensated for the earlier effect of H. avenae and other environmental factors were mainly responsible for the fluctuations in yield of wheat.

Previous results (Section 3.2.2) have shown that the final size of the leaves of wheat was affected by H. avenae and have also suggested that the rate of growth of plants was faster. Floral initiation occurred within 20 days of sowing when wheat was grown in a controlled environment (Williams, 1966) and within 42 days of sowing in

the field (Symes, 1974). Therefore, floral initiation occurred early in the season when the larvae of H. avenae were likely to be active in the soil (Fig. 1), invading plants and affecting growth. The effects of H. avenae on the growth of leaves, stem and inflorescence of resistant and susceptible cultivars of wheat and barley are described and compared in this section.

4.1 Materials and Methods

4.1.1 Growth room study

Materials and methods used were similar to those described in Section 3.1.1.1.

Growth of inoculated and uninoculated plants of susceptible cultivars of wheat (cv. Halberd) and barley (cv. Clipper) and resistant cultivars of wheat (cv. AUS 10894) and barley (Morocco) was measured. Inoculated and uninoculated plants were treated with similar solutions (water or nutrient) throughout the growth of plants. The number of larvae (± S.E.) inoculated onto seedlings were 332±9 at sowing and then 544±18, 504±7, 800±32, 792±22 and 938±27 successively at 5 day intervals following sowing.

Plant height and length of lamina of expanding and expanded leaves were measured 11 days after sowing and then at 3 or 4 day intervals until 45 days after sowing, after which measurements were on days 52 and 73. Each leaf was designated as L1, L2, etc. and the number referred to order of emergence. Plant height was recorded for the first tiller of each plant and was the height of the top of the sheath of the latest expanded leaf above the sand surface until emergence of the inflorescence, when height was to the top of the inflorescence and did not include awns. Length of lamina of leaves was either the length of lamina of expanding leaves visible above the newest leaf or the length of lamina of expanded

leaf. Lamina length of individual leaves was measured until this length was similar on three successive occasions. Breadth of expanded lamina of leaves was measured at the time of the final measurement of lamina length.

Growth of plants was terminated 73 days after sowing and the other parameters were then measured. The number of tillers, total number of spikelets and number of fertile spikelets on the inflorescence and the number of female nematodes on roots were counted. After collecting the female nematodes, plants were oven dried at 80°C for 12 hours and the dry weight of stems and roots were measured.

Plants were grown on one tray in the growth room, arranged in a randomised block design of five replicates and analyses of variance were done on the raw data.

4.1.2 Field Study

Cultivars of cereals resistant to *H. avenae* are of commercial value if they reduce the effects of nematodes on crop growth and increase yields in later crops. An indication of the commercial value of resistance in wheat was obtained by growing AUS 10894 for one season on an area infected with *H. avenae*, then measuring the growth of a susceptible wheat (cv. Halberd) in the following season and comparing this with the results on areas where Halberd was grown in both seasons. Medic clover (*Medicago littoralis*) was grown for commercial seed production in close proximity to these areas and between the plots of Halberd and AUS 10894 were areas sprayed with non-residual weedicides (fallow), so a comparison was made of the effects of resistant wheat, medic clover and fallow with that of a susceptible wheat on the growth of Halberd in the following season.

From a field near Windsor in South Australia (FIG. 22) in which Halberd infected with *H. avenae* was grown during 1973, a site was selected on the

basis of soil uniformity and the evenness of nematodes throughout the site which was assessed by an examination of the roots of stubble in March, 1974.

During 1974, Halberd and AUS 10894 were planted in plots 3m. x 3.5m.; there were two plots per block and each of the three plots was separated by a path 1.5 m. wide. The cultivars were assigned to plots within blocks at random and arranged as shown in Fig. 10. The number of blocks was limited by the availability of seed of AUS 10894. Seeding was on 12th June and the rows were in an east to west direction. In 1975, the whole site was sown with Halberd on 22nd May with the rows at right angles to those in 1974.

Seed bed preparation and sowing of cultivars were similar in 1974 and 1975, and followed the normal crop management program for the district, except that in 1974, all plots were sprayed the same way on two occasions during cereal growth to control weeds. On 5th August, 1974, the path areas were sprayed with paraquot and amitrole ammonium thiocyanate (Weedazole TL plus*) to kill all plants.

During 1975, plants of Halberd were sampled twice following emergence of the inflorescence, when grain development was at the 'dough' stage and when the grain was mature just prior to harvest. At each time, five random samples were taken from the areas of plots of Halberd, AUS 10894, paths (fallow) and medic clover in 1974, with at least one sample from each plot and pathway. Samples from areas with medic clover were from the southern side and close to the area of plots. For samples at the 'dough' stage of grain development, plants in 30 cm. of row with 15 cm. depth of soil were collected with a shovel. Tillers of plants within a pair of rows 1.75 m. long were harvested when the grain was ripe

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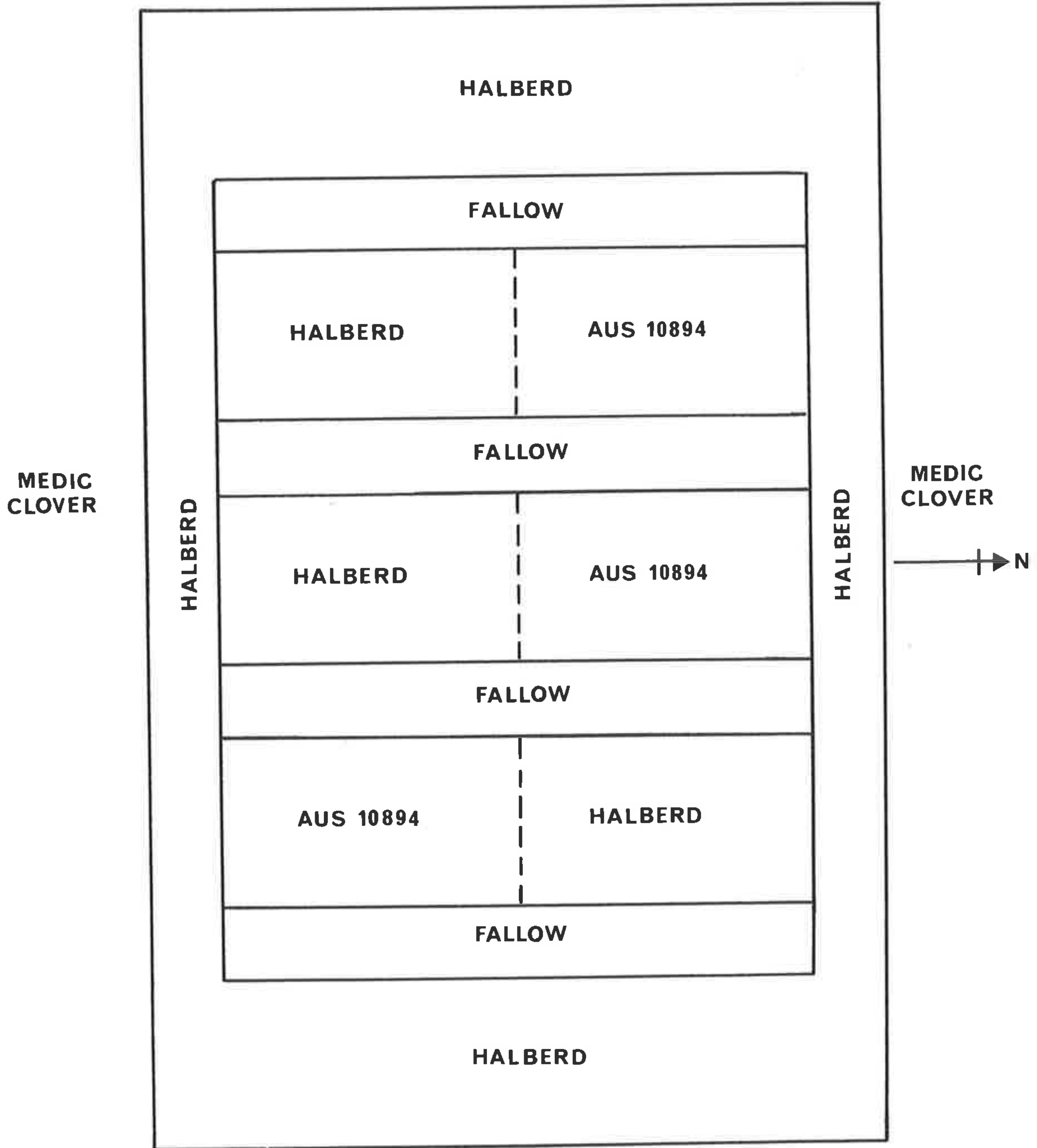
* trade name

Figure 10. Arrangement of plots of Halberd and AUS 10894 with associated areas of fallow and medic at the infested field site near Windsor.


MEDIC CLOVER
HALBERD
FALLOW
HALBERD
AUS 10894
FALLOW
MEDIC
CLOVER
HALBERD
HALBERD
AUS 10894
HALBERD
MEDIC
CLOVER
N
FALLOW
AUS 10894
HALBERD
FALLOW
HALBERD

and gave a total row length of 3.5 m. per sample.

From plants collected at the 'dough' stage, the number of female nematodes were counted and the fresh weight of roots was measured after washing plants (Section 3.1.1.1) and the number of females per gram of fresh root within a sample was calculated. Then the number of stems and subcrown internodes were counted to give the total number of tillers and plants per sample respectively, from which the average number of tillers per plant within a sample was calculated. Incidence of fungal diseases, mainly the 'take all' fungus (Gaeumannomyces graminis var. tritici), on roots of plants was assessed visually as the fraction of diseased roots to total roots on each plant within a sample. This was then expressed as the average fraction of root affected per plant. Eight plants without fungal infection were selected from each sample and the average height, length of flag leaf and number of spikelets per plant were calculated from the main tiller of these plants. Height was from the basal node to the top of the inflorescence, length of flag leaf was both the length of lamina and sheath, and the number of spikelets was both the total number and number of fertile spikelets.

At harvest, the number of inflorescences on the mature plants was counted, the plants were then thrashed and the weight of grain per sample was measured. From these measurements, weight of grain per inflorescence was calculated and recorded as weight of grain per tiller. Grain weight was also measured.

Analyses of variance were done on data of the five replications of all the parameters measured at each time of sampling.

4.2 Results

4.2.1 Growth room study

A comparison of inoculated and uninoculated plants of the same

cultivar provided an assessment of the effect of inoculation on plant growth. Leaf growth expressed as lamina length was affected by nematodes and because the rate of exponential growth of corresponding leaves was similar, nematodes were usually associated with either reduced size of leaf (e.g. Fig. 11, L3) or delayed emergence of the leaves (e.g. Fig. 11, L7) but occasionally leaves showed both of these effects (e.g. Fig. 11, L6) because the differences in lamina length during exponential growth was very significant. Normally the earlier leaves were smaller while emergence of the later leaves was delayed.

Inoculation of Halberd reduced the length of mature laminae of L2, L3, L6 (Fig. 11), reduced the breadth of mature laminae of L2, L3, L4 (Table 21) and delayed the time of emergence of L4, L5, L6, L7 and L8

TABLE 21.

EFFECT OF *H. AVENAE* ON BREADTH (MM.) OF LAMINAE OF EXPANDED LEAVES OF SUSCEPTIBLE (CV. HALBERD) AND RESISTANT (CV. AUS 10894) WHEAT.

		Halberd		AUS 10894	
		Control	Inoculated	Control	Inoculated
Leaf:	L1	4.1	4.0	4.1	4.0
	L2	3.8	3.4	3.9	3.1
	L3	5.0	4.0	5.0	4.0
		L.S.D. (P = 0.05) = 0.2			
	L4	6.6	5.2	6.4	5.0
	L5	7.3	7.0	7.0	6.1
	L6	8.8	8.3	7.6	7.0
		L.S.D. (P = 0.05) = 0.6			
	L7	9.2	8.6	8.0	7.7
	L8	9.6	10.1	8.8	8.2
	Flag	10.3	10.7	8.8	8.2
		L.S.D. (P = 0.05) = 1.2			

Figure 11. Effect of *H. avenae* on growth and final length of lamina of leaves of susceptible wheat (cv. Halberd).

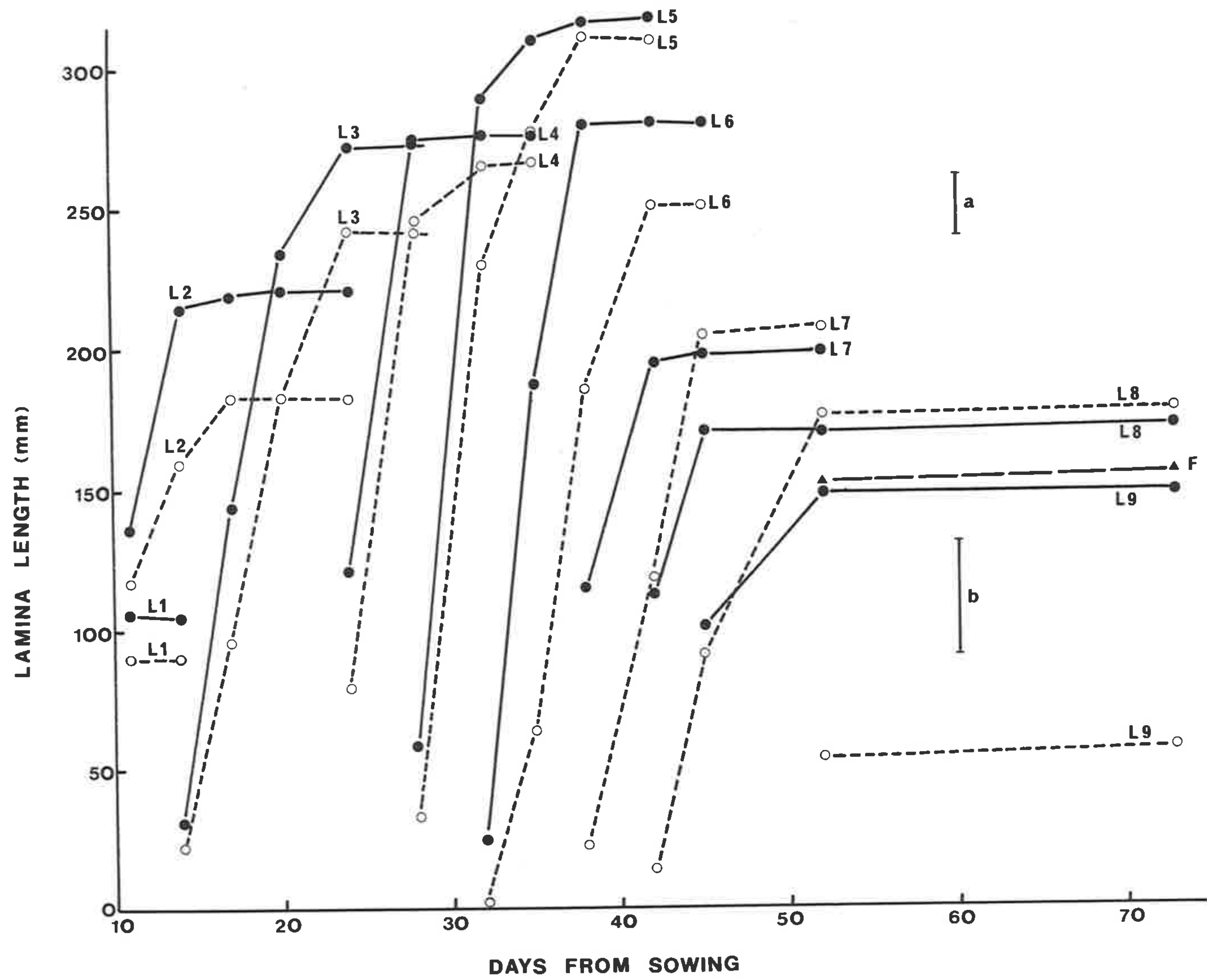
●——● Uninoculated

o----o Inoculated

I (a) L.S.D. (P = 0.05) of expanded lamina

I (b) L.S.D. (P = 0.05) of expanding lamina

L1, L2, L3 etc. - refer to leaves numbered in order of development.



LAMINA LENGTH (mm)
DAYS FROM SOWING
0
50
100
150
200
250
300
10
20
30
40
50
60
70
L1
L1
L2
L2
L3
L3
L4
L4
L5
L5
L6
L6
L7
L7
L8
L8
F
L9
L9
a
b

(Fig. 11). With L6, both the emergence was delayed and length of the mature lamina was shortened by inoculation. Only two of the inoculated plants developed L9 while all uninoculated plants developed L9 so the mean length of the mature lamina of L9 was shorter on inoculated plants. The last leaf to develop on a plant was the flag leaf and emergence of this leaf was apparently not delayed because 3 of the inoculated plants developed L8 and all uninoculated plants developed L9 as the flag leaf.

When AUS 10894 was inoculated, the length of mature laminae of L1, L2, L3, L4, L5, L6 (Fig. 12) and the breadth of mature lamina of L2, L3, L4, L5 (Table 21) were reduced. Emergence of L7 and probably L8 were delayed (Fig. 12). L8 was the flag leaf on both the inoculated and uninoculated plants. Therefore, leaf size was affected more and the time of emergence of leaves was affected less by inoculation than Halberd (Fig. 11).

Inoculation of Clipper had little effect on the length of mature laminae and only L2 was significantly shorter (Fig. 13), but the breadth of mature laminae was reduced on L2, L3, L4, L5, L6 (Table 22) and emergence of L4, L5, L6, L7, L8, L9 (Fig. 13) was delayed. L9 was the flag leaf on both the inoculated and uninoculated plants.

Both the length (Fig. 14) and breadth (Table 22) of mature laminae of Morocco were not affected by inoculation, but emergence of L4, L5, L6, L7, L8, L9 was delayed (Fig. 14). Because either L9 or L10 was the flag leaf on both the inoculated and uninoculated plants, inoculation delayed the emergence but did not affect the size of the flag leaf.

Inoculations reduced the height of seedlings of Halberd (Fig. 15 a), AUS 10894 (Fig. 15 b) and Clipper (Fig. 15 c) at various times during the first 30 days of growth, but did not affect Morocco (Fig. 15 d). These effects were likely to be associated with the effects of inoculations on the size, time of emergence and other parameters of growth of the early leaves.

Figure 12. Effect of *H. avenae* on growth and final length of lamina of leaves of resistant wheat (cv. AUS 10894).

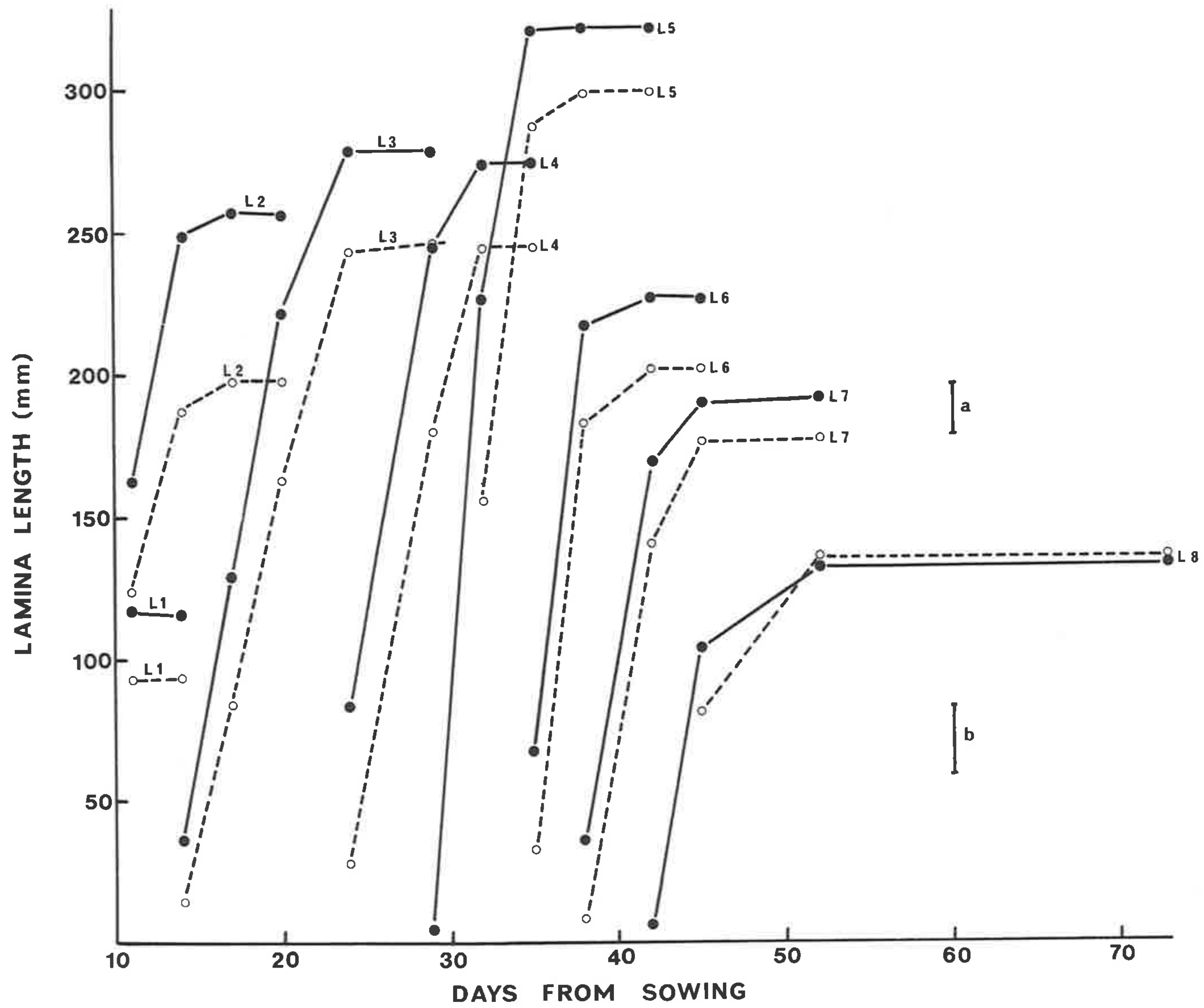
●——● Uninoculated

o----o Inoculated

I (a) L.S.D. (P = 0.05) of expanded leaves

I (b) L.S.D. (P = 0.05) of expanding leaves.

