TOLERANCE IN WHEAT TO *HETERODERA AVENAE*

by

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SUMMARY

Field and laboratory studies were done to establish the existence of tolerance in wheat to *H. avenae*, to determine whether tolerant cultivars had any characteristics of growth which distinguished them from less tolerant cultivars and to develop a quick, simple and non-destructive tolerance assay for use by plant breeders.

A field trial using ethylene dibromide (EDB) to reduce the nematode population in some plots revealed that EDB delayed hatching of *H. avenae* by about 6 weeks but did not alter % hatch.

A second field trial, to assess the effect of nematode density (number of larvae per plant 2 weeks after sowing) on yield of two cultivars, provided the first direct evidence that tolerance to *H. avenae* exists in wheat cultivars. The difference between the cultivars was related to other yield parameters (number of heads per plot, number of fertile spikelets per plot and number of grains per plot) and the length of leaf 4.

Development of a laboratory technique using plants inoculated at sowing and grown at 10°C, showed that tolerance could be assessed in the early stages of growth. When growth parameters of 11 wheat cultivars, with a range of tolerance based on yield, were analysed, significant correlation coefficients showed that uninoculated roots of more tolerant cultivars grew more slowly up to 29 days after sowing than did those of less tolerant cultivars. Moreover, this characteristic was not related to weight of the original seed. Furthermore, plants whose uninoculated roots grew more slowly in the first 29 days were less affected by inoculation at sowing than were those with faster growing roots. This difference was apparent in roots (primary seminal, seminal lateral and total root lengths) 29 days after sowing and in shoots (shoot dry weight) 52 days after sowing.

A test to determine the influence of the endosperm on initial root growth rate showed that roots of a tolerant cultivar grew more slowly for the first 13 days of growth than did a less tolerant cultivar. After 13 days roots of the two cultivars grew at the same rate. Inoculation of the two cultivars while roots were growing at the same rate revealed that tolerance was not reliant on initial root growth rate but was associated with reduced response to the nematodes. The tolerant cultivar required seven times the inoculum density of the intolerant cultivar to produce a significant quantity of gall tissue. Direct assessment revealed that heavier galls were produced on the intolerant cultivar than on the tolerant cultivar. Thus it is possible that diversion of metabolites from other plant parts to gall tissue was less in the tolerant cultivar, a feature that might favour increased top growth and yield.

Tolerance of heterogeneous plant populations may be assessed using a visual rating of early top growth in the field at 6 weeks after sowing. Tolerance of homogeneous wheat lines may also be determined in the laboratory by assessing the reduction in length of primary seminal roots or root dry weight 29 days after inoculation at sowing or reduction in shoot dry weight after 52 days.

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.

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J.M. STANTON

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CHAPTER I

INTRODUCTION

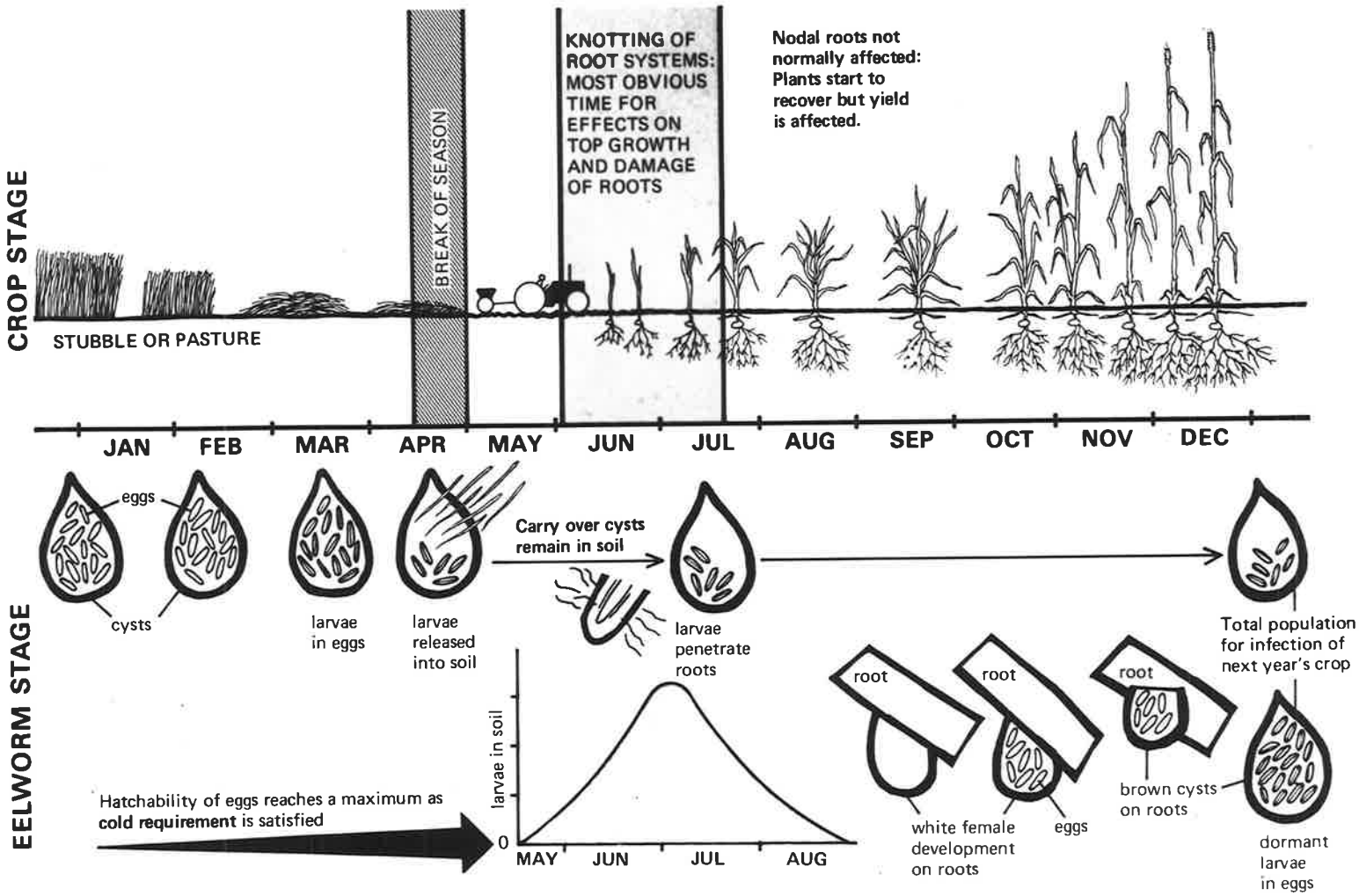
Heterodera avenae Wollenweber, 1924 was first recorded in Australia and South Australia by Davidson in 1930. Since then it has been reported in Victoria (Millikan, 1938a) but only in the Mallee and Wimmera districts (Meagher, 1968), Western Australia (MacNish, 1964) and New South Wales (McLeod 1968). *H. avenae* may be the most important organism damaging wheat, barley and oats (Banyer, 1966; Mathison, 1966; Hickinbotham, 1930; Robinson, 1961) in those states. Annual loss due to *H. avenae* is estimated at $20-40m. in South Australia and $30m. in Victoria (Rovira, 1982).

1.1 Life cycle of *H. avenae*

In the cereal regions of southern Australia, with a Mediterranean climate of hot, dry summers and cool, wet winters, cysts mature on host roots in late spring (November) and eggs survive over the summer (Fig. 1.1). Hatching and invasion occur after opening rains in autumn and winter (May to July). There is no evidence of an inherent seasonal hatching cycle (Banyer & Fisher, 1971a) but there are specific temperature requirements for hatching.

Cotton (1962) showed that hatching of English populations occurs with a temperature rise after a period of low temperature which suggests that hatching is most likely to occur in spring. This is not an adequate explanation as some larvae hatch in autumn to produce new cysts in spring (Coppock & Winfield, 1959; Vernon, 1962; Kerry & Hague, 1974). While Juhl (1968) obtained the best hatch with alternating temperatures, Fushtey and Johnston (1966) suggested that a pre-incubation period of 8 weeks at 0-7^{o}C was required for hatching Canadian populations for which the optimum temperature was 10-15^{o}C. However, this was not substantiated by Banyer and Fisher (1971b).

Fig. 1.1: Life cycle of *H. avenae* in relation to growth of wheat in South Australia (modified from Dubé *et al.*, 1979).


CROP STAGE
STUBBLE OR PASTURE
BREAK OF SEASON
KNOTTING OF ROOT SYSTEMS: MOST OBVIOUS TIME FOR EFFECTS ON TOP GROWTH AND DAMAGE OF ROOTS
Nodal roots not normally affected: Plants start to recover but yield is affected.
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
EELWORM STAGE
eggs
cysts
larvae in eggs
larvae released into soil
Carry over cysts remain in soil
larvae penetrate roots
Total population for infection of next year's crop
root
root
root
white female development on roots
eggs
brown cysts on roots
dormant larvae in eggs
Hatchability of eggs reaches a maximum as cold requirement is satisfied
larvae in soil
0
MAY
JUN
JUL
AUG

Hatching of Australian populations occurs in two phases (Banyer & Fisher, 1971b): (i) a period of larval development with an optimum temperature of 10°C and (ii) eclosion with an optimum temperature of 20°C. Phase 1 must be completed before phase 2 can start but both may occur over the range 5-20°C (Banyer & Fisher, 1971b). As temperatures drop below 20°C after summer to about 10°C at a depth of 10-15 cm in autumn and winter, both hatching phases may proceed. Rivoal (1978, 1979) showed a similar hatching pattern for two French populaions but with slightly different low temperature optima. The interpretation of English data by Williams and Beane (1972, 1979), suggesting a pre-incubation period of 8 weeks at 0-7°C, was inadequate in view of the two phase system suggested by Banyer and Fisher (1971b).

With early opening rains, eggs, under the influence of temperature, hatch as development is completed. If opening rains are delayed hatching occurs as soon as moisture is available resulting in a 'mass' hatch which coincides with germination and root emergence. Thus, damage is more severe with late opening rains (Banyer & Fisher, 1971a,b).

In England, Williams and Beane (1972, 1979) showed that at 10 and 15°C hatching was stimulated by root exudates of resistant and susceptible wheat, oat and barley cultivars and Kerry and Jenkinson (1976) obtained similar results with oats and winter barley in pots out-of-doors. Winslow (1955) and Hesling (1957), however, found no stimulation by root exudates of grasses and cereals at 20-25°C ~~nor~~ neither did Banyer and Fisher (1971b) in South Australia with wheat at 15-22°C. Stimulation by root exudates may affect initial density of larvae invading roots when cysts or eggs are used as inoculum.

The optimum temperature for invasion by *H. avenae* is 20°C (Davies & Fisher, 1976a) which is the same as that for motility (Banyer & Fisher, 1972). The number of larvae invading roots increases linearly with

inoculum density up to a maximum (O'Brien & Fisher, 1978a). Penetration is also affected by time of exposure of roots to larvae, number of root tips and distance of inoculum from roots (Davies & Fisher, 1976a). Individual larvae are able to penetrate and emerge from roots twice without loss of infectivity (Davies & Fisher, 1976a) and at 15°C most larvae are established in the roots within 17 days (Fisher, unpubl. data).

Continuous feeding by the female is necessary for maximum production (Cook, 1977), survival and development (Banyer & Fisher, 1976) of eggs. Fecundity and egg contents of females are not affected by time of maturity of the host (Cook, 1977) but, by delaying maturation of a particular host, the rate of egg production is reduced with an increase in the number of eggs produced (Banyer & Fisher, 1976).

1.2 Pathotypes

Most European countries have at least two pathotypes of *H. avenae*. The first report of pathotypes was on barley in Denmark (Andersen, 1959). Four pathotypes in Netherlands (Kort *et al.*, 1964) and at least two in England (Cotten, 1963; Fiddian & Kimber, 1964) and Wales (Fiddian & Kimber, 1964) have been recognised. Meagher (1974a) and McLeod and Khair (1978) showed that the Australian population is the same species as that in Europe and Canada but it is a different pathotype (Brown, R., 1969; Brown & Meagher, 1970). Ellis & Brown (1976) consider that the Australian population may consist of a mixture of pathotypes but there is little evidence for this. According to Meagher (1968), *H. avenae* is found only on sandy and friable soils in Victoria but the nematode has been reported in all cereal districts of South Australia on all soil types, including red-brown earths and heavy clays (Banyer, 1966). In

contrast to the work of Meagher (1974b)in Victoria, studies by Banyer and Fisher (1971a,b) in South Australia showed that hatching responds to low temperature. Despite possible behavioural differences, populations from Victoria and South Australia react similarly to an "International Test Range" of resistant and susceptible cultivars (O'Brien & Fisher, 1979).

1.3 Effects of *H. avenae* on wheat

A systemic nematicide applied 6 weeks after sowing to soil infested with *H. avenae* had no effect on wheat growth suggesting that a large amount of damage probably occurs during early growth of the plant (Brown, 1972). Rovira *et al.* (1981) found that by controlling *H. avenae* with aldicarb, which they claimed does not reduce fungal damage, the number of plants surviving, the number of fertile tillers, the number of grains per head, top weight at the three-leaf stage and leaf area at tillering were increased and concluded that *H. avenae* affects the plant between germination and tillering. Plant weight and grain weight per plant are reduced, ear emergence delayed (Meagher *et al.*, 1978) and the lengths of the first three leaves reduced by *H. avenae*, but time of stem elongation and, therefore, duration of vegetative growth and spikelet initiation are unaffected (O'Brien & Fisher, 1981).

Larvae usually penetrate the root just behind the growing root tip. This halts growth of the root which then becomes thickened at the penetration site and divides to produce a mass of short, thickened side branches (Gair, 1965). Giant cells or syncytia are formed and this becomes the feeding site for the larva. Thus, effects of *H. avenae* have been attributed to nutrient and water stress due to a reduction in development of seminal, seminal lateral and nodal roots (O'Brien & Fisher, 1981) and aerial effects are identical with symptoms of nitrogen and other mineral deficiences (Gair, 1965). Plants may

be able to recover or compensate for damage if water and nutrients are readily available (O'Brien & Fisher, 1981). Because of the association between nematode infection and nutrient deficiency in the wheat plant, it will be useful to describe briefly the influence of nutrient deficiency on uninfected plants.

1.4 The influence of nutrient deficiencies on wheat and subsequent growth responses

Nitrogen and phosphorus deficiencies reduce the number of seminal lateral (Tennant, 1976) and nodal (Drew *et al.*, 1973) roots and tiller number (Drew *et al.*, 1973; Tennant, 1976) with delay in tillering (Tennant, 1976). Low nitrogen levels also reduce the shoot : root ratio due to reduction in shoot growth (Brouwer, 1966; Drew *et al.*, 1973) and increased root extension (Brouwer, 1966; Tennant, 1976). Nitrogen deficiency just prior to floral initiation is reflected in reduced spikelet number (Single, 1964). Water stress retards shoot apex development (Angus & Moncur, 1977) and reduces shoot growth whereas roots are relatively insensitive (Brouwer, 1966).

Low leaf area index before ear emergence reduces grain number per spikelet and mean grain weight following reduction in rate of shoot apex development (Davidson, 1965). The final size (Williams, 1960) and dry weight (Williams, 1964 in Williams & Rijven (1965)) of a leaf increases with leaf number until floral initiation when leaves become progressively shorter (Jewiss, 1966). This change appears to be related to stem elongation. Lengths of successive sheaths, leaf area and leaf width, however, increase progressively (Jewiss, 1966).

Rapid leaf appearance is associated with more spikelets and faster tillering (Jewiss, 1966; Syme, 1974) and earlier tillers are more likely to become fertile (Ryle, 1966; Rawson, 1971). Tillering of temperate cereals

reaches a peak in spring and falls to a minimum before ear emergence (Thorne, 1962; Watson *et al.*, 1963). Duration of the period from floral initiation to terminal spikelet formation determines the number of spikelets which is increased only by extension of this period (Rawson, 1970). Thus, by ear emergence, yield potential, number of ears, number of spikelets per ear (Alston, 1979), number of florets per spikelet (Davidson, 1965; Alston, 1979), i.e. the number and potential size of sites at which starch can accumulate (Thorne, 1966), has been determined. Grain weight, however, is dependent on carbohydrate assimilated after ear emergence (Thorne, 1966).

Although a close relationship between the number of nodal roots and the number of tillers is expected this is often not found suggesting the involvement of other factors (Brouwer, 1966). It is generally accepted that effectiveness per g of the seminal root system is much greater than that of the nodal roots (Brouwer, 1966). Therefore, damage to seminal roots might be expected to have more effect on the plant than damage to nodal roots. Brouwer and Kleinendorst (1965, in Brouwer, 1966) showed that this may be related to compensatory growth. By pruning seminal roots, fineness and density of branches increased although weight increased only slightly.

Such observations indicate that damage to roots, such as that caused by nematodes, is likely to influence top growth in a variety of ways. In studies of tolerance, it will, therefore, be necessary to take various growth characteristics into account although it seems likely that yield is the integration of all responses.

1.5 Tolerance

Tolerance of a plant to an organism has received divergent opinions depending on the organism being studied. With pathogens other than nematodes, tolerance has been used to describe a level of resistance between immunity and full susceptibility (Schafer, 1971). That is, it has been used to

describe a resistant or partially resistant reaction although Rohde (1972) suggested that intolerance should be confined to the hypersensitive type of resistance reaction. Some authors (Caldwell *et al.*, 1958), however, state that tolerance should not be confused with resistance and nematologists generally tend to agree with the latter idea. Thus, although different terms may be applied to the character (e.g. host sensitivity (Cook, 1974)), there seems to be general agreement that a tolerant plant is one which suffers less from an equivalent nematode density than an intolerant plant. This is the definition that will be used in this thesis.

As tolerance is dependent on host response to invasion by, and subsequent growth and development of, the organism, variation between hosts is expected to exist (Cook, 1974). Tolerance is a relative concept and may occur in varying amounts (Schafer, 1971). Thus, the description (Dropkin, 1955; Caldwell *et al.*, 1958; Schafer, 1971; Rohde, 1972) that a tolerant plant may be subjected to heavy attack by the organism without suffering high yield loss may be too difficult to attain with some disease associations at the present time. There are different levels of tolerance in cereals to *H. avenae* when measured over a limited range of densities (Fisher *et al.*, 1981) but these may change when the density of *H. avenae* is altered. Seinhorst (1961) proposed a model to describe the relationship between nematode density and yield. He concluded that damage by nematodes occurred only when the nematode density exceeded the tolerance limit (T). Yield was not affected below this density because either damage occurred only to tissue that was not essential for plant growth or the plant was able to recover (Seinhorst, 1965). Although the concept of a tolerance limit provides a useful way of determining an acceptable nematode density, no experimental evidence has given it unequivocal support.

An important advantage which tolerance has over resistance is that it does not exert selection pressure to change the virulence of the pathogen but if tolerance is unlikely to provide sufficient protection, especially with a heavy attack, then it should be combined with other control measures such as resistance (Schafer, 1971; Fisher *et al.*, 1981).

One of the problems with tolerance is that its use leads to an increase in the numbers of the organism. During development of populations of *H. avenae*, for example, on a particular host, one of the factors regulating numbers is the amount of damage caused to the host (Jones & Perry, 1978). The reduction in damage due to tolerance will increase the amount of root material available for reproduction resulting in higher multiplication rate or a higher ceiling level (Andersson, 1982; Cook & York, 1982; Gair, 1965; Grosse *et al.*, 1982; Seinhorst, 1961). The effect of such an increase may overcome tolerance (Cook & York, 1982) and might be disastrous if an intolerant crop followed.

There is confusion among mycologists on the use of tolerance. Part of this arises because of the difficulty in assessing the numbers of fungi present in a plant. Thus, symptoms (e.g. lesions, pustules, etc.) have been used as an indirect measure of the fungus (Schafer, 1971). Symptom expression and yield may be closely related, however, particularly where the plant part harvested is the same as that diseased, so that the amount of fungus and yield become almost synonymous and lead to ambiguity in assessing tolerance. Another difficulty with fungi and also with some nematodes arises in those cases where the organism reproduces continuously in its association with the plant e.g. *Ditylenchus dipsaci* on oats (Stanton, 1979) or rusts on wheat. A comparison of tolerance between two cultivars depends on yield reduction due to equivalent densities of the organism. Although initial densities of the organism may be the same, small differences in

resistance may vastly alter numbers by the end of the season, so that tolerance cannot be assessed at equivalent densities. Either cultivars with the same degree of resistance must be compared or yields must be assessed by comparing a parameter related to yield during the first generation of the organism. Most of the information on tolerance that has been published deals with *Heterodera* spp. or *Globodera* spp., i.e. those nematodes which produce a single generation during the life of the host.

Of primary importance in assessing tolerance, in order to eliminate the effects of resistance, is to establish equivalent growth of the pathogen on the cultivar (Schafer, 1971). This may be done by counting pathogens (e.g. nematodes in roots) but often initial density of the pathogen is used to estimate the intensity of pathogen attack (e.g. fungal pathogens). The latter method is not sufficient to remove the effects of different levels of resistance.

Ideally, assessment of tolerance should be made by comparing slopes of regression lines of yield on initial population density (Fisher *et al.*, 1981). This is particularly difficult to measure in the field so resistance is more often sought (O'Brien & Fisher, 1974). Evans (1982b) stated that the only satisfactory method of assessing tolerance of a cultivar is to grow it with other cultivars in plots of varying nematode densities. He described four experimental designs which have been used to produce a range of population densities in order to eliminate laborious sampling of small plots:

1. Use of two or more sites with different nematode populations but which are otherwise similar (e.g. Evans & Franco, 1979; Fisher *et al.*, 1981). Although this method gives no control over environmental differences between sites, the use of several sites may indicate

those environmental factors affecting plant growth and nematode populations that can be taken into account in assessing tolerance.

2. Use of heavily infested fields with nematicide on some plots (e.g. Whitehead *et al.*, 1980). This is complicated by the effects of the nematicide on factors other than nematodes.

3. Use of plots widely spaced across an infested field to give a range of densities (e.g. Brown, E., 1969). In South Australia, where soils are extremely variable, environmental differences may obscure treatment effects.

4. Use of preparatory treatments (e.g. with resistant and susceptible cultivars) to produce a population range (e.g. Evans, 1982b). This method requires 2 years and produces differences in nutrient content of the soil.

The way in which yield and other differences are expressed may cause variation in tolerance assessment (Fisher *et al.*, 1981). For example, if the yield loss, in absolute terms, of two cultivars is similar with a given pathogen density, i.e. regression lines comparing yield and pathogen density are parallel, the higher yielding one will appear to be more tolerant if differences are expressed as percentage yield reduction. Absolute yield loss expresses the effect of the disease alone whereas percentage yield loss includes the effect of the cultivar's inherent yielding potential. In this thesis tolerance will be expressed as absolute yield loss.

For practical plant breeding purposes it is desirable to assess tolerance early in growth and by non-destructive means.

1.6 Reports of tolerance to nematode attack

In 1959, Mountain and Patrick showed that the peach cultivar Shalil,

is more tolerant to *Pratylenchus penetrans* than is the cultivar Lovell. They claimed that tolerance to attack and subsequent root degeneration, which is mainly due to production of phytotoxic substances through hydrolysis of amygdalin by the nematode, may depend on the amount of amygdalin in the root system.

Fox and Spasoff (1976) reported tolerance of tobacco cultivars to *Globodera solanacearum*.