L1, L2, L3 etc., leaves numbered in order of development.



LAMINA LENGTH (mm)
300
250
200
150
100
50
10
20
30
40
50
60
70
DAYS FROM SOWING
L1
L1
L2
L2
L3
L3
L4
L4
L5
L5
L6
L6
L7
L7
L8
a
b

Figure 13. Effect of *H. avenae* on growth and final length of lamina of leaves of susceptible barley (cv. Clipper).

●———● Uninoculated

o-----o Inoculated

I (a) L.S.D. (P = 0.05) of expanded leaves

I (b) L.S.D. (P = 0.05) of expanding leaves

L1, L2, L3 etc., leaves numbered in order of development.

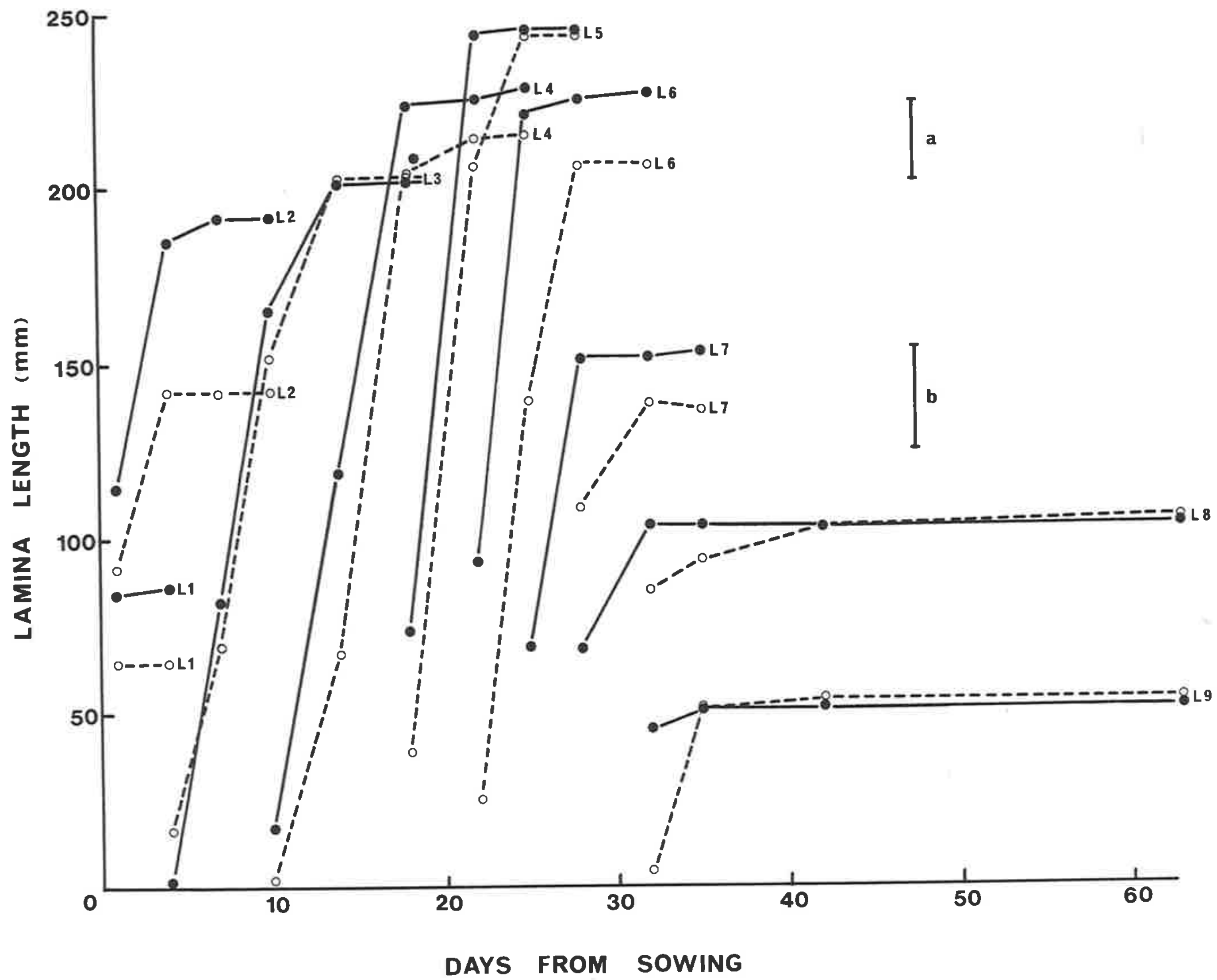

LAMINA LENGTH (mm)
250
200
150
100
50
0
10
20
30
40
50
60
DAYS FROM SOWING
L1
L1
L2
L2
L3
L4
L4
L5
L6
L6
L7
L7
L8
L9
a
b

Figure 14. Effect of *H. avenae* on growth and final length of lamina of leaves of resistant barley (cv. Morocco).

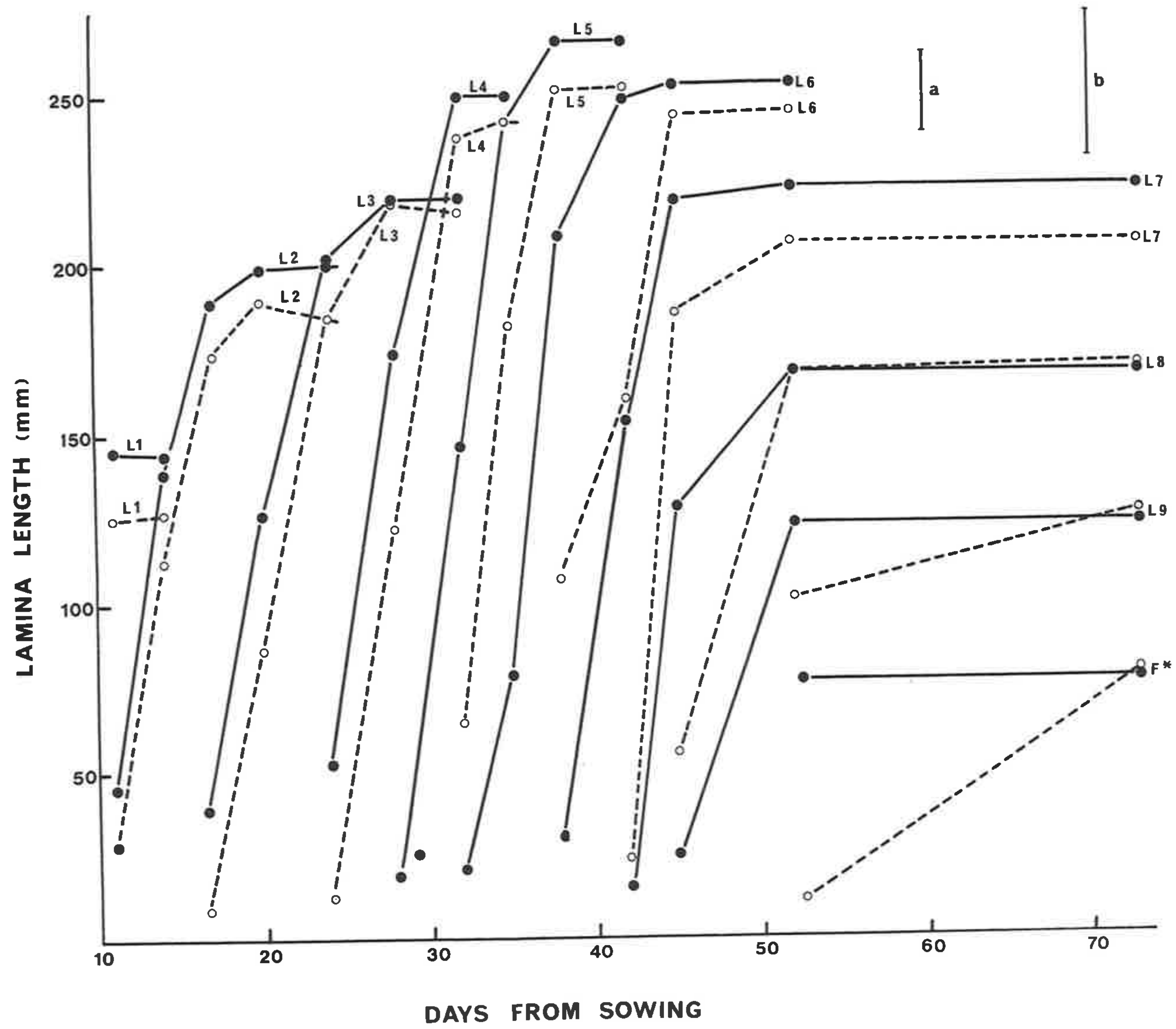
●——● Uninoculated

o----o Inoculated

I (a) L.S.D. (P = 0.05) of expanded leaves

I (b) L.S.D. (P = 0.05) of expanding leaves

L1, L2, L3, etc. leaves numbered in order of development.



LAMINA LENGTH (mm)
DAYS FROM SOWING
L1
L1
L2
L2
L3
L3
L4
L4
L5
L5
L6
L6
L7
L7
L8
L9
F*
a
b
250
200
150
100
50
10
20
30
40
50
60
70

Figure 15. Effect of *H. avenae* on plant height of wheat and barley during the first 30 days of growth.

●——● Uninoculated

o- - -o Inoculated

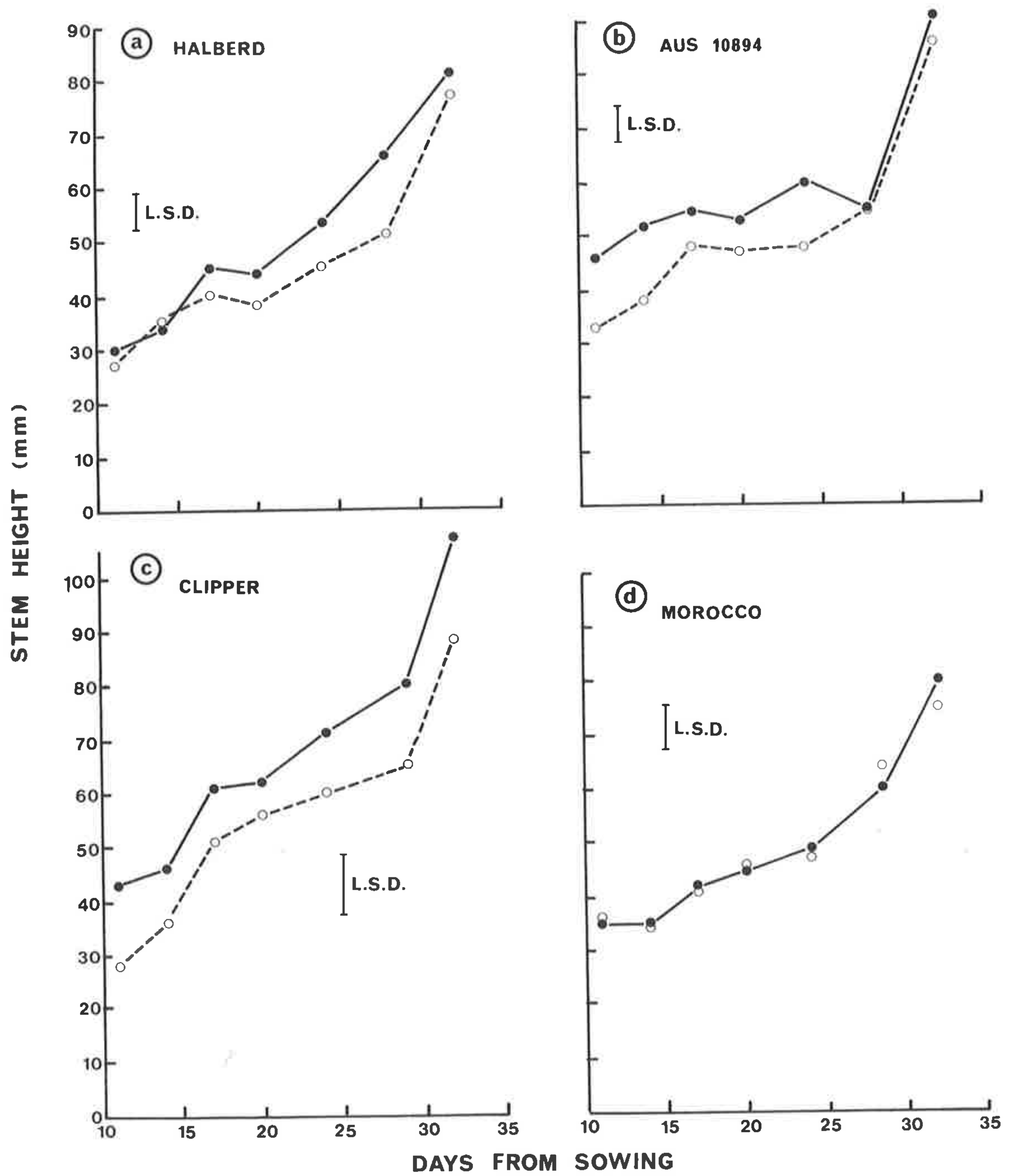
(a) Susceptible wheat (cv. Halberd)

(b) Resistant wheat (cv. AUS 10894)

(c) Susceptible barley (cv. Clipper)

(d) Resistant barley (cv. Morocco)

L.S.D. (P = 0.05)



a
HALBERD
b
AUS 10894
c
CLIPPER
d
MOROCCO
L.S.D.
L.S.D.
L.S.D.
L.S.D.
STEM HEIGHT (mm)
DAYS FROM SOWING
0
10
20
30
40
50
60
70
80
90
100
10
15
20
25
30
35

TABLE 22.

EFFECT OF H. AVENAE ON BREADTH (MM.) OF LAMINAE OF EXPANDED LEAVES OF SUSCEPTIBLE (CV. CLIPPER) AND RESISTANT (CV. MOROCCO) BARLEY.

Leaf	Clipper		Morocco	
	Control	Inoculated	Control	Inoculated
L1	6.1	5.6	7.6	7.4
L2	5.2	4.2	7.2	6.5
L3	7.4	5.6	7.9	7.0
	L.S.D. (P = 0.05) = 0.6		N.S.	
L4	8.3	7.1	8.1	7.7
L5	8.6	7.3	9.2	9.0
L6	8.7	7.9	10.2	10.3
	L.S.D. (P = 0.05) = 0.6		L.S.D. (P = 0.05) = 0.8	
L7	7.7	7.4	10.2	11.6
L8	7.3	6.8	12.0	12.3
L9	4.5	3.9	10.2	10.6
Flag	4.5	3.9	8.0	7.9
	L.S.D. (P = 0.05) = 1.3		L.S.D. (P = 0.05) = 1.4	

Inoculation of Halberd did not affect the exponential rate of elongation of stems, but the start of exponential elongation and the 'boot' stage of growth were delayed and the final height of stems was reduced (Fig. 16 a, Plate 3 a). When AUS 10894 was inoculated, elongating stems tended to be shorter, but the effects were small and the mature plants attained similar heights (Fig. 16 b, Plate 3 b). With the inoculation of Clipper, the start of exponential elongation was not affected, but the rate of this elongation was slower, emergence of the inflorescence was delayed and the final height of stems was reduced

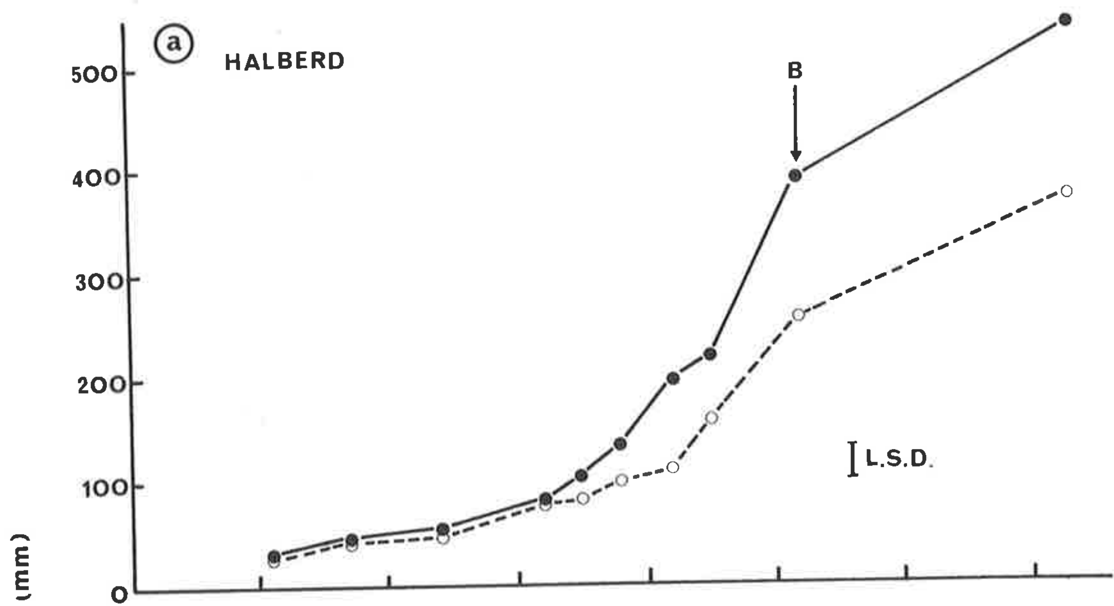
Figure 16. Effect of *H. avenae* on height of susceptible (cv. Halberd) and resistant (cv. AUS 10894) wheat.

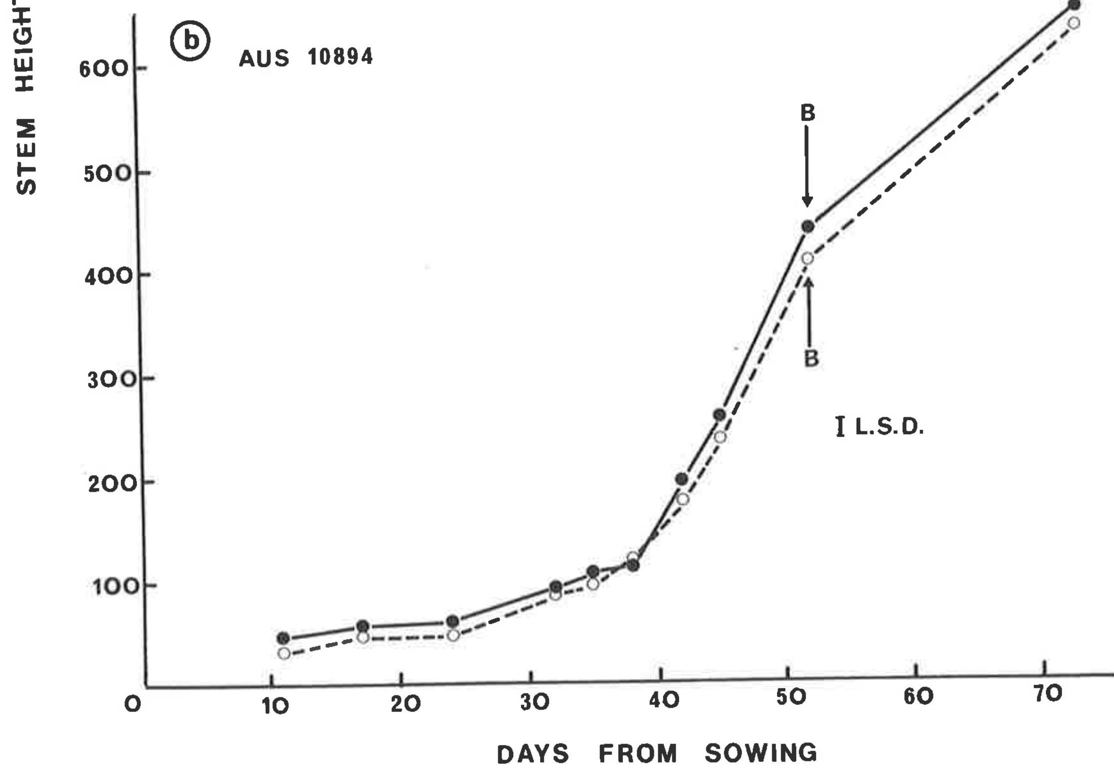
●——● Uninoculated

o----o Inoculated

B – the 'boot' stage of plant growth

L.S.D. (P = 0.05)





DAY OF INOCULATION

Plate 3. Comparison of plants inoculated with *H. avenae* and uninoculated plants of wheat and barley after 73 days growth.

(a) Susceptible wheat (cv. Halberd)

(b) Resistant wheat (cv. AUS 10894)

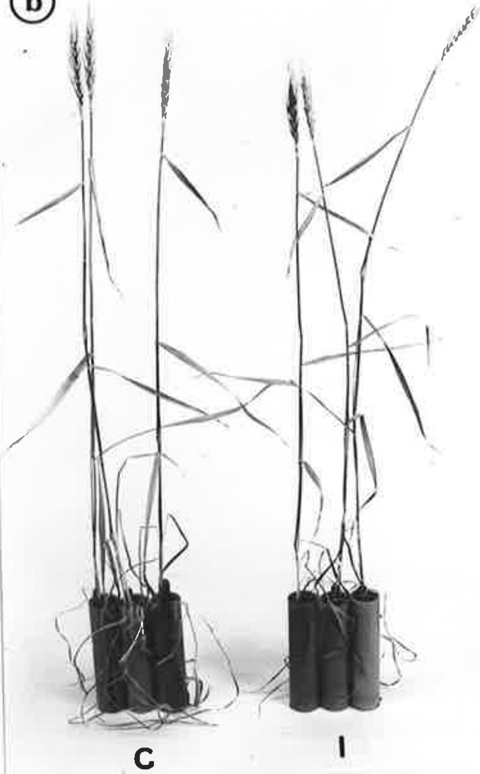
(c) Susceptible barley (cv. Clipper)

(d) Resistant barley (cv. Morocco)

C - Uninoculated plants

I - Inoculated plants.

a
C
I


b
C
I

c
C
I

d
C
I

(Fig. 17 a, Plate 3 c). The effects of inoculation on Morocco were probably similar to those with Clipper, except that mature plants attained a similar height (Fig. 17 b, Plate 3 d).

Inoculation reduced the numbers of tillers developed on AUS 10894 but not on Halberd, Clipper and Morocco (Table 23). Root

TABLE 23.

EFFECT OF H. AVENAE ON NUMBER OF TILLERS, AND DRY WEIGHT OF STEM AND ROOT PER PLANT OF WHEAT (CVS. HALBERD, AUS 10894) AND BARLEY (CVS. CLIPPER, MOROCCO).

	D.W. Stem (g.)		D.W. Root (g.)		Nos. of Tillers	
Inoculation	–	+	–	+	–	+
Halberd	0.86	0.46	0.15	0.11	1.0	1.6
AUS 10894	0.91	0.69	0.13	0.09	2.8	1.0
Clipper	0.69	0.49	0.18	0.16	3.6	3.2
Morocco	0.73	0.60	0.17	0.18	1.9	1.2
L.S.D. (P = 0.05)	= 0.12		= 0.06		= 1.1	

growth apparently recovered for all cultivars following inoculation because the dry weight of roots of mature plants was similar within cultivars, but roots on inoculated wheat (cv. Halberd and cv. AUS 10894) tended to be lighter (Table 23). The dry weight of stems was reduced on all inoculated plants, but resistant barley (cv. Morocco) seemed less affected than Clipper and resistant wheat (cv. AUS 10894) was less affected than Halberd (Table 23).

An effect of the inoculation on the differentiation of the

Figure 17. Effect of *H. avenae* on height of susceptible (cv. Clipper) and resistant (cv. Morocco) barley.

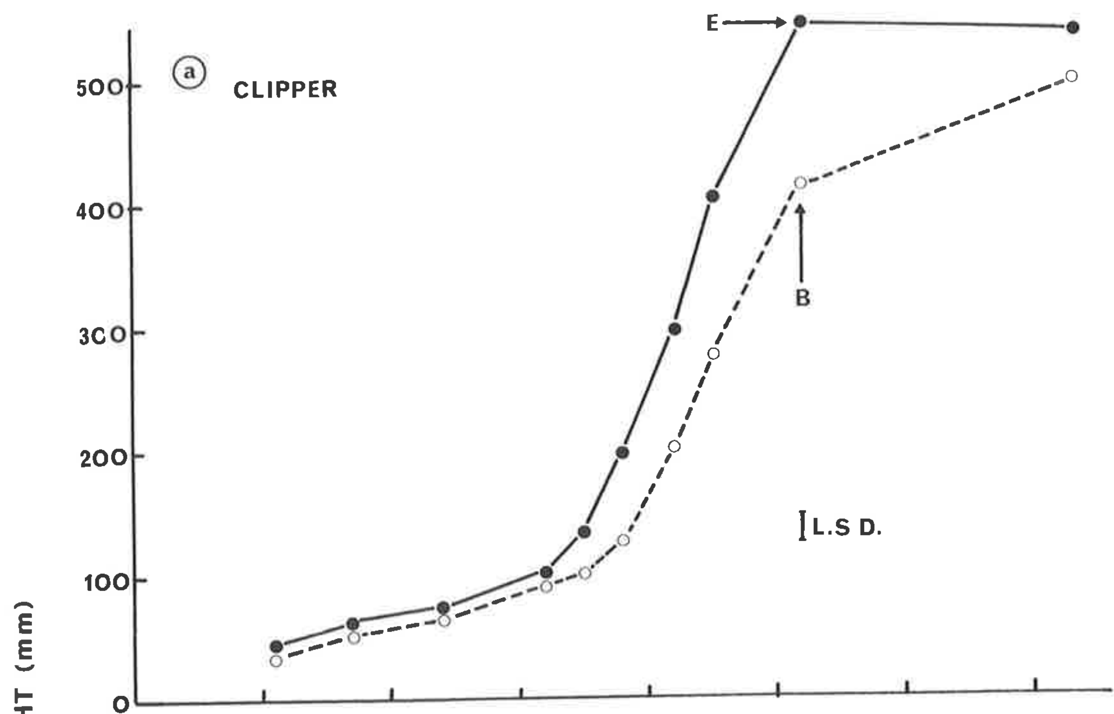
●——● Uninoculated

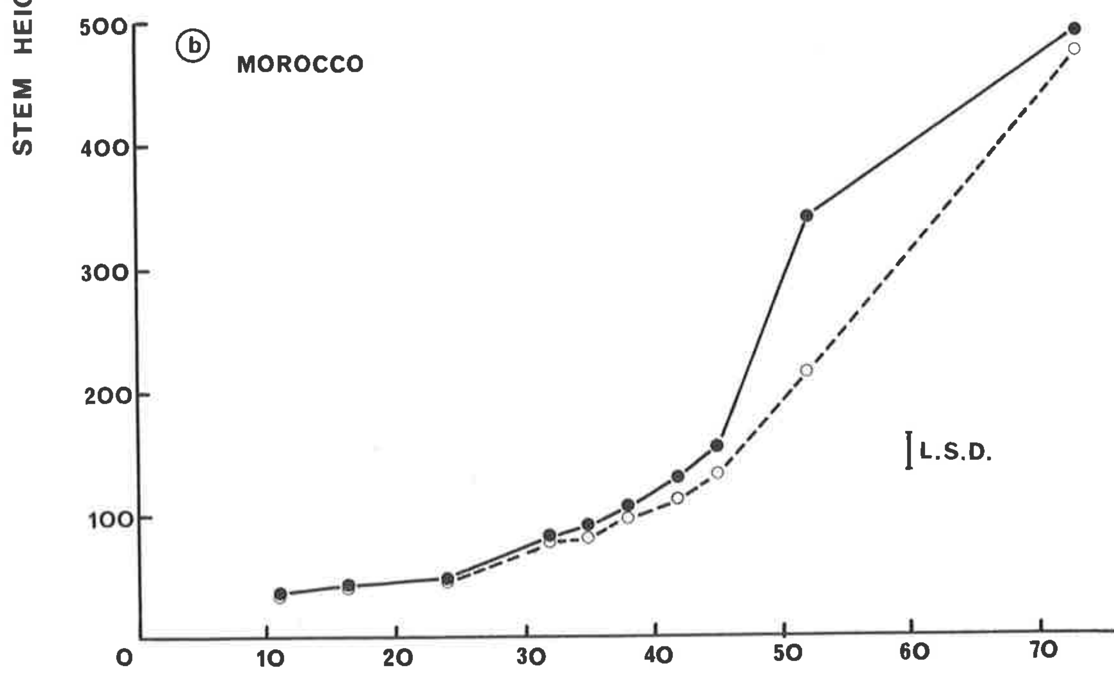
o----o Inoculated

B - the 'boot' stage of plant growth

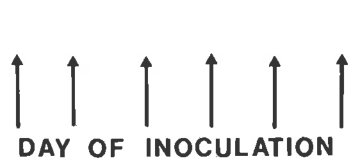
E - the emergence of the inflorescence from the flag leaf sheath.

L.S.D. (P = 0.05)





DAYS FROM SOWING



inflorescence could affect the total number of spikelets. The total number of spikelets (Table 24) was reduced proportionally between the

TABLE 24.

EFFECT OF *H. AVENAE* ON NUMBER OF SPIKELETS PER INFLORESCENCE OF WHEAT (CVS. HALBERD, AUS 10894) AND BARLEY (CVS. CLIPPER, MOROCCO).

	Number of Spikelets			
	Total		Fertile	
	Control	Inoculated	Control	Inoculated
Halberd	15.0	12.6	12.0	8.5
AUS 10894	13.4	11.6	10.6	9.2
L.S.D. (P = 0.05)	= 1.2		= 1.2	
Clipper	11.6	10.0	6.6	5.6
Morocco	17.8	16.6	11.2	10.2
L.S.D. (P = 0.05)	= 1.7		= 1.5	

two cultivars of wheat (cvs. Halberd & AUS 10894) following inoculation and although the number of fertile spikelets was also reduced in both cultivars by inoculation, AUS 10894 was less affected than Halberd. Total number and number of fertile spikelets on barley (cvs. Clipper & Morocco) were not significantly affected by inoculation, but the inoculated plants tended to have fewer spikelets and perhaps the effect of inoculation on the number of spikelets was masked by some other factor (e.g. nutrients) affecting growth of uninoculated barley.

Due to the number of larvae used in inocula, competition between larvae reduced the number of females which developed on the susceptible cultivars. Susceptible wheat (cv. Halberd) averaged 31 females and susceptible barley (cv. Clipper) averaged 21 females per plant, and these

numbers were fewer than had been obtained previously (Section 3.1.2). Few females developed on resistant cultivars; resistant wheat (cv. AUS 10894) averaged 2 females and resistant barley (cv. Morocco) averaged 1 female per plant.

4.2.2 Field Study

Because the plots of Halberd and AUS 10894 grown in 1974 were separated by paths without plants and surrounded by medic (Fig. 10), the opportunity was taken to compare the effect of these additional sites on the growth and grain yield of Halberd in 1975 with that of Halberd and AUS 10894. The effects of Halberd and AUS 10894 were statistically analysed and then the four treatments were analysed together. Both analyses gave similar results, therefore, the complete analysis is presented in Tables 25-27.

The number of plants and incidence of fungal disease on roots of these plants collected at the 'dough' stage of plant growth were similar for each of the areas, as were both the fresh weight of roots and number of female nematodes per sample because high residual variance within areas prevented the analyses showing any significant difference (Table 25). Root weight in grams varied from 6.9 to 14.3, 8.9 to 10.8, 9.2 to 23.5 and 7.1 to 17.0 while the number of females varied from 648 to 1082, 652 to 743, 443 to 1897 and 670 to 1499 for the areas of Halberd, AUS 10894, fallow and medic during 1974 respectively. However, when both of these parameters were combined to give the number of females per gram of root, differences occurred between the treatments and showed that the management in 1974 affected the initial density of nematodes in soil in 1975, with the highest densities occurring after growth of Halberd and the lowest densities occurring after fallow. AUS 10894 and medic had similar effects on the density of nematodes and these densities

TABLE 25.

EFFECT OF FIELD MANAGEMENT OF *H. AVENAE* DURING 1974 ON VARIOUS PARAMETERS OF HALBERD AT THE 'DOUGH' STAGE OF GROWTH DEVELOPMENT DURING 1975.

	Management in 1974				
	Halberd	AUS 10894	Fallow	Medic	L.S.D. (P = 0.05)
No. of plants	12.8	12.4	13.2	11.8	N.S.
Root disease*	0.16	0.14	0.10	0.12	N.S.
Fw.** of roots (g.)	8.4	9.4	13.9	12.2	N.S.
No. of females	877	700	792	970	N.S.
No. females per g. of root	107	76	58	81	17.5

* Root disease - expressed as the fraction of plants per sample with disease

** Fresh weight.

were intermediate to those for Halberd and fallow.

A comparison of the effects of AUS 10894 and Halberd during 1974 on growth of Halberd in 1975 showed that the height, number of tillers and total number of spikelets were similar, but the length of flag leaf and number of fertile spikelets were increased following AUS 10894 (Table 26). When fallow was compared with Halberd, the number of tillers were similar, but fallowing increased all the other aspects of growth that were measured. The effect of medic on growth of Halberd was similar to fallow and both increased the length of flag leaf and number of fertile spikelets more than AUS 10894.

The components of grain yield of Halberd were measured at maturity (Table 27). Weight of 1,000 grains was similar following AUS 10894 and Halberd, but the total grain weight per harvest and grain weight per tiller were increased following AUS 10894 because the number

TABLE 26.

EFFECT OF FIELD MANAGEMENT OF H. AVENAE DURING 1974 ON PARAMETERS OF TILLER GROWTH OF HALBERD AT THE 'DOUGH' STAGE OF GRAIN DEVELOPMENT DURING 1975.

	Management in 1974				
	Halberd	AUS 10894	Fallow	Medic	L.S.D. (P = 0.05)
Height (mm.)	638	686	735	731	52
Length of flag leaf					
Lamina (mm.)	117	142	176	166	23
Lamina & sheath (mm.)	245	286	338	325	36
No. of tillers	1.0	1.1	1.3	1.3	N.S.
No. of spikelets					
Total	13.5	14.1	14.6	14.9	0.9
Fertile	9.1	10.4	12.0	12.0	0.9

TABLE 27.

EFFECT OF FIELD MANAGEMENT OF H. AVENAE DURING 1974 ON COMPONENTS OF GRAIN YIELD OF HALBERD AT MATURITY DURING 1975.

	Management in 1974				
	Halberd	AUS 10894	Fallow	Medic	L.S.D. (P = 0.05)
Grain weight (g.)					
Total	79	103	150	139	23
per Tiller	0.47	0.58	0.74	0.72	0.10
per 1,000 grains	33	34	38	39	2
No. grain/tiller	14.0	17.0	19.3	18.5	2.1

of grains per tiller was increased. Fallow and medic increased all components of yield over that obtained following AUS 10894 and Halberd with the exception of an intermediate effect of medic to fallow and AUS 10894 on the number of grains per tiller.

4.3 Discussion

Serial inoculations of Halberd with *H. avenae* until after floral initiation affected vegetative growth and the number of spikelets on the spike. These effects on plant growth can be explained by the reduced supply of metabolites and nutrients within the plant following invasion by nematodes.

Growth of the first four leaves of wheat, although dependent to some extent on the carbohydrate and nutrient reserves of the endosperm (Williams, 1960), was reduced on Halberd by the inoculation with nematodes. If larvae secrete either a growth substance or precursor (Krusberg, 1963; Johnson & Viglierchio 1969 b) when establishing and forming syncytia (Johnson & Fushtey, 1966), then they may disrupt the gradients of growth substances within plants and have a direct influence on plant growth. Therefore, an effect additional to that of nematodes on root growth and function may be involved during the early growth of plants. This aspect needs further examination.

The longest leaf on a cereal plant was often the one emerging at the initiation of floral development (Borrill, 1959) and the transfer of apical dominance from the leaf to spikelet development progressively reduced the rate of early growth of leaves, i.e., before emergence, because of reduced nutrient supply to the leaves (Williams, 1960). Therefore, as the growth of the longest leaf was similar for Halberd with and without nematodes, but emergence of the following leaves was progressively delayed and fewer leaves were developed on plants with

nematodes, nematodes have not affected the time of floral initiation, but they have reduced the rate of differentiation of leaves before floral initiation and have reduced the rate of growth of leaves before their emergence following floral initiation. These effects were similar to those of nutrient supplies on the number of leaves and leaf area (Single, 1964; Langer, 1966) and this has suggested nematodes affected plant growth by reducing the supply of nutrients. Floral initiation and stem growth of Halberd have also been affected in a similar way by nematodes as the number of spikelets per spike were reduced and the rate of early growth of the stem was reduced and this delayed the period of rapid elongation, delayed emergence of the spike and reduced plant height.

With AUS 10894, nematodes had little effect on the rate of growth of leaves and stem, but the reduced leaf area resulting from the reduction in the size of mature leaves in infected plants was probably the cause of fewer spikelets being initiated (Davidson, 1965; Puckridge, 1968). Although nematodes had similar effects on the root growth of AUS 10894 and Halberd, growth of the stem and the number of spikelets were not so severely affected and therefore, AUS 10894 seemed more tolerant to nematodes than Halberd.

Apart from the nematodes having no effect on the number of leaves and spikelets, growth of susceptible barley (cv. Clipper) inoculated with nematodes was similar to Halberd. Vegetative growth of resistant barley (cv. Morocco) inoculated with nematodes was not affected before floral initiation, but the rate of growth of leaves before emergence was decreased after floral initiation and emergence of leaves was delayed. This later effect of nematodes on growth of Morocco explained the reduced effect of nematodes on the top weight of the mature plants. No clear effect of nematodes on the number of spikelets was obtained for either of the barley cultivars. This may be the result of compensatory growth in

the inoculated plants during the indeterminate growth of the spike, but as the root weight of mature plants was not affected by nematodes, a restriction on the growth of roots of uninoculated plants by the growth container may have confounded any reduction in spikelet number due to infection with nematodes.

Although nematodes have affected vegetative and reproductive growth of cereals grown in a controlled environment by restricting nutrient supply, this needed confirmation in a field situation to assess the likely effect of nematodes on grain yield in relation to other environmental factors. Areas of different initial densities of *H. avenae* were developed in the field by growing susceptible (cv. Halberd) and resistant (cv. AUS 10894) cultivars of wheat. Numbers of nematodes infecting Halberd grown within these areas during the following season were assessed from the number of female nematodes per gram of fresh root. This assessment of nematode density was more meaningful than cysts or eggs per unit weight of soil because it assessed the actual number of larvae hatching and invading plants, thereby reducing some of the uncertainty about the number of larvae hatched from cysts or eggs.

When Halberd followed Halberd, more nematodes infected the plants and smaller flag leaves were produced by the plants than when Halberd followed AUS 10894. It was also likely that the increase in nematodes infecting the plants reduced the size of most of the leaves, lowered the leaf area index and the capacity of plants to utilise light, which could be a cause of the reduction in both the number of spikelets and fertile spikelets, and grains per spikelet (Davidson, 1965; Aitken, 1966; Frankel & Roskams, 1975). But grain weight was not affected in Halberd, therefore the reduction in grain yield was due to the effects of environmental factors on the early growth of plants, and the number of nematodes infecting plants was the main difference between the two sites.

Therefore, growing the resistant wheat (cv. AUS 10894) lowered the number of nematodes infecting plants in the following season and this increased grain yield of Halberd.

Fallow, by increasing moisture and nutrients available to the plants, and medic, by increasing the nitrogen content of soils, improved the soil environment for the growth of Halberd in the following season over that obtained by growing Halberd or AUS 10894. Leaf area index and all the components of grain yield measured for Halberd following fallow and medic were increased over that for Halberd following Halberd by both changing the soil environment and reducing the density of nematodes in the soil. A similar number of nematodes invaded Halberd following medic and AUS 10894, but fewer nematodes invaded following fallow. However, similar growth and grain yield of Halberd occurred following fallow and medic but, apart from total spikelets per spike, growth and yield of Halberd following AUS 10894 were poorer. Therefore, an interaction between nematode density and environment, especially nutrient supply, has occurred and these field results have supported the conclusions of the growth room study that H. avenae affects plant growth by affecting nutrient and moisture supply.

Seasonal rainfall during 1975 was above average at Windsor, especially in the spring (September to November), while the synoptic study by Stynes (1975) from which models of factors affecting growth of wheat in South Australia were calculated, was done during a season of below average rainfall. Due to the complexity of the relationship between factors of the environment (including disease organisms) and grain yield of cereals, the relative importance of any factor would change from season to season. Therefore, further synoptic studies are needed to assess the importance of H. avenae over a wide range of seasonal conditions, but additional aspects of plant growth and yield to those used by Stynes (1975) should be

included.

From the information available at this time, the use of resistant cultivars within the normal agricultural practice of rotation, which includes a season of pasture, would reduce nematode densities, increase nutrient supply and therefore increase grain yields.

5. MECHANISM OF RESISTANCE TO *H. AVENAE* IN WHEAT

Biotypes of *H. avenae* capable of breaking resistance in cereals have been reported in Europe (Andersen, 1959; Cotten, 1963; Kort *et al.*, 1964; Neubert, 1966) and although there may be only one biotype in Australia (Brown, R., 1969), a few female nematodes usually develop on the resistant cultivars of wheat (O'Brien & Fisher, 1974); these may represent a different biotype. If this is so, using resistant cultivars commercially would temporarily control the nematode, increase the proportion of resistant breaking biotypes and eventually resistance would be lost.