The degree of tolerance of potato cultivars to *G. rostochiensis* was found to be related to accumulation of calcium in the plant on uninfested soil and this may reflect water use efficiency (Evans & Franco, 1979). Later, Evans (1982a) found a correlation between transpiration per g plant dry weight and % calcium in total dry matter. Furthermore, nematode infestation induced an increase in abscisic acid (ABA) levels which halted growth. It is possible that nematode-induced increase in ABA levels has less effect on nematode tolerant cultivars. Evans (1982b) concluded that the simplest assays for tolerance were stomatal resistance, which was lower in more tolerant cultivars, and potassium and calcium accumulation which were higher and lower respectively in more tolerant cultivars. Moreover, as shoot : root ratio is constant for a given nematode population, root growth, which is greater in more tolerant cultivars (Stone, 1981; Evans, 1982b) may be assessed by monitoring shoot growth (Evans, 1982b).

Seinhorst (1979) hypothesized, after studying *H. avenae* on oats, "..... that nematode attack slows down the development of the plant in such a way that plants of the same size remain identical in form and stage of physiological development irrespective of the time they require to attain that size". Thus, tolerance would be expressed as reduction of growth

delay. In 1981(b) Seinhorst described two other mechanisms by which nematode attack affects plant growth; (i) growth reduction associated with reduced water consumption and increased dry matter content (this is common with tylenchids) and (ii) increase in shoot : root ratio by decreasing root weight and decrease of water consumption which may possibly lead to reduction in growth rate (e.g. *Longidorus elongatus* on *Lolium perenne*).

Howard (1965 in Schafer 1971) thought that tolerance to nematodes might depend on strong root systems or on drought resistance. Evans (1982b) found that potato cultivars which are more tolerant to *G. rostochiensis* do produce larger root systems. Differences in water supply, however, do not affect tolerance of oats to attack by *H. avenae* (Seinhorst, 1981a). In fact, drought accelerated but nematode attack delayed emergence of the first panicle. Thus, effects of nematode attack cannot be explained simply in terms of water stress.

It is generally accepted that barley cultivars are more tolerant to *H. avenae* than wheat which is more tolerant than oats (Hesling, 1959; Stone, 1960). Grosse *et al.* (1982) found that tolerance of oats cv. Hedwig was overcome by a density of 39 eggs and larvae/g of soil whereas barley cv. Gitte was still tolerant at this density. Seinhorst (1981a) claimed that the tolerance limit for oats was the same as that for wheat (Meagher & Brown, 1974) but it is difficult to equate these two findings because of the difference in technique. Meagher and Brown studied the effect of encysted eggs in microplots on grain yield whereas Seinhorst used hatched larvae, unevenly distributed, as inoculum for pot experiments and assessed the effects on yield indirectly by measuring early growth characters.

1.7 Mechanisms of resistance to *H. avenae*

By definition (Fisher *et al.*, 1981), resistance reduces development and reproduction of the nematode. However, resistance presents no barrier to initial larval penetration (Williams, 1970; Cotten, 1967, 1970a,b; Cook *et al.*, 1974; O'Brien & Fisher, 1977, 1978b; Johnson & Fushtey, 1966). After initial penetration by *H. avenae* of resistant (AUS 10894) and susceptible (Halberd) wheat genotypes, the number of galls developing and the number of nematodes increased on Halberd but the number of nematodes in AUS 10894 decreased throughout the growing season and the number of galls remained the same (O'Brien & Fisher, 1977). Although the evidence is not convincing, the authors' explanation of the decrease in total number of *H. avenae* in AUS 10894 roots was the induction of resistance in the seminal roots which were available to the nematodes when invasion began. Either physiological changes occurred within the plant or resistance was induced into later roots to prevent invasion and development. According to O'Brien and Fisher (1978b), resistance is induced into inoculated roots of AUS 10894 within 12 hours but this resistance is not transferred to uninoculated roots of the same seedling. Development of *H. avenae* is delayed similarly in resistant barley genotypes and this is noticeable 14 days after sowing (Cotten, 1970b). Fewer *H. avenae* are found in resistant than susceptible oat roots 30 days after sowing (Cook *et al.*, 1974). Fewer nematodes are found in these resistant cultivars either because they fail to establish feeding sites and leave the roots or they die and become unidentifiable (Cook *et al.*, 1974). Development of the male of *H. avenae* is unaffected by resistant wheat and barley cultivars but few or no viable females are produced (Brown, 1974). Corn, which is resistant to *H. avenae*, permits maturation of females which then do not break through the root surface to be

fertilised and, therefore, fail to produce viable cysts (Fushtey, 1965; Johnson & Fushtey, 1966). Necrotic reaction of resistant wheat (Brown, 1974) and corn (Johnson & Fushtey, 1966) cultivars may inhibit development of *H. avenae*.

1.8 Resistance to *H. avenae*

Resistance to *H. avenae* has been known in barley since 1920 (Nilsson-Ehle, 1920; Andersen, 1961; Gair *et al.*, 1962; Ellis & Brown, 1976) and has been found in oats (Andersen, 1961; Cotten, 1963; Mathison, 1966; Brown & Meagher, 1970), rye (Brown & Meagher, 1970) and wheat (Nielsen, 1966). The first evidence of field resistance to an Australian population was found in oats and barley (Millikan, 1938b). Wheat cv. Loros is resistant in Denmark (Nielsen, 1966) but only moderately resistant to the South Australian (O'Brien & Fisher, 1974) and Victorian (Brown, 1974) populations. Moderate resistance was also found in Spring wheat, AUS 10894 (O'Brien & Fisher, 1974).

Inheritance of resistance to *H. avenae* differs between cultivars. In *Avena sterilis* I.376 it is controlled by two dominant genes, in *A. sativa* cv. Mortgage Lifter by two recessive genes and in *A. byzantina* PI 175021 by one dominant gene (Cotten & Hayes, 1972). Inheritance of resistance in barley is controlled by one dominant gene but there are different genes in some cultivars so three to five genes may be involved (O'Brien *et al.*, 1979). Resistance in Loros and Spring wheat (AUS 10894) to the Australian population has been attributed to the same single major dominant gene although there may be modifier genes in Loros (O'Brien *et al.*, 1980).

1.9 Relationship between resistance and tolerance

Resistance of tobacco to *G. solanacearum* is genetically independent of tolerance (Fox & Spasoff, 1976). This has also been claimed for potato and *G. rostochiensis* (Evans & Franco, 1979), wheat and *H. avenae* (Fisher *et al.*, 1981) and various host species and *H. marioni* (Christie, 1946). The tobacco cultivar, Dixie Bright 101, is resistant and intolerant to *Pratylenchus* spp. (Drolsom & Moore, 1955) as corn is to *H. avenae* (Fushtey, 1965; Johnson & Fushtey, 1966). Thus, tolerance may be considered separately from resistance. Many workers have found that tolerance is associated with resistance, e.g. *H. avenae* on barley (Cotten, 1970b) and *Ditylenchus dipsaci* on various host species (Stanton, 1979), but in both of these examples the association was due to differences in resistance of host plants which altered the nematode density so that the effects of tolerance were obscured.

1.10 Aim of experimental work

Incorporation of resistance to *H. avenae* into current wheat cultivars (e.g. Condor, Halberd, Egret and Oxley) is not likely to be entirely satisfactory because these cultivars are damaged severely. The present work, therefore, has a number of aims both practical and academic; (i) the clear establishment of tolerance to nematodes in wheat cultivars or lines, (ii) an understanding of the nature and assessment of tolerance and (iii) a non-destructive and simple test for tolerance that can be used by plant breeders, particularly in the early stages of growth.

Two field assays were tested: (i) use of nematicide on field plots and (ii) use of initial density estimate on a series of small plots. The effect of the nematode on growth processes of tolerant and intolerant wheat cultivars was studied intensively both in the field and in a controlled en-

vironment. The influence of a nematicide on both the nematode and wheat plants was assessed in a field trial.

CHAPTER II

EFFECTS OF *H. AVENAE* ON TWO WHEAT CULTIVARS, EGRET AND COOK, IN A FIELD TRIAL WITH NEMATICIDE TREATMENT

In 1978, Fisher *et al.* (1981) compared yield and early growth parameters of many cultivars and breeders' lines of wheat on a site heavily infested with *H. avenae* with the average on five other sites in South Australia. They found that, with the exception of cv. Cook, tolerance, as measured by yield, and early growth were correlated. It subsequently appeared that Cook was severely damaged in early growth but recovered to become the most tolerant of commercial cultivars tested. The following trial was designed to examine the nature of recovery and to glean some information on tolerance.

Chemical control became commercially feasible in 1978 with the development of an applicator for ethylene dibromide (EDB) (Gurner *et al.*, 1980) so this system was used to produce 'nematode-free' control plots and also to examine the effect of EDB on *H. avenae* and host plants.

2.1 Materials and Methods

The trial was conducted in a sloping field of sandy soil near Murray Bridge, 80 km south-east of Adelaide, South Australia, in an area with average annual rainfall of approximately 300 mm falling mainly between May and October. The field was to be returned to natural pasture after one year of Halberd wheat, a susceptible cultivar. The land was harrowed by the farmer on 1/5/80 to a depth of 5 cm and plots were sown on 28/5/80 using a Connor Shea combine. Sowing and fertilizing with superphosphate were conventional for the area. EDB was applied at sowing with a Jectarow* at the

*Registered trade name.

rate of 3.7 1/ha. Herbicides MCPA* and Dicamba*, were applied at recommended rates by hand spray on 29/7/80.

Five treatments, fallow (F), Egret (E) (an intolerant commercial cultivar), Cook (C), Egret treated with EDB (EN) and Cook treated with EDB(CN), were replicated four times and arranged in a randomised complete block design with plots 2 x 5m with a 30 cm pathway between plots in the same replicate and a 1m pathway between replicates. Each replicate was positioned across the slope. Each plot was divided into 10 sub-plots of 1 x 1m using wooden pegs. One sample was taken from each sub-plot at every sampling time. In order to minimise the effects of destructive sampling each sub-plot was divided visually into nine equal squares. These were sampled at random throughout the trial but each was used only once. An auger of 5 cm diameter and 15 cm in length was used for sampling to obtain soil and root material. When plants were taken, the auger was placed over the plant so that it was in the centre. The plant and soil obtained was used as one sample. In all, 39 parameters were measured on each sub-plot at various times throughout the year.

Four weeks before sowing, initial density of the nematode was determined in a sample from each of the sub-plots (i.e. 200 samples) by the following method: first, the % soil water content was estimated from three 50g soil samples. Cysts were extracted from a 200g soil sample (adjusted for soil water) in a 500 ml flask. Organic matter and cysts were floated off and decanted on to a 22 mesh sieve (with openings of 710 µm) over a 44 mesh sieve (with openings of 355 µm). After washing, material in the top sieve was discarded and that in the lower sieve was washed on to a piece of 11 cm filter paper with lines spaced to fit the field of vision of the

*Registered trade names.

microscope, i.e., approximately 12 mm. The filter paper had been moistened and placed in a Büchner funnel. The water was removed under vacuum and the filter paper transferred to a glass plate. Under 12.5 x power, cysts were picked out and put into water in a glass block. Cyst walls were broken and eggs removed. All healthy eggs and larvae in three 1 ml aliquots of a suitable dilution were counted and numbers of eggs per g and eggs per cyst determined.

Four, ten and sixteen weeks after sowing, free larvae in the soil were counted and height and growth stage of plants recorded. Larvae and some organic matter were extracted from a 300g soil sample (adjusted for soil water) using a Seinhorst elutriator. Larvae were separated from organic matter using a modified Baerman funnel. Growth stage was measured using the code of Zadoks *et al.* (1974). Height, to the nearest 5 mm, was measured from the ground to the tip of the longest leaf.

Larval penetration in roots, numbers of knots per root system and length of the longest primary root, were measured 4 weeks after sowing. Roots were washed and then stained by boiling for 3 minutes in lactophenol cotton blue (Southey, 1970). After clearing for 3-4 days in clear lactphenol, the roots were homogenised at high speed for 10 minutes and larvae counted microscopically. Ten and sixteen weeks after sowing, root lengths and % of root-tips invaded were measured. A line intersect method modified by Tennant (1975) was used to measure the total length of the nodal and seminal root systems. It was impractical to count the total number of larvae in the root system at these times because of the large number involved and because the whole root system could not be collected. Furthermore, early mature males may have left the root system and mature females may have

been dislodged during sampling so that counting would have been inaccurate. An estimation of larval invasion at these two sampling times was obtained by sampling 50 root-tips at random on each of the nodal and seminal root systems and recording the % of root-tips with one or more larvae present.

An estimate of germination was obtained, 7 weeks after sowing, by counting the number of plants in the middle row of each sub-plot. Seven, eight and nine weeks after sowing, the presence of tillers, nodal roots and damage to nodal roots, on one plant selected at random from each sub-plot, was recorded.

For the final sampling, 30 weeks after sowing, cysts were extracted from 200g of soil as described above, but new and old cysts and eggs were counted separately. Percent hatch and multiplication rate of eggs were calculated using the following formulae:

1. % hatch = $100 (P_i - C)/ P_i$

   P_i, mean initial population density;

   C, mean carryover population density of 10 plot values.

2. multiplication rate = P_f / P_i

   P_f, mean final population density of 10 plot values.

Larvae collected from the soil 4, 10 and 16 weeks after sowing were pooled in each treatment and tested for infectivity. In the first two tests, only larvae from fallow (F) and untreated Egret (E) plots were used; in the third, larvae from EDB-treated Egret (EN) and Cook (CN) plots were also tested. Freshly hatched larvae (Chapter 4.1.1.1) were used throughout in control treatments. Pre-germinated Egret seedlings were sown in short tubes (Chapter 4.1.1.1) and inoculated with 100 larvae in 5 ml water (six replicates were used) and grown at 15°C with a 14 hour day. After 17 days

the roots were washed, stained with lactophenol cotton blue (Southey, 1970), macerated and the larvae counted. Infectivity was defined as the number of test larvae which had penetrated expressed as a % of the number of control larvae which had penetrated.

No yield data were available from this trial because of damage by rabbits.

An analysis of variance using a 2 x 2 factorial design was used to analyse data comparing cultivars and EDB treatment. Data from plots untreated with EDB (E, C and F plots) were compared using an analysis of variance with one-way classification. It would have been possible to analyse all data together in a 3 x 2 factorial design if a treatment FN (i.e. fallow plots treated with EDB) had been included. This treatment was considered to be redundant and, therefore, not a practical use of the time available. Thus, the analysis was split into two parts to assess EDB treatment x cultivar interactions and also the effect of the presence of a host on *H. avenae*. A 3 x 2 x 2 factorial design for analysis of variance was used to determine cultivar x EDB x time interactions for data pertaining to number of larvae in soil at 4, 10 and 16 weeks after sowing. A 3 x 2 factorial design was used to determine cultivar x time interactions for data pertaining to % infectivity at 4, 10 and 16 weeks after sowing. Appropriate data transformations were used as required.

2.2 Results

From the 200 samples, the mean initial density of *H.avenae* of the experimental area was 23.6 eggs/g with variation in different plots from 8.3 - 51.0 eggs/g and between replicates from 17.4 - 27.3 eggs/g. There was no significant difference in initial density between treatments or cultivars (Tables 2.1, 2.4).

TABLE 2.1: Effect of the presence of host plants and of cultivar on *H. avenae*.

Parameter	Host Effect[a]	Egret	Cook	Fallow	L.S.D. (P=0.05)
Initial density (eggs/g)	NS	26.2 (1.29)[b]	24.8 (1.26)	27.3 (1.26)	-
Larvae in 300g soil 4 weeks after sowing	NS	168 (1.77)	299 (2.35)	259 (2.31)	-
Larvae in 300g soil 10 weeks after sowing	NS	177 (2.12)	130 (2.01)	136 (2.05)	-
Larvae in 300g soil 16 weeks after sowing	NS	33 (1.33)	30 (1.17)	41 (1.39)	-
Number new cysts in 300g soil[c]	*	7 (0.77)	10 (0.73)	2 (0.41)	0.15
Number old cysts in 300g soil[c]	NS	4	6	5	-
Total number cysts in 300g soil[c]	NS	12 (0.99)	16 (1.04)	7 (0.88)	-
Eggs/new cyst[c]	NS	321	288	364	-
Eggs/old cyst[c]	NS	175	172	180	-
Total eggs/cyst[c]	NS	250	229	229	-
New eggs/g[c]	*	11 (0.90)	11 (0.81)	4 (0.55)	0.17
Old eggs/g[c]	NS	4 (0.58)	6 (0.70)	5 (0.70)	-
Total eggs/g[c]	NS	15 (1.07)	18 (1.07)	8 (0.91)	-

[a] NS - F value not significant ($P<0.05$); * F value significant ($P<0.05$)

[b] $\log_{10}$ transformation

[c] assessed at end of growing season.

2.2.1 Effect of presence of host plants on *H. avenae*

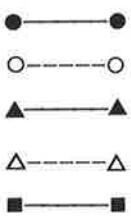
The number of free larvae present in the soil for each treatment at each sampling time is shown in Fig. 2.1. However, because numbers are complicated by interactions and different methods of analysis, an overall least significant difference cannot be given but differences will be discussed below.

At any one of the three sampling times the number of free larvae in 300g soil in fallow (F), untreated Egret (E) and untreated Cook (C) plots did not differ (Table 2.1). There was no significant interaction ($P<0.05$) between time and host (F, E or C plots). There was a significant effect ($P<0.01$) of time (Table 2.2) on the number of free larvae, which remained the same at 4 and 10 weeks after sowing but was reduced at 16 weeks.

There was a significant interaction ($P<0.05$) between time and presence of host plants on % infectivity (Fig. 2.2). At 4 weeks after sowing, % infectivity of larvae from E plots was significantly less than that from F plots; at 10 weeks there was no significant difference; at 16 weeks % infectivity of larvae from E plots was significantly higher than that from F plots.

The only effects of host on final measurements (i.e. 30 weeks after sowing) of *H. avenae* were on number of new cysts and eggs/g of soil (Table 2.1) and these were due to a reduction of these parameters in F plots compared to E and C plots. No significant differences were found between % hatch or multiplication rate in F, E or C plots (Table 2.3).

Fig. 2.1: Hatching pattern of *H. avenae* showing relationship between mean number of free larvae/g of soil from five treatments and time after sowing.



Egret untreated
Egret treated with EDB
Cook untreated
Cook treated with EDB
Fallow

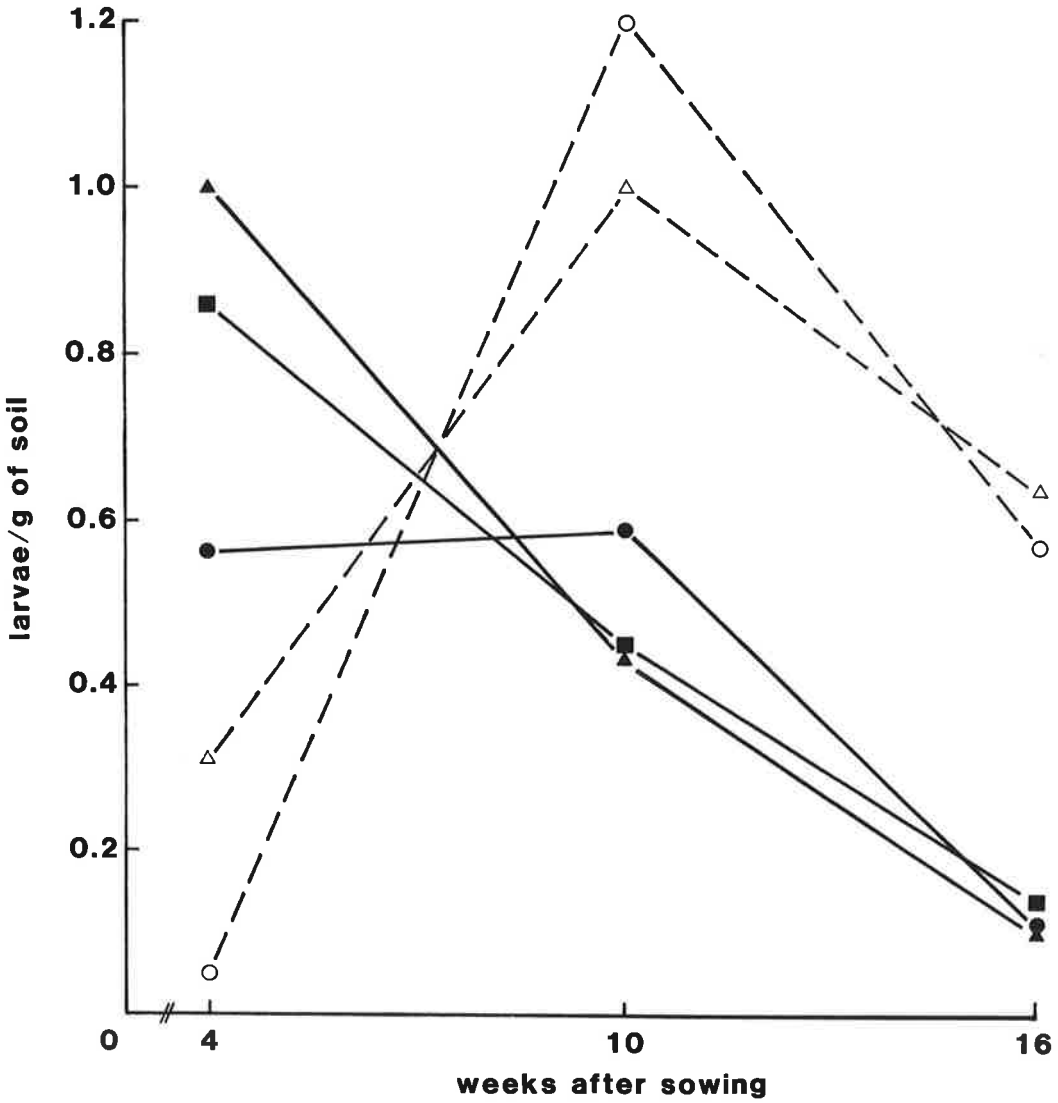

1.2
1.0
0.8
0.6
0.4
0.2
0
4
10
16
larvae/g of soil
weeks after sowing

TABLE 2.2: Log_e of mean number of *H. avenae* larvae in 300g soil from untreated Egret, Cook and fallow plots at 4, 10 and 16 weeks after sowing.

	Weeks after sowing		
	4	10	16
Larvae in 300g soil (log_e)	4.95	4.75	3.00

L.S.D. ($P<0.05$) = 0.51
($P<0.01$) = 0.72

Fig. 2.2: Changes in % infectivity of free *H. avenae* larvae from soil of fallow and Egret-sown plots 4, 10 and 16 weeks after sowing.