Because of the limited time available to investigate the probability of more than a single biotype occurring within a nematode population, this aspect was examined by investigating the mechanism of resistance in the laboratory, growth room and the field rather than by investigating the continual growth of the resistant cultivars on the same nematode population. This section describes these studies on some aspects of the mechanism of resistance of wheat to *H. avenae* and the results are discussed in relation to both previous studies and the possibility of resistance breaking biotypes.

5.1 Materials and Methods

Twelve experiments are described in this section and, unless otherwise stated, the tips of the first seminal roots of pregerminated seeds were covered with sand for inoculation with larvae in 0.15% distilled water agar as described in Section 2.1.1.1. Also, the number of nematodes other than males and females within roots was counted by the method for counting the number of larvae penetrating roots (Section 2.1.1.1), and the number of adult nematodes was counted as described for females (Section 3.1.1.1) and males (Section 3.2.1).

To examine the number of larvae of *H. avenae* penetrating roots of susceptible (cv. Halberd) and resistant (cvs. AUS 10894 & AUS 90248) wheat, seedlings were inoculated in petri dishes with 17($\pm$1), 31($\pm$3), 63($\pm$3), 116($\pm$2), 216($\pm$11), 429($\pm$17), 560($\pm$19) or 777($\pm$10) larvae ($\pm$S.E.), incubated at 15°C for 24 hours and then the number of larvae within the inoculated roots was counted. One pregerminated seed of each cultivar was placed in a petri dish and the 3 cultivars in a dish were inoculated with the same density of larvae. Analyses of variance were claculated on the 8 randomised replicates of each density of inoculum.

The number of nematodes penetrating, establishing and developing in roots of Halberd, AUS 10894 and AUS 90248 were compared following inoculations with 33($\pm$3), 123($\pm$8) or 398($\pm$12) larvae and, Halberd and AUS 10894 were also compared following an inoculation of 792($\pm$31) larvae. One seedling of each cultivar was placed in a petri dish and inoculated with the same density of larvae, therefore most dishes contained 3 seedlings but at the highest density each dish contained 2 seedlings. Twelve replicates were randomised and incubated at 15°C for 18 hours then 20°C for 18 hours before 6 of the replicates were used to count the number penetrated. Seedlings in the other 6 replicates were washed and transplanted to 120 mls. of Hoaglands solution (Section 3.1.1.1) in polystyrene sample jars of 45 mm. in diameter and 100 mm. high, and were covered with brown paper to exclude light. These seedlings were grown in a growth room (Section 3.1.1.1) and the number of larvae which emerged from the roots during the first 7 days was counted by exchanging the nutrient solution, and counting the number of larvae within the original solution. The seedlings continued growth in the growth room until 20 days after incubation when both the number of established nematodes and the number of nematodes which had developed beyond the second stage were counted before any males emerged (Trudgill, 1967;

O'Brien, 1972). Analyses of variance were done on these results.

Further information on the number of larvae establishing and developing, plus the number becoming female in roots of Halberd, AUS 10894 and AUS 90248 was obtained by inoculating the roots with 5, 10, 20, 50, 100 or 300 larvae in petri dishes as described above, but incubation was at 15°C for 24 hours before transplanting the seedlings to growth tubes of sand (Section 3.1.1.1). Because a smaller number of larvae was expected to invade roots with the low than the high densities of inoculum, fluctuations of only one or two nematodes at the lowest densities would cause a greater proportional variation than a similar number at the highest densities. This effect was minimised within the limited space of a growth room by inoculating groups of 10, 5, 4, 2, 2 and one seedling for densities of 5, 10, 20, 50, 100 and 300 larvae respectively. Growth tubes were arranged in a randomised block design of 10 blocks within the growth room, and 5 of the blocks were sampled 20 days after incubation to count both the number of established nematodes and number developed beyond the second stage of growth, while the remaining seedlings continued growth until 35 days after incubation when the number of females per inoculated root was counted. All results obtained were transformed to the equivalent number of nematodes per 10 inoculated roots and analyses of variance were on these results.

Because of the experimental design with an unequal number of plants at different densities, the previous experiment was repeated using an equal number of plants. The number of larvae penetrating, establishing, developing and becoming females were assessed for roots of Halberd, AUS 10894 and AUS 90248 inoculated with 7(±1), 12±1), 25(±1), 56(±4), 81(±3) and 110(±7) larvae, then incubated at 20°C for 36 hours before the seedlings were grown in either sand or water culture. Following incubation, 5 replicates of each density were used to count the number of

larvae penetrating roots, 8 replicates of densities of 25, 56, 81 and 110 larvae were grown in water culture for 20 days before counting both the number of established larvae in roots and the number of these larvae which had begun development, and 8 replicates of all the densities were grown in growth tubes with sand for 36 days before counting the number of females. Materials and methods were as described previously and analyses of variance were done on the results.

To examine the number of males and females on Halberd and AUS 10894, seedlings were germinated and inoculated in growth tubes (Section 3.1.1.1) at one time with 500 larvae at sowing, or two times with 500 larvae at sowing and 750, 1500, 3000 or 6000 larvae 12 days after sowing. Seedlings were grown in the growth room (Section 3.1.1.1) for 42 days (i.e. 30 days after the second inoculation), when the number of males and females were counted in the 5 replicates distributed at random on a tray. Analyses of variance were done on these results.

A further examination of male and female nematodes was made on susceptible (cv. Halberd) and resistant (cv. AUS 10894) wheat and on susceptible (cv. Clipper) and resistant (cv. Morocco) barley. Seeds were germinated, sown, inoculated and maintained in a growth room as described above, but the inoculations of 5 replicates were with 332($\pm$6) larvae at sowing, or 504 ($\pm$7) larvae 10 days after sowing or 332($\pm$6) larvae at sowing and 504($\pm$7) larvae 10 days after sowing. The number of females and males were counted 30 days after the second inoculation and analyses of variance were done on these results.

To compare the effect of Halberd and AUS 10894 on _H. avenae_ under field conditions, the plants grown at Windsor during 1974 were examined (Section 4.1). Ten plants per cultivar, with at least 3 from each of the 3 plots sown, were randomly collected 22 (July 4), 39 (July 21) and 63 (August 14) days after sowing by placing an auger, which gave a

core of both the soil and associated roots for each plant of 6.5 cm. diameter by 15 cm. deep, over each plant. Soil was washed from the roots, and the roots were weighed (Section 3.1.1.1) before the number of galls and number of both second-stage and developing larvae of H. avenae, with the number of foreign nematodes within roots, were counted. Analyses of variance were done on these results.

The effects of resistant wheat (cv. AUS 10894) on development of H. avenae during the experiments already described in this section suggested an effect of the initial infections of H. avenae on penetration and establishment of larvae inoculated onto roots after the initial inoculation. Therefore 5 experiments were done on seedlings in petri dishes to examine this effect further.

To examine the effect of duration of invasion of an initial inoculation with H. avenae on the penetration of larvae from a second inoculation on the same root, the first seminal root of Halberd and AUS 10894 was inoculated and incubated at 20°C. One seedling of each cultivar was placed in each petri dish and treated the same way. For the single inoculations, enough seedlings were inoculated with 297(±7) larvae to give 5 replicates of each duration of 4 or 10 hours incubation before the penetrated larvae were counted, or 5 replicates were inoculated with 70(±4) larvae and incubated for 12 hours before counting. For the double inoculation, seedlings were inoculated with 297(±7) larvae and after 4 or 10 hrs, the second inoculation of 70(±4) larvae was done and the number of penetrated larvae in 10 replicates was counted after a further 12 hours incubation. Analyses of variance were carried out.

A further examination of the effect of an initial inoculation of H. avenae on the penetration of larvae from a second inoculation onto the same root of Halberd and AUS 10894, plus the effect on the establishment

of larvae was done. The procedure was similar to that described above except that there was only one duration of incubation of the first inoculation; whenever seedlings were harvested for counting the number of penetrated larvae, an equal number of seedlings were placed in a Seinhorst mistifier for 4 days to determine the number of larvae which became established, and there were 10 replicates of all treatments. As controls, a single inoculation of 338($\pm$15) larvae was left in association for 8 hours with the roots, which were then washed to remove the larvae external to the roots and the seedlings were placed on fresh agar for 4 or 16 hours before the number of larvae penetrated and established were determined. As a control for the second inoculation, 96($\pm$5) larvae were added 12 hours after the start of the experiment, i.e., fresh seedlings were inoculated when the first plants were harvested for counting, and the number of penetrated and established larvae were determined 12 hours later. For the double inoculation, both procedures were combined, i.e., seedlings were inoculated with 338($\pm$15) larvae for 8 hours, then they were washed and placed on fresh agar for 4 hours before the second inoculation of 96($\pm$5) larvae was added and the number of penetrated and established larvae in the roots were counted 12 hours later. Analyses of variance were done on the results.

To examine the effect of inoculating the first seminal root of Halberd and AUS 10894 on a later inoculation of the second or third root, the first root was inoculated with 338($\pm$15) larvae and following 13 hours incubation at 20°C, the roots were washed, transferred to new agar and either the second or third root was inoculated with 78($\pm$3) larvae. Extension of (Section 2.1.1.1), and both the number of penetrated and established larvae in, the inoculated second or third root were measured after 24 hours incubation at 20°C. The number of established larvae was counted following 4 days in a Seinhorst mistifier. As the controls, the

first root only, or either the second or third root only, or none of the roots on a seedling were inoculated with the same density and incubated for the same duration as the equivalent roots when the seedlings were inoculated at two times. Extension of the second or third root, and both the number of penetrated and established larvae in all the inoculated roots were measured. One seedling of each cultivar was placed in a petri dish as described previously and there were 10 randomised replications of each treatment. Analyses of variance were done on the results.

The effect of time of inoculation of the first three seminal roots of AUS 10894 on extension of, and both the penetration and establishment of larvae in the fourth or fifth seminal root was examined. Each of the first three roots were inoculated before the fourth and fifth roots had emerged (0 days) with 209($\pm$8) larvae, or when the fourth and fifth root were approximately 1 mm. long (2 days) with 195($\pm$6) larvae, or when they were approximately 10 mm. long (3 days) with 204($\pm$12) larvae. Invasion of larvae into the first three roots was terminated after 24 hours by washing the larvae off the roots with water and the seedlings were transferred to fresh agar. The fourth or fifth root of each seedling was inoculated on day 4 with 100($\pm$9) larvae and invasion was terminated 24 hours later when the extension of, and both the number of penetrated and established larvae in these roots were measured as described above. The number of established larvae in the uninoculated fourth or fifth root was also counted and as controls, the extension of the fourth or fifth root on uninoculated seedlings and the number of larvae penetrated when the fourth or fifth root was the only root inoculated on a seedling, were measured at the same time as the measurements on seedlings inoculated at two times. Seedlings were incubated at 20°C and 8 replicates were used for each treatment. Analyses of variance were done on these results.

A comparison was then made of the effect of inoculation of each

of the first three seminal roots of AUS 10894 and Halberd with 24($\pm$3) larvae on the reaction of the fourth or fifth root to inoculation with 16($\pm$1) larvae. The first three roots were inoculated before the next pair of roots were visible and one of this next pair was then inoculated when they had grown onto the agar surface. On both occasions, invasion was terminated 24 hours after inoculation as described previously. Incubation was at 20°C throughout the experiment. Eight replicates were used for both the seedlings inoculated at two times and the control seedlings with the fourth or fifth root only inoculated. Extension of each of the first three roots, with and without nematodes, and of the one inoculated root of the next pair was measured as described above. Then the number of larvae in each of the first three inoculated roots and in the one inoculated root of the next pair were counted following the termination of invasion, while the other uninoculated root of the next pair was also examined for larvae as a check on possible contamination by larvae from the first inoculation. Analyses of variance were done on these results.

Because of the effects of the different methods of inoculating Halberd and AUS 10894 on the establishment and development of _H. avenae_, a further examination was made of the effect of _H. avenae_ on the growth of the two cultivars. Seedlings were germinated and the seedlings inoculated in growth tubes (Section 3.1.1.1) with 425($\pm$11), 532($\pm$20) and 786($\pm$30) larvae 0, 5 and 15 days after sowing respectively, were compared with the uninoculated seedlings. The number of females was counted and both the wet roots (Section 3.1.1.1) and fresh stems were weighed 50 days after sowing for the 5 replicates distributed at random on a tray. A 'Students' t test was done to compare each result of the inoculated and uninoculated control plants of each cultivar.

5.2 Results

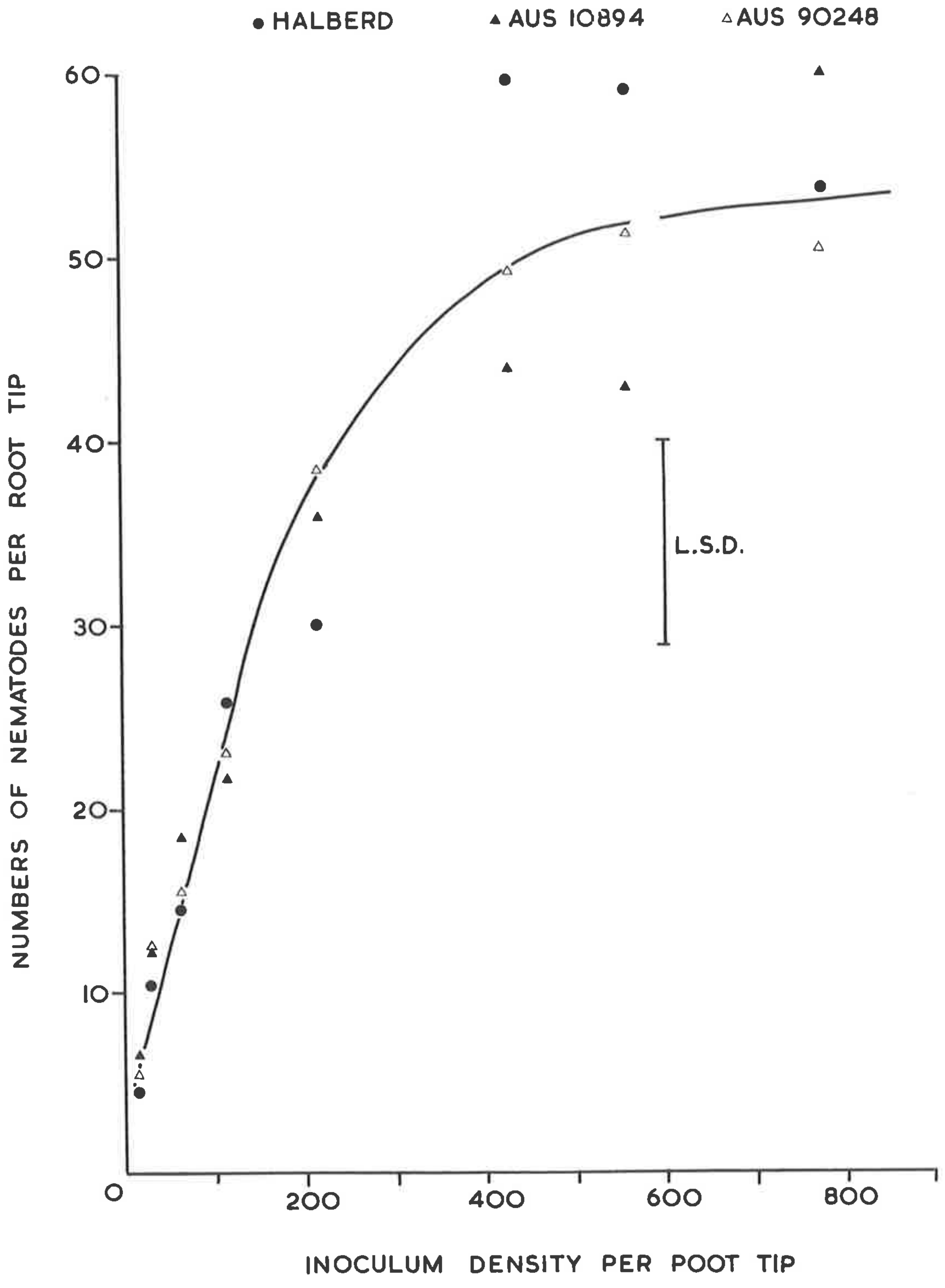
The numbers of larvae penetrating susceptible (cv. Halberd) and resistant (cvs. AUS 10894 & AUS 90248) wheat during 24 hours showed increased variation within cultivars as the density of inoculum increased. Therefore, statistical analyses were on treatments inoculated with less than or with more than 300 larvae and on the combined treatments. A similar number penetrated the different cultivars at each density of inoculum (Fig. 18) and a phase of linear increase was followed by a phase of a constant number penetrating as the density of inoculum increased.

Three separate experiments were done to compare the effects of density of inoculum on the establishment and development of larvae in susceptible (cv. Halberd) and resistant (cvs. AUS 10894 & AUS 90248) wheat. In the first experiment, the number of larvae penetrating during 1½ days (Table 28) and leaving over the following 7 days were similar for the three cultivars, and both of these numbers of larvae increased with increasing density of inoculation. After 18 days, a similar number of nematodes established in the roots of each cultivar at the lowest density of inoculation, but fewer established in the roots of the resistant cultivars at the higher densities. The number of established nematodes increased up to an inoculum density of 123 larvae and then remained constant in Halberd, but in the resistant cultivars the increase continued to an inoculum density of 398 larvae. At the lowest density of inoculum, the number of larvae which developed was similar in all cultivars, but fewer developed in the resistant cultivars than in Halberd at the higher densities, and fewer tended to develop in AUS 10894 than AUS 90248 at an inoculum density of 398 larvae. In Halberd and AUS 90248, the number developing increased with density up to an inoculation of 398 larvae, but this increase in AUS 10894 was only up to a density of 123 larvae.

Figure 18. Effect of density of *H. avenae* on number of larvae penetrating roots of susceptible (cv. Halberd) and resistant (cvs. AUS 10894 & AUS 90248) wheat.

L.S.D. (P = 0.05) of all treatments

Analysis of numbers of larvae penetrating roots with densities greater than 300 larvae showed no significant difference between cultivars.



HALBERD
AUS 10894
AUS 90248
60
50
40
30
20
10
0
200
400
600
800
NUMBERS OF NEMATODES PER ROOT TIP
INOCULUM DENSITY PER POOT TIP
L.S.D.

Table 28.

Effect of density of H. avenae on number of larvae penetrating, establishing and developing in Halberd, AUS 10894 and AUS 90248.

	No. of Larvae in inoculum			
	33	123	398	792
1½ days after inoculation:	No. of larvae penetrated			
Halberd	7	28	49	53
AUS 10894	8	25	43	57
AUS 90248	6	20	46	-
	L.S.D. (P = 0.05) = 12			
7 days after inoculation:	No. of larvae emerged			
Halberd	1	5	19	31
AUS 10894	2	6	20	34
AUS 90248	1	6	20	-
	L.S.D. (P = 0.05) = 9			
18 days after inoculation:	No. of nematodes established			
Halberd	5	21	24	24
AUS 10894	5	12	17	18
AUS 90248	6	12	17	-
	L.S.D. (P = 0.05) = 5			
18 days after inoculation:	No. of nematodes developing			
Halberd	4	12	16	16
AUS 10894	4	9	8	8
AUS 90248	4	8	11	-
	L.S.D. (P = 0.05) = 3			

In the second experiment, a narrower range of densities of inoculation was used and the number of females was counted. The number of larvae which established or developed showed the same trends with increase in density of inoculum (Fig. 19 a, b), a phase of linear increase in the number of nematodes followed by a plateau phase in which the number remained constant. Each cultivar was similar with respect to the number established or developed at each density of inoculation. At low densities, most of the larvae which established were able to develop but as density of inoculum increased, a smaller proportion of the established larvae were able to develop until the number of established larvae was constant. The number of females also showed a linear and plateau phase with increasing density of inoculation (Fig. 19 c), but in AUS 10894, the number decreased at the highest density. The change from the linear to the plateau phase of the number of females was at a lower density than that for the number of developing nematodes. Although a similar number of females developed on the three cultivars at the lowest densities, fewer developed on AUS 90248 than Halberd and even fewer developed on AUS 10894 than AUS 90248 as the density of inoculation increased.

In the third experiment, the same pattern of penetration of larvae was obtained (Fig. 20), a linear increase in the number penetrated, which was followed by a plateau phase as density of inoculation increased, and the number of penetrated larvae was similar in the three cultivars at each density. However, the plateau phase was reached at a much lower density of inoculum than in the previous experiment on penetration (Fig. 18). The number of established and developed nematodes in the three cultivars were similar at each density of inoculation, and although the number established and developed were similar at the low density (Fig. 20), more were established than developed at the higher densities. There was no significant change in the number of larvae established or developing as

Figure 19. Effect of density of *H. avenae* on number of established, developing and female nematodes in susceptible (cv. Halberd) and resistant (cvs. AUS 10894 & AUS 90248) wheat.

(a) Number of nematodes in roots 20 days after sowing.

(b) Number of nematodes beyond the second stage of development 20 days after sowing.