L.S.D.	(P<0.05)
●———●	Egret untreated
○-----○	Fallow

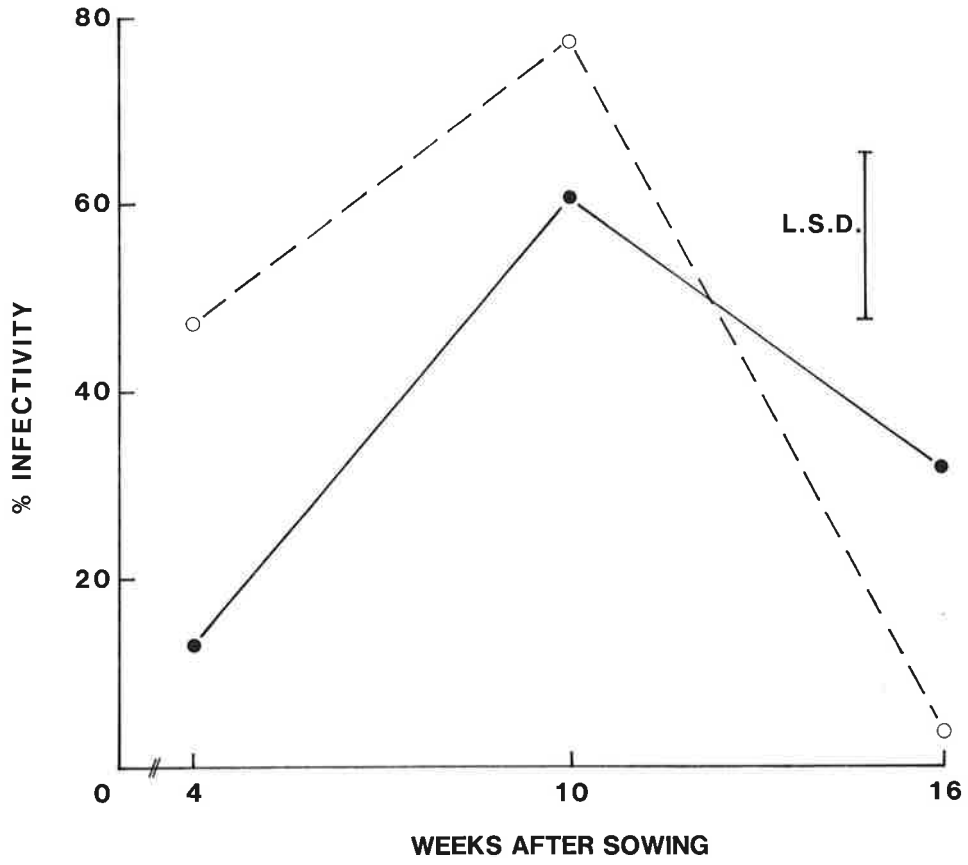

80
60
40
20
0
4
10
16
% INFECTIVITY
L.S.D.
WEEKS AFTER SOWING

TABLE 2.3: Mean plot values of % hatch and multiplication rate of *H. avenae* in Egret (E), Cook (C) and fallow (F) treatments[a].

	Fallow	Egret	Cook
% hatch	78.7	81.2	76.3
Multiplication rate	0.38	0.71	0.86

[a]treatment effects not significant ($P<0.05$).

2.2.2 Effect of cultivar and time after sowing on *H. avenae*

There was no cultivar effect on the number of larvae in roots 4 weeks after sowing nor on the % of seminal root-tips invaded at 10 and 16 weeks or % of nodal root-tips invaded at 16 weeks after sowing (Table 2.4). At 10 weeks after sowing, however, more root-tips of Egret plants (E and EN plots) contained larvae than did those of Cook plants (C and CN plots). There were no cultivar effects on final counts of cysts and eggs (Table 2.4) or on % hatch or multiplication rate of *H. avenae* (Table 2.5).

A significant cultivar x time effect was found on free larvae in 300g of soil (Table 2.6). Although no effect of cultivar on number of larvae in soil was observed when data were analysed at each time for cultivar x EDB interactions (Table 2.4), when these data were pooled to test for cultivar x time interactions (Table 2.6), 4 weeks after sowing, the number of free larvae in soil of all plots sown with Egret (E and EN plots) was less than that in Cook-sown (C and CN) plots. Significant differences between cultivars were not found for this character at the other two sampling times. When looking at the differences over time for each cultivar (Table 2.6), the number of free larvae in soil of Egret-sown plots increased between weeks 4 and 10 and then decreased by week 16 to the same level as that at week 4. Cook-sown plots, however, contained the same number of free larvae at weeks 4 and 10 and the number was reduced by week 16. The overall effect, when looking at all plots not treated with EDB (F, E and C plots)(Table 2.2), shows that the number of free larvae in soil was similar at weeks 4 and 10 but was reduced ($P < 0.01$) at week 16.

TABLE 2.4: Effects of cultivar and EDB treatment on *H. avenae*

Parameter	Effects[a]			Treatment[b]			
	Cultivar	EDB	Cultivar x EDB	E	C	EN	CN
Initial density (eggs/g)	NS	NS	NS	26.2	24.8	15.0	24.5
Larvae in 300g soil at week 4	NS	*	NS	168 (4.07)[c]	299 (5.41)	15 (2.27)	94 (3.25)
Larvae in 300g soil at week 10	NS	**	NS	177 (4.88)	130 (4.62)	359 (5.55)	309 (5.28)
Larvae in 300g soil at week 16	NS	**	NS	33 (3.06)	29 (2.69)	172 (4.62)	192 (4.31)
Larvae in roots at week 4	NS	NS	NS	41 (2.88)	80 (3.15)	7 (1.25)	46 (2.27)
% seminal root-tips invaded at week 10	NS	NS	NS	25.8	35.8	35.2	33.2
% seminal root-tips invaded at week 16	NS	NS	NS	27.9	28.7	27.1	33.6
% nodal root-tips invaded at week 10	**	*	NS	35.5	29.4	40.1	33.9
% nodal root-tips invaded at week 16	NS	NS	NS	26.9	24.6	26.9	22.6
Number new cysts in 300g soil[d]	NS	*	NS	7 (1.78)	10 (1.68)	3 (0.98)	4 (1.27)
Number old cysts in 300g soil[d]	NS	NS	NS	4	6	5	5

continued/...

TABLE 2.4 (continued)

Parameter	Effects[a]			Treatment[b]			
	Cultivar	EDB	Cultivar x EDB	E	C	EN	CN
Total number cysts in 300g soil[d]	NS	NS	NS	12 (2.28)	16 (2.40)	7 (1.97)	9 (2.05)
Eggs/new cyst[d]	NS	NS	NS	321	288	291	302
Eggs/old cyst[d]	NS	**	NS	175	172	110	150
Total eggs/cyst[d]	NS	**	NS	250	229	166	201
New eggs/g[d]	NS	*	NS	11 (2.08)	11 (1.85)	4 (1.10)	6 (1.46)
Old eggs/g[d]	NS	NS	NS	4 (1.34)	6 (1.61)	3 (1.14)	4 (1.32)
Total eggs/g[d]	NS	**	NS	15 (2.46)	18 (2.48)	6 (1.77)	9 (2.03)

[a] NS - F value not significant ($P<0.05$); * F value significant ($P<0.05$); ** F value significant ($P<0.01$)
[b] E - Egret untreated; C - Cook untreated; EN - Egret treated with EDB; CN - Cook treated with EDB
[c] - Log_e transformation
[d] - Assumed negative binomial distribution and deviances compared to χ^2.

TABLE 2.5: Mean plot values of % hatch and multiplication rate of *H. avenae* in Egret- and Cook-sown plots, untreated or treated with EDB[a].

	Untreated	EDB-treated
% hatch		
Egret	81.2	76.3
Cook	83.1	77.0
multiplication rate		
Egret	0.71	0.86
Cook	0.80	0.69

[a]EDB-treatment, cultivar and EDB-treatment x cultivar effects not significant ($P<0.05$).

TABLE 2.6: $\log_e$ of mean number of *H. avenae* larvae in 300g soil at 4, 10 and 16 weeks after sowing untreated and EDB-treated Egret and Cook plots to show EDB x time[a] and cultivar x time[b] effects.

Weeks after sowing	Untreated	EDB-treated	Egret	Cook
4	4.77	2.76	3.20	4.33
10	4.76	5.42	5.22	4.96
16	2.89	4.47	3.84	3.52

[a] EDB x time effect significant ($P<0.01$)
[b] Cultivar x time effect significant ($P<0.05$)
L.S.D. for comparing between times for untreated or EDB-treated plants or for same cultivar:
($P<0.05$) = 0.72
($P<0.01$) = 1.04
L.S.D. for comparing between untreated and EDB-treated plants or cultivars at same time:
($P<0.05$) = 0.66
($P<0.01$) = 0.94

2.2.3 Effect of EDB treatment on *H. avenae*

Significant effects of EDB treatment were found at weeks 4, 10 and 16 on numbers of free larvae in 300g soil (Table 2.4). At week 4 there were fewer larvae present in EDB-treated soil (EN and CN plots) than in untreated soil (E and C plots). At weeks 10 and 16 there were more larvae present in EDB-treated than in untreated soil. There was a significant EDB x time interaction on numbers of free larvae in soil (Table 2.6). In untreated plots (E and C) the number of larvae present in soil at weeks 4 and 10 were the same and then decreased by week 16. This was also noted in F plots (Table 2.2). EDB-treated plots (EN and CN), however, had fewer larvae at week 4 than at week 10 (Table 2.6). At week 16 the number of larvae was less than at week 10 but was still greater than at week 4.

Infectivity at week 16 was unaffected by EDB treatment as larvae from E, EN and CN plots were similar in this character (Table 2.7). Larvae from these plots were all more infective than those from F plots.

There was no significant effect of EDB treatment on number of larvae in roots at week 4, on % of seminal root-tips invaded at weeks 10 and 16 or on % of nodal root-tips invaded at week 16 (Table 2.4). However, EDB treatment increased the % of nodal root-tips invaded at week 10.

Some parameters of the final population were affected by EDB treatment. There were fewer new cysts, fewer eggs remaining in old cysts, and reductions in total eggs per cyst, new eggs/g and total eggs/g following EDB treatment. Percent hatch and multiplication rate were unaffected by EDB treatment (Table 2.5).

TABLE 2.7: Percent infectivity of free *H. avenae* larvae from F, E, EN and CN plots 16 weeks after sowing

	F[a]	E	EN	CN
% infectivity	9	39	38	36

L.S.D. (P<0.05) = 15
(P<0.01) = 24

[a]F - fallow; E - Egret untreated; EN - Egret treated with EDB; CN - Cook treated with EDB.

2.2.4 Effect of EDB treatment on host

No significant effect of EDB was found on growth stage at weeks 4 and 16 (Table 2.8) but at week 10 EDB increased growth stage. At week 10, when leaves were emerging, Egret plants were more mature than Cook plants. Plant height was increased by EDB treatment at weeks 4 and 10 but not at week 16. At the latter two times Egret plants were taller than Cook plants.

The number of plants surviving to week 7 in the middle row of each sub-plot was affected by both cultivar and EDB treatment. EDB treatment decreased the number of plants remaining and there were fewer Egret than Cook plants (Table 2.8).

At week 4, EDB decreased the number of knots on roots but had no significant effect on the length of the longest root at that time (Table 2.8).

Seminal root length at weeks 10 and 16 was unaffected by EDB treatment and both cultivars had the same length roots (Table 2.8). The % of seminal root-tips invaded at these times was also unaffected by cultivar or EDB treatment (Table 2.4).

At weeks 7 and 9, EDB treatment increased the % of plants with nodal roots and more Egret plants had nodal roots than did Cook (Table 2.8). At week 8 there was a cultivar x EDB interaction affecting the % of plants with nodal roots. This character was increased by the use of EDB but more so in Cook-sown plots and fewer Cook than Egret plants had nodal roots. When curves in Fig. 2.3 were extrapolated by sight, 50% of EN plants had nodal roots at 40 days, E plants

TABLE 2.8: Effects of cultivar and EDB treatment on plant growth

Parameter	Effects[a]			Treatment[b]			
	Cultivar	EDB	Cultivar x EDB	E	C	EN	CN
Growth stage at week 4[c]	NS	NS	NS	12	12	12	12
Growth stage at week 10	*	**	NS	16 (2.83[d])	14 (2.72)	21 (3.07)	17 (2.89)
Growth stage at week 16	NS	NS	NS	27	17	35	29
Height at week 4 (cm)	NS	*	NS	10.3	8.9	11.3	10.9
Height at week 10 (cm)	**	*	NS	23.0	14.0	23.7	19.3
Height at week 16 (cm)	*	NS	NS	25.4	12.7	30.0	21.8
Number plants in middle row at week 7	*	**	NS	17	19	14	17
Number knots per plant at week 4	NS	*	NS	54 (3.41)	120 (4.44)	13 (2.02)	42 (2.49)
Length of longest primary root at week 4 (cm)	NS	NS	NS	4.6	3.2	5.1	5.0
Seminal root length at week 10 (cm)	NS	NS	NS	354	290	297	300
Seminal root length at week 16 (cm)	NS	NS	NS	469 (5.45)	295 (5.48)	349 (5.45)	341 (5.33)
% plants with nodal roots at week 7[e]	**	**	NS	45.0	2.5	87.5	50.0
% plants with nodal roots at week 8[e]	-	-	*	70.0	17.5	100.0	52.5

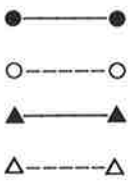
(continued)

TABLE 2.8 (continued)

Parameter	Effects[a]			Treatment[b]			
	Cultivar	EDB	Cultivar x EDB	E	C	EN	CN
% plants with nodal roots at week 9[e]	**	**	NS	77.5	27.5	100.0	72.5
Nodal root length at week 10 (cm)	NS	*	NS	41 (2.28)	28 (0.85)	78 (3.45)	52 (2.53)
Nodal root length at week 16 (cm)	**	*	NS	350 (4.38)	33 (1.22)	518 (5.63)	135 (3.35)
% plants with nodal root damage at week 8[e]	-	-	*	30.0	5.0	17.5	20.0
% plants with nodal root damage at week 9[e]	-	-	**	60.0	7.5	62.5	60.0
% plants with tillers at week 7[e]	*	**	NS	12.5	0.0	45.0	27.5
% plants with tillers at week 8[e]	*	NS	NS	35.0	0.0	77.5	42.5
% plants with tillers at week 9[e]	-	-	**	47.5	0.0	85.0	60.0

[a] NS - F value not significant ($P<0.05$); * F value significant ($P<0.05$); ** F value significant ($P<0.01$)
[b] E - Egret untreated; C - Cook untreated; EN - Egret treated with EDB; CN - Cook treated with EDB
[c] Assumed Poisson distribution and deviances compared to χ^2
[d] Log_e transformation
[e] Assumed negative binomial distribution and deviances compared to χ^2.

Fig. 2.3: Pattern of nodal root emergence shown by mean percentage of plants with nodal roots under four treatments against time after sowing. The times for 50% of plants to have nodal roots are shown.

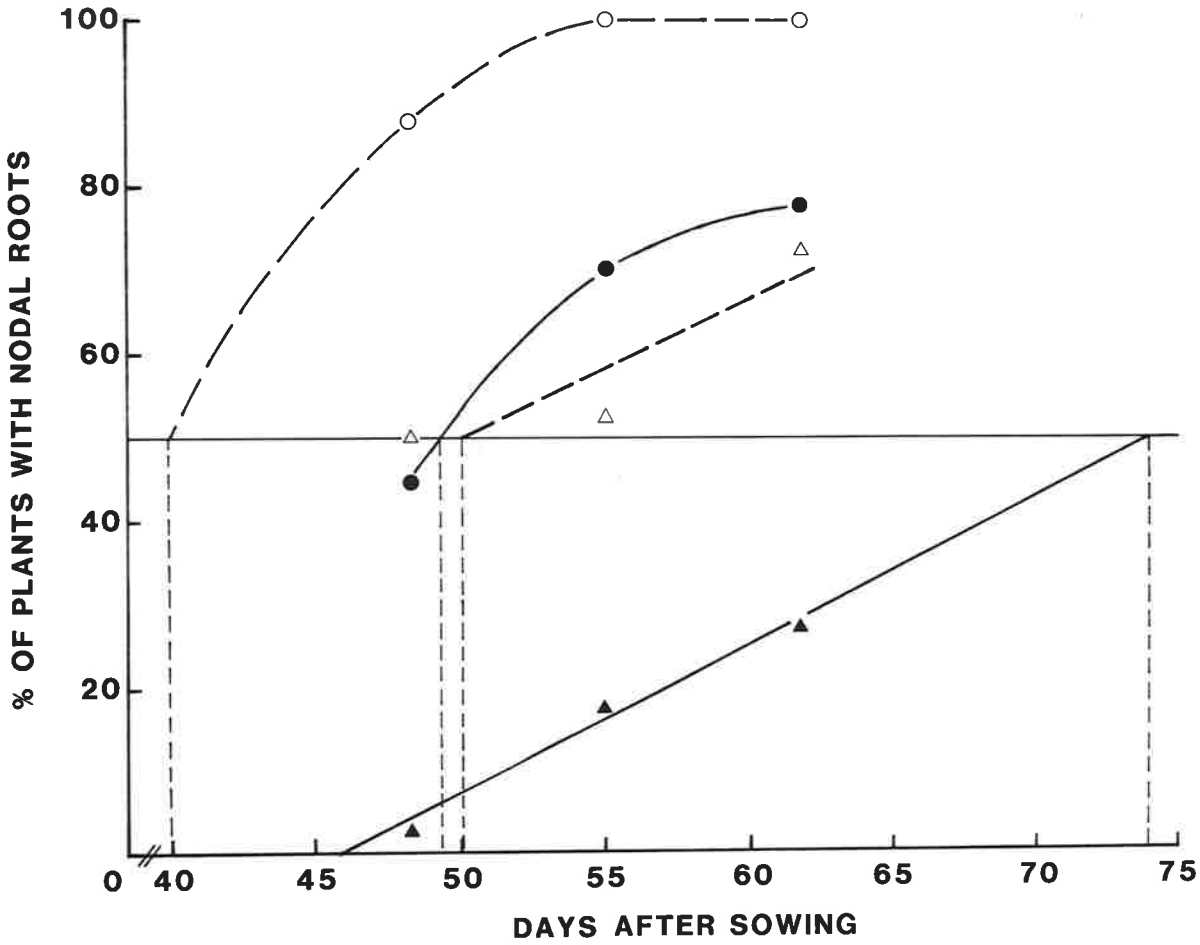


Egret untreated

Egret treated with EDB

Cook untreated

Cook treated with EDB


100
80
60
40
20
0
40
45
50
55
60
65
70
75
% OF PLANTS WITH NODAL ROOTS
DAYS AFTER SOWING

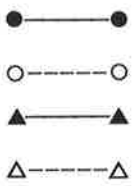
at 49 days (a delay of 9 days due to *H. avenae*), CN plants at 50 days and C plants at 74 days (a delay of 24 days due to *H. avenae*) after sowing.

Nodal root length at weeks 10 and 16 was increased by the use of EDB. At week 16, Cook plants had significantly shorter nodal roots than did Egret (Table 2.8). At weeks 8 and 9 there were significant cultivar x EDB interactions on the % of plants with nodal root damage (Table 2.8). At week 8, the % of Egret plants with damaged nodal roots was decreased by EDB treatment but that of Cook plants was increased. At week 9, EDB treatment increased the % of Cook plants with damaged nodal roots with very little change in Egret plants.

There was no effect at week 16 of EDB on the % of nodal root-tips invaded (Table 2.4) but at week 10, EDB increased invasion of nodal roots.

More Egret than Cook plants had tillered at seven, eight and nine weeks after sowing and EDB treatment increased this in both cultivars (Table 2.8). At week 9 there was a significant interaction between cultivar and EDB treatment when tillering of Cook plants was increased more by EDB than was that of Egret plants. By extrapolating tillering curves, Fig. 2.4 shows that 50% of EN plants had tillered 48 days, E plants 64 days, and CN plants 58 days after sowing. Untreated Cook (C) plants had not tillered by week 9.

Fig. 2.4: Tillering pattern shown by mean percentage of plants with tillers under four treatments against time after sowing. The times for 50% of plants to tiller are shown.



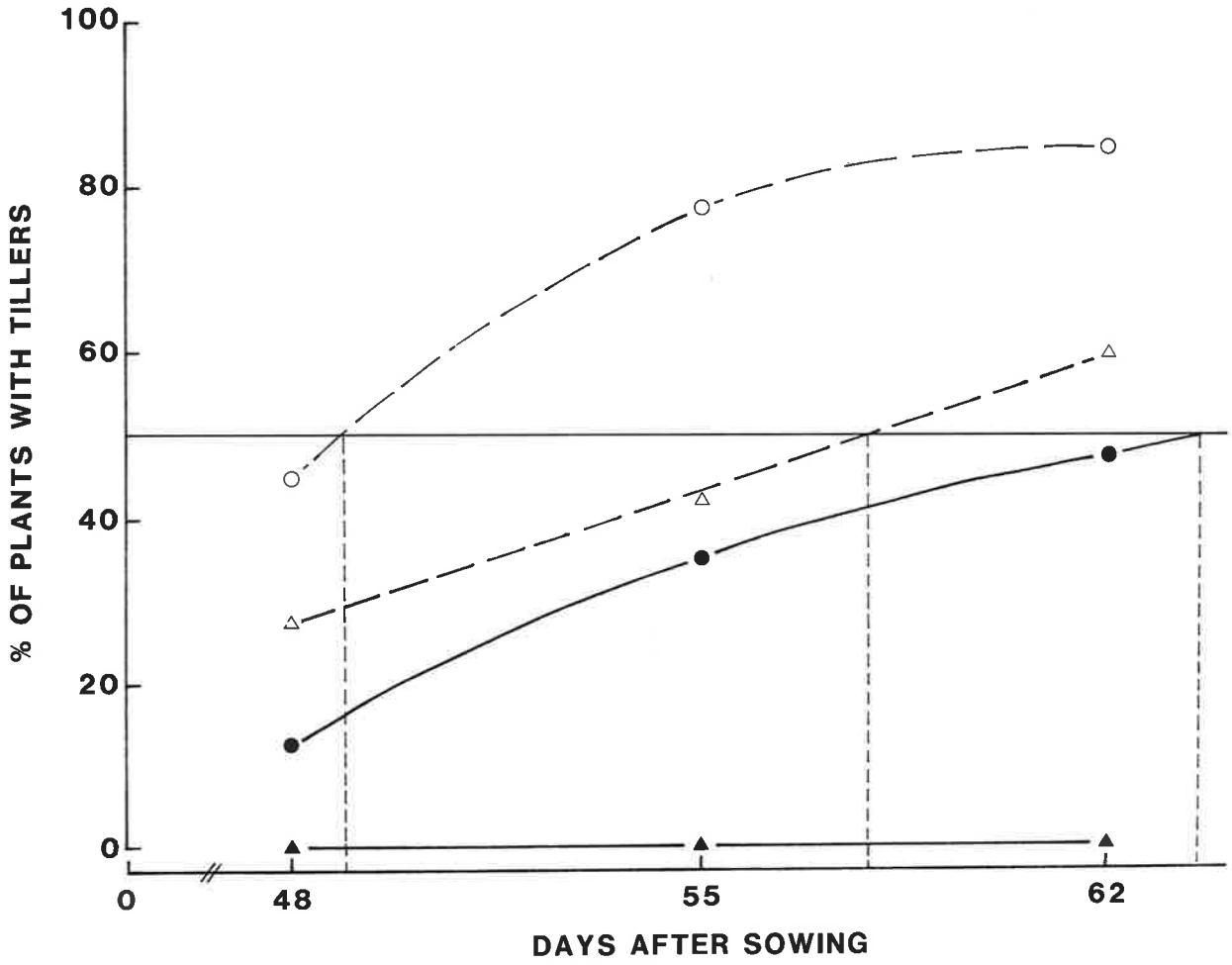

100
80
60
40
20
0
% OF PLANTS WITH TILLERS
0
48
55
62
DAYS AFTER SOWING

TABLE 2.9: Monthly rainfall (mm) at the site of the 1980 trial near Murray Bridge

Month	Rainfall	Month	Rainfall
January	2	July	13
February	3	August	14
March	2	September	18
April	69	October	84
May	50	November	10
June	49	December	17

2.3 Discussion

Three important factors affect interpretation of the results. Little rain fell at the site between June and October (Table 2.9). The rain that fell in October was too late to influence growth of the crop markedly so that from August to the end of September, the period of tillering, elongation and anthesis, the plants were suffering increasingly from the drought andthis obviously affected their growth and development. The same is true of the nematode. From August onwards males are usually free in the soil so that lack of soil moisture undoubtedly limited their movement, interrupted copulation and interferred with egg deposition.