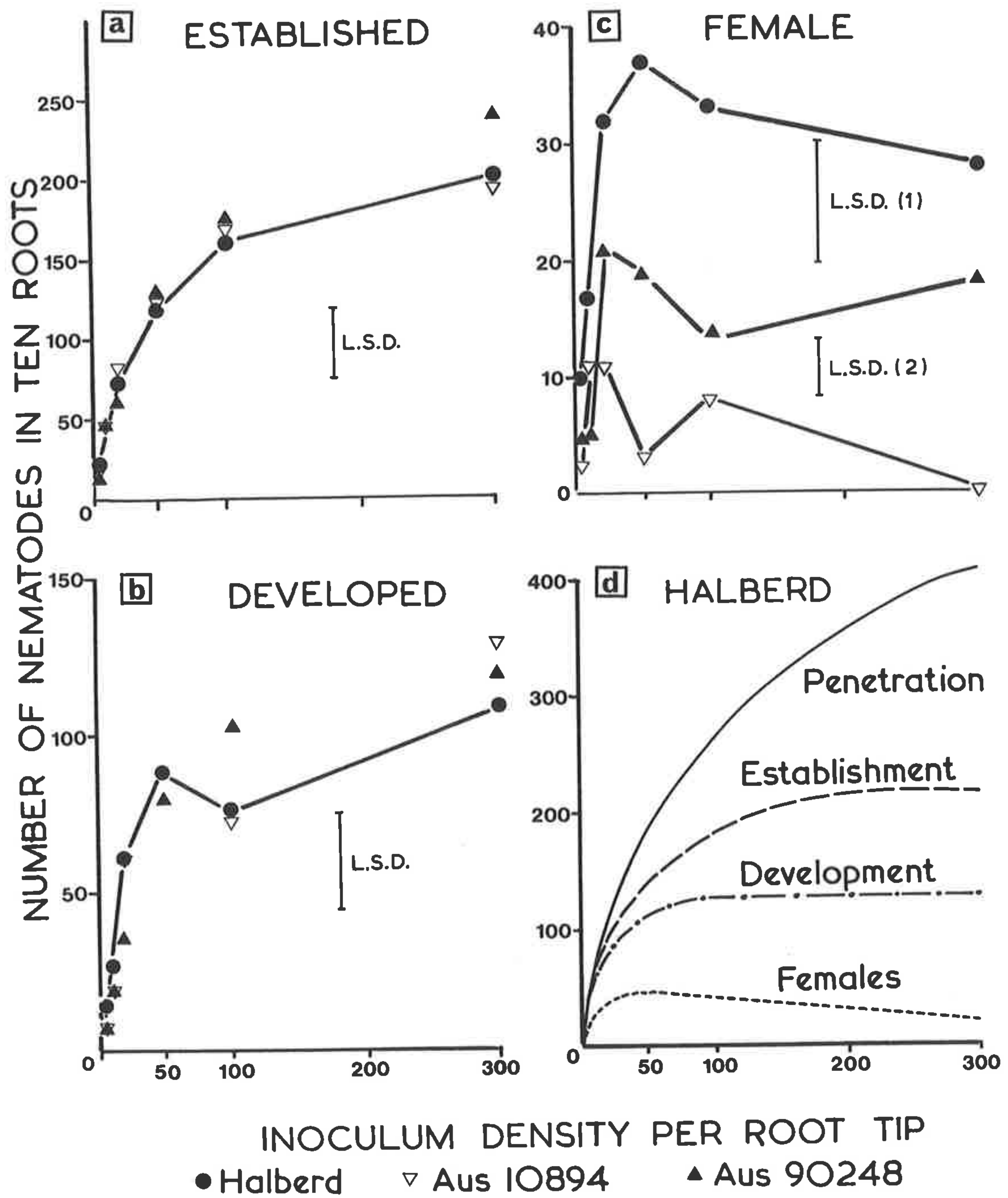
(c) Number of females 35 days after sowing.

I (1) L.S.D. following analysis of Halberd, AUS 10894 and AUS 90248.

I (2) L.S.D. following analysis of AUS 10894 and AUS 90248.

(d) Proposed relation between stages of nematode action in roots of Halberd.

L.S.D. (P = 0.05)



a
ESTABLISHED
250
200
150
100
50
0
L.S.D.
b
DEVELOPED
150
100
50
0
50
100
200
300
L.S.D.
c
FEMALE
40
30
20
10
0
L.S.D. (1)
L.S.D. (2)
d
HALBERD
400
300
200
100
0
50
100
200
300
Penetration
Establishment
Development
Females
NUMBER OF NEMATODES IN TEN ROOTS
INOCULUM DENSITY PER ROOT TIP
Halberd
Aus 10894
Aus 90248

Figure 20. Effect of density of *H. avenae* on penetration, establishment and development of larvae and female development in susceptible (cv. Halberd) and resistant (cvs. AUS 10894 & AUS 90248) wheat.

L.S.D. (P = 0.05)

(1) Analysis of the number of larvae penetrating roots.

(2) Analysis of the number of established and developed nematodes.

(3) Analysis of the number of females on the three cultivars.

(4) Analysis of the number of females on AUS 10894 and AUS 90248.

Penetration ●———●
Establishment ●— —●
Development ●—·—●
Females ●----●

200
150
100
50
0

NUMBER OF NEMATODES IN TEN ROOTS

L.S.D (1)
L.S.D. (2)
L.S.D. (3)
L.S.D. (4)

0
50
100

INOCULUM DENSITY PER ROOT TIP

● HALBERD ● ▲AUS 10894▲ △AUS 90248△

density of inoculum increased and these numbers were less than that obtained in the previous experiment (Fig. 19). However, the number of females and the pattern of production with increasing inoculum density (Fig. 20) were similar to the previous experiment (Fig. 19), except that the number of females on AUS 90248 was reduced to equal the number on AUS 10894 at the higher densities of inoculum. The number of females on Halberd at the two lowest densities of inoculum was approximately half that of the number of larvae penetrating (Fig. 20) and at the next highest density, were approximately half that of the number of developing larvae.

Two separate experiments examined the effect of different inoculations on the number of male and female nematodes developed on susceptible and resistant cultivars. In the first of these, the number of males and females on Halberd and AUS 10894 were compared. The number of females on Halberd increased initially when larvae were added 12 days after sowing (Table 29) but then remained constant while the number of

Table 29.

Effect of density of *H. avenae* on number of male and female nematodes on Halberd and AUS 10894.

No. of larvae in inoculum		No. of females		No. of males	
Day 0	Day 12	Halberd	AUS 10894	Halberd	AUS 10894
500	0	22	1	28	10
500	750	34	2	64	15
500	1,500	37	3	65	13
500	3,000	39	2	80	14
500	6,000	39	1	114	13
L.S.D. (P = 0.05)		= 11		= 31	

males not only increased initially but also continued to increase as the density of the second inoculation increased. With larvae added only at sowing, a similar number of males and females occurred in Halberd, but with a second inoculation, the male to female ratio gradually increased with the density of larvae. However, AUS 10894 had fewer males and females than Halberd and these numbers were similar in all the inoculation treatments.

In the other experiment, the number of males and females on susceptible and resistant cultivars of wheat and barley were compared. The number of males on the four cultivars, except between AUS 10894 and Morocco with the double inoculation, was similar within each of the inoculations (Table 30) and between the single inoculation 10 days after

Table 30.

Effect of time and density* of inoculation of *H. avenae* on number of adult nematodes on susceptible (cv. Halberd) and resistant (cv. AUS 10894) wheat, and susceptible (cv. Clipper) and resistant (cv. Morocco) barley.

* No. larvae in inoculum: 332 ± 6 on Day 0
504 ± 7 on Day 10

Inoculation	No. of females			No. of males		
(Days from sowing)	0	10	0&10	0	10	0&10
Halberd	14	3	21	23	50	58
AUS 10894	1	0	1	25	60	41
Clipper	14	1	19	22	50	59
Morocco	0	0	0	14	64	71
L.S.D. (P = 0.05)		= 5			= 23	

sowing and inoculation at both times, but fewer males developed following the single inoculation at sowing. There were no females on the resistant barley (cv. Morocco), very few on resistant wheat (cv. AUS 10894) with each inoculation and very few also on susceptible wheat (cv. Halberd) and susceptible barley (cv. Clipper) with the single inoculation 10 days after sowing. More females developed on both susceptible cultivars when inoculated twice than with either of the single inoculations, and the single inoculation at sowing produced more females than 10 days later. With each type of inoculation, the number of females on Halberd and Clipper was similar.

The effects of Halberd and AUS 10894 on H. avenae were compared under field conditions. At 22 days after sowing the total number of H. avenae in the roots was similar for both cultivars and all the nematodes were second stage larvae (Fig. 21). Following further growth of Halberd, the total number and number of developing nematodes increased and the number of second-stage larvae remained the same for at least 17 days before decreasing. However, with further growth of AUS 10894, the number of second-stage larvae decreased, the total number of nematodes tended to decrease and only a few of the nematodes began development. Root weight was similar in both cultivars (Table 31) until 64 days after

Table 31.

Effect of H. avenae on fresh root weight and number of galls on Halberd and AUS 10894 grown under field conditions.

	Fresh root weight (g)			No. galls/plant	
Days from sowing	22	40	64	22	64
Halberd	0.11	0.11	0.43	1.1	53
AUS 10894	0.11	0.16	0.31	0.2	3
L.S.D. (P = 0.05)		= 0.08		= 7	

Figure 21. Effect of growing susceptible (cv. Halberd) and resistant (cv. AUS 10894) wheat in the field on number of *H. avenae* in roots at different times after sowing.

L.S.D. (P = 0.05)

sowing when the roots of Halberd were heavier than AUS 10894. This increase in root weight was probably due to the increased number of galls induced by the nematodes on Halberd, as this was the only time when either cultivar showed a significant correlation coefficient ($r_{[8]} = 0.9814$) between the number of developing nematodes and root weight, and this was obtained with Halberd. All the seminal roots of Halberd were infected by nematodes several times but only the first three or four seminal roots appeared to be infected once on AUS 10894 and the other roots did not appear affected (Plate 4). The number of nematodes other than H. avenae was also counted, and although the number of these increased during the growth of plants, no significant difference occurred between the number on Halberd and AUS 10894.

Because of the appearance of the roots of AUS 10894 examined from the field, a further five laboratory experiments examined the effect of inoculation of AUS 10894 on the later penetration and establishment of larvae.

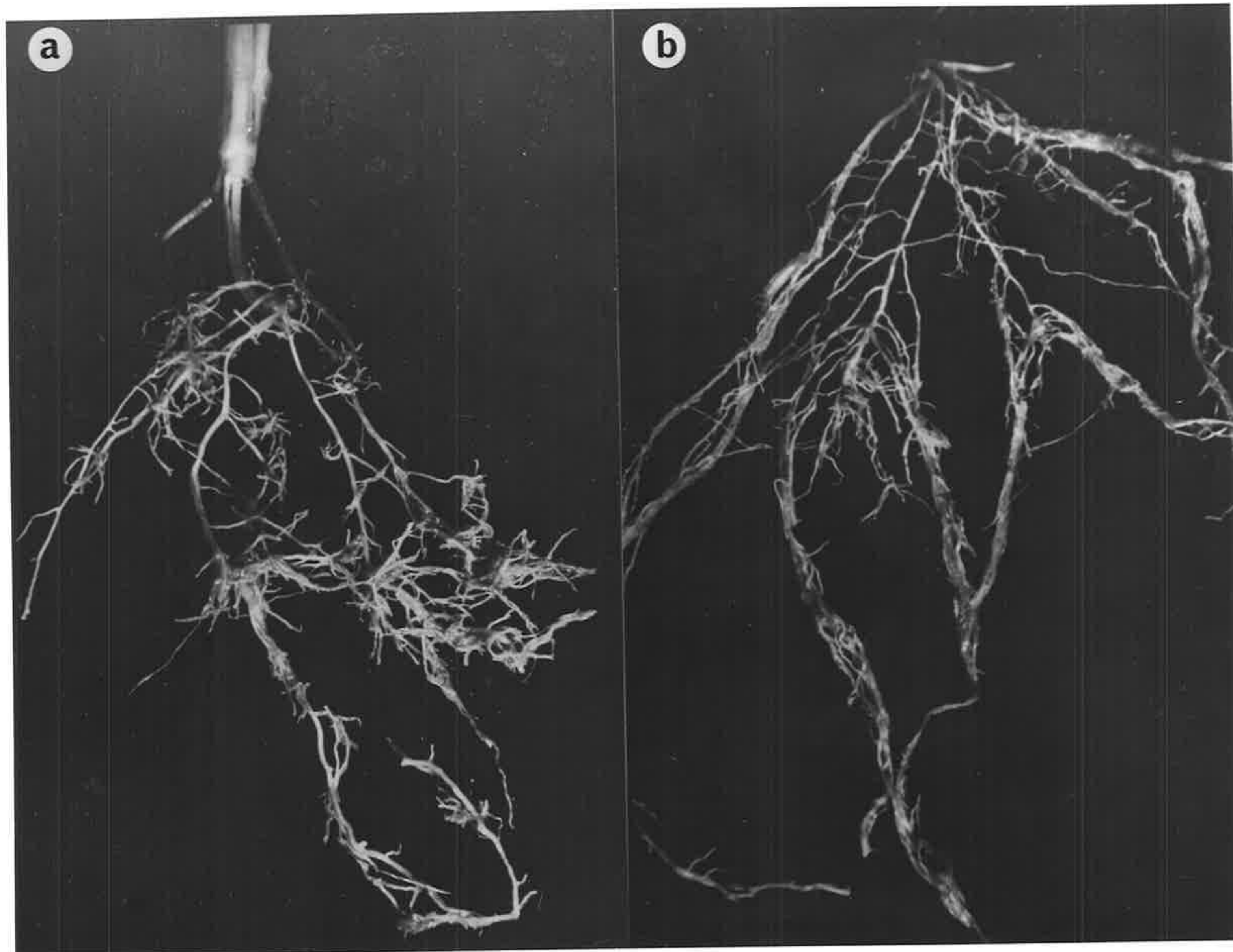
Two experiments examined the localised effect by inoculating the same root on two separate occasions. In the first experiment, there was no difference in the number of larvae penetrating the first seminal root of Halberd and AUS 10894 within each inoculation (Table 32). However, some effect of the first inoculation on penetration of larvae in the second inoculation showed, because with the double inoculations, the difference between the number of larvae which penetrated Halberd, when invasion of larvae in the first inoculation increased from 4 to 10 hours, was greater than that for AUS 10894.

In the second experiment there were no differences in the number of larvae penetrating or establishing in Halberd or AUS 10894 following inoculations at only one time (Table 33), but both numbers were greater in Halberd than AUS 10894 following the inoculation at two times. The number of larvae in roots 12 and 24 hours after starting the first

Plate 4. Infested seminal roots of susceptible and resistant wheats collected 63 days after sowing near Windsor during 1974.

(a) Susceptible - cv. Halberd

(b) Resistant - cv. AUS 10894.



a
b

Table 32.

Effect of density, duration and number of inoculations of *H. avenae* on penetration of larvae into the first seminal root of Halberd and AUS 10894.

INOCULATION 1					
- No. of larvae	296	296	0	296	296
- Duration (Hrs.)	4	10	0	4	10
INOCULATION 2					
- No. of larvae	0	0	70	70	70
Nos. of larvae in					
- Halberd	4	21	10	18	26
- AUS 10894	5	20	12	20	22
L.S.D. (P = 0.05)		= 6		= 5	

Table 33.

Effect of density, number of inoculation and duration after inoculation of *H. avenae* on penetration and establishment of larvae within the first seminal root of Halberd and AUS 10894.

INOCULATION 1				
No. of larvae	338	338	338	0
Duration (Hrs.)	12	24	0	0
INOCULATION 2				
No. of larvae	0	0	96	96
No. Penetrated				
Halberd	21	21	35	15
AUS 10894	19	20	23	17
	L.S.D. (P = 0.05) = 8			
No. Established				
Halberd	12	17	19	13
AUS 10894	8	12	12	8
	L.S.D. (P = 0.05) = 6			

inoculation were the same in both cultivars and similar to the number in AUS 10894 following inoculation at two times, but in Halberd, the number following inoculation at two times seemed to be the additive effect of the two single inoculations. Therefore, penetration of larvae in the second inoculation was inhibited in AUS 10894 but not in Halberd. No additive effect occurred with the number of larvae established and this number was not increased in either cultivar by the second inoculation, however, fewer larvae tended to be established 12 hours after the first inoculation than after 24 hours.

One of the experiments examined the effect of inoculating the first seminal root on growth of roots and both the penetration and establishment of larvae following inoculation of one of the next pair of roots. Extension of equivalent roots (Table 34) and both the number of

Table 34.

Effect of an initial inoculation of the first and a later inoculation of the second or third seminal root with *H. avenae* on extension (mm.) of the later inoculated root of Halberd and AUS 10894.

No. of larvae				
Seminal 1	0	338	338	0
Seminal 2 or 3	0	0	78	78
Root Extension (mm.)				
Halberd	18	16	4	5
AUS 10894	18	16	6	5

L.S.D. ($P = 0.05$) = 3

penetrated and established larvae in the roots (Table 35) were the same for both cultivars. There was no influence of the inoculated first seminal root on larvae invading one of the next pair of roots.

Table 35.

Effect of an initial inoculation of the first and a later inoculation of the second or third seminal root with *H. avenae* on penetration and establishment of larvae in the later inoculated root of Halberd and AUS 10894.

Nos. of larvae				
Seminal 1	0	338	0	338
Seminal 2 or 3	78	78	78	78
	No. Penetrated		No. Established	
Halberd	11	11	10	12
AUS 10894	10	12	9	9
			(N.S.)	

Therefore, two experiments examined the effect of inoculating the first three seminal roots on the fourth and fifth root. In the first experiment, there was no significant effect of delaying inoculation of the first three roots from before, at and after appearance of roots 4 and 5 on the extension of roots 4 and 5 following their inoculation (Table 36), but extension tended to be reduced with each delay. Both the number of penetrated and established larvae in either the fourth or fifth root increased with each delay in inoculation of the first three roots. However, increased cross contamination by larvae following each delay in inoculation of the first three seminal roots to roots 4 and 5 was shown by an increase in the number of established larvae in the uninoculated root 4, or 5, on a seedling.

In the second experiment, the first three seminal roots of both Halberd and AUS 10894 were inoculated before the appearance of roots 4 and 5. Root extension of, and the number of penetrated larvae in the first three seminal roots of Halberd and AUS 10894 were the same (Table 37) and

Table 36.

Effect of time of an initial inoculation of the first three and a later inoculation of the fourth or fifth seminal root with *H. avenae* on extension of, and penetration and establishment of larvae in the later inoculated root of AUS 10894.

						L.S.D. (P = 0.05)
INOCULATION 1 (Seminal 1, 2, 3) No. of larvae	0	0	209	195	204	
time (Days)	0	0	0	2	3	
INOCULATION 2 (Seminal 4 or 5) No. of larvae	0	100	100	100	100	
Root Extension (mm.)	18	5	5	3	2	3
Penetration (No.)		14	11	16	22	6
Establishment (No.) Inoculated root			6	7	9	2
Uninoculated root			1	3	4	

Table 37.

Effect of an initial inoculation of the first three and a later inoculation of the fourth or fifth seminal root with *H. avenae* on the extension (mm.) of, and penetration and establishment of larvae in, the inoculated roots of Halberd and AUS 10894

	Root 1,2,3		Root 4 or 5	
INOCULATION 1 (Seminal 1, 2, 3) No. of larvae	0	24	0	24
INOCULATION 2 (Seminal 4 or 5) No. of larvae	16	16	16	16
Root Extension (mm.) Halberd	18	3	7	7
AUS 10894	19	3	7	8
			(N.S.)	
Penetration (Nos.) Halberd		6	4	5
AUS 10894		6	5	5
			(N.S.)	

there was no difference between these three roots on a seedling. When root 4, or 5 was inoculated at a later time, root extension of, and the number of penetrated larvae were the same for both cultivars and was not affected by the earlier inoculation. There were no larvae in the uninoculated roots when they were examined.

When the effect of H. avenae on the growth of Halberd or AUS 10894 was examined, wet root weight in both cultivars was similar (Table 38) and while fresh weight of stem was not significantly less in

Table 38.

Effect of H. avenae on number of females and growth of Halberd and AUS 10894.

	Halberd		AUS 10894	
	Control	Inoculated	Control	Inoculated
No. of females	0	36*	0	2
Root Weight (g.)	1.9	2.0	1.6	1.5
Stem Weight (g.)	3.1	2.7	3.1	2.3*

* Significant at P = 0.05

Halberd, it was less in AUS 10894 following a comparison of the inoculated and uninoculated plants 50 days after sowing. Many females developed on Halberd, but except for one plant which produced 5 females, either 0 or 1 female developed on AUS 10894. Also, the fresh stem weight of the one plant of AUS 10894 with 5 females was similar to that of the uninoculated plants.

5.3 Discussion

Invasion of resistant plants by nematodes has often been confirmed so that resistance does not depend on barriers obstructing the entry of nematodes. Sometimes invasion was less (Shepherd, 1959; Dropkin & Webb, 1967) but often was not affected by resistance of the host

(Endo, 1965; Reynolds et al., 1970; McClure et al., 1974 a). Resistant barley had no effect on invasion of H. avenae (Cotton, 1967) and there was no effect of resistant cultivars of wheat on invasion of this nematode. When invasion was affected, e.g., incubation at 20°C instead of 15°C following inoculation, the same effect occurred on the resistant and susceptible cultivars, and this effect was difficult to explain because the motility of larvae was likely to be similar at both temperatures (Banyer & Fisher, 1972). Therefore, invasion of larvae into a single root of resistant cultivars of wheat was probably at random and similar to that described for susceptible wheat in Section 2.4.