The second factor which appears in some of the data is that the cultivar Cook is later maturing than Egret. Normally this would not affect the results too drastically but the onset of drought conditions in August caught Cook at an earlier stage of development and so probably had a greater effect on Cook than on Egret.

The third factor was variation. In field experiments, particularly with nematodes, variation is to be expected. Normally, by increasing the number of samples taken, accuracy can be improved. If this had been possible, then some of the differences in results, which were not quite significant, may have become so and some of the difficulties in interpretation would have been reduced. To increase the number of samples, when such a large number of parameters were to be examined, was not possible with the time and resources available. Interpretation of results is undertaken with this in mind.

2.3.1 Effects of EDB treatment, cultivar and presence of host on *H. avenae*

Hatching under fallow shows the normal hatching pattern for eggs

of *H. avenae* under the environmental conditions for the area. Numbers of larvae free in the soil of F plots did not change between weeks 4 and 10 (i.e. between the third week of June and the first week of August) suggesting that peak numbers occurred between these times (Dubé *et al.*, 1979). By week 16 (mid-September) there were few free larvae in the soil so that hatching had probably ceased at or before this time.

Neither Cook nor Egret had any significant effect on this pattern of hatching suggesting that root secretions (Williams & Beane, 1972, 1979) had no effect on hatching or had an effect that was too small to be measured by the sampling method used here. The cultivar x time effect on numbers of free larvae in the soil that was found when analysing data from E, EN, C and CN plots (Table 2.6) (showing that the number of larvae in Egret-sown plots was less than that in Cook-sown plots at week 4) may have been produced by the inclusion of all data in the analysis and, therefore, by the EDB x time effect (Table 2.6). The analysis in Table 2.1, showing no significant difference due to cultivar was more direct and, therefore, probably examines the situation more precisely. The presence of wheat roots did not affect the % hatch or multiplication rate.

Conversely, EDB treatment showed some surprising results. Although the reduction in numbers of larvae due to EDB at week 4 was expected the significant increase in numbers at 10 weeks was not. EDB probably had two effects on the population; it killed a proportion of those larvae already hatched and free in the soil and delayed hatching of encysted eggs by about 6 weeks. This has yet to be

confirmed in laboratory trials but it is possible that reversible narcosis was induced, as has been noted for *Aphelenchus avenae* (Evans & Thomason, 1970) and several species (Ferguson, 1939) resulting in reduced motility (Marks *et al.*, 1968) which may be associated with reduced respiration as has been found with *A. avenae* (Marks, 1971). Motility is essential for hatching of *H. avenae* (Banyer & Fisher, 1972). A consequence of the peak in numbers of larvae in EDB-treated soil at week 10 was that EDB increased the % of nodal root-tips invaded at that time.

Four weeks after sowing, the % infectivity of free larvae in fallow soil was greater than that in E soil. With 259 larvae in 300g of fallow soil and 47% infectivity, a total of 122 larvae were infective. Thirteen % of the 168 free larvae in 300g of E soil, i.e. 22 larvae, were infective. An average of 41 larvae were found in E roots at that time so a total of 63 larvae in 300g of roots and soil, or 38%, were infective. This reduction in infectivity of larvae in E plots at 4 weeks may be significant. If so, the reduction must have been due to the presence of host roots and probably to penetration of the root system. Larvae may penetrate only twice before losing infectivity (Davies & Fisher, 1976a) so that the loss in infectivity of larvae remaining in E soil at week 4 could well have been due to penetration of the roots. This occurred early in the season when soil temperature was relatively high and so infectivity was lost more rapidly (Davies & Fisher, 1976b). The effect of lower soil temperature and soil water may have been responsible for the increased infectivity at week 10. As this occurred under both F and E treatments the effect was general and not specific to the treatment. By week 10, the root

system of the plant had extended beyond the volume sampled by the auger so that few root-tips would have been present in the sample. This would have greatly reduced the possibility that larvae free in the soil at that time had previously penetrated plant roots. The large decrease in infectivity at 16 weeks was expected for a number of reasons. Hatching had probably ceased some time prior to sampling, so that there was a considerable interval during which the larvae would lose infectivity. Furthermore, the drought had increased in severity almost to the stage of killing the plants. Soil in E plots would have been drier during the drought than soil in F plots because of transpiration by plants. Thus, larvae in E plots had probably ceased movement some time before those in F plots and would, therefore, have retained infectivity for a longer time resulting in a higher % infectivity of E larvae at week 16.

Although EDB delayed hatching, once hatched the larvae retained infectivity and as might be expected were more infectious at 16 weeks than larvae from F plots.

The data on % hatch and multiplication rate suffered from insufficient samples. No effect of EDB treatment, cultivar or presence of host roots on % hatch could be shown. The number of eggs remaining in old cysts was reduced by EDB treatment suggesting that more eggs had hatched. These two findings are not consistent as there was no difference in the initial density of eggs. Accurate estimates of % hatch are not available for Australian conditions. Banyer & Fisher (1971b) showed that, with fluctuating temperatures similar to those that occur in the field, 86% of eggs hatched and this is similar to that observed in this trial (Tables 2.3, 2.5). Whether a greater hatch occurred in EDB-treated than untreated plots remains to be confirmed.

The absence of host roots significantly reduced the production of new eggs/g of soil. EDB treatment had the same effect and also reduced the final population (total number of eggs/g). These observations were expected and suggest that multiplication rate would have been reduced by EDB treatment and lack of host. Surprisingly, however, there was no significant difference in multiplication rate due to EDB treatment or presence of host. The multiplication rates in Tables 2.3 and 2.5 show that populations were reduced even under the susceptible Egret and Cook. A number of factors may have contributed to this. The initial population was relatively high, having been produced on the semi-tolerant cultivar Halberd, so that a high multiplication rate from this trial could not be expected. Ceiling levels on intolerant cultivars such as Egret are expected to be lower than on the more tolerant Halberd (Andersson, 1982). The onset of the drought during egg deposition may have reduced fecundity of the nematode so that a low multiplication rate resulted.

That the susceptible cultivars Egret and Cook reduced the population as much as fallow was unexpected. There were few grass plants (mainly barley grass) in the fallow plots and, therefore, fewer new cysts and fewer new eggs/g in fallow than in the untreated E and C plots. For these reasons it would be expected that the multiplication rate in fallow soil would be less than that in E and C soil. This seems to be an example of inaccuracy due to insufficient sampling. If drought had not intervened the differences between fallow and crop may have been significant. The data for the EDB-treated plots should also be treated with caution. The concentration of EDB used was determined on a cost/benefit basis (Gurner *et al.*, 1980) and not on

the basis of population control so that it may not have given sufficient control. Because of the delayed hatching, drought may have affected multiplication more severely in treated than in untreated plots.

2.3.2 Effects of EDB treatment and *H. avenae* on plant growth

EDB treatment (and, therefore, *H. avenae*) had similar but opposite the same effects on the growth stage and height of both Egret and Cook, i.e. cultivar x EDB interactions were not significant and, therefore, these two characters were not useful in detecting differences in tolerance. However, they do indicate that Cook was slower to produce leaves than was Egret and that, without EDB treatment, leaf production was delayed in both cultivars. As expected (Jewiss, 1966; Syme, 1974), this was reflected in the rate of tiller production of plants in each treatment. Tillering of Cook was later than Egret but the delay in 50% tiller formation, a method for removing the effects of cultivar differences in time of development, indicated that Cook was more affected by nematodes than Egret. This is shown also by the cultivar x EDB interactions at weeks 10 and 16. Cook appeared to be extremely intolerant as measured by tillering because the untreated plants had failed to tiller by the time sampling for tillers had finished.

Similarly, the time for 50% of the plants to have nodal roots was delayed more by nematodes in Cook than in Egret. Even by week 16 the nodal root length of Cook plants was only 10% of that of Egret plants. Consequently, more untreated Egret than Cook plants had nodal root damage at weeks 8 and 9 and nodal root damage was increased more in Cook plants by EDB treatment than in Egret plants.

At week 7, the density of Cook plants was significantly greater than that of Egret plants which may have been a contributing factor in

reduction of tillering. Survival of plants to week 7 was reduced by EDB treatment presumably due to some phytotoxic effect. Gurner *et al.* (1980) found that EDB was not phytotoxic but their assessment was based on seedling emergence. Other effects of control of *H. avenae* by EDB treatment (Gurner *et al.*, 1980) are consistent with the findings of this trial, *viz.* an increase in tillering and root length and a decrease in the number of knots per plant 8 weeks after sowing.

2.3.3 Conclusions

The aim of this trial was to examine the growth of Cook for indications of tolerance. Fisher *et al.*, (1981) reported that Cook was damaged early (almost to the extent of Egret) but recovered later to yield as well as the best of the commercial cultivars. They included this as a tolerant reaction. This trial could not confirm recovery because of drought conditions but the data suggest that Cook was wrongly classified.

The early growth studies, suggest that both cultivars are intolerant but Cook is more so. This difference between cultivars was apparent up to the time of tillering and nodal root formation in a number of characters. It is not possible to observe recovery without yield data. If Cook does have the ability to recover then it may be associated with its later maturing genotype which delayed formation of nodal roots until larval numbers in soil of untreated plots had been reduced and consequently they might have escaped penetration. Escaping damage at this time, however, would contribute little to the recovery because the data presented here show that most damage occurs early in the plant's growth. This can be seen from the delayed hatching of eggs in EDB-treated plots. Although, many infective larvae

were present in the soil of EDB-treated plots from week 10 onwards, this was not reflected in height, growth stage, tillering, nodal root formation or length of nodal or seminal roots. Furthermore, EDB has been in commercial use for some years now and the presence of these larvae from the beginning of August has not been reflected in yield (Gurner *et al.*, 1980).

Thus, the ability to recover is related mostly to the plant's genotype. Late maturing cultivars are not of great benefit in the South Australian environment because the probability of drought increases greatly towards the end of the year. Thus it would be preferable to look for tolerance to the early damage in a cultivar that matures earlier.

The usefulness of EDB to simulate a nematode-free control needs to be assessed. EDB probably reduced numbers of larvae for a very short time at the beginning of the season but did not reduce the total number of larvae throughout the season. The effects of delayed hatching could not be adequately assessed but it seems preferable to avoid the use of EDB treatments in the further examination of tolerance.

CHAPTER III

EFFECT OF *H. AVENAE* ON GROWTH AND YIELD VARIABLES OF TWO WHEAT CULTIVARS, CONDOR AND RAC311, IN A FIELD TRIAL

The previous trial was deficient in that yield data could not obtained. It was determined, however, that Cook was probably intolerant and, therefore, was not typical of other tolerant cultivars in that its early growth was greatly affected by *H. avenae*. Furthermore, EDB treatment did not produce nematode-free controls. Therefore, in an attempt to examine tolerance more directly the relationship between yield and initial density had to be studied. Such information for Australian field conditions was not known at this time.

Usually an estimate of initial nematode density is based on initial numbers of eggs/g of soil. Many factors may affect hatching and penetration so that experiments using the same initial density based on this estimate are not necessarily directly comparable. Therefore, a direct count of the number of larvae in the roots, i.e. the exact pathogen density on the plant, was considered preferable. It was decided to undertake an intensive study of growth and yield parameters of a tolerant cultivar, RAC311, and the intolerant cultivar, Condor, with initial density of nematodes determined by numbers in the roots. Resistance was eliminated as a yield-determining factor by choosing these two cultivars which have approximately the same level of resistance (Fisher, pers. comm.; Dubé, pers. comm.).

3.1 Materials and Methods

An experimental site was chosen in a previously infested paddock at Charlick Experimental Station 60 km south-east of Adelaide. In the previous year the area had been sown with the susceptible cultivar, Halberd. A random assessment of eight soil samples in the test area revealed a range of approximately 0-80 eggs/g of soil with nematode density increasing from the

lower right corner to the upper left corner in Fig. 3.1a. Condor, an intolerant cultivar (Fisher *et al.*, 1981) and RAC311, a tolerant line (Wilson unpubl. data), were tested in 20 blocks of two plots each, sown at 50 kg/ha. Plots were 50 cm long and four rows wide. Rows were 15 cm apart. Cultivars were allotted randomly to blocks (Fig. 3.1a). Plots were arranged in pairs of one cultivar so that one plot ('growth' plot) was used throughout the year to assess plant parameters and the other ('yield' plot) was used for the final assessment of yield parameters. Initial population density was measured 2 weeks after sowing by removing plants A to F (Fig. 3.1b) and counting the number of larvae present in roots stained with lactophenol cotton blue (Southey, 1970). Plants A, B, C and D were used for a population density estimate of the 'growth' plot and plants C, D, E and F of the 'yield' plot. In order to identify plants for future measurement, five plants in each 'growth' plot were tagged at random 2 weeks after sowing.

Blocks were sown on 9 June 1981. Plants for initial nematode density estimate were taken 14 days later, and root length of these plants recorded. Zadoks (Zadoks *et al.*, 1974) growth stage was recorded 27, 41, 57, 97, 113 and 128 days after sowing. Tillers were counted on the last three occasions and heads on the last two. Maximum length of the first four leaves was measured between 27 and 57 days after sowing. After this time it was too difficult to determine the leaves on the main stem of the plant. Each of the above measurements was on the five tagged plants in each 'growth' plot. Five times between 27 and 57 days after sowing, one plant was taken at random from each 'growth' plot. Seminal and nodal roots were counted at each sampling time. Seminal root length was measured 97 and 113 days after sowing and nodal roots at the latter time. After this time, root systems were too large to be taken without disturbing adjacent plants.

Fig. 3.1a: Layout of experimental area at Charlick Experimental Station showing random allottment of cultivars to blocks.

R. RAC311
C. Condor

Fig. 3.1b: Design of experimental blocks showing spacing and division into two plots, each consisting of four rows.
Plants A to F were used to estimate initial nematode density.

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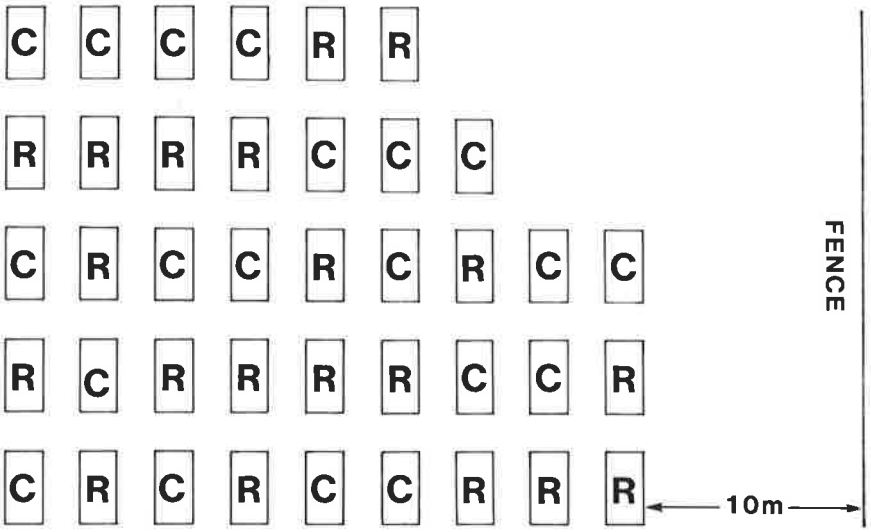
C C C C R R
R R R R C C C
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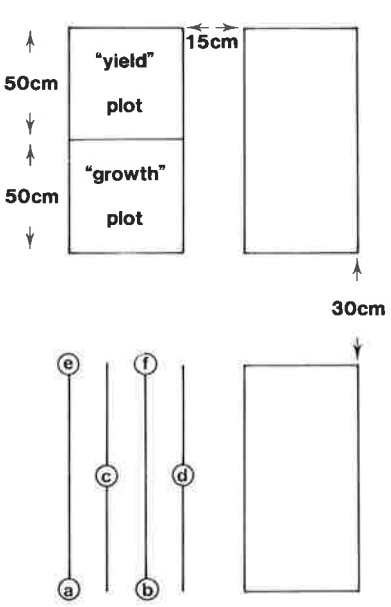

50cm
"yield" plot
15cm
50cm
"growth" plot
30cm
e
f
c
d
a
b

Apical meristems were examined 27 and 35 days after sowing for number of leaves initiated, on day 41 for number of floral ridges, on day 57 for total number of primordia and on day 69 for total number of double ridges and spikelets initiated.

Yield was assessed 198 days after sowing using all plants in the 'yield' plots. Number of heads, number of tillers, % fertile tillers, number of fertile spikelets, number of grains, number of fertile spikelets per head, 1000 grain weight and total grain weight were recorded for each plot. It was not possible to distinguish accurately between adjacent plants at that time so parameters could not be assessed on a per plant basis.

Regression lines for each cultivar for all parameters against initial population density were compared.

3.2 Results

Slopes and Y-intercepts of regression lines relating root length of the two cultivars to number of larvae in the roots 2 weeks after sowing were compared and found to be not significantly different ($P<0.05$). This implies that cultivar differences in root growth did not affect the number of larvae in roots at that time. Therefore, the initial population density estimates for each cultivar are directly comparable.

There were no significant differences between regression slopes for any parameter assessed before harvest except for maximum length of leaf 4 (Table 3.1). In that case, the slope for RAC311 was greater than that for Condor but Y-intercepts were not significantly different.

Slopes and Y-intercepts for parameters measured at harvest are listed in Table 3.2. In all cases where slopes were significantly different (number of heads per plot, number of fertile spikelets per plot, number of grains per plot, total grain weight per plot) that for Condor was negative

TABLE 3.1: Slopes and Y-intercepts of regression lines relating various parameters of Condor and RAC311 to number of *H. avenae* larvae in roots 2 weeks after sowing (initial density)

Parameter	Days after sowing	Slope		Y-intercept	
		Condor	RAC311	Condor	RAC311
Maximum length leaf 1 (cm)	-	0.02	0.01	7.4	8.0
Maximum length leaf 2 (cm)	-	0.01	0.01	10.2	10.3
Maximum length leaf 3 (cm)	-	0.00	0.02	8.2	9.8
Maximum length leaf 4 (cm)	-	-0.01(NS)[a]	0.05*[b](NS)	10.5	7.5
Number of seminal roots/plant	27	0.00	-0.01	5	5
Number of seminal roots/plant	35	0.00	0.00	5	6
Number of seminal roots/plant	41	-0.01	-0.01	6	7
Number nodal roots/plant	41	0.00	0.00	2	2
Number nodal roots/plant	57	0.00	-0.02	4	4
Number nodal roots/plant	69	-0.03	-0.01	9	11
Seminal root length (cm)/plant	14	0.07	0.34	58.4	50.3
Seminal root length (cm)/plant	35	0.57	-0.07	78.7	125.7
Seminal root length (cm)/plant	41	0.27	-0.39	141.4	163.9
Nodal root length (cm)/plant	41	-0.03	0.07	6	2
Growth stage	27	0.00	0.00	11	11
Growth stage	41	-0.03	-0.02	18	19
Growth stage	57	-0.02	0.00	22	22
Growth stage	97	-0.02	-0.01	29	32
Growth stage	113	-0.04	-0.04	53	57
Growth stage	128	-0.01	-0.01	69	71
Number leaves initiated/plant	27	0.00	0.01	7	7
Number leaves initiated/plant	35	0.00	-0.02	9	10

(continued)

TABLE 3.1 (continued)

Parameter	Days after sowing	Slope		Y-intercept	
		Condor	RAC311	Condor	RAC311
Number floral ridges initiated/ plant	41	-0.01	0.00	2	2
Number leaf and floral primordia/ plant	57	0.00	0.02	18	19
Number spikelets initiated/plant	69	-0.02	-0.05	12	19
Number double ridges/plant	69	-0.03	-0.04	6	17
Number tillers/plant	97	-0.01	-0.01	3	4
Number tillers/plant	113	-0.01	0.00	3	4
Number tillers/plant	128	-0.01	0.00	4	5
Number heads/plant	113	-0.01	-0.01	2	2
Number heads/plant	128	-0.01	0.00	4	4
% fertile tillers	113	0.00	0.00	60	50
% fertile tillers	128	0.00	0.00	90	70

[a](NS) - Not significantly different from zero ($P<0.05$)

[b] *- Cultivars significantly different ($P<0.05$) (where slopes were not significantly different, difference between Y-intercepts was not tested).

TABLE 3.2: Slopes and Y-intercepts of regression lines relating various parameters of RAC311 and Condor measured at harvest to number of *H. avenae* larvae in roots 2 weeks after sowing (initial density)

Parameter	Slope		Y-intercept	
	Condor	RAC311	Condor	RAC311
Number of tillers/plot	-0.19	0.01	106	107
Number of heads/plot	-0.12(NS)[a]	0.11*[b](NS)	91	88
% fertile tillers	0.06	0.10	86	82
Number of fertile spikelets/plot	-1.61(*)	3.46**(NS)	1157	1051*
Number of fertile spikelets/head	0.00	0.01	13	13
Number of grains/plot	-2.94(*)	5.22*(*)	2279	2132*
1000 grain weight (g)	0.00	0.02	36	41
Total grain weight (g)/plot	-0.10(NS)	0.26**(*)	81	88**

[a](NS) - Slope not significantly different from zero (P<0.05)
(*) - Slope significantly different from zero (P<0.05)
[b]* - Cultivars significantly different (P<0.05)
** - Cultivars significantly different (P<0.01)
(Where slopes were not significantly different, difference between Y-intercepts was not tested).

and for RAC311 positive although slopes were not always significantly different from zero. The regression lines and data for total grain weight per plot (yield) against initial nematode density are plotted in Fig. 3.3 to show distribution of points. The slope of the line for RAC311 is significantly greater than zero but that for Condor is not different from zero. By comparing Y-intercepts, it is shown that the yield of Condor in the absence of nematodes was less than that of RAC311.

3.3 Discussion

The normal reduction in yield that one might expect with increasing nematode density did not occur in either Condor or RAC311, i.e. regression slopes were not significantly less than zero but were significantly different from each other. The initial density of nematodes in the blocks increased from the lower right corner to the upper left corner in Fig. 3.2. This was probably due to a change in fertility or a slight difference in soil type across the plot area. In previous years, more fertile areas could have maintained better growth of susceptible plants than less fertile areas. With better growth and, therefore, larger root systems, plants could support more nematodes resulting in greater final population densities (Andersson, 1982; Gair, 1965; Hesling, 1959; Seinhorst, 1961). Thus, in those areas more suitable for plant growth, crops in the following year would experience a greater initial population density than in other areas. This trial showed greater yield of RAC311 at larger than at smaller initial nematode densities and no apparent effect of initial density on yield of Condor.