Although some symptomatic response, such as a necrotic or hypersensitive reaction, is usually shown by resistant roots it is not essential for the expression of resistance by the plant (Dropkin, 1969) and does not always occur (Reynolds et al., 1970; Fassuliotis, 1970), but resistance is induced soon after infection begins (Dropkin, 1969). Larvae of H. avenae had established in susceptible and resistant roots of wheat within 12 hours, cytological studies showed resistance in cotton to Meloidogyne incognita occurred in 48 hours (McClure et al., 1974 b) and larvae of Meloidogyne incognita acrita left roots of resistant alfalfa after 3 days (Reynolds et al., 1970). Also, fewer nematodes developed in resistant than susceptible roots because larvae migrated from the roots (Reynolds et al., 1970), second stage larvae died in the roots (Shepherd, 1959) or syncytia were initiated and then degenerated before larval development began (Endo, 1965). A similar number may develop with fewer nematodes becoming female because inadequate syncytia were developed (Fassuliotis, 1970) or syncytia degenerated and the nematodes died before they become adult (McClure et al., 1974 b).

No cytological studies were done on resistance in wheat to H. avenae, but the same number of larvae migrated out of resistant and

susceptible roots, resulting in the same number of larvae remaining in the roots and the same number of nematodes developing beyond the second stage of development at each density of inoculation. The number of female nematodes were similar at low densities, but then more females developed on Halberd than the resistant cultivars and more developed on AUS 90248 than AUS 10894 as density of inoculation was increased. Therefore, not all larvae were able to initiate suitable syncytia in resistant roots to develop into females and either some development of these nematodes occurred before they died or they developed into males. Although sex reversal may have occurred, the greater ratio of males to females in resistant than susceptible roots was more likely the result of the reduction in the number of females, and if more males developed, this was probably due to the selective establishment of male larvae in the same manner as that described for the changes in the sex ratio on susceptible plants as inoculum density increased (Section 3.2.3).

Therefore, the mechanism of resistance occurring at a single root tip of wheat following a single inoculation with larvae of _H. avenae_ was induced following invasion of the root by larvae. Invasion by the nematode was random and when small numbers invaded, the larvae established and developed normally; however, as the number invading increased, the first larva into the root established, developed normally and induced a resistant mechanism which prevented female larvae entering after them from forming suitable syncytia to become females. These later larvae, potentially female, either remained and died or migrated out of the root, while those larvae which were potentially males, were able to establish and develop because their requirements were less than that for development of female larvae. So far, no symptomatic response would be shown by the plant, but as the number of larvae invading roots increased, eventually a hypersensitive reaction occurred and the degeneration of

syncytia prevented female but allowed male development. Thus, two phases in the resistance mechanism seemed likely and the number of larvae invading roots to induce these mechanisms differed for AUS 90248 and AUS 10894 as they supported a different number of females as density of inoculation increased.

The number of *H. avenae* within roots of Halberd and AUS 10894 were counted from plants grown in the field and initially both cultivars contained the same number of second-stage larvae. From then on, the number of nematodes developing in Halberd continued to increase as larvae continually invaded the roots while the number of larvae in AUS 10894 decreased and only a few of the larvae began development. Also, there was an average of 53 galls on roots of Halberd and only 3 galls on AUS 10894, suggesting the first three seminal roots of the resistant cultivar were invaded similarly to those of the susceptible cultivar, but the roots which emerged after these either escaped invasion or continued to grow despite invasion. A similar observation on AUS 10894 was made by Brown, J. (1974), and therefore, not only had a localised mechanism as described for inoculation of a single root occurred on the first seminal roots, but also the resistance had probably already been induced into the other roots. In addition to this, field observations and growth studies (Section 4) suggested AUS 10894 was more tolerant to *H. avenae* than Halberd, and this tolerance was likely to relate to the induced resistance of all roots following the initial invasion of nematodes.

Although a similar effect occurred in one growth room study in which there was no increase in the number of males on AUS 10894 following inoculation at two separate times with different densities of larvae, this was not repeated in a later experiment. Also, the laboratory studies in which different roots were inoculated with a delay between inoculations did not give any indication of a transfer of resistance from

one seminal root to another. Because these were preliminary studies and time did not permit continued investigations, there were many possible explanations of this failure to demonstrate the translocation of resistance within AUS 10894 and further studies need to be done on this aspect. However, a high initial density of inoculum prevented invasion of larvae from a second inoculum on the same root and demonstrated a change in the mechanism of local resistance with increased density of nematodes. Therefore, the reaction of a resistant wheat to infection was also likely to change with the initial density of nematodes invading the plant.

In histological investigations of the host-nematode relationship of H. avenae and resistant cultivars of barley and oat (Cook, 1974), larvae invading barley stimulated the initiation of syncytia and the cytoplasm of these cells became sparse and vacuolated, while invasion of oat was normally followed by hyperplasia and necrosis which prevented the initiation of syncytia. No experimental details were given with these conclusions and different histological responses by barley and oat to nematode invasion may occur with different densities of inoculum, i.e., a higher density of nematodes inoculated onto barley may cause a similar histological reaction to that reported on oat, and a lower density of nematodes with oat may give a similar histological response to that on barley. However, two different histological responses within resistant cereals to H. avenae have been reported and the response may be dependent upon the density of nematode invading the roots.

The following sequence of responses of resistant wheat probably occurred as the density of nematodes increased. When a low number of larvae invaded the early seminal roots during germination of the host, penetration, establishment and development of larvae to adults was unaffected, but sufficient reaction by the host may have occurred to

induce resistance in the roots which developed later. As this initial density of nematodes increased, a localised resistance was induced which inhibited female development and there was no further increase in the number of females, while resistance was probably still induced into the later roots. This inhibition of female development was possibly a delayed introduction of the localised mechanism of resistance which was expressed by either a degeneration of syncytia or hyperplasia and necrosis preventing the initiation of further syncytia. Further increases in the initial density of nematodes continued to affect female development, and although the number of females was constant at first, a density was reached when the number of females decreased and eventually no females developed on the roots. These changes implied an earlier introduction of the localised mechanism within the root with the increased rate of invasion as the initial density increased until no females developed and no resistance was transferred to the other roots. The importance of female development in the transfer of resistance between roots was also implied and favoured the earlier introduction of a hypersensitive reaction as initial density of nematodes increased.

Further, similar studies with histological and biochemical studies are required to verify these changes in the resistance mechanism of wheat with increasing density of nematodes. The time of the initial invasion by the nematode determined the number of roots per plant infected on resistant cultivars by the nematode as each root infected at this time reacted independently of the other roots. Changes in the initial density and time of invasion caused anomolies in the results, e.g., an increase in the number of males following two inoculations, the effect on the penetration of larvae in a later inoculum and the change from a tolerant to an intolerant reaction in AUS 10894, and these can be explained by the mechanisms of resistance which have been proposed.

The few females which normally develop on the resistant cultivars of wheat can be explained by random establishment and development of female larvae. Therefore, these females are a result of the mechanism of resistance and not the selection and development of a minor biotype within the nematode population. Also, O'Brien & Fisher (1974) suggested Loros (AUS 90248) may have been susceptible to one population and that two biotypes of H. avenae may occur in South Australia. The higher number of females counted on AUS 90248 could have resulted from a late infection of a low density of nematodes and this provides a more likely hypothesis to that of a biotype effect.

Much of this discussion is hypothetical, but is consistent with the results obtained and shows more is involved in the mechanism of resistance in wheat to H. avenae than the localised and hypersensitive reactions of hosts to fungal infection (Day, 1973). Also, due to the relationship between female development and the transfer of resistance between roots of the host, growth regulators and the balance between growth regulators and phenolics (Wallace, 1973) may be involved in more than necrotic and hypersensitive responses of hosts to invasion.

6. EXAMINATION OF H. AVENAE FROM DIFFERENT SITES FOR BIOTYPES

Different biotypes of *H. avenae* have been identified within Europe (Andersen, 1959; Cotten, 1963; Kort *et al.*, 1964; Neubert, 1966), but when the European test cultivars were used in Victoria, one biotype which differed from the European biotypes was established (Brown, R., 1969) and additional support for the single biotype has been reported (Brown & Meagher, 1970; Brown, R., 1974). Two biotypes in South Australia were possible (O'Brien & Fisher, 1974), but an environmental effect was the more likely explanation of the variation in reaction reported for AUS 90248.

Because of the possibility of biotypes of *H. avenae* breaking down the resistance introduced into a new cultivar, further assessments were made of the presence of biotypes within South Australia. These assessments are discussed in this section.

6.1 Materials and Methods

Three different assessments were done of the reaction of cereal cultivars to *H. avenae* from different sites throughout the cereal growing areas of South Australia. All assessments were on the number of females per plant following the inoculation and growth of plants in the growth room (Section 3.1.1.1).

Between the 'boot' to 'anthesis' stages of cereal growth during 1973, all cereal growing areas were visited and 26 sites of *H. avenae* (Fig. 22) were selected. Selection was on the basis of both the number of females on cereal roots and variation in environment, i.e., selection was biased to give a wide range of soil and climatic conditions under which the nematode had developed. This, and the collection of mature cysts with associated organic matter between February and early May during 1974, was done with the aid of Officers of the State Department of

Figure 22. Distribution of sites of the populations of *H. avenae* sampled throughout the cereal growing areas of South Australia in the search for different biotypes of the nematode.

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Agriculture. The soil samples containing the mature cysts were stored at ambient temperature until the 22nd May, 1974, when they were transferred to a temperature of 10°C for 6 weeks before the cysts were separated from the soil, washed from the roots and the hatching larvae were then collected and stored at 5°C (Section 2.1.1.1).

In the first assessment, the reaction of 6 cereal cultivars to H. avenae from the 26 sites (Fig. 22) was assessed and another cultivar of barley (cv. No. 191) was intended but then omitted because of poor germination. Four of the cultivars, 3 wheat and 1 barley, were selected from the cereals tested in previous experiments (Section 3.1.1.2). These were Halberd as the susceptible standard and AUS 10894, AUS 90248 and Morocco as the resistant cultivars. In addition, an oat (cv. Sun II) and 2 barleys (cvs. Drost and No. 191) obtained from the Victorian Plant Research Institute at Burnley were included. The 5 replicates of each site were arranged in a randomised block design, inoculated with 300 larvae at sowing, 400 larvae 7 days after sowing and 600 larvae 14 days after sowing, and the number of females per plant was counted 50 days after sowing. No analyses were done, but the reaction of the cultivars was assessed on the mean number of females per plant. A cultivar was rated as resistant when there were fewer than 5 females per plant and susceptible when there were 5 or more females per plant.

In the second assessment, the reaction of 9 barley, 8 oat, 7 wheat and 1 rye cultivars to H. avenae from sites 14, 19, 20 and 21 (Fig. 22) was assessed at the same time and by the same procedure as in the first assessment. With the exception of Halberd which was included as a susceptible standard, the cultivars tested (Table 40) comprised an 'International' test range of cultivars used to identify different biotypes. Except for three of the wheat cultivars, Halberd and AUS 10894 which were used previously (Section 3.1.1.2) and Psathias (AUS 881) which

was obtained from the Victorian Wheat Research Institute at Horsham, the cultivars were obtained from C.H. Nielsen at Tylstrup in Denmark. Analyses of variance were done separately for the barley, wheat and oat cultivars on both the raw and $\log_e$ transformed data.

For the third assessment, the reaction of cultivars of 3 barley, 2 oat and 1 wheat cultivar selected from the 'International' range of cultivars to H. avenae from sites 12, 19, 20 (Fig. 22) was assessed and compared with the reaction of a susceptible standard for each of the genera. Clipper for barley, Halberd for wheat and Early Kherson for oat were the susceptible standards as these three cultivars are susceptible cultivars grown throughout South Australia. Bajo Aragon -1-1, Dalmatishe and P 31322-1 for barley and Psathias for wheat were selected to be re-tested because they were often resistant in European tests (Nielsen, pers. comm.), while Sun II and 640318-40-2-1 for oat were selected to reassess the difference obtained between these two cultivars to the nematode from site 19 in the previous assessment. There were 7 replicates of each site arranged in a randomised block design. The wheat and oat cultivars were inoculated with 250 larvae at sowing and 500 larvae 7 and 21 days after sowing, while the barley cultivars were inoculated with 500 larvae at sowing, 7, 14 and 21 days after sowing. The number of females per plant was counted 60 days after sowing and an analysis of variance was done on the raw data.

6.2 Results

Mature cysts of H. avenae were collected over a long duration (February to early May), in wet soil from 12 of the sites and in dry soil from the other 14 sites. These variations in collection may relate to the irregular hatching of larvae which occurred between the sites and because of this, no assessment was possible for sites 2, 5, 15 and 23 (Table 39), only 3 replicates were possible for sites 7, 11 and 25, and

Table 39.

Effect of different populations of *H. avenae* on number of females per plant of Halberd, AUS 10894, AUS 90248, Morocco, Sun II and Drost.

Results presented are;

(a) The average number of females from 5 replicates, with the rating of the reaction of each cultivar as either susceptible (S) or resistant (R).

(b) Numbers in brackets are the range of number of females per plant.

* Three replicates only of each cultivar tested against the population.

? an uncertain classification of the host reaction.

Site (Fig. 22)	Halberd	AUS 10894	AUS 90248	Morocco	Sun II	Drost
1	11 (7-14) S	3 (0-6) R	1 (0-4) R	0 (0-2) R	9 (6-13) S	9 (7-10) S
2	No result					
3	13 (8-20) S	2 (0-4) R	2 (1-3) R	0 (0) R	10 (5-17) S	11 (9-13) S
4	13 (12-14) S	2 (0-3) R	2 (1-2) R	0 (0-2) R	10 (7-12) S	12 (6-18) S
5	No result					
6	11 (7-17) S	1 (0-2) R	1 (1-2) R	0 (0-1) R	7 (5-9) S?	11 (8-15) S
7*	14 (13-15) S	2 (0-4) R	1 (0-3) R	0 (0) R	3 (1-6) R?	5 (3-8) S ?
8	14 (8-25) S	2 (1-4) R	3 (1-5) R	0 (0) R	19 (11-38) S	12 (7-19) S
9	19 (10-35) S	1 (0-3) R	1 (0-2) R	0 (0-1) R	12 (6-16) S	10 (7-12) S
10	12 (7-16) S	1 (0-2) R	1 (0-1) R	0 (0-1) R	10 (8-13) S	9 (6-12) S
11*	15 (9-25) S	2 (2-5) R	2 (1-3) R	0 (0-1) R	4 (3-6) R?	10 (7-12) S
12	20 (13-26) S	3 (1-8) R	4 (1-5) R	2 (0-5) R	13 (7-18) S	14 (8-19) S
13	13 (10-17) S	1 (0-1) R	1 (0-2) R	0 (0-1) R	11 (6-21) S	11 (7-16) S
14	13 (10-23) S	2 (0-7) R	4 (1-6) R	0 (0-2) R	13 (8-17) S	12 (9-15) S
15	No result					
16	30 (21-41) S	4 (0-9) R	3 (1-5) R	0 (0) R	18 (13-28) S	13 (9-28) S
17	11 (7-15) S	1 (0-2) R	1 (0-1) R	0 (0) R	9 (6-14) S	9 (5-13) S
18	13 (10-14) S	0 (0-1) R	1 (0-3) R	0 (0) R	4 (2-6) R?	8 (4-10) S
19	14 (11-17) S	3 (0-6) R	3 (1-4) R	0 (0) R	15 (9-20) S	13 (8-15) S
20	21 (10-34) S	2 (0-4) R	4 (0-8) R	1 (0-2) R	17 (11-21) S	14 (11-18) S
21	16 (10-23) S	3 (1-4) R	4 (1-6) R	0 (0) R	11 (7-13) S	10 (8-15) S
22	17 (12-22) S	3 (1-4) R	2 (1-3) R	2 (0-9) R	11 (5-16) S	
23	No result					
24	15 (6-24) S	1 (0-2) R	2 (0-5) R	1 (0-1) R	3 (1-5) R?	10 (5-14) S
25*	12 (10-16) S	1 (1-2) R	2 (0-3) R	0 (0-1) R		
26	17 (7-33) S	2 (1-3) R	2 (1-4) R	2 (0-6) R	11 (6-20) S	9 (5-15) S

larvae stored for different durations were used in the inoculations. However, the reaction of Halberd and Drost was rated as susceptible and for AUS 10894, AUS 90248 and Morocco was rated as resistant at all of the sites tested, while the reaction of Sun II was usually rated as susceptible, but was rated as resistant to larvae from sites 7, 11, 18 and 24.

The assessment of the reaction of an 'International' range of cultivars to 4 sites of the nematode was done with larvae collected at the same time as those for the previous assessment. Therefore, the differences which occurred between sites 14, 19, 20 and 21 (Table 40) were probably related to the duration of storage of the larvae. However, no significant interaction occurred between sites and cultivars, there were only minor changes in the ranking of the reaction of cultivars between sites and no biotype effect was recognised. Within the barley cultivars, Martin 403-2 and Morocco were resistant and the reaction of Dalmatishe was variable between the sites. The reaction of rye (cv. Rogo) was variable within replications. For wheat, AUS 10894 and the Loros cross (63/1-7-15-12) were resistant while Psathias and Iskamish -K-2-light gave variable reactions between sites. Within the oat cultivars, *A. sterilis* and Silva were resistant while the reaction of 640318-40-2-1 was variable between sites. Plants of TY 72-42-1 and TY 72-49-1 grew poorly and the number of plants assessed to give the mean number of females per plant varied from 1 to 4.

In the third assessment, when the reaction of cultivars selected from the 'International' range was tested, a significant interaction between cultivars and sites occurred as well as differences between cultivars and sites (Table 41). The differences between cultivars showed variations in susceptibility of the cultivars tested and no plants were resistant, while the differences between sites showed variations in

Table 40.

EFFECT OF H. AVENAE FROM FOUR SITES ON NUMBER OF FEMALES PER PLANT OF CEREALS IN THE 'INTERNATIONAL' RANGE FOR IDENTIFICATION OF BIOTYPES.

	Site of population of *H. avenae* (Fig. 22)				
	20	19	21	14	
BARLEY					L.S.D. (P=0.05) between sites
Varde	38	20	18	14	
Siri (101 cross)	24	15	15	12	
Emir	21	14	9	7	
Ortolan	21	13	9	9	
P31322-1	20	11	7	7	= 3
Bajo Aragon-1-1	18	10	12	8	
Dalmatische	13	7	4	1	
Martin 403-2	3	0	2	1	
Morocco	0	0	0	0	
	L.S.D. (P=0.05) between cultivars = 4				
RYE					
Rogo	9	4	4	1	
WHEAT					
Iskamish-K-2-Dark	36	25	21	20	L.S.D. (P=0.05) between sites
Halberd	30	26	16	18	
Capa	23	19	20	17	
Psathias - AUS 881	13	13	7	3	= 4
Iskamish-K-2-Light	8	10	4	7	
63/1-7-15-12 (Lones cross)	7	2	3	1	
AUS 10894	5	3	4	2	
	L.S.D. (P=0.05) between cultivars = 5				
OAT					
Nidar II	31	26	22	23	L.S.D. (P=0.05) between sites
Sun II	21	14	11	11	
640318-40-2-1	19	6	10	15	
IGV H 72-646*	17	10	13	10	= 3
Silva	3	6	3	2	
A. sterilis cc 4658	0	0	1	0	
	L.S.D. (P=0.05) between cultivars = 4				
TY 72-42-1	17	0	15	9	
TY 72-49-1	12	5	13	12	

* (*A. sterilis* (A:n) x Sun II^{7})

Table 41.

EFFECT OF H. AVENAE FROM THREE SITES ON NUMBER OF FEMALES PER PLANT OF CULTIVARS OF CEREALS FROM THE 'INTERNATIONAL' RANGE FOR IDENTIFICATION OF BIOTYPES.

	Site of population of H. avenae (Fig. 22)			
BARLEY	20	19	12	L.S.D. (P=0.05) between sites
Clipper	50	19	29	
Bajo Aragon-1-1	24	7	14	
Dalmatishe	15	7	10	
P31322-1	18	6	10	
WHEAT				
Halberd	16	15	14	= 3
Psathias - AUS 881	17	13	18	
OAT				
Early Kherson	27	13	17	
Sun II	32	13	20	
640318-40-2-1	21	11	16	

L.S.D. (P=0.05) between cultivars = 4
" " of interaction (cultivar x sites) = 8

the effectiveness of the inoculations.

6.3 Discussion

The variations in the number of females per plant of individual cultivars, between sites and different assessments, demonstrated the problems in comparing the effects of nematodes from different sites on the host reaction. Irrespective of the technique used for inoculation, i.e., naturally infested soil, mature cysts or larvae, it would be very difficult to manage the nematode from the different sites to give similar infections of the test plants. Therefore, separation of the reactions of plants on the number of females as highly resistant, resistant, moderately resistant, poorly resistant, moderately susceptible and susceptible were impractical because most of the variations were due to

the testing methods and not differences in the nematode. However, better management of the mature cysts before and during hatching so that fresh larvae could be used in the inocula (Section 2.3) and the use of lower densities of larvae in the inocula (Section 3.2) would minimise the variations in reactions of host plants to nematodes from different sites.