The significant difference between the slopes of regression lines of yield against initial nematode density shows that RAC311 is more tolerant than Condor. Regression lines relating number of heads per plot, number of fertile spikelets per plot and number of grains per plot to initial nematode

Fig. 3.2: Plot means of six plant samples of number of *H. avenae* larvae in roots 2 weeks after sowing (initial nematode density) showing population distribution in the experimental area.

1) 0-49
2) 50-99
3) 100-149
5) 200-249 larvae per root system.

3 5 2 3 3 2

3 2 2 1 3 3 2

3 3 2 2 2 1 1 2 2

2 3 2 1 1 1 1 1 1

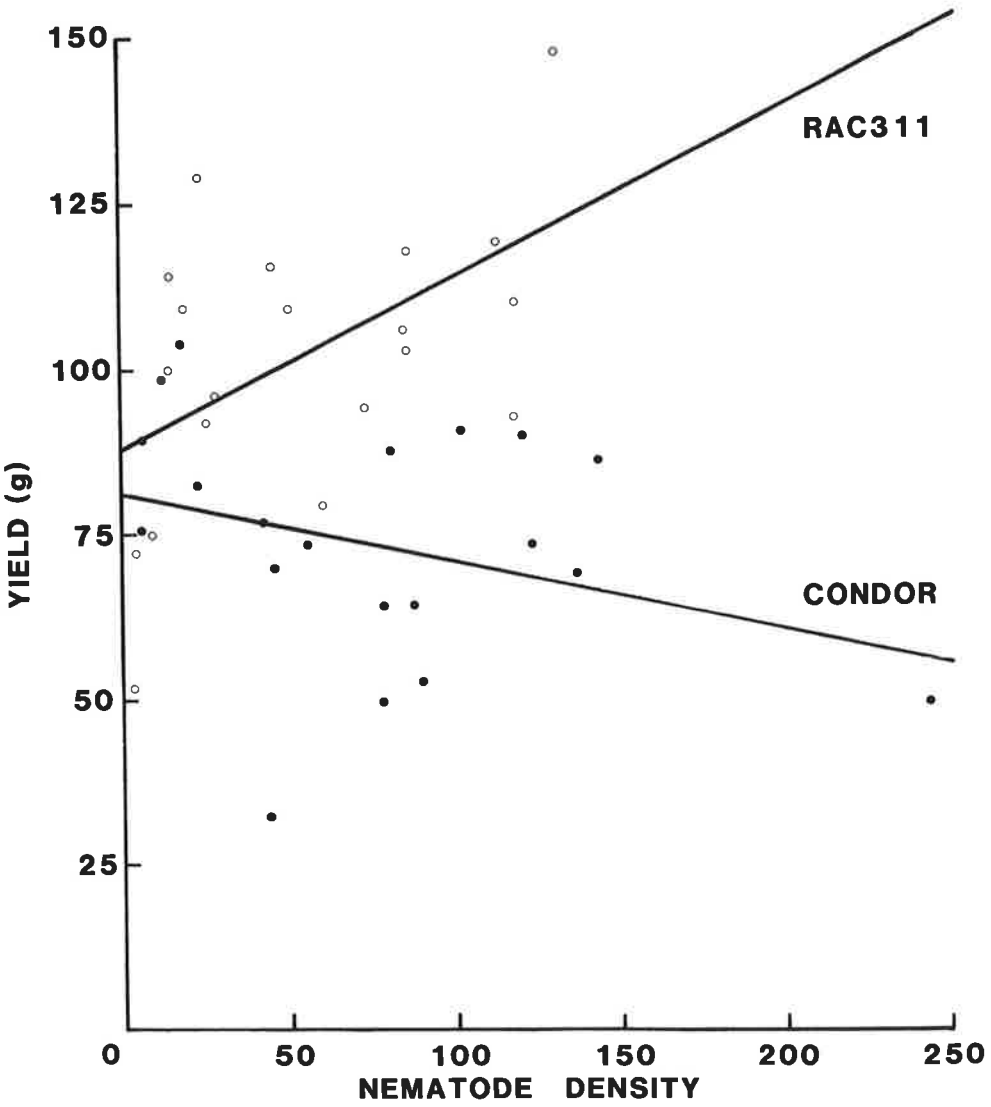
2 2 1 1 1 1 1 1 1

FENCE

Fig. 3.3: Effect of density (number of larvae in roots at 2 weeks after sowing) of *H. avenae* on yield (total grain weight (g)/plot) of Condor and RAC311. Solid lines are calculated regression lines.

● Condor

○ RAC311


150
125
100
75
50
25
YIELD (g)
RAC311
CONDOR
0
50
100
150
200
250
NEMATODE DENSITY

density show a similar relationship between Condor and RAC311 as that found for yield indicating that these factors were responsible for the difference.

The number of heads per plot represents the number of fertile tillers at the end of the season. The difference between the two cultivars could have arisen in two ways; (i) RAC311 produced more tillers than Condor at a given initial nematode density and the same proportion in each became fertile or (ii) each line produced the same number of tillers but a higher percentage of tillers of RAC311 than of Condor became fertile. However, there was no significant difference between slopes of regression lines for number of tillers per plot or for % fertile tillers. The difference in slopes for number of fertile spikelets per plot was not due to a difference in number of fertile spikelets per head so must have been the result of a difference in number of heads per plot. Similarly, the difference in slopes for total grain weight per plot (yield) between cultivars was not due to a difference in the weight of each grain but to the difference in number of grains per plot. This in turn was due to the difference in number of fertile spikelets per plot and supports the difference found between cultivars in number of heads per plot. A higher F ratio, despite greater residual variation, when comparing slopes of regression lines for number of tillers per plot, suggests that this may have been the factor controlling number of heads per plot rather than % fertile tillers. Also, the absolute difference in slopes was greater for number of tillers per plot than for % fertile tillers. If this were the case, then early growth, i.e. factors affecting tillering, was very important in determining yield. This is consistent with the work of Fisher *et al.* (1981) where tolerance was correlated with an early growth rating.

If the number of tillers per plot was the controlling factor, then the difference in yield between the two cultivars was the direct result of either a difference in number of tillers per plant or in number of plants per plot. It was not possible at harvest to distinguish one plant from another so that

plants could not be counted. Rovira *et al*. (1981) found that, by controlling *H. avenae* with aldicarb, the number of plants surviving was increased. However, it is very unusual for *H. avenae* to kill plants except when subjected to other environmental stresses (Dropkin, 1980) so increased survival as reported by Rovira *et al*. may have been due to factors other than aldicarb. The possibility of a difference between cultivars in density of plants, however, should not be discounted entirely as no significant difference was found in number of tillers per plant at 97, 113 or 128 days after sowing.

The only parameter measured before harvest which produced a significant difference for Condor and RAC311 was maximum length of leaf 4. The difference shows the same relationship as that of other significant parameters, i.e. the slope for Condor was less than that of RAC311. It was not expected that the first three leaves would be affected as these are already initiated in the embryo (Williams, 1960) so that only their growth could be influenced. Leaf 4 is initiated after germination so that both initiation and growth can be affected by many factors. The same is true of subsequent leaves. The four-leaf stage occurred beween 27 and 41 days after sowing so the fact that leaf 4 was affected indicates that early growth was important in tolerance. Later leaves should also be studied to determine their possible effect on supply of assimilates to developing tillers and thus on yield. Tillering rate has been associated with rate of leaf appearance (Jewiss, 1966; Syme, 1974) which in turn should be reflected in growth stage. Differences between the two cultivars in growth stage were not seen in this trial but this may have been due to the insensitivity of the Zadoks growth scale. For example, the three-leaf stage encompasses plants with the third leaf just opened as well as plants with the fourth or even fifth leaves emerged. However, in the previous trial (Chapter 2.2.4) an increase in rate of leaf emergence was found with EDB treatment.

Because of the probable association between soil fertility and initial nematode density it is difficult to determine whether slopes of early parameters differed between cultivars or even if they changed with initial density of the nematode. At higher initial densities, fertility might have been high enough to compensate for nematode damage resulting in a zero or positive slope for a VARIABLE against initial nematode density and masking differences between cultivars. Yield characters, however, showed significant differences between cultivars because they integrate the effects of all of the earlier differences in growth response.

This field trial provided the first direct evidence that tolerance to *H. avenae* exists in wheat cultivars. Although the work of Fisher *et al.* (1981) suggested this, there was no direct relation of yield to nematode densities. Laboratory trials are required to control unwanted variables and to examine, in particular, root growth which could not be studied closely in the field. Experimental work is also needed to determine the usefulness of characters such as shoot : root ratio (Seinhorst, 1979) for examining tolerance simply and rapidly.

CHAPTER IV

TOLERANCE ASSAYS IN CONTROLLED ENVIRONMENT

The previous trial showed that RAC311 was more tolerant than Condor under field conditions and a number of yield parameters, nearly all of which occurred late in the development of the plant, were associated. The possibility that early parameters of growth contributed to this difference was strong but variation in growth in the field obscured the differences between cultivars. It is necessary to examine early growth, particularly, under controlled conditions to determine whether tolerance might be assessed early in the plant's growth. For plant breeding purposes, the sooner after sowing that tolerance can be assessed, the better.

A number of variables which possibly affected the field study, such as soil type, nutrition and water, could easily be controlled in the laboratory. However, size of container and growth conditions might affect plants so that tolerance would be masked. Initial density of nematodes might also be an important factor in that the difference in tolerance between two cultivars could increase to a certain nematode density and then disappear as initial density increased past that limit.

With these qualifications in mind this section deals with attempts to find an assay adequate for examining tolerance.

4.1 Comparison of the effects of *H. avenae* on two wheat cultivars grown in short tubes at 15°C

The first attempt to find a suitable laboratory method for examining tolerance was a modification of a technique used by O'Brien (1976).

4.1.1 Materials and Methods

4.1.1.1 General

Seeds for pre-germination were selected initially for uniformity of size and lack of damage. To enable selection of uniform seedlings, three times the number required were pre-germinated. Seeds were surface sterilized for 5 minutes in 1% sodium hypochlorite solution and then washed with tap water until the chlorine odour was no longer present. Seeds were then placed separately onto 7 cm filter paper in 9 cm Petri dishes to which 2 ml of sterile distilled water had been added. Thirty seeds were spaced uniformly in each dish and then kept in the dark at 15°C until three roots appeared each of which was 1-2 cm long.

The method for growing seedlings was similar to that described by O'Brien (1976). One end of opaque, plastic conduit - 12 cm long by 27 mm internal diameter - was sealed with Parafilm* and the tubes were filled with half-strength John Innes soil without peat. Pre-germinated seedlings were sown 1 cm deep and inoculated with the required number of larvae in 1 ml of tap water. One ml of tap water was used as the control.

Larvae were recovered from soil by sieving, placed in bolting silk in a Petri dish, moistened and incubated at 10°C. Once hatched, larvae emerged into the water in the Petri dish and were collected daily. If not used immediately, larvae were stored at 5°C in shallow water. No larvae were stored for longer than 3 days.

*Registered trade name.

4.1.1.2 Experimental

The cultivars, RAC311 and Condor, were grown by the method described in Chapter 4.1.1.1. One hundred and twenty control plants and 120 plants inoculated at sowing with 75 larvae were grown for each cultivar. Ten plants of each cultivar in each treatment were harvested 3,7,11,14,21,28,35,42,49,56,63 and 119 days after sowing. At all but the last sampling time shoot and root dry weights were recorded along with root length (using the line intersect method modified by Tennant (1975)), height, growth stage (Zadoks, 1974), number of nodal roots and leaf length. At maturity, 119 days after sowing, number of fertile spikelets, number of grains, total grain weight, 1000 grain weight and shoot and root dry weights were recorded.

In order to assess quickly the effect of *H. avenae* on each variable a *t*-test was used to test for significant differences between control and inoculated plants at each sampling time.

4.1.2 Results

Many of the parameters measured (mean root dry weight (Table 4.1.1), mean height (Table 4.1.4), mean growth stage (Table 4.1.5), mean shoot : root ratio (Table 4.1.6), mean maximum leaf lengths (Table 4.1.7), mean number of nodal roots (Table 4.1.8) and mean number of fertile spikelets, number of grains, total grain weight and 1000 grain weight (Table 4.1.9)) showed no or few significant differences following inoculation. However, inoculation more consistently reduced shoot dry weight of Condor than that of RAC311 (Table 4.1.2). Mean total root length of Condor and RAC311 were consistently reduced over the first 14 or 21 days of growth, respectively (Table 4.1.3).

TABLE 4.1.1: Mean root dry weight (mg) per plant of ten Condor and RAC311 plants either uninoculated (C), or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C.

Days after sowing	Condor		RAC311	
	C	I	C	I
3	5.0	4.0*	2.9	2.6
7	10.5	11.3	6.9	8.8
11	11.0	11.6	13.0	11.2
14	16.2	18.7	15.9	17.2
21	26.6	24.7	25.9	30.9
28	63.1	74.6	70.3	72.6
35	104.9	88.4	94.1	80.1
42	137.5	106.4	139.5	128.4
49	124.3	154.5	146.8	155.3
56	189.4	158.6	155.3	161.2
63	145.0	133.6	157.8	196.3*
119	206.0	153.8	172.0	177.0

*Difference due to inoculation significant ($P<0.05$).

TABLE 4.1.2: Mean shoot dry weight (mg) per plant of ten Condor and RAC311 plants either uninoculated (C) or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C

Days after sowing	Condor		RAC311	
	C	I	C	I
3	2.9	2.2**	2.9	2.6
7	7.3	5.9*	5.9	4.5
11	12.9	9.6*	16.5	9.1***
14	22.1	19.9	19.3	18.4
21	42.7	32.8	41.0	34.4*
28	71.5	68.2	66.6	65.6
35	107.0	92.1	98.0	88.0
42	138.6	119.4	140.1	131.0
49	177.6	199.6	192.8	191.8
56	280.7	246.7	239.3	228.0
63	320.1	305.5	298.1	344.5
119	371.0	326.0	406.0	421.0

*;**;*** Difference due to inoculation significant ($P<0.05$, $P<0.01$, $P<0.001$, respectively).

TABLE 4.1.3: Mean total root length (cm) per plant of ten Condor and RAC311 plants either uninoculated (C) or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C

Days after sowing	Condor		RAC311	
	C	I	C	I
3	19.6	9.2***	8.6	4.2**
7	39.0	19.4***	31.0	12.5*
11	111.5	47.6***	148.9	57.3***
14	238.2	170.0*	202.4	155.3*
21	477.4	323.1	512.9	388.3**
28	1028.2	1071.1	1263.2	1037.9
35	1551.7	1448.5	1332.2	1406.7
42	1667.0	1327.3	1814.0	1802.2
49	1647.6	1755.6	1912.6	1882.3
56	2275.6	1797.9	1980.5	1887.3
63	1923.4	1913.8	2046.9	2576.3*

*, **, *** Difference due to inoculation significant ($P<0.05$, $P<0.01$, $P<0.001$ respectively).

TABLE 4.1.4: Mean height (cm) to tip of youngest leaf of ten Condor and RAC311 plants either uninoculated (C) or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C

Days after sowing	Condor		RAC311	
	C	I	C	I
3	4.2	2.9**	1.2	1.1
7	8.4	7.6	7.6	5.5
11	11.2	9.3	14.9	10.9***
14	16.1	15.0	13.1	13.9
21	20.6	18.5	19.2	18.5
28	25.8	26.3	24.8	26.1
35	25.8	25.1	23.8	24.7
42	24.5	23.7	24.2	23.6
49	23.0	24.3	22.2	23.4
56	28.2	26.8	23.6	24.0
63	30.3	31.6	27.9	31.9

, * Difference due to inoculation significant ($P<0.01$, $P<0.001$ respectively).

TABLE 4.1.5: Mean growth stage of ten Condor and RAC311 plants either uninoculated (C) or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C

Days after sowing	Condor		RAC311	
	C	I	C	I
3	10.0	10.0	8.5	8.0
7	9.9	10.0	9.7	9.9
11	10.9	10.9	11.0	10.3***
14	11.0	12.4	11.0	11.0
21	13.1	12.0	12.8	12.0
28	13.9	13.9	13.6	13.6
35	14.1	15.1	15.5	14.4
42	15.1	15.2	19.8	18.5
49	18.9	28.0	17.9	20.6
56	42.8	43.0	37.6	38.8
63	49.8	52.4	45.0	46.9

*** Difference due to inoculation significant ($P<0.001$).

TABLE 4.1.6: Mean shoot : root ratio of ten Condor and RAC311 plants either uninoculated (C) or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C

Days after sowing	Condor		RAC311	
	C	I	C	I
3	0.592	0.528	0.441	0.555
7	0.743	0.550	0.830	0.571*
11	1.225	0.848*	1.313	0.919*
14	1.286	1.077	1.217	1.071
21	1.538	1.251	1.310	1.140
28	1.145	0.919	0.960	0.910
35	1.031	1.087	0.968	1.136
42	1.021	1.148	1.014	1.039
49	1.431	1.271	1.324	1.222
56	1.665	1.570	1.530	1.407
63	2.288	2.318	1.917	1.860
119	1.963	2.248	2.547	2.482

* Difference due to inoculation significant ($P<0.05$).

TABLE 4.1.7: Mean maximum leaf length (cm) of ten Condor and RAC311 plants either uninoculated (C) or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C

Leaf number	Condor		RAC311	
	C	I	C	I
1	8.6	7.0*	11.2	9.6*
2	13.4	13.3	14.8	14.6
3	19.5	20.1	18.9	20.6
4	17.4	17.8	16.1	16.0
5	13.4	12.7	13.2	13.3
6	9.7	9.6	9.8	9.7
7	7.8	7.5	7.6	8.2
8	5.8	6.3	5.1	6.5

*Difference due to inoculation significant ($P<0.05$).

TABLE 4.1.8: Mean number of nodal roots per plant of ten Condor and RAC311 plants either uninoculated (C) or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C

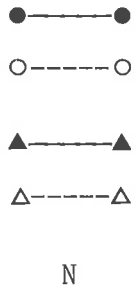
Days after sowing	Condor		RAC311	
	C	I	C	I
3	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0
14	0.0	0.0	0.0	0.0
21	1.6	1.1	1.4	0.8
28	3.7	3.7	2.9	2.8
35	4.1	3.8	4.2	2.7*
42	4.9	4.8	5.3	4.2
49	5.0	5.4	5.5	5.3
56	5.4	5.9	5.7	6.2
63	6.1	5.8	6.0	6.0

*Difference due to inoculation significant ($P<0.05$).

TABLE 4.1.9: Mean number of fertile spikelets, number of grains and total grain weight (mg) per plant and 1000 grain weight (g) of ten Condor and RAC311 plants either uninoculated (C) or inoculated with 75 *H. avenae* larvae at sowing (I) and grown at 15°C

	Condor		RAC311	
	C	I	C	I
No. fertile spikelets per plant	3.9	4.9	2.3	3.4
No. grains per plant	4.6	5.7	2.9	3.4
Total grain weight per plant	154.7	181.9	92.8	112.8
1000 grain weight	35.3	32.7	34.3	34.4

Fig. 4.1.1: Mean total root length of Condor and RAC311 plants, grown at 15°C, at several times after sowing.



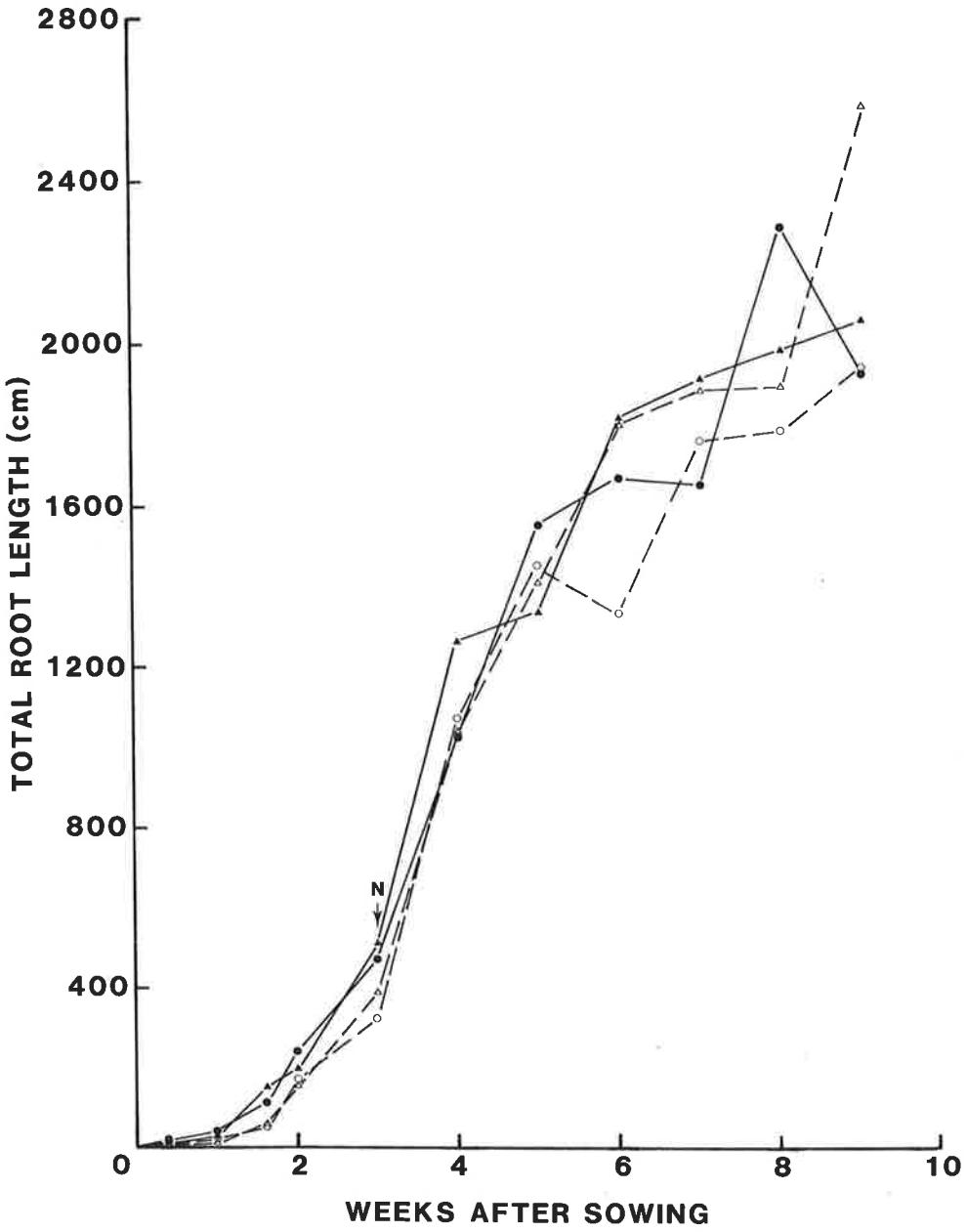
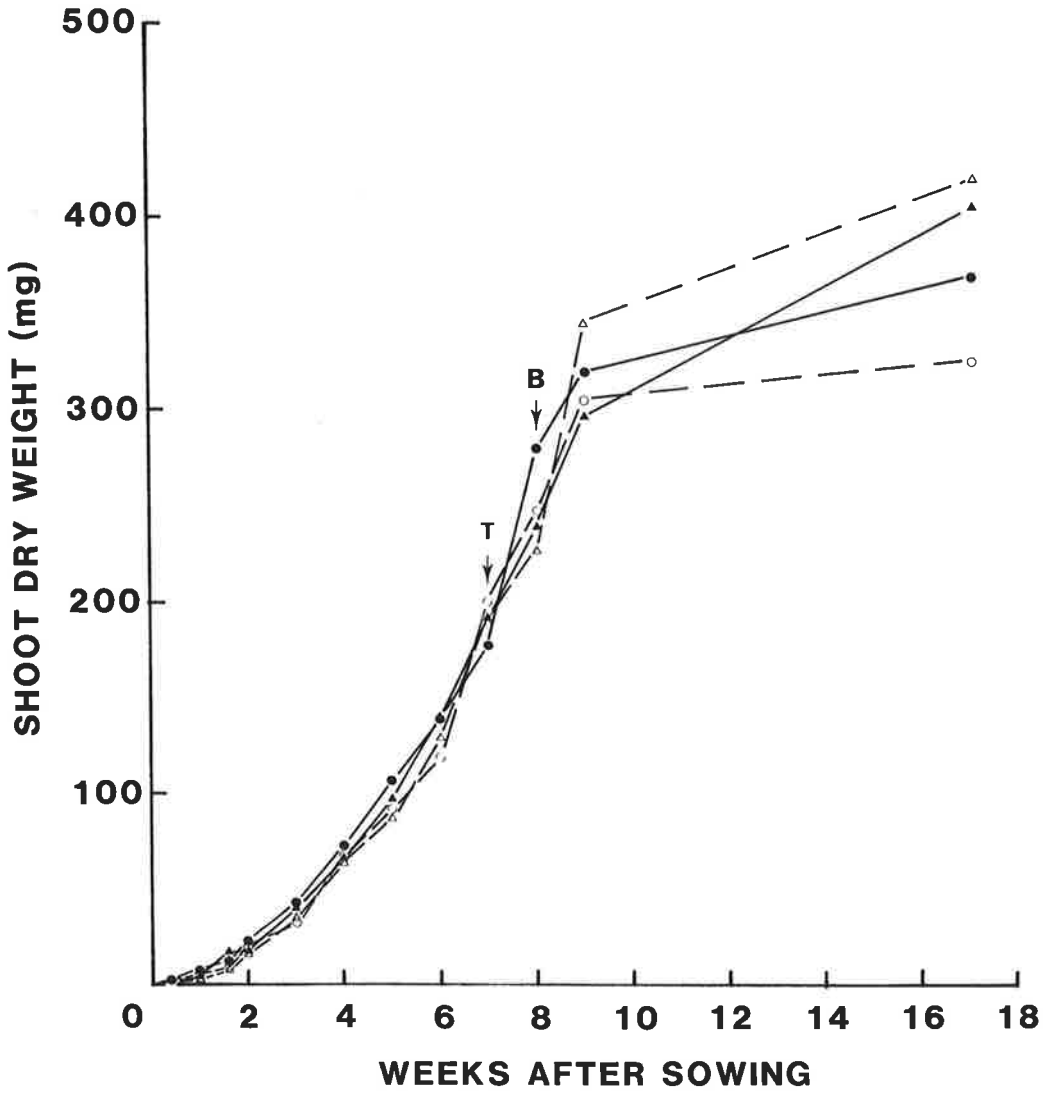

2800
2400
2000
1600
1200
800
400
0
2
4
6
8
10
TOTAL ROOT LENGTH (cm)
WEEKS AFTER SOWING
N

Fig. 4.1.2: Mean shoot dry weight of Condor and RAC311 plants, grown at 15°C, at several times after sowing.