A comparison of nematodes from Watchman in South Australia (O'Brien & Fisher, 1974) and Sea Lake in Victoria (Brown, R., 1969) with those tested in this section was attempted. However, insufficient larvae were collected from the mature cysts from the two sites to conduct a meaningful test on the reaction of the different hosts to these nematodes. Therefore, only one biotype of H. avenae seemed likely for South Australia and whether this was the same as the one biotype suggested for Victoria (Brown, R., 1969; 1974) was not determined. However, the susceptible reaction of Psathias may have suggested different biotypes because the reactions of Psathias and AUS 10894 were similar against Victorian populations of the nematode (Brown, R., 1974). Ellis & Brown (1976) suggested the possibility of resistance-breaking biotypes within Victorian populations of the nematode, but the host reactions were variable and this was likely to be due to the method used to assess the reactions. The reactions of Sun II, Drost and Morocco suggested the biotype in South Australia was different from the European biotypes (Cook & Williams, 1972), but this could not be established definitely because of the absence of No. 191 from the assessment. However, the susceptible reactions of Bajo Aragon-1-1, Dalmatishe, P 31322-1, Psathias and 640318-40-2-1 suggested this biotype was different from those of Europe (Nielsen, pers. comm.). Further testing of nematodes from different localities by the same method of assessment would be needed to establish the relationship between the different nematodes.

Some doubt may exist on the ability to transfer the resistance

from one cultivar to another because one barley; cv. P 31322-1 (selected from crosses which included Drost and Morocco) and three oat; cvs. IGV H 72-646, TY 72-42-1 and TY 72-49-1 (selected from crosses with _A. sterilis_) were susceptible. This was probably due to an inability to maintain resistance to the Australian biotype when the selection was made against the European biotypes. However, the one wheat cultivar; 63/1-7-15-12 (selected from a cross with Loros) was resistant and the Loros used was likely to be similar to AUS 90248. Therefore, the resistance of AUS 10894 and AUS 90248 to the nematode at all sites used in the assessments, and the probability of a single biotype in South Australia, supported the suitability of these cultivars for inclusion in a breeding programme. Before other resistant cultivars are included in a breeding programme, it would be desirable to test their reaction to nematodes from a wide range of sites throughout the areas intended for their use.

7. INHERITANCE OF RESISTANCE TO *H. AVENAE* IN WHEAT AND BARLEY

The inheritance of resistance to *H. avenae* in wheat (Nielsen, 1966; Slootmaker *et al.*, 1974), oat (Cotten & Hayes, 1972) and barley (Andersen & Andersen, 1968; Cotten & Hayes, 1969) has been studied, but no information is available on inheritance in these crop species to Australian populations of the nematode. Resistance is determined by the number of females on plant roots (Andersen, 1961) and progeny with 0 or 1 female were rated as resistant in the study on inheritance in wheat (Slootmaker *et al.*, 1974). However, in the studies on inheritance in barley (Cotten & Hayes, 1969) and oat (Cotten & Hayes, 1972) cultivars, a bimodal distribution was determined for resistant and susceptible reactions of the progeny, but not by selecting an absolute number of females below which plants were resistant. The difficulty in assessing the reaction of progeny was attributed partly to environmental variation during the tests and partly to genetic variation in the aggressiveness of the nematode (Cotten & Hayes, 1969).

In Section 3.1, a technique was described in which both the environmental conditions and the number of larvae inoculated onto the plants were controlled, and in Section 6, the probability of only one biotype of the nematode in South Australia was determined. Therefore, this technique was used in an attempt to establish the mode of inheritance of resistance in wheat and barley cultivars to this biotype.

7.1 Materials and Methods

The assessments were by the method described in Section 3.1.1.1 and 156 plants were grown on each tray (Section 3.1.1.2) with plants of the parents, F1 and F2 progeny arranged at random throughout all the trays needed to accommodate the plants within a test. The wheat and barley were grown in separate trials.

With wheat, seeds of the susceptible (cv. Halberd) and resistant (cvs. AUS 10894 and AUS 90248) parents were from the same source as those used previously (Section 3.1.1.2) and seeds of both the F1 and F2 progeny of Halberd x AUS 10894, Halberd x AUS 90248 and AUS 10894 x AUS 90248 were provided by Dr. A.J. Rathjen. Approximately 30 plants of Halberd, AUS 10894 and progeny from the F1 of each of the three crosses, 15 plants of AUS 90248, 180 plants from the F2 generation of both the Halberd x AUS 90248 and AUS 10894 x AUS 90248 crosses, and 370 plants from the F2 generation of Halberd x AUS 10894 were inoculated with 256(±56), 590(±33) and 733(±51) larvae (±S.E.) at 0, 7 and 21 days following sowing respectively.

Barley plants from different crosses involving Clipper (C) as the susceptible parent and Athinais (A), CI 8147 (CI), Marocaine 079 (Ma), Morocco (Mo), Nile (N) and WI 2231 (W) as the resistant parents were assessed by the number of females on roots. Except for Ma (WI 2397, CI 8334) which was obtained from the Victorian Wheat Research Institute, Horsham, Victoria, the other cultivars were from the same source described in Section 3.1.1.2. Approximately 10 plants from the F1 and 70 plants from the F2 of A x C, CI x C, Ma x C, Mo x C, N x C and W x C were tested. However, there were the following exceptions; 5 plants from the F1 of CI x C, 162 plants from the F2 of A x C and no plants from the F1 of Ma x C. Also, 30 to 40 plants from (A x Ma) x C, (A x Mo) x C, (A x N) x C, (CI x Mo) x C, (Mo x C) x C, (Ma x C) x C, (N x Mo) x C and (W x Mo) x C, and 20 plants from (A x C) x C were tested. All parents and progeny were provided by Dr. D. Sparrow, and were inoculated with 231(±8), 463(±12) and 454(±11) larvae at 0, 7 and 20 days following sowing respectively.

For both the wheat and barley, the raw data and both the square root and logarithmic transformations were used to determine the 95%

confidence limits of the distribution of the reaction of the susceptible parents and of the resistant parents combined with the F1 progeny. This was similar to the procedure used by Cotten & Hayes (1969) to determine the bimodal distribution of resistant and susceptible genotypes.

7.2 Results

The distribution of F2 and intercross progeny of wheat and barley in these tests was not bimodal, and therefore, some of the genotypes could not be classified as either resistant or susceptible.

Distribution of the F1 progeny of Halberd x AUS 10894 (Fig. 23 b) was between that of the two parents (Fig. 23 a) and the mean of this distribution ($\bar{x}$ = 12) was significantly greater than that of AUS 10894 ($\bar{x}$ = 3) and smaller than that of Halberd ($\bar{x}$ = 39). With the F2 progeny (Fig. 23 c), the distribution was continuous and skewed towards that of the resistant parent (cv. AUS 10894).

Progeny of Halberd x AUS 90248 showed a similar pattern of distribution (Fig. 24) to that of the progeny of Halberd x AUS 10894 (Fig. 23). The mean of the distribution of the F1 progeny ($\bar{x}$ = 16) was significantly greater than that of AUS 90248 ($\bar{x}$ = 4), smaller than that of Halberd and similar to that of the F1 progeny of AUS 10894 x Halberd.

There was no difference in the distribution of parents and both the F1 and F2 progeny of AUS 10894 x AUS 90248 (Fig. 25), and the mean of each of these distributions was less than that for the F1 progeny of Halberd x AUS 10894 and Halberd x AUS 90248.

The susceptible parent (cv. Clipper) had low numbers of females per plant in the test on barley and this distribution overlapped the distribution of 5 of the resistant parents; Athinais and Nile (Fig. 26), Marocaine 079 (Fig. 27), WI 2231 and CI 8147 (Fig. 28), but not Morocco (Fig. 26). Although all resistant and susceptible genotypes were not

Figure 23. Frequency distribution of number of females of *H. avenae* per wheat plant of parents and progeny of Halberd x AUS 10894.

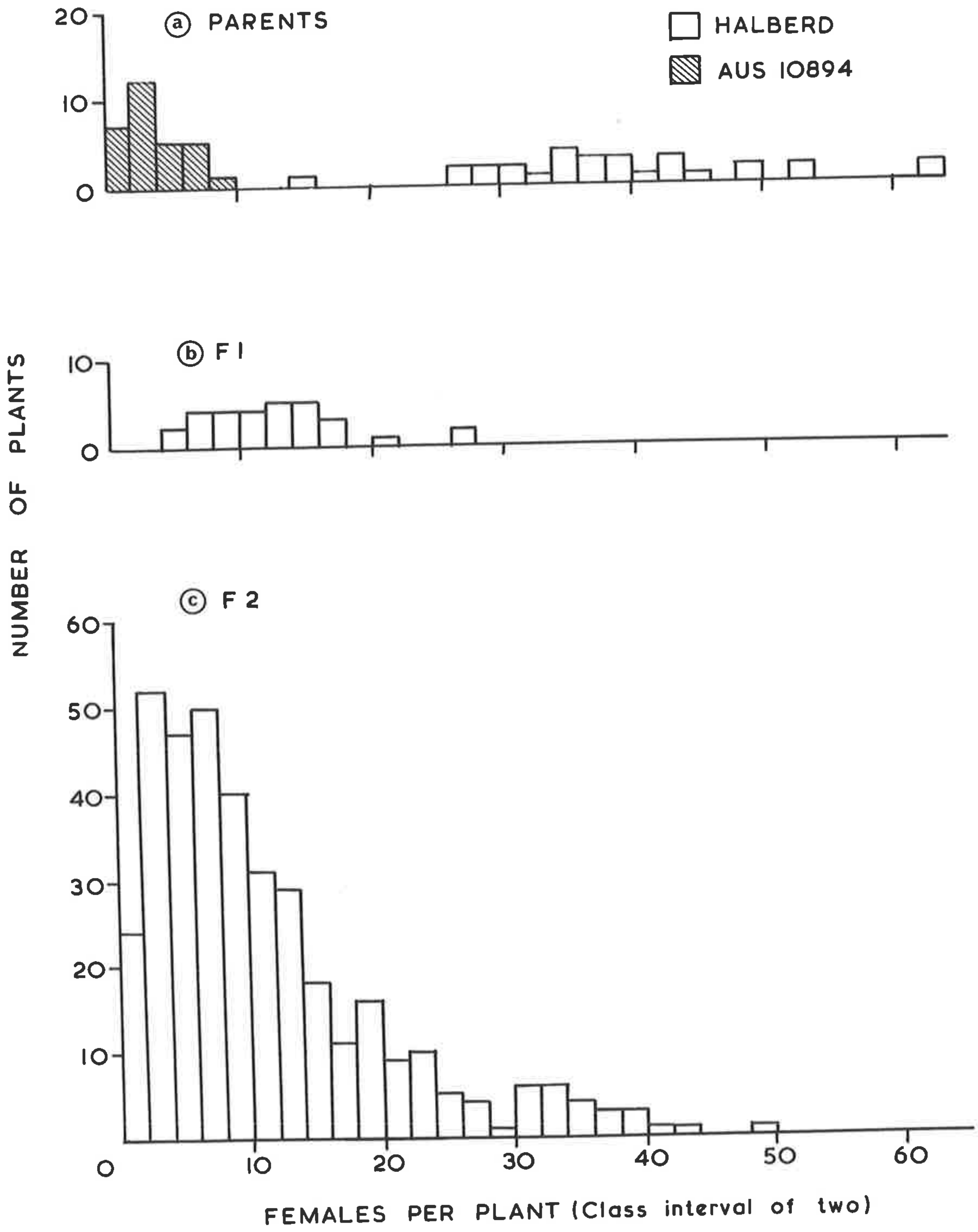

HALBERD X AUS 10894
(a) PARENTS
HALBERD
AUS 10894
20
10
0
(b) F1
10
0
(c) F2
60
50
40
30
20
10
0
10
20
30
40
50
60
NUMBER OF PLANTS
FEMALES PER PLANT (Class interval of two)

Figure 24. Frequency distribution of number of females of *H. avenae* per wheat plant of parents and progeny of Halberd x AUS 90248.

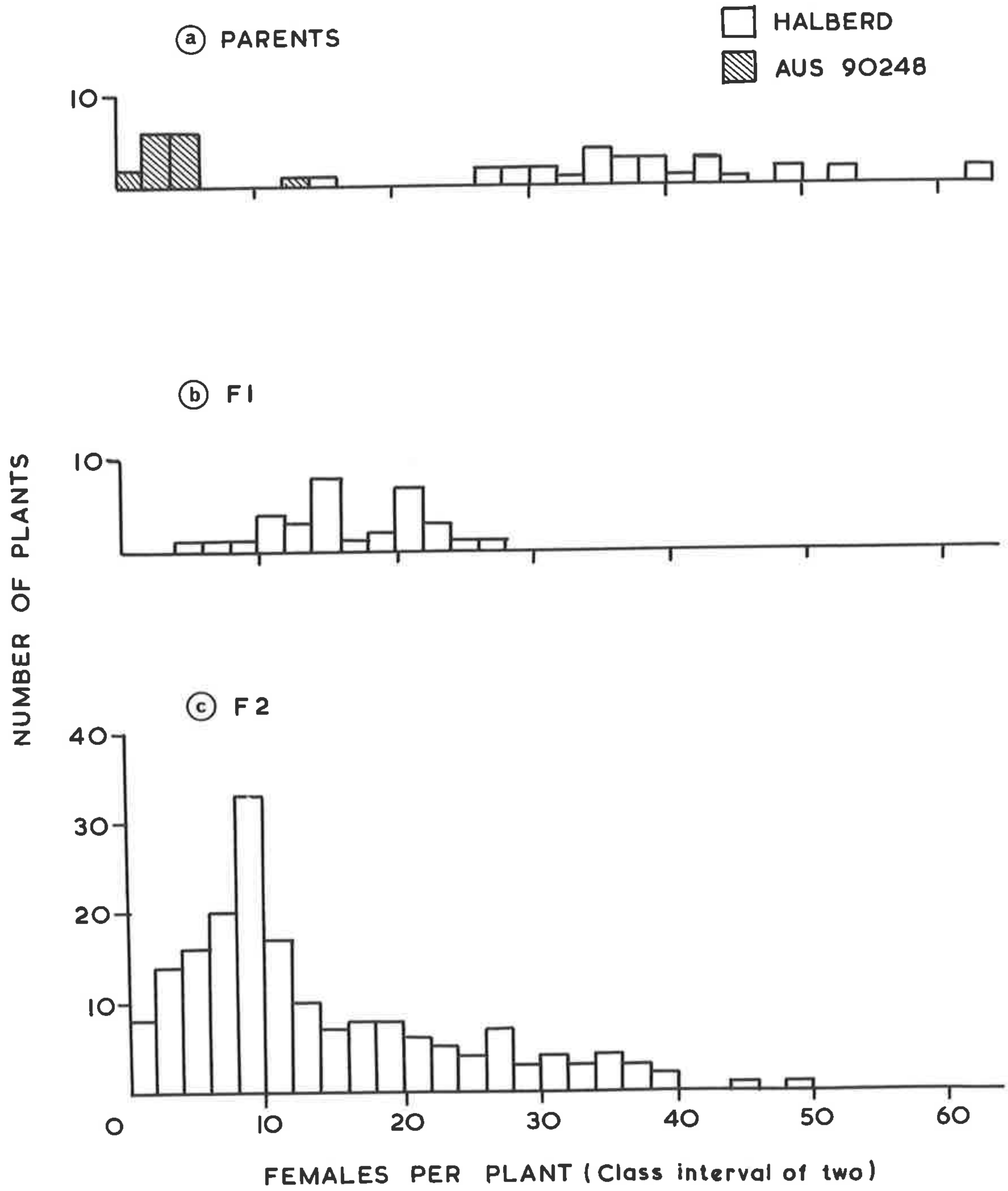
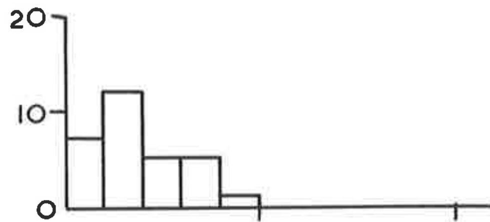
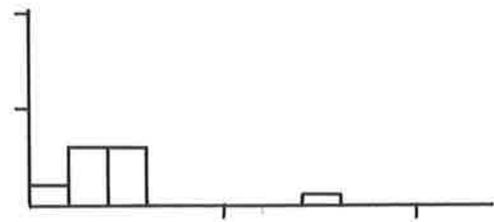

HALBERD X AUS 90248
ⓐ PARENTS
HALBERD
AUS 90248
10
ⓑ F1
10
ⓒ F2
40
30
20
10
0
10
20
30
40
50
60
NUMBER OF PLANTS
FEMALES PER PLANT (Class interval of two)

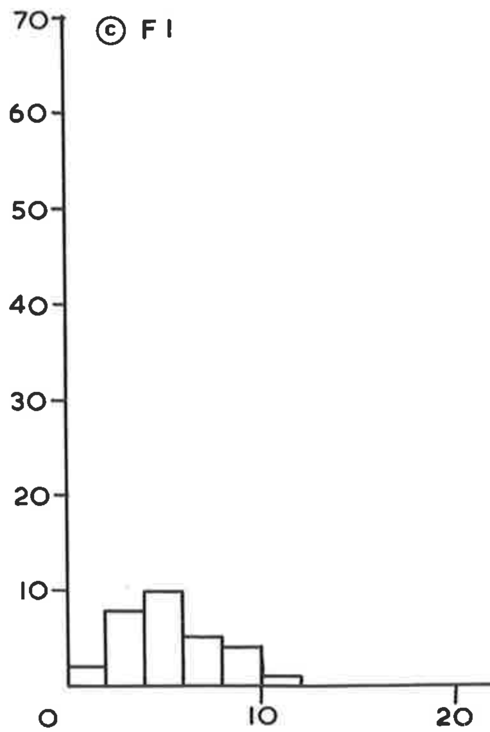
Figure 25. Frequency distribution of number of females of *H. avenae* per wheat plant of parents and progeny of AUS 10894 x AUS 90248.

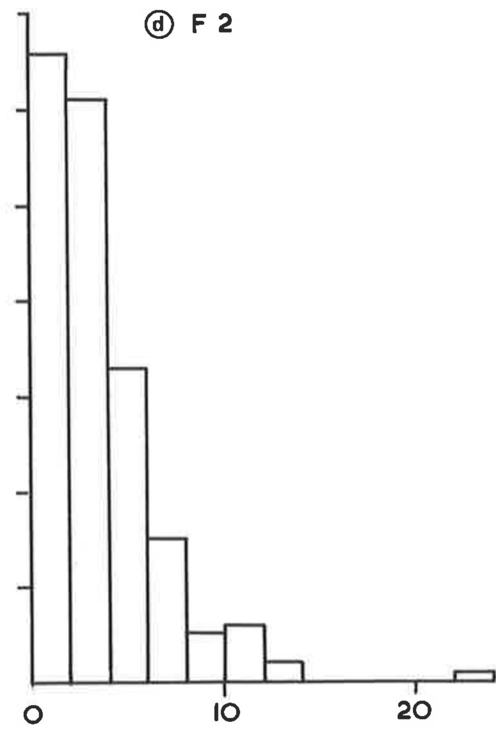
AUS 10894 X AUS 90248





NUMBERS OF PLANTS





FEMALES PER PLANT (Class interval of two)

distinguished, the distribution of the F1 progeny (Fig. 26, 27, 28) suggested only partial dominance as its mean was greater than that of the resistant parent, and this was similar to the results which occurred with wheat (Fig. 23, 24).

When Athinais and Nile (Fig. 26), Marocaine 079 (Fig. 27) and CI 8147 (Fig. 28) were the resistant parents, the distributions of the F2 progeny were similar and skewed to give a similar distribution to that for the F2 progeny of wheat (Fig. 23, 24). The progeny from (A x C) x C and (Ma x C) x C (Fig. 27) produced distributions which more closely resembled the distribution of Clipper than the resistant parent.

Most of the F2 progeny from Mo x C (Fig. 27) seemed resistant and the lack of genotypes as susceptible as Clipper was unexpected. This was anomolous and further investigations would be needed to confirm this result by remaking the cross. The distribution of progeny from (Mo x C) x C was not sufficiently distinct to provide definite conclusions on the resistance of Morocco.