●———●	Condor uninoculated
o-----o	Condor inoculated with 75 *H. avenae* larvae at sowing
▲———▲	RAC 311 uninoculated
△-----△	RAC311 inoculated with 75 *H. avenae* larvae at sowing
T	Tillering
B	Booting


500
400
300
200
100
SHOOT DRY WEIGHT (mg)
B
T
0
2
4
6
8
10
12
14
16
18
WEEKS AFTER SOWING

4.1.3 Discussion

When assessing many parameters at many sampling times, isolated significant differences should be treated with caution as they may occur by chance. In this trial, for many parameters, significant differences were sometimes found on a single occasion only and it seems wiser to regard these as chance occurrences rather than to place too much credence on their significance. The two characters which may have more relevance are shoot weight and total root length as differences between cultivars were recorded for these parameters on more than one occasion.

The aim of this experiment was to decide whether the small plastic tubes were suitable for experimental work on tolerance. They have proved suitable for work on resistance (O'Brien, 1976) and have many features to recommend them. They are small, use a minimum of soil, can be inoculated simply and successfully and many can be fitted into a confined space. Maintenance and watering of them can be handled with ease. Yet some of these characters were disadvantageous for an examination of tolerance under the conditions of this experiment. When increases in seminal root length were plotted against time (Fig. 4.1.1) for Condor and RAC311 both inoculated and uninoculated, some of the reasons for the unsuitability of the small tubes are apparent. By about 5 weeks (35 days) after inoculation, the seminal root system of each treatment had reached its maximum length; that is, the seminal root system had occupied all the volume of soil in the tube. Probably tube size had limited root growth before this time so that from about day 28, tube size may have been masking expression of tolerance in the root system. As root growth and shoot growth are

related, shoot growth may also have been affected by tube size after day 28.

From Fig. 4.1.1 seminal root length became much more variable after day 28 than before and this variation is probably responsible for some of the spurious significant differences that were obtained after that time. The volume of soil in the tubes was not measured accurately; the soil was simply poured into the tube until it was filled. With watering, the soil tended to settle to a volume consistent with the amount of soil in the tube. As roots grew to occupy the soil volume present, the maximum length of roots (and of other parameters) was probably determined more by the volume of soil than by other characters such as inoculation or tolerance and this probably caused many of the individual significant differences in later measurements.

The rate of increase in shoot dry weight declined in the same way as did seminal root length (Fig. 4.1.2) but from day 63 onwards, i.e. 4 weeks later, indicating that the plant can store nutrients to maintain shoot growth for 4 weeks during periods of stress. In fact, reduction in rate of shoot weight increase may not have been the result of nutrient deficiency but the stage of plant development. At day 63, plants had reached the mid- to late-boot stage, leaves had ceased to emerge and some inflorescences were beginning to emerge. It is not expected that shoot weight would increase greatly after this time. Perhaps, if roots had stopped growing earlier, i.e. at an earlier stage of development, shoots may have continued growing for more than 4 weeks longer than the roots. In either case, this excess of nutrients, which may not be essential for normal growth of the plant, may enable both cultivars to tolerate some amount of nematode damage.

In examining the effects of nematodes over the first 4 weeks of growth, few consistent differences were obtained; root length was affected and possibly shoot dry weight as well. These differences were expected to be more consistent. This might suggest that nematode density, or at least the number of larvae invading plant roots, was too small to produce substantial changes in the growing pattern. In resistance testing in these tubes a 60% penetration is normally obtained. This suggests that larvae are used efficiently and that the initial nematode density as well as tube size need further examination.

4.2 Comparison of the effects of *H. avenae* on three wheat cultivars grown in long tubes at 15°C

The field trial, described in Chapter 3, did not provide much information on root growth and a large amount of variation was found in other characters. A study of plant growth in short, narrow tubes in the previous experiment showed that tube size had a large influence on the growth of the plants after about 4 weeks. Variation in plant growth was considerable with few consistent effects of inoculation with nematodes. Destructive sampling, which was necessary to take the desired measurements, probably added to the variation and also required a large number of plants. A system in which root measurements could be made continually without destroying the plants and which would allow unrestricted root growth for longer than 4 weeks was desirable.

The effect of inoculation was not consistent in the previous experiment and this needed further examination to determine a satisfactory initial nematode density for the conditions of growth. Another highly tolerant cultivar, Gerek (Wilson *et al.*, 1983), had become available and this was examined as well in the following trial.

4.2.1 Materials and Methods

Seedlings were grown in 900 cm long P.V.C.* storm drain pipe with an internal diameter of 870 mm. The pipe sections were cut in half lengthwise and a sheet of perspex was cut to fit along the pipe, i.e. 900 cm x 900 mm. The perspex was secured by a strip of plastic tape, 2 cm wide, along each side and three strips across the bottom to close off the end.

Tubes were filled to 4 cm below the top with moist John Innes soil with half-strength nutrients but no peat. A pre-germinated seed (Chapter 4.1.1.1) was placed on the surface of the soil close to the perspex and was then covered with 1 cm of soil. The required number of larvae in 5 ml of water was added to the soil surface directly over the seedling. Tubes were kept in a frame approximately 700 cm high, 50 cm wide and 75 cm deep. Tubes were placed with the perspex downward and at an angle of 30° from the vertical so that roots would grow along the inner surface of the perspex and would thus be visible and easily measurable.

The three cultivars, Condor, RAC311 and Gerek, were inoculated with nematode densities of 0, 50, 100, 150 or 200 larvae per tube. Treatments were replicated six times and tubes arranged randomly. Height to the tip of the youngest leaf and Zadoks growth stage were recorded 15, 22, 29 and 36 days after sowing. At the first sampling time the length of leaves 1 and 2 were measured and leaves 2,3 and 4, respectively, were measured at the other three times. On day 32 nodal roots were counted and the depth to the lowest visible root was measured. Forty-three days after sowing, tillers were counted and on days 50 and

*Registered trade mark.

57, Zadoks growth stage was recorded. When tops were completely dry, plants were harvested. Top dry weight, number of heads, number of tillers, number of grains and weight of grain (yield) were recorded.

Data were analysed using an analysis of variance with a 3 x 5 factorial design to determine the effects of cultivar, nematode density and the cultivar x density interactions.

4.2.2 Results

Cultivars and nematode densities produced significant effects in most parameters (Table 4.2.1). Examination of cultivar effects showed that Gerek had shorter leaves 1 and 2 than did Condor and RAC311 (Table 4.2.2). Until day 36, with the exception of day 29, Gerek was more mature than the other two cultivars but after this time was less advanced than Condor (Table 4.2.2). Gerek was shorter than RAC311 at both day 22 and day 29 and shorter than Condor at day 22 (Table 4.2.2). At day 43, Gerek had more tillers than RAC311 which had more than Condor (Table 4.2.2). In all of the parameters assessed at harvest, RAC311 and Condor did not differ. Gerek, however, had higher values for all characters measured (Table 4.2.2).

In parameters where there was an effect of different nematode densities (Table 4.2.3), no significant difference, except in growth stage at day 15, was found between uninoculated plants and those with 50 larvae added at sowing. Significant reductions in these parameters were found when inoculated with 100 larvae at sowing but there were no further reductions with greater inoculum densities.

The only significant cultivar x density interactions occurred with growth stage at day 15 and maximum length of leaf 2 (Table 4.2.1).

TABLE 4.2.1: Variance ratios related to cultivar, density and cultivar x density interaction effects when Gerek, RAC311 and Condor were inoculated in long tubes with 0, 50, 100, 150 or 200 *H. avenae* larvae

Parameter	Variance ratios		
	Cultivar	Density	Cultivar x Density
Max. length of leaf 1	10.39***	1.15	1.59
Max. length of leaf 2	8.70***	4.65**	2.56*
Max. length of leaf 3	3.61*	2.83*	2.01
Max. length of leaf 4	12.12***	1.88	0.94
Max. length of leaf 5	5.07**	1.61	1.26
Growth stage, day 15	3.18*	2.76*	2.22*
Growth stage, day 22	7.01**	1.22	0.69
Growth stage, day 29	10.45***	3.61*	2.06
Growth stage, day 36	17.73***	1.43	0.92
Growth stage, day 50	6.90**	0.32	0.30
Growth stage, day 57	30.23***	0.07	1.55
Height, day 15	2.13	4.29**	1.24
Height, day 22	3.16*	4.43**	1.07
Height, day 29	4.84*	3.29*	1.58
Height, day 36	2.74	0.97	0.52
Number of nodal roots, day 32	1.03	0.96	0.98
Root depth, day 32	1.80	1.71	2.10
Number of tillers, day 43	28.73***	0.53	0.60
Shoot dry weight[a]	5.64**	1.12	1.04
Number of tillers[a]	27.35***	0.61	0.78

continued/..

TABLE 4.2.1 (continued)

Parameter	Variance ratios		
	Cultivar	Density	Cultivar x Density
Number of heads[a]	10.31***	2.32	0.51
Number of infertile tillers[a]	26.36***	0.46	0.61
Number of grains[a]	3.66*	1.95	1.18
Grain weight[a]	0.41	0.99	1.12

[a]Parameters assessed at harvest on a per plant basis

*, **, ***Significant ($P<0.05$, $P<0.01$, $P<0.001$ respectively).

TABLE 4.2.2: Mean values of ~~parameters~~ VARIABLES where significant variance ratios for cultivar effects were found when Gerek, RAC311 and Condor were grown in long tubes (Table 4.2.1).

Parameter	Gerek	RAC311	Condor	L.S.D. (P<0.05)
Max. length of leaf 1 (cm)	5.3[a]	6.9	7.7	1.1
Max. length of leaf 2 (cm)	13.6	17.3	16.2	1.8
Max. length of leaf 3 (cm)	27.0	29.0	27.5	1.7
Max. length of leaf 4 (cm)	30.2	32.0	28.9	1.2
Max. length of leaf 5 (cm)	32.5	33.4	31.0	1.5
Growth stage, day 15	12.6	11.9	11.9	0.6
Growth stage, day 22	17.9	15.6	14.2	2.0
Growth stage, day 29	22.0	21.2	19.4	1.2
Growth stage, day 36	24.1	22.6	21.6	0.9
Growth stage, day 50	26.5	26.8	30.1	2.2
Growth stage, day 57	31.3	36.1	40.9	2.5
Height (cm), day 22	31.1	34.0	35.5	2.3
Height (cm), day 29	35.2	37.8	35.8	1.7
Number of tillers, day 43	5.1	2.8	1.7	0.9
Shoot dry weight (g)[b]	6.9	4.9	5.0	1.3
Number of tillers[b]	7.3	3.4	2.9	1.3
Number of heads[b]	4.0	2.6	2.5	0.8
Number of infertile tillers[b]	3.3	0.8	0.5	0.8
Number of grains[b]	75	50	61	18

[a]Values are means of 6 replicates
[b]~~Parameters~~ VARIABLES assessed at harvest on a per plant basis.

TABLE 4.2.3: Mean values of various ~~parameters~~ VARIABLES where significant variance ratios for density effects were found when Gerek, RAC311 and Condor were grown in long tubes (Table 4.2.1).

Parameter	Inoculum Density 0	50	100	150	200	L.S.D. (P<0.05)
Max. length of leaf 2 (cm)	17.7[a]	17.5	14.3	15.3	13.8	2.4
Max. length of leaf 3 (cm)	29.7	28.9	26.6	27.2	27.0	2.2
Growth stage, day 15	13.0	11.9	11.9	11.9	11.9	0.8
Growth stage, day 29	22.1	21.8	19.9	20.6	20.1	1.5
Height (cm), day 15	22.3	21.7	18.7	19.6	17.6	2.7
Height (cm), day 22	35.5	34.2	30.9	30.9	31.0	3.0
Height (cm), day 29	38.3	37.2	35.7	34.9	35.3	2.2

[a]Values are means of 6 replicates.

The former interaction (Table 4.2.4) was produced by the mean value for uninoculated Gerek. No trends were obvious in the data for maximum length of leaf 2 (Fig. 4.2.1). Therefore, it was considered unnecessary to attempt to fit linear regressions to these data.

4.2.3 Discussion

This trial was not successful as a tolerance assay or for studying root systems throughout the growing season. It was intended that root length would be measured weekly but roots did not grow along the perspex as expected, possibly because no attempt was made to exclude light, and so were barely visible. Furthermore, it was found that by moving the tubes soil was severely compacted. This would have restricted root growth and, therefore, was avoided wherever possible.

Coefficients of variation for some parameters ranged up to 108% showing that variation may have been the major reason for lack of significant differences in the data and, therefore, for the failure of the technique as a tolerance assay. This might have been overcome by using many more samples but would have required more soil and space than was available. The major source of variation was probably in the soil profile. With a large quantity of soil, such as that required in these tubes, compaction was a problem as this restricted root extension and possibly nematode movement as well. Therefore, a successful tolerance assay would probably not involve large tubes and their inherent problems and further investigations should be concentrated on the use of smaller tubes.

The data from different initial densities (Table 4.2.3) showed that the number of larvae in inocula should be changed to show the

TABLE 4.2.4: Means of six replicates showing growth stage 15 days after inoculation of Gerek, RAC311 and Condor with five densities of *H. avenae* in long tubes

Cultivar \ Density	0	50	100	150	200
Gerek	15.0	12.0	12.0	12.0	12.0
RAC311	12.0	11.7	12.0	12.0	11.8
Condor	12.0	12.0	11.7	11.8	12.0

L.S.D. ($P<0.05$) = 1.43

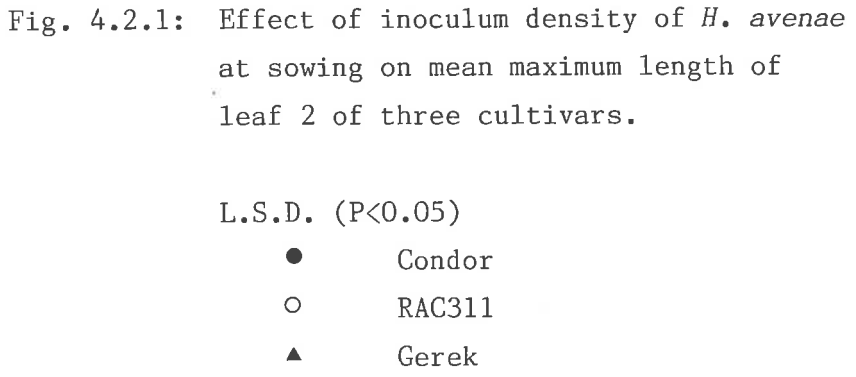
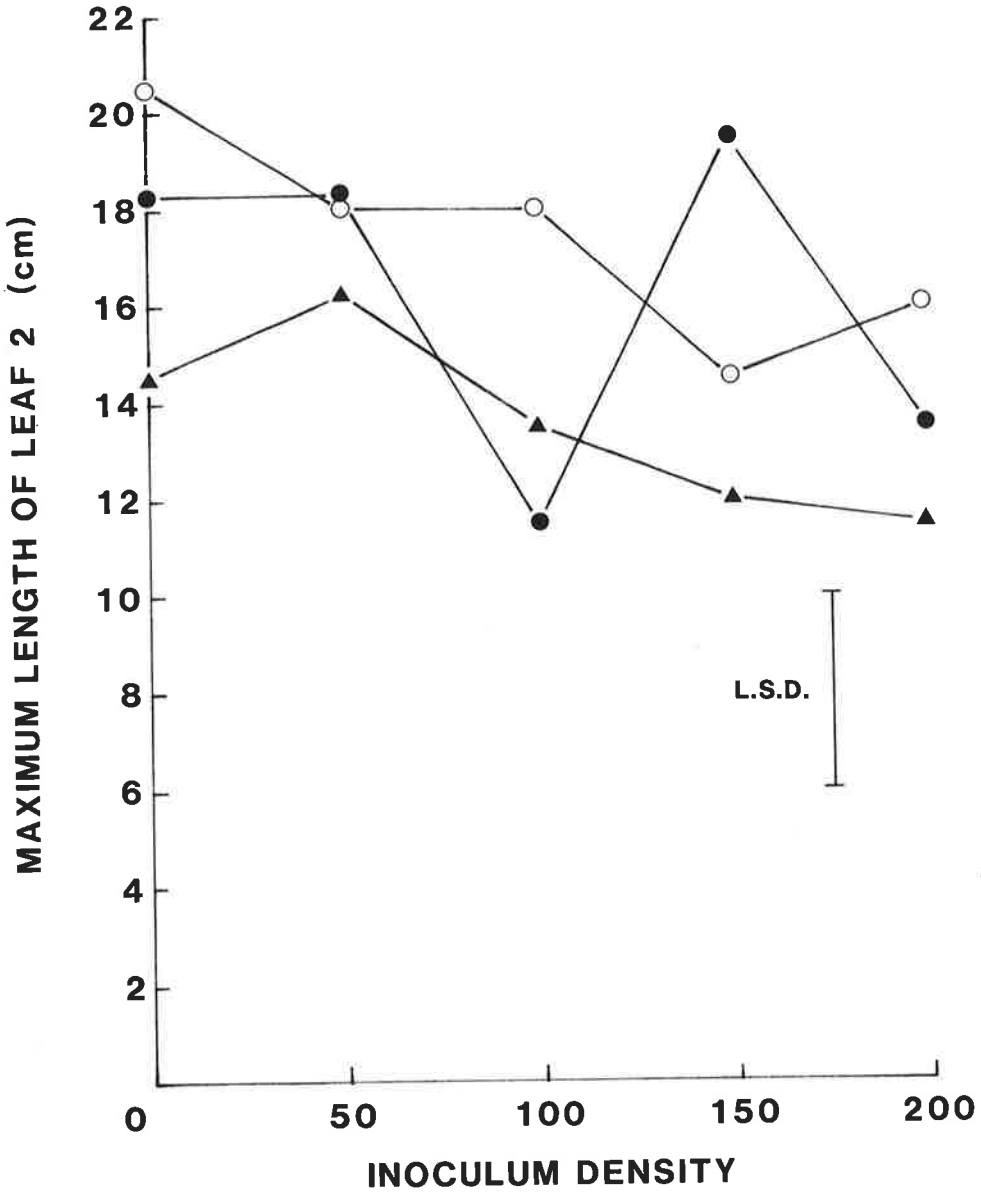


Fig. 4.2.1: Effect of inoculum density of *H. avenae* at sowing on mean maximum length of leaf 2 of three cultivars.


MAXIMUM LENGTH OF LEAF 2 (cm)
22
20
18
16
14
12
10
8
6
4
2
0
50
100
150
200
INOCULUM DENSITY
L.S.D.

effect of a range of initial densities. Few differences were found between 0 and 50 larvae and none between 100, 150 and 200 larvae per tube. One hundred larvae per tube should be the minimum number used as inoculum and other densities need to be greater than two hundred to gain information relating to density effects.

No cultivar x density interactions were of use in tolerance assessment but it is interesting to note that Gerek produced a very large number of tillers and, also, more infertile tillers than the other two cultivars. Thus, the total number of tillers of Gerek may be reduced more than that of the other two cultivars without loss of yield if the tillers that were lost were infertile. Therefore, a simple count of total tiller number may not assess tolerance accurately. The number of heads (fertile tillers) may be a more useful character.

4.3 Comparison of the effects of *H. avenae* on three wheat cultivars grown in short tubes at 10°C

Attempts to find a satisfactory assay for differences in tolerance in the early stages of growth of wheat have, so far, failed. Growth in the small tubes (Chapter 4.1), provided variation could be controlled, was satisfactory for a short period of time up to about 4 weeks. However, the initial density of larvae should be increased to at least 100 to obtain greater nematode effects. The large tubes (Chapter 4.2), because of the effect of soil compaction on movement and watering, were unsatisfactory and did not allow continuous assessment of root growth.

It was decided to persevere with the small tubes, as these were much more convenient to handle, but to attempt to extend the time over which observations could be made by lowering the temperature for growth to 10°C and reducing the daylength to 10 hours. It seemed likely that the rate of

plant growth would be affected to a greater extent than the activities of the nematode and might, therefore, give more consistent effects. This temperature, 10°C, is closer to that in the field at the time of the early assessment of Fisher *et al.* (1981).

4.3.1 Materials and Methods

The method used was similar to that in Chapter 4.1.1 except that plants were kept at 10°C with a 10 hour daylength. The cultivars used were RAC311, Condor and Halberd, a similarly susceptible (Fisher, 1982) but semi-tolerant cultivar (Fisher *et al.*, 1981), and these were inoculated at sowing with 100 larvae. Twenty inoculated and twenty uninoculated seedlings of each cultivar were sown except for Halberd where there were only ten seedlings in each treatment. At 29 and 52 days after sowing, ten plants of each cultivar in each treatment were harvested but Halberd was harvested only at 29 days. Seminal root length, root dry weight, shoot dry weight, and shoot : root ratio were assessed at each time and at 52 days nodal roots were counted and measured. During growth, height, Zadoks growth stage and maximum lengths of the first three leaves were recorded at various times after sowing.

4.3.2 Results

Total root length of both Halberd and Condor were reduced at day 29 by inoculation while RAC311 was unaffected (Table 4.3.1). However, the root dry weight of RAC311 was increased and shoot dry weight remained unchanged so that shoot : root ratio was decreased at 29 days after sowing. Halberd and Condor were not affected in these

TABLE 4.3.1: Mean values of parameters measured at 29 days after sowing three cultivars either inoculated with 100 *H. avenae* larvae at sowing (I) or uninoculated (C)

Parameter		RAC311	Halberd	Condor
Total root length (cm)	C	108[a]	125[a,b]	162[b]
	I	98	90**	104**
Root dry weight (mg)	C	11[a]	14[a,b]	17[b]
	I	18*	17	16
Shoot dry weight (mg)	C	34[a]	31[a]	32[a]
	I	33	30	27
Shoot : root ratio	C	3.3[a]	2.1[b]	1.8[b+]
	I	1.9***	1.8	1.4

*, **, *** Values for control and inoculated plants in each parameter significantly different ($P<0.05$, $P<0.01$, $P<0.001$ respectively)

a,b Values for uninoculated plants in each parameter followed by the same letter are not significantly different ($P<0.05$)

\+ RAC311 and Condor are significantly different ($P<0.01$).

three characters and shoot dry weight was not changed by *H. avenae* in any of the three cultivars at that time.

At 52 days after sowing, seminal root length, root dry weight and shoot : root ratio were unaffected in both cultivars. Shoot dry weight of Condor was reduced but that of RAC311 was unchanged. The number of nodal roots was not affected but *H. avenae* reduced the length of nodal roots of RAC311 at 52 days after sowing (Table 4.3.2).

Of the first three leaves, only the maximum length of leaf 1 of Condor was reduced by inoculation (Table 4.3.3). Height of RAC311 was unaffected by inoculation but that of Condor was reduced at 23 days after sowing (Table 4.3.4). Growth stage was not affected by inoculation ($P<0.05$) at any time in any of the three cultivars.

Roots of uninoculated Condor grew faster till day 29 than did those of RAC311 while those of Halberd were intermediate. There was no difference in shoot dry weight between cultivars so that root growth was reflected in shoot : root ratio (Table 4.3.1). By day 52 there were no differences between cultivars in any of these parameters. There was no significant difference ($P<0.05$) between the shoot : root ratio of Condor at 29 and 52 days after sowing but that of RAC311 did decrease ($P<0.05$) between the two times indicating an increased rate of root growth of RAC311. Root dry weight for RAC311 and Condor has been plotted against time (Fig. 4.3.1) to illustrate the difference between the two cultivars.

4.3.3 Discussion

The results obtained from using this technique appeared to be more consistent than from the previous two methods (Chapters 4.1; 4.2) and

TABLE 4.3.2: Mean values of parameters measured at 52 days after sowing two cultivars either inoculated with 100 *H. avenae* larvae at sowing (I) or uninoculated (C)

Parameter		RAC311	Condor
Seminal root length (cm)	C	810[a]	944[a]
	I	898	908
Root dry weight (mg)	C	48[a]	49[a]
	I	42	41
Shoot dry weight (mg)	C	112[a]	119[a]
	I	103	101**
Shoot : root ratio	C	2.0[a]	1.8[a]
	I	2.3	2.2
Number of nodal roots	C	3[a]	4[a]
	I	3	3
Nodal root length (cm)	C	21[a]	19[a]
	I	12*	16

*, **Values of inoculated and uninoculated plants in each parameter were significantly different ($P<0.05$, $P<0.01$ respectively)

[a]Values for uninoculated plants in each parameter did not differ significantly ($P<0.05$).

TABLE 4.3.3: Mean maximum lengths of the first three leaves of three cultivars either inoculated with 100 larvae at sowing (I) or uninoculated (C)

Leaf number		RAC311	Halberd	Condor
1	C	10.0	13.2	11.6
	I	9.8	13.0	10.8**
2	C	15.5	-	16.9
	I	14.2	-	16.6
3	C	26.9	-	27.2
	I	25.2	-	27.4

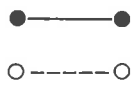
**Values of inoculated and uninoculated plants were significantly different ($P<0.01$).

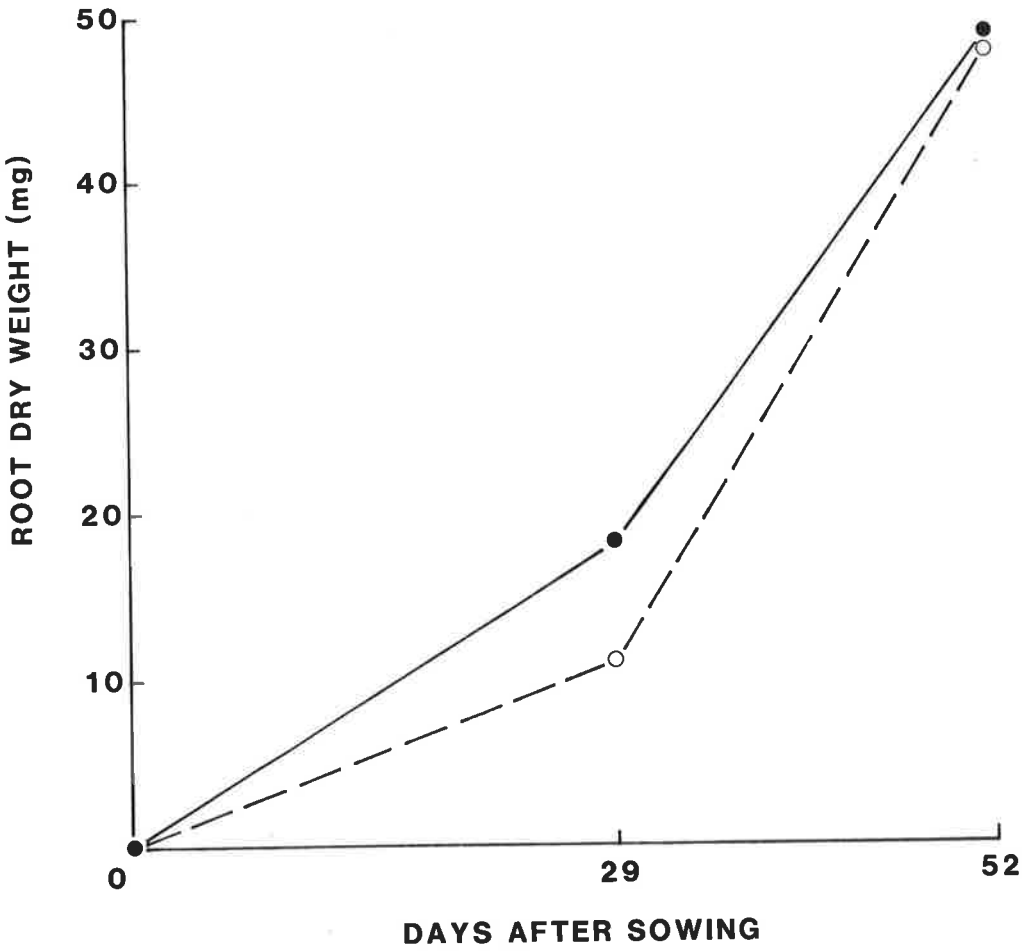
TABLE 4.3.4: Mean height at three times after sowing of two cultivars either inoculated with 100 larvae at sowing (I) or uninoculated (C)

Height (cm)		RAC311	Condor
23 days after sowing	C	11.5	12.4
	I	10.4	11.0**
33 days after sowing	C	20.7	22.8
	I	19.9	21.1
52 days after sowing	C	38.9	38.4
	I	37.5	38.5

**Values for inoculated and uninoculated plants were significantly different ($P<0.01$).

Fig. 4.3.1: Mean root dry weight of uninoculated Condor and RAC311 plants, grown at 10°C, at two times after sowing.




50
40
30
20
10
0
ROOT DRY WEIGHT (mg)
29
52
DAYS AFTER SOWING

confirmed the data on the comparison of RAC311 and Condor from the field experiment (Chapter 3). Condor appeared intolerant and RAC311 more tolerant with Halberd occupying an intermediate position.

The root systems of RAC311, Condor and Halberd reacted differently to infection by *H. avenae*. In RAC311, total root length was not affected significantly at day 29 so that galling increased the dry weight of roots. In Condor and Halberd, however, root length was reduced significantly so that galling merely maintained root dry weight. The overall effect was that infection actually increased the production of root tissues of RAC311 (i.e. a stimulation) while the quantity of root material of Condor and Halberd remained approximately the same but in a shortened, swollen form.

The other effects that were observed probably resulted from these initial effects on root growth, e.g. the reduction in the shoot : root ratio of RAC311 is a direct result of the changes in root dry weight. No changes above-ground were found in RAC311 either at 29 or 52 days after sowing. Above-ground growth of Condor, however, was reduced at 52 days after sowing and this was probably due to decreases in the height and length of the first leaf. Although roots of Halberd reacted similarly to those of Condor, its first leaf was not shortened by inoculation.

Seminal root systems of Condor and RAC311 at 52 days after sowing had recovered from the early effects of infection suggesting either that the plant could compensate for the damage caused to the roots or that root systems had fully occupied the tubes. However, Condor plants had not recovered entirely as shoot growth was reduced at that time by inoculation.

Nodal roots of RAC311 were shortened by inoculation but this may reflect delayed emergence rather than reduced growth rate. Further investigation is required to determine whether this might affect yield.

The value of some of the parameters examined could now be questioned. Zadoks growth scale did not give any significant results. Either it is too crude to assess the differences in development that did occur or none occurred. It will no longer be used. The value of leaf measurements after leaf 1 and height have so far not given any useful results so these will not be used again; shoot dry weight seems to cover most aspects of above-ground growth that are necessary at this stage. Should further investigation of shoot weight be required later, then it may be necessary to return to some of these criteria.

The technique appeared satisfactory and the results suggested that differences in tolerance were apparent early in the growth of the seedling. Thus, further investigation, using this method, of the effect of *H. avenae* on a range of tolerant and intolerant cultivars was warranted.

It is now possible to assess the validity of Seinhorst's (1979) hypothesis. He proposed that nematode attack merely delays plant development and that, at a given shoot weight, the shoot : root ratio is the same for plants with and without nematodes. No effect of nematodes on growth stage was found in the current trial indicating that delay in plant development was not great. Furthermore, at least for RAC311 at day 29, although shoot weight remained the same, root weight was altered by nematodes thereby decreasing the shoot : root ratio. The shoot : root ratio may be constant after day 52 which

would be consistent with Fatemy & Evans (1982), Trudgill & Cotes (1982) and Evans (1982b), who found that nematodes reduced the shoot: root ratio but this remained constant at a given nematode density. It appears that the situation is not as simple as Seinhorst has described, at least in early growth.

An observation from this trial which may or may not be important in tolerance was the way in which uninoculated plants grew. The fact that there appeared to be a relationship between initial root growth of uninoculated plants and known tolerance of the three cultivars tested suggests that this may be significant and will be examined further.

CHAPTER V

RELATIONSHIP BETWEEN EARLY GROWTH PARAMETERS AND OBSERVED TOLERANCE IN SEVERAL CULTIVARS

At 10°C (Chapter 4.3) it was found that, at day 29, total root length of Condor and Halberd was reduced when inoculated at sowing whereas root dry weight of RAC311 was increased. Fifty-two days after sowing, the shoot dry weight of Condor had been reduced by inoculation. If this response to *H. avenae* i.e. initial reduction in root growth with later reduction in shoot growth, is typical of the intolerant reaction then a continuum of cultivars from tolerant to intolerant should show a gradation of reaction. Thus, 11 cultivars, showing a range of tolerance on the basis of yield (Fisher *et al.*, 1981), were examined as in Chapter 4.3 for effects on early growth following inoculation. The only available estimate of tolerance in wheat is that of Fisher *et al.* (1981) taken under field conditions and is based largely on yield i.e. on growth over the whole season. Already it is known that the yield of Cook, a cultivar of intermediate tolerance, is obtained in a different manner from other cultivars (Chapter 2.3). It may be that there are other unknown mechanisms conferring tolerance. Nevertheless, it seemed worthwhile to test these early estimates of tolerance against the field reactions of a known range of cultivars.

5.1 Materials and Methods

Cultivars were chosen to include a range from tolerant to intolerant. The cultivars - ((Siete Cerros x Mengavi) x Crim) x Hazera, (MMC x Hazera); (Mexico x Koda) x Raven, (MKR) (Fisher *et al.*, 1981); RAC311 and Gerek (Wilson *et al.*, 1983) - are considered to possess a high degree of tolerance. (Siete Cerros x Mengavi) x Crim, (MMC); Condor; Warigal and Egret are highly intolerant (Fisher *et al.*, 1981) while Cook, Halberd and

Bindawarra appear to be intermediate between these two groups although Cook did not rate well in early growth (Fisher *et al* ., 1981) or in my early tests (Chapter 2).

Methods used were similar to those in Chapter 4.3.1. Twenty plants of each cultivar were inoculated with 100 larvae and twenty were used as controls Ten plants in each treatment for each cultivar were harvested 29 days after sowing and the other ten 52 days after sowing. On the first occasion leaf 1 and the roots were measured and root dry weight was recorded as these characters had shown significant differences with inoculation in Chapter 4.3.2. At this sampling time roots were divided into primary seminal roots and seminal lateral roots in order to determine where the effect on total root length arose. At the later sampling time root and shoot dry weight were recorded and shoot : root ratio determined.

Student's t-test was used in each parameter for each cultivar to determine the significant difference between control and inoculated plants. Pearson correlation coefficients were determined for pairs of all parameters measured and for % tolerance as determined by Fisher *et al*. (1981).

5.2 Results

At day 29 all cultivars had significantly shorter primary roots due to inoculation but the reductions for the intolerant cultivars were greater than for the tolerant (Table 5.1). Few cultivars showed significant reduction in total root length and no cultivar showed any significant change in the length of seminal lateral roots following inoculation (Table 5.1).

Of the parameters measured at 52 days after sowing, the shoot : root ratio was affected most by inoculation but was reduced significantly in only half of the cultivars (Table 5.2). In only two of the cultivars was root dry weight increased while shoot dry weight was not affected significantly (Table 5.2).

TABLE 5.1: Effects, at 29 days after sowing, of inoculation of several cultivars with 100 *H. avenae* larvae at sowing; C - control; I - inoculated; and % tolerance

Cultivar		Primary seminal root length (cm)	Seminal lateral root length (cm)	Total root length (cm)	Root dry weight (mg)	Length leaf 1 (cm)	% Tolerance
Egret	C	63	58	121	14	9	17.2
	I	34***	48	82	18	9	
Condor	C	69	92	161	17	10	30.6
	I	40***	86	126	27***	10	
MMC	C	58	21	79	12	12	13.4
	I	31***	28	59*	19	12	
Warigal	C	71	132	203	17	11	43.5
	I	48***	83	131**	24*	8*	
Halberd	C	56	38	94	11	11	54.9
	I	43**	32	75*	15*	11	
Cook	C	61	76	137	13	8	56.1
	I	43*	70	113	18	8	
MKR	C	60	28	88	10	10	96.5
	I	46*	40	86	19***	10	
Gerek	C	49	51	100	12	8	_[a]
	I	36*	32	68*	15	7	
RAC311	C	56	63	119	13	9	_[a]
	I	45*	51	96	21***	10	
Bindawarra	C	54	40	94	10	7	58.5
	I	43*	38	81	16**	9	
MMC x Hazera	C	45	45	90	9	6	101.1
	I	35*	41	76	21***	8*	

*, **, *** Differences due to inoculation significant ($P<0.05$, $P<0.01$, $P<0.001$ respectively)

[a] Not tested by Fisher *et al.* (1981).

TABLE 5.2: Effects, at 52 days after sowing, of inoculation of several cultivars with 100 *H. avenae* larvae at sowing: C - control I - inoculated

Cultivar		Shoot dry weight (mg)	Root dry weight (mg)	Shoot : Root ratio
Egret	C	79	44	1.9
	I	79	54	1.5*
Condor	C	103	62	1.7
	I	106	73	1.5
MMC	C	62	25	2.6
	I	57	32*	1.8**
Warigal	C	86	43	2.0
	I	82	50	1.7**
Cook	C	68	45	1.6
	I	71	50	1.5
MKR	C	68	41	1.7
	I	60	36	1.8
Gerek	C	60	33	1.9
	I	56	37	1.6*
RAC311	C	92	45	1.9
	I	88	50	1.8
Bindawarra	C	79	52	1.5
	I	75	55	1.4
MMC x Hazera	C	56	24	2.4
	I	63	39*	1.7***

*, **, *** Differences due to inoculation significant ($P<0.05$, $P<0.01$, $P<0.001$ respectively)
(Halberd was not included as very few pre-germinated seedlings emerged).

The correlation coefficients (Table 5.3) show some unexpectedly significant results. Some were expected, e.g. the positive correlations between total root length, root dry weight and primary root length of control plants at day 29. Percent tolerance was negatively correlated with reduction in length of primary seminal roots at day 29 and positively correlated with increase in shoot dry weight at day 52 due to inoculation.

Percent tolerance was negatively correlated with dry weight of roots of control plants at day 29. Reduction in length of primary seminal roots at day 29 due to inoculation was positively correlated with root weight and length of primary roots of control plants at day 29. Seminal lateral root length increase at day 29 due to inoculation was negatively correlated with total root length of control plants at day 29. Reduction in total root length at day 29 was positively correlated with total length, length of primary and dry weight of roots of control plants.

Because initial seed weight might have been responsible for some of these correlations, the weight of 100 seeds (based on three replicates of 100 of the seeds used in this trial) was tested for correlation with all other parameters. None of these correlations was significant (Table 5.3).

5.3 Discussion

The ranking of cultivars on the basis of % tolerance from field data (Fisher *et al.*, 1981) was the first attempt to interpret tolerance in wheat and results from the current trial largely confirm the field estimate. Furthermore, some useful correlations were found between very early growth of inoculated seedlings and the tolerance ranking which was based on yield of plants grown in infested soil. Changes in primary seminal root length at day 29 and shoot dry weight at day 52 were related to tolerance. The previous

TABLE 5.3: Matrix of Pearson correlation coefficients of variates 1 to 13 measured on 11 cultivars

1													
2	NS												
3	-0.6698	0.8734											
4	NS	0.7762	0.8350										
5	NS	NS	NS	NS									
6	-0.8263	NS	0.7438	0.7459	NS								
7	NS	NS	NS	NS	NS	NS							
8	NS	0.7414	0.6797	0.7392	NS	NS	NS						
9	NS	-0.7862	NS	NS	NS	NS	NS	NS					
10	0.7607	NS	NS	NS	NS	NS	NS	NS	NS				
11	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS			
12	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS		
13	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
	1	2	3	4	5	6	7	8	9	10	11	12	13

1 - % tolerance (Fisher *et al.*, 1981)
2 - total root length of control plants, day 29
3 - root dry weight of control plants, day 29
4 - primary seminal root length of control plants, day 29
5 - 100 grain weight
6 - primary seminal root length reduction with inoculation, day 29
7 - leaf 1 length reduction with inoculation, day 29
8 - total root length reduction with inoculation, day 29
9 - seminal lateral root length increase with inoculation, day 29
10 - root dry weight increase with inoculation, day 29
11 - shoot dry weight increase with inoculation, day 52
12 - root dry weight increase with inoculation, day 52
13 - shoot : root ratio increase with inoculation, day 52

RAC311 and Gerek were not included in variate 1 as they were not tested by Fisher *et al.* (1981)
Halberd was not included in variates 11 to 13 as very few pre-germinated seedlings emerged
NS - correlation not significant ($P<0.05$). Where a significant result was obtained the correlation coefficient is given.

trial (Chapter 4.3) also suggested that these characters might be related to tolerance and this trial has confirmed that. The greater the tolerance of a cultivar the smaller the reduction in primary seminal root length (at day 29) and the greater the increase in shoot dry weight (at day 52). Both of these reactions suggest that reduction of growth due to inoculation is minimised in tolerant seedlings.

The relation between the field ranking of cultivars for tolerance and the reduction in primary seminal root length following inoculation was not complete as there was some variation. This may have been caused by the difference between field and laboratory conditions; it may have been due to different mechanisms involved in tolerance, e.g. the cultivar, Cook, seems not to possess tolerance when assessed in early growth (Chapter 2) but it still yielded well in the field (Fisher *et al.*, 1981); or it may have been due to variation in the laboratory trial, e.g. in this trial no reduction in total root length was observed for Condor as occurred in the previous trial.

Another feature was the apparent recovery of plants. The effects on root growth visible at day 29 had largely disappeared by day 52. The same occurred in the previous trial. In intolerant plants, at day 52, the effects of infection were transferred to shoots. Whether shoot growth would recover was not determined as later samples would be required. Recovery is difficult to interpret. It may be an innate characteristic of the cultivar, in which case, variation in the ability to recover or to compensate for the damage may be expected or it may be an artifact of the inoculation method, nematode density or the conditions of growth for the seedlings.

The fact that seminal lateral roots were unaffected by *H. avenae* was probably due to the method of inoculation. A result of the single inoculation at sowing may have been that all the infective larvae had established before the lateral roots had emerged so that they largely escaped attack.

This may have enabled the seedlings to recover. However, the lack of apparent effects on seminal lateral roots even of tolerant cultivars indicates that tolerance is not due to compensation for damaged primary roots by root proliferation, i.e. adventitious rooting. Rather, rate of elongation of primary seminal roots of tolerant cultivars was not reduced as much by inoculation as was that of intolerant cultivars.

A further feature was the correlation of % tolerance with root dry weight of the uninoculated plants, i.e. the slower the initial growth of roots of a cultivar the greater the tolerance. This was not due to differences in weight of the original seed as % tolerance was not significantly correlated with seed weight. Therefore, early growth rate of roots is very important in tolerance. Evans (1982b) suggested that tolerance of potato cultivars to *Globodera rostochiensis* may be assessed on the basis of high root vigor in the absence of nematodes, the opposite idea from that obtained here. In potatoes the shoot : root ratio remained constant at a given nematode density (Evans, 1982b) suggesting that root growth and, therefore, tolerance could be assessed on the basis of shoot growth. However, in my experiment, shoot : root ratios differed between Condor and RAC311 at day 29 but not at day 52 (Tables 4.3.1; 4.3.2). The difference between the ratios at the two times was mainly due to changes in root weight of RAC311 and the effect on this of the plant's ability to recover. Potatoes and wheat seem to differ but this may simply reflect sampling at different times. The same may be true of Seinhorst's hypothesis (1979) which was proposed on the basis of sampling between 3 and 14 weeks after sowing. If growth continues as shown in Fig. 4.3.2, then RAC311 would have more vigorous root growth in later samples than would Condor and possibly a constant shoot : root ratio after day 52. In the tests carried out at 15°C, these changes were not recorded because growth rate of the plants was too fast to allow separation. However, at 10°C, tolerance

could be assessed in the absence of nematodes by measuring very early growth rate of roots of different cultivars.

This difference in early growth rates of roots is important but needs to be checked further. The slower rate of root growth of RAC311 up to day 29 means that it has a lesser capacity to respond to infestation by *H. avenae* so that root growth (and subsequently top growth) is not altered as much as in Condor. The faster rate of root growth after day 29 (Fig. 4.3.2) may enable RAC311 to recover or compensate more rapidly. These effects will be examined in more detail in the following Chapter.

CHAPTER VI

ROLE OF THE ENDOSPERM IN TOLERANCE

A difference in root growth up to day 29 between uninoculated RAC311, Halberd and Condor plants, which was related to tolerance, was observed in Table 4.3.1. This was supported by a negative correlation between root dry weight of uninoculated plants and % tolerance (Table 5.3). Thus, tolerance may be dependent in some way on reduced early growth, i.e. on the hormonal condition which affects the release of endosperm reserves to the root as growth of the first five primary roots is dependent mainly on the endosperm (Williams, 1960). If this hypothesis is correct then, if RAC311 were inoculated when its roots were growing at the same rate as those of Condor, it should not appear more tolerant than Condor. To test this hypothesis the most suitable time for inoculation, i.e. when endosperm release to Condor and RAC311 roots is occurring at the same rate, must be determined.

6.1 Examination of release of endosperm reserves

6.1.1 Materials and Methods

Seeds of Condor and RAC311, which had been pre-germinated as before (Chapter 4.1.1) until all three roots were about 1 cm long, were plated separately, on day 0, onto 9 cm Petri dishes containing 7 cm diameter filter paper and 2 ml sterile distilled water. Plants were kept in a light proof cardboard box at 10°C and more water was added as required to keep the filter paper moist. Seven replicates were used and on days 0,3,6,10,13,17,20,23,41 and 48, root length, root dry weight, shoot dry weight and shoot : root ratios were recorded. Differences between Condor and RAC311 were determined by analysing data using a *t*-test.

6.1.2 Results

There were no significant differences throughout the experiment between the shoot dry weights of RAC311 and Condor except for the single result on day 41 (Table 6.1.1). On days 3 and 6, Condor had longer roots than did RAC311 but after that time no significant difference was found between the two cultivars for this character (Table 6.1.2). Root dry weight of Condor was significantly greater than that of RAC311 until day 13 (Fig. 6.1.1) but they did not differ significantly after that time. Slopes of lines on Fig. 6.1.1 up till day 13 were 0.28 for Condor ($r = 0.997$, $P<0.001$) and 0.19 for RAC311 ($r = 0.996$, $P<0.001$) showing that until day 13 roots of RAC311 grew more slowly than did those of Condor and these slopes were significantly different ($P<0.05$). No significant difference ($P<0.05$) was found between slopes of lines for RAC311 and Condor between days 13 and 48 (Fig. 6.1.1). After day 13, slopes of lines became increasingly closer to zero as endosperm reserves were depleted. Because of the differences in root dry weight between the two cultivars, the shoot : root ratio of RAC311 to day 13 was significantly greater than that of Condor (Table 6.1.3).

6.1.3 Discussion

It was not expected that shoot dry weight would vary between RAC311 and Condor as no difference was found earlier between these cultivars when uninoculated (Tables 4.3.1; 4.3.2). The difference between rate of root growth of RAC311 and Condor was expected and is consistent with results previously obtained for uninoculated plants (Table 4.3.1) and confirms the initially slower rate of growth of RAC311 roots. The effect of root dry weight on shoot : root ratio to day 13 was also consistent with earlier results (Table 4.3.1).

TABLE 6.1.1: Shoot dry weight (mg), measured at several times, of RAC311 and Condor seedlings grown in Petri dishes in the dark at 10°C

Day	RAC311	Condor
0	1.4	1.6
3	2.7	2.9
6	4.4	4.9
10	6.4	6.9
13	8.0	8.4
17	9.8	9.4
20	10.2	10.5
23	10.8	10.1
41	15.8	13.3*
48	12.8	13.4

*Cultivars significantly different ($P<0.05$).

TABLE 6.1.2: Total root length (cm), measured at several times, of RAC311 and Condor seedlings grown in Petri dishes in the dark at 10°C

Day	RAC311	Condor
0	5.6	8.0
3	7.7	12.9**
6	11.0	18.2*
10	17.1	24.6
13	24.5	30.9
17	34.8	32.1
20	30.6	37.1
23	39.4	38.4
41	61.3	56.7
48	54.1	57.8

*, **Cultivars significantly different ($P<0.05$, $P<0.01$ respectively).

Fig. 6.1.1: Change with time of root dry weight of RAC311 and Condor seedlings grown in Petri dishes in the dark at 10°C.

●———● Condor

o-----o RAC311

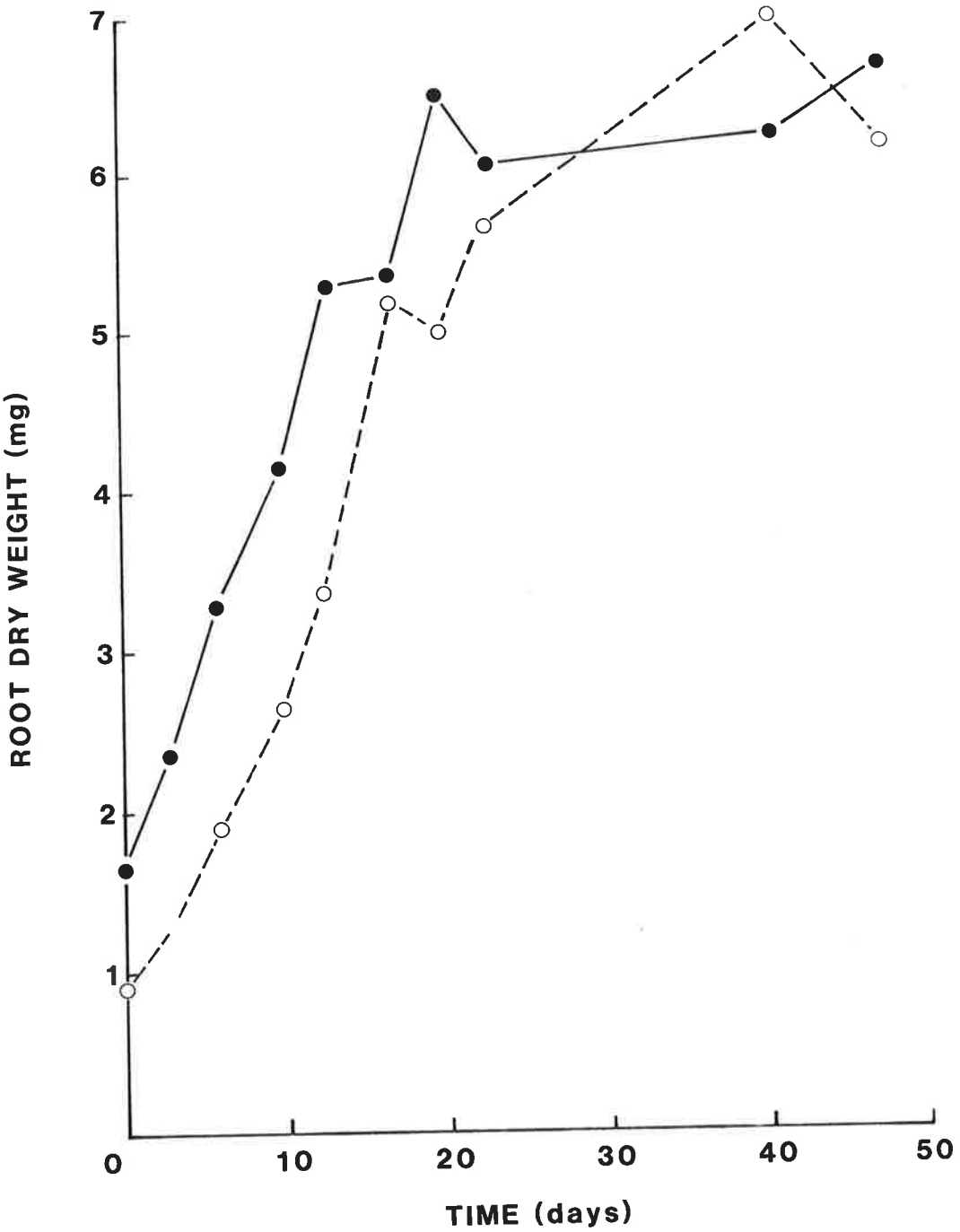

ROOT DRY WEIGHT (mg)
7
6
5
4
3
2
1
0
10
20
30
40
50
TIME (days)

TABLE 6.1.3: Shoot : root ratio, measured at several times, of RAC311 and Condor seedlings grown in Petri dishes in the dark at 10°C

Day	RAC311	Condor
0	1.4	1.0*
3	2.3	1.3**
6	2.4	1.5***
10	2.6	1.7**
13	2.6	1.6**
17	1.9	1.8
20	2.2	1.6*
23	2.1	1.7
41	2.3	2.1
48	2.3	2.0

*, **, *** Cultivars significantly different ($P<0.05$, $P<0.01$, $P<0.001$ respectively).

Results support the hypothesis that the differences in the use of endosperm by the roots may be partially responsible for the difference in early root growth between uninoculated RAC311 and Condor. Roots grew similarly from day 13 onward so that, in determining the importance of the endosperm in tolerance, inoculation at day 17, when roots of each cultivar weighed the same, would be the most suitable time.

6.2 The response to *H. avenae* of intolerant and tolerant plants growing at the same rate

In order to determine whether the mechanism responsible for the reduced rate of early root growth of RAC311 when compared to Condor is also responsible for its observed tolerance, inoculation should occur at a time when roots of both cultivars are growing at the same rate, i.e. after day 13. The aim was to determine the lowest inoculum density which would produce an intolerant reaction in Condor; at day 17, many more root-tips would be available for penetration than at sowing, and this suggests that a density greater than 100 larvae per plant would be required. If RAC311 was also intolerant at that density then one could conclude that time of inoculation and, therefore, rate of early root growth was involved in the tolerance mechanism. If so, the tolerance mechanism would only operate during very early growth but its effects might be carried through to maturity.

6.2.1 Materials and Methods

Plants were grown at 10°C in short plastic tubes (Chapter 4.3.1). Fifty plants each of RAC311 and Condor were sown. Plants were inoculated at 17 days after sowing with 0, 100, 300, 500 or 700 larvae applied to the soil surface. Ten plants were used in each treatment. Thirty-four days after sowing, plants were harvested and the following

parameters measured - primary seminal root length, seminal lateral root length, total root length, root dry weight, shoot dry weight and shoot : root ratio. A t-test was used to find the significance of differences between inoculated and control plants for each parameter.

6.2.2 Results

A stimulation of all parameters of Condor, except shoot dry weight, occurred when inoculated with 100 larvae (Table 6.2.1). Condor's growth was reduced at densities of 500 and 700 larvae. At both densities, total and seminal lateral root lengths were reduced and, when inoculated with 700 larvae, shoot dry weight was reduced. No effect of inoculation on growth of RAC311 was observed until the inoculum density reached 700 larvae when root growth was stimulated (Table 6.2.2). The difference in response of roots of RAC311 and Condor to infection is illustrated in Fig. 6.2.1.

6.2.3 Discussion

Data for various root parameters of Condor show the sequence of events which has come to be regarded as normal for parasitic nematodes (Seinhorst, 1961; Oostenbrink, 1966) - a stimulation of growth at relatively low densities followed by a reduction in growth as initial density increases. The primary seminal root length was not reduced significantly because, when inoculated 17 days after sowing, their root-tips would have been too deep in the soil for a significant number of larvae to reach them. Nevertheless, the trend was present.

The response of roots of RAC311 appeared to be quite different. Only at the highest density was root weight increased suggesting that, to produce the normal sequence of stimulation followed by growth re-

TABLE 6.2.1: Effects of inoculum density of *H. avenae* on growth ~~parameters~~ VARIABLES of Condor plants inoculated 17 and harvested 34 days after sowing

Growth ~~parameter~~ VARIABLE	Inoculum Density				
	0	100	300	500	700
Primary seminal root length (cm)	50	61*	51	44	43
Seminal lateral root length (cm)	133	211**	119	77*	33**
Total root length (cm)	183	272**	170	121*	76**
Shoot dry weight (mg)	36	43	36	32	30*
Root dry weight (mg)	14	24**	18	15	11
Shoot : root ratio	2.7	1.9**	2.2	2.4	2.9

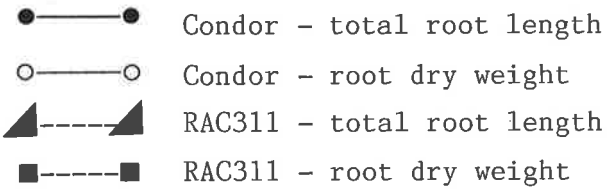
*, ** Significantly different from uninoculated control ($P<0.05$, $P<0.01$, respectively).

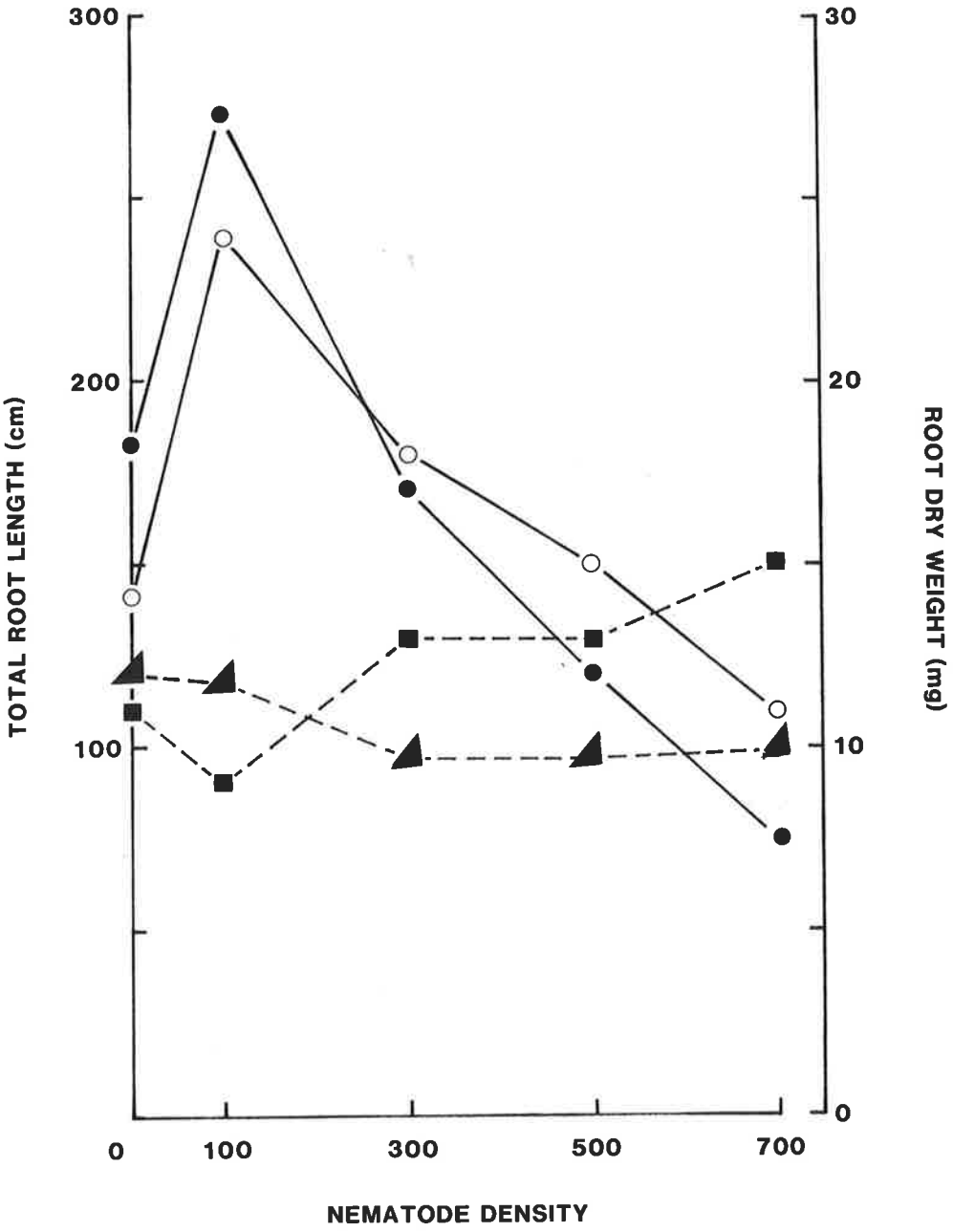
TABLE 6.2.2: Effects of inoculum density of *H. avenae* on growth ~~parameters~~ VARIABLES of RAC311 plants inoculated 17 and harvested 34 days after sowing

Growth ~~parameter~~ VARIABLE	Inoculum Density				
	0	100	300	500	700
Primary seminal root length (cm)	53	52	44	50	49
Seminal lateral root length (cm)	67	66	53	47	50
Total root length (cm)	120	118	97	97	99
Shoot dry weight (mg)	34	32	35	34	37
Root dry weight (mg)	11	9	13	13	15*
Shoot : root ratio	3.2	3.4	2.8	2.8	2.6*

*Significantly different from uninoculated control ($P<0.05$).

Fig. 6.2.1: Effect of inoculum density of *H. avenae* on total root length (left hand scale) and root dry weight (right hand scale) of Condor and RAC311 plants inoculated 17 days and harvested 34 days after sowing.




300
200
100
30
20
10
0
0
100
300
500
700
TOTAL ROOT LENGTH (cm)
ROOT DRY WEIGHT (mg)
NEMATODE DENSITY

duction, densities from 700 larvae upward would be required.

The difference in response of RAC311 and Condor can easily be seen by comparing the number of larvae required, per cm of root of uninoculated plants measured at 34 days after sowing (roots were not measured at inoculation) to produce stimulation of root growth. Although uninoculated primary seminal roots of each cultivar were the same length at 34 days after sowing, Condor's seminal lateral roots were about twice as long as those of RAC311. It would be expected, therefore, that, to produce the same response, RAC311 would require fewer larvae than would Condor. However, in this test, RAC311 needed seven times more larvae than did Condor to produce a stimulation of growth. This is equivalent to 5.8 nematodes/cm of total root length of uninoculated plants measured at 34 days after sowing (or 63.6 nematodes/g of root dry weight) to produce an increase in the root dry weight of RAC311 while Condor only required 0.6 nematodes/cm of total root length (or 7.1 nematodes/g of root dry weight).

Increase in root dry weight due to infection can be attributed to production of galls on the roots. It appears that galls produced on roots of RAC311 are smaller than those of Condor. Gall production is presumably a plant response to disturbed hormonal balance, secondary to syncytium production, and not required by the nematode. RAC311 may have properties which minimize disturbance of the plant's physiology by the nematode and, therefore, reduce gall production. This would reduce competition with other plant parts for assimilates, i.e. the 'metabolic sink' effect would be reduced.

These differences between RAC311 and Condor, a clear expression of tolerance, were obtained with inoculation at a time which was separated from a relationship with germination and the use of the endosperm. Thus tolerance probably operates over a considerable part of the growth of the

plant is not dependent on the reduced initial root growth rate.

6.3 Differences in galls produced on tolerant and intolerant plants

In Chapter 6.2, after inoculation with 100 larvae 17 days after sowing the root dry weight of Condor plants increased, whereas that of RAC311 plants was not increased with fewer than 700 larvae. This suggests that RAC311 did not produce as much gall tissue per nematode as did Condor. This section examines gall production directly. As initial root growth rate was not involved in the tolerance mechanism (Chapter 6.2.3), plants were inoculated at sowing to make the assay quicker.

6.3.1 Materials and Methods

Condor and RAC311 seeds were pregerminated and ten plants of each cultivar were inoculated with 100 larvae at sowing and grown at 10°C with a 10 hour daylength (Chapter 4.3.1). Plants were harvested 38 days after sowing. Galls on primary roots which had associated lateral root proliferation ('primary' galls) were cut free from roots and their dry weight determined. Younger ('secondary') galls, on primary or lateral roots, without associated lateral root proliferation, were counted. Total root length and root dry weight were recorded. Cultivars were compared using a *t*-test.

6.3.2 Results

Both cultivars had the same number of 'primary' galls but those of Condor were heavier (Table 6.3.1). Condor plants had more 'secondary' galls than did RAC311. Roots of Condor were longer and heavier than roots of RAC311 (Table 6.3.1).

6.3.3 Discussion

The results of this test support the hypothesis that Condor

TABLE 6.3.1: Mean values of ~~parameters~~ VARIABLES of Condor and RAC311 plants 38 days after inoculation at sowing with 100 *H. avenae* larvae.

~~Parameter~~ VARIABLE	RAC311	Condor
Number of 'primary' galls	2.8	3.4
Dry weight of 'primary' galls (mg)	1.0	2.1*
Number of 'secondary' galls	6.2	12.6*
Root length (cm)	46.0	90.7**
Root dry weight (mg)	6.7	9.9***

*, **, *** Cultivars significantly different ($P<0.05$, $P<0.01$, $P<0.001$ respectively).

produces more gall tissue per nematode than does RAC311. The fact that approximately three 'primary' galls had developed on each plants by 38 days after sowing indicates that these galls were probably produced by the initial invasion of the three primary seminal roots present at sowing. The reduced weight of 'primary' galls on RAC311 plants may be partially due to the slower initial growth of roots of RAC311 compared to Condor. Previously, a difference between cultivars in increase of root weight was found following inoculation at a time when roots of both cultivars were growing at the same rate (Table 6.2.1; 6.2.2). Therefore, at least for galls on primary roots, a mechanism other than rate of root growth might determine the amount of gall tissue produced Little is known of increase in size of galls with time. Presumably galls reach a maximum size. Rate of root growth may influence the time required for galls to achieve maximum size. To eliminate the effects of rate of root growth, gall weights should be compared when at maximum size. Time did not permit further examination of this aspect but at 38 days after inoculation galls might be at their maximum size. Further measurements of older galls would confirm this.

The difference between cultivars in the number of 'secondary' galls may be the result of growth rate of roots. Although the roots of Condor were twice as long as those of RAC311 they were not twice as heavy. This was probably due to more lateral roots, which were thinner than primary roots, on Condor than on RAC311 plants. If production of seminal lateral roots of RAC311 were slower than that of Condor then it would have fewer root tips for invasion by larvae and, therefore, would produce fewer galls. Later, when roots of both cultivars are the same size and growing at the same rate, the ultimate number of galls produced by each cultivar may be the same but the size attained by these galls might be determined by a mechanism other than root growth rate.

This mechanism resulted in reduced response of RAC311 plants to nematodes and might be controlled hormonally. If so, further examination of the mechanism of tolerance will involve a study of the physiological control of gall formation as well as that of the movement of substances between the endosperm, shoot and roots. The