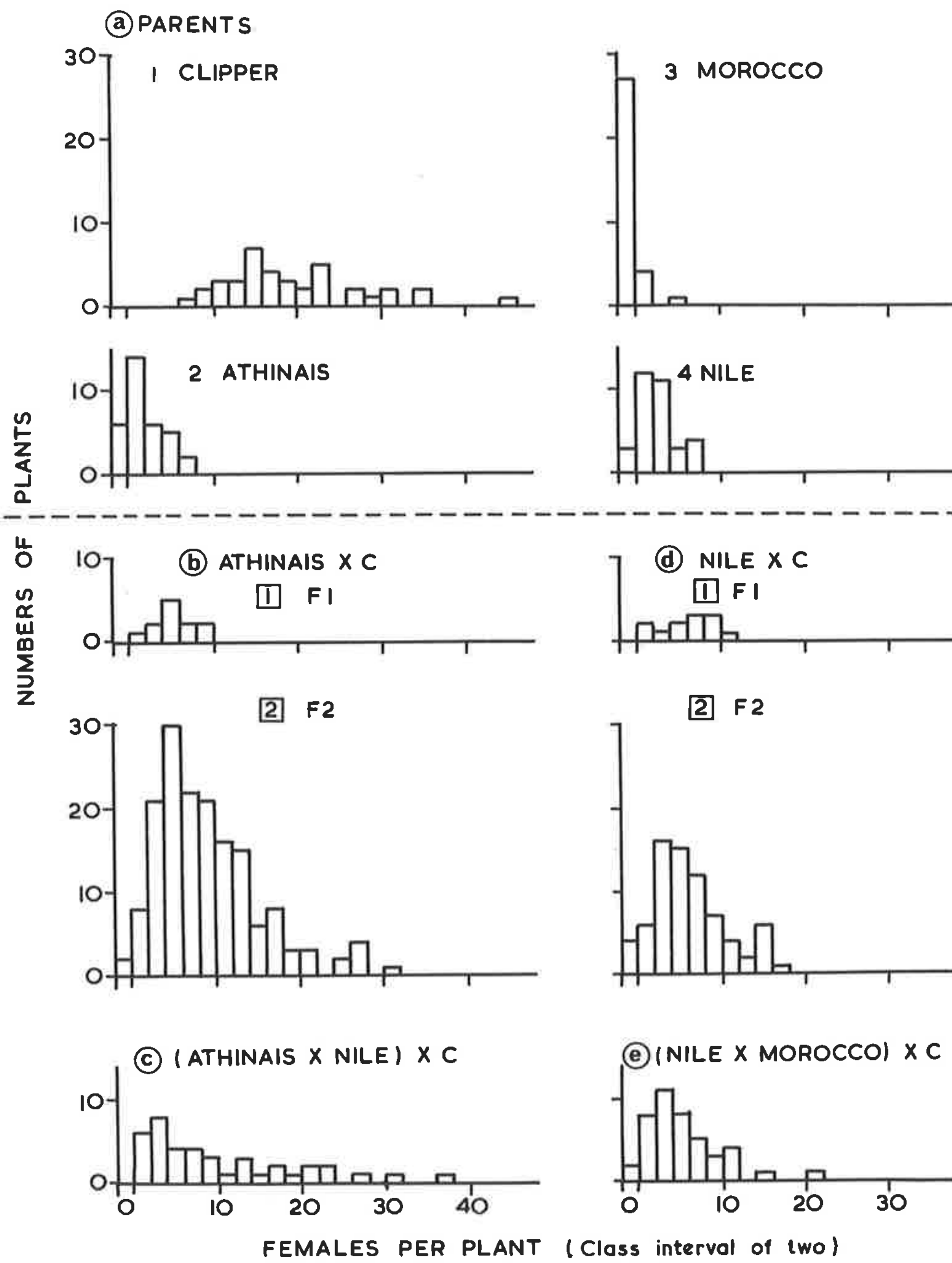
Whenever WI 2231 was used as a parent (Fig. 28), discussion of resistance was not needed as the differences in distributions between WI 2231 and Clipper were negligible.

The distributions for (A x N) x C and (N x Mo) x C (Fig. 26) resembled those for the F2 which suggested that the resistances in Athinais and Nile, and Nile and Morocco were not the same. Conversely, the distribution for (A x Ma) x C (Fig. 27) resembled an F1 and suggested that the resistance in Athinais and Marocaine 079 was the same. With (A x Mo) x C (Fig. 27) and (CI x Mo) x C (Fig. 28), the distributions were not distinct, but they seemed more likely to resemble those for the F2 than the F1, which suggested that the resistances in Athinais and CI 8147 were not the same as the resistance in Morocco.

Figure 25. Frequency distribution of number of females of *H. avenae* per barley plant of parents, F1 and F2 progeny of A x C and N x C, and progeny of (A x N) x C and (N x Mo) x C.

Parent cultivars of barley:

A - Athinais	Mo - Morocco
C - Clipper	N - Nile

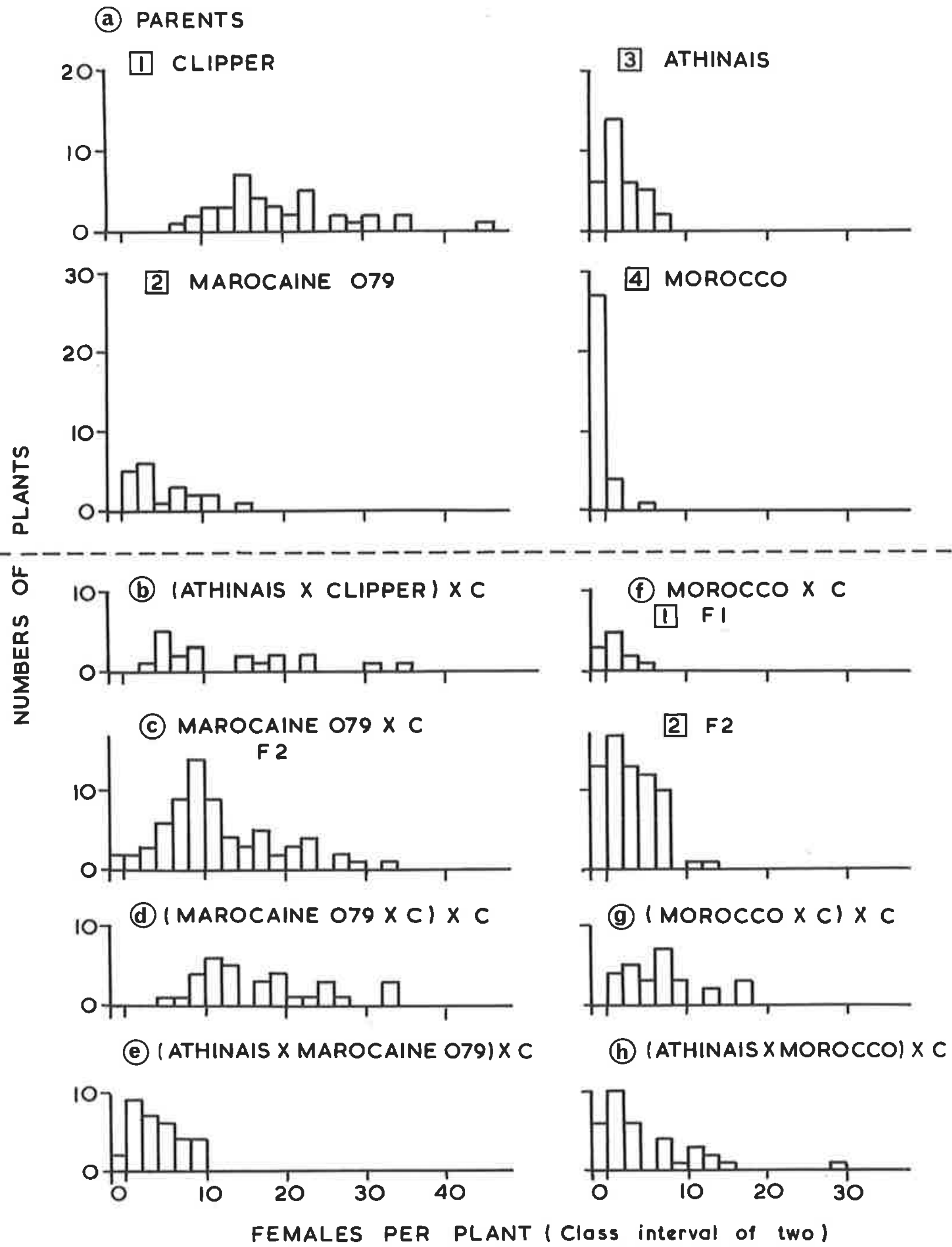


ⓐ PARENTS
1 CLIPPER
3 MOROCCO
2 ATHINAIS
4 NILE
NUMBERS OF PLANTS
ⓑ ATHINAIS X C
1 F1
ⓓ NILE X C
1 F1
2 F2
2 F2
ⓒ (ATHINAIS X NILE) X C
ⓔ (NILE X MOROCCO) X C
FEMALES PER PLANT (Class interval of two)

Figure 27. Frequency distribution of number of females of *H. avenae* per barley plant of parents, F1 progeny of Mo x C, F2 progeny of Mo x C and Ma x C, and progeny of (A x C) x C, (Ma x C) x C, (Mo x C) x C, (A x Ma) x C and (A x Mo) x C.

Parent cultivars of barley:

A - Athinais	Ma - Marocaine 079
C - Clipper	Mo - Morocco

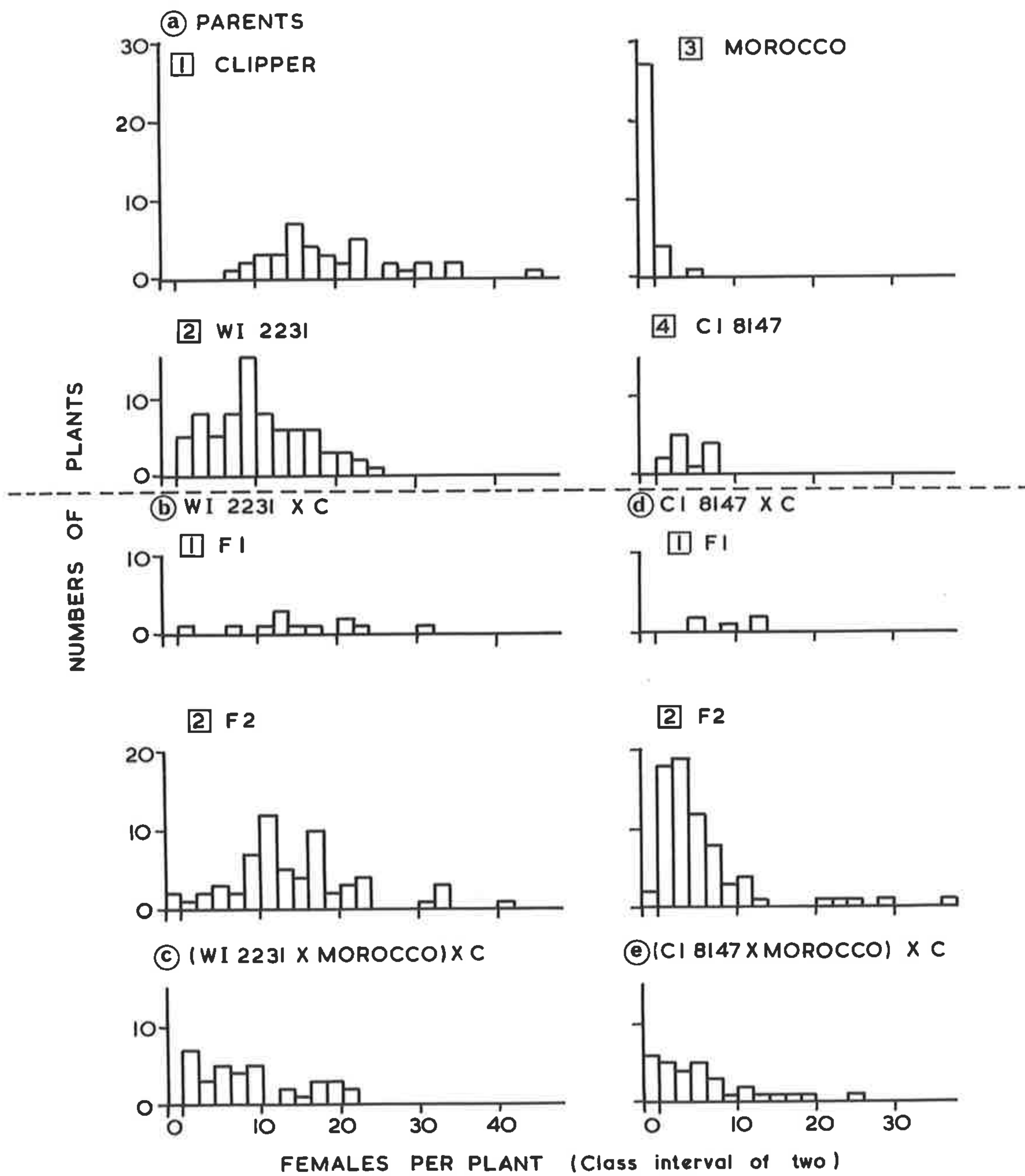


(a) PARENTS
1 CLIPPER
3 ATHINAIS
2 MAROCAINE 079
4 MOROCCO
NUMBERS OF PLANTS
(b) (ATHINAIS X CLIPPER) X C
(f) MOROCCO X C
1 F1
(c) MAROCAINE 079 X C
F2
2 F2
(d) (MAROCAINE 079 X C) X C
(g) (MOROCCO X C) X C
(e) (ATHINAIS X MAROCAINE 079) X C
(h) (ATHINAIS X MOROCCO) X C
FEMALES PER PLANT (Class interval of two)

Figure 29. Frequency distribution of number of females of *H. avenae* per barley plant of parents, F1 and F2 progeny of W x C and CI x C, and progeny of (W x Mo) x C and (CI x Mo) x C.

Parent cultivars of barley:

C - Clipper	Mo - Morocco
CI - CI 8147	W - WI 2231


a PARENTS
1 CLIPPER
3 MOROCCO
2 WI 2231
4 CI 8147
b WI 2231 X C
1 F1
d CI 8147 X C
1 F1
2 F2
2 F2
c (WI 2231 X MOROCCO) X C
e (CI 8147 X MOROCCO) X C
NUMBERS OF PLANTS
FEMALES PER PLANT (Class interval of two)
0
10
20
30
40

7.3 Discussion

Although resistance in oats (Cotten & Hayes, 1972) and barley (Andersen, 1961; Cotten & Hayes, 1969; Hayes & Cotten, 1970) included both the dominant and recessive expression of one or more genes, a monogenic and dominant expression of resistance was usually involved. Resistance in wheat (Nielsen, 1966; Slootmaker et al., 1974) has also been attributed to a single dominant gene. These conclusions on the inheritance of resistance would only be valid if a bimodal distribution of the reaction of resistant and susceptible genotypes occurred, i.e., the distribution of the resistant genotypes differed from that of the susceptible genotypes, but were similar to that of the resistant parent. Bimodal distributions of the resistant and susceptible genotypes may have occurred in the previous studies on the inheritance of resistance to H. avenae in cereals, but often the resistance rating given to genotypes was determined from an absolute number of females and insufficient data was included to validate this assessment of resistance, e.g., Slootmaker et al. (1974) rated genotypes of wheat as resistant when 0 or 1 female was counted on the roots, no data was presented for the reaction of genotypes in the F1 and only the rating of resistant or susceptible was given for genotypes in the F2.

The distributions of resistant and susceptible genotypes of oats (Cotten & Hayes, 1972) and barley (Cotten & Hayes, 1969) were only separated after the calculation of the 95% confidence limits of the square root transformation for the distribution of both the susceptible parent and the resistant parent combined with that of the F1 progeny. All the genotypes of barley were then rated as either resistant or susceptible, but some of the genotypes of oats were still intermediate to the two distributions and could not be rated. Similar calculations were done in an attempt to separate resistant and susceptible genotypes during these

studies on resistance in wheat and barley, but too many of the genotypes were intermediate to the two distributions and could not be rated because a bimodal distribution did not occur in the F2.

Failure to distinguish between resistant and susceptible reactions was probably due to the inability of the technique used to give sufficient females on the susceptible genotypes. Therefore, if fresh larvae in the inocula improves both the establishment and development of larvae invading the plants (Section 2.3), and a lower number of larvae in the inocula reduces the competition between larvae and has less effect on root growth (Section 3.2), this method of assessment could be modified to increase the number of females on the susceptible parents in further studies. However, except for the inability to establish bimodal distributions, the distribution of these genotypes of wheat and barley seemed similar to those for barley (Cotten & Hayes, 1969) and oats (Cotten & Hayes, 1972), even though there were 220 to 767 females on the susceptible barley and 43 to 873 females on the susceptible oats in those studies. Therefore, although variations within the methods used affected the reactions of the genotypes, the mode of resistance within the genotypes has also probably affected the reactions to infection.

Because the F1 distributions were intermediate to that of the resistant and susceptible parents, only partial dominance has been expressed, and because the F2 distributions were not bimodal, it was invalid to draw any firm conclusions on Mendelian inheritance. However, the F2 distributions encompassed both parents and pointed clearly to only one or two genes being involved, as the F2 range would not be so extensive if many genes were involved.

During an early assessment of resistance in wheat in Australia (Brown & Meagher, 1970), Loros was susceptible. But there are 5 accessions of Loros within the Australian Wheat Collection at Tamworth in

New South Wales. When 4 of these were tested by Brown, J. (1974), 3 were susceptible and 1 (cv. AUS 11577) was resistant and the other accession (cv. AUS 90248) was also resistant (O'Brien & Fisher, 1974). AUS 90248 may be the same as AUS 11577. Therefore, the relation between AUS 90248 and the Loros used in the genetic studies in Europe (Nielsen, 1966; Slootmaker et al., 1974) is uncertain. However, a single major gene was also likely in AUS 90248 and the resistance in AUS 10894 appeared to be the same, because no susceptible genotypes were segregated in the F2 of AUS 90248 x AUS 10894. The partial dominance of this gene, the continuity in the F2 distributions and the consistent differences between the two cultivars during studies on the mechanism of resistance (Section 5) have suggested polygenes, or modifier genes, (Rieger et al., 1968) were associated with the major gene in the expression of resistance in wheat.

With barley, a similar relation between major and modifier genes was also possible. Resistance in Athinais, CI 8147, Marocaine 079 and Nile was likely to be associated with one major gene, while in Morocco, either two major genes were probably associated with resistance or an incorrect cross was tested in the F2 as only a single gene has previously been identified (Hayes & Cotten, 1970). Although the same gene may have been involved in Athinais and Marocaine 079, a different gene was associated with resistance in Nile than Athinais and these differed from the resistance in Morocco. Resistance in CI 8147 may have also differed from that in Morocco. Therefore, at least 2 and possibly 4 different major genes were involved in the expression of resistance within barley to the population of H. avenae used in these tests.

8. GENERAL DISCUSSION

These studies have shown *H. avenae* affected the early seedling growth of wheat, suppressed the grain yield and that significant reduction of grain yield could occur when no symptoms were obvious on the plant. A wider distribution of an economic reduction in the yield of wheat, as a result of this organism than was previously recognised in South Australia, has been implied. Therefore, the importance of the incorporation of resistance to the nematode into commercial cultivars of cereals was re-emphasised.

Two techniques were developed and widely used throughout these studies on different aspects associated with the resistance in cereals to the nematode. The results were often variable because insufficient data was available on the density of larvae normally associated with invasion of either individual or entire root systems of seedlings at different stages of growth. Often the density of larvae used in the inoculations were abnormally high and therefore, competition occurred between larvae invading, establishing and developing within roots, and although more practical methods of inoculation were determined, they were recognised too late for use in these investigations.

However, studies on the relation of the nematode to both resistant and susceptible cultivars of wheat showed that the invasion of root tips by different larvae within the nematode population was at random, and that approximately an equal number of male and female nematodes developed at low densities of inoculation. As the density of inoculation increased, either resistance was induced in the resistant cultivars or competition eventually occurred between larvae in the susceptible cultivar; female development was then restricted and more of the male than female larvae developed into adult nematodes.

The field study in which AUS 10894 was compared with Halberd showed the resistant cultivar reduced the density of the nematode and increased the yield of the susceptible cultivar in the following season. An examination of the relation of the nematode to the two cultivars during the first year of this study suggested that the resistance induced into the first seminal roots on AUS 10894 was also transferred to the roots which developed later. This effect of resistance and a growth room study, which compared the effect of the nematode on the growth of Halberd and AUS 10894, suggested tolerance may normally be associated with the resistance in AUS 10894. This was an important conclusion because it has implied that the selection of the highest yielding genotypes during the latter stages of selection in a breeding programme would also normally be a selection for resistance to the nematode.

Although the same monogenic resistance with partial dominance occurred in AUS 10894 and AUS 90248, the mechanism of resistance in AUS 10894 was consistently better than that in AUS 90248. This difference between the two resistant cultivars of wheat suggested a difference in the polygenes or modifier genes likely to be associated with the single major gene in the inheritance of resistance. Therefore, AUS 90248 would be a suitable cultivar to use as a resistant parent, but the better resistance in AUS 10894 would favour its selection as the cultivar to use in the transfer of resistance to commercial cultivars of wheat within a breeding programme.

Only one biotype seemed likely within South Australia, and both AUS 10894 and AUS 90248 were resistant to the nematode at all sites tested during this study. Although variations in the number of females occurred on the indicator plants, the relation of the nematode to resistant and susceptible cultivars showed these variations were likely

to be the result of variations in the density of active larvae used in the assessments, and not in the virulence of the nematode.

Therefore, the resistance available to the nematode in wheat cultivars would be beneficial if transferred to commercial cultivars. Increased grain yield would result from both the reduction of the density of the nematode population and the tolerance associated with the mechanism of resistance. The resistance would be retained for a long time as there is probably only one biotype in South Australia and also, there is unlikely to be any resistance-breaking nematodes within this population.

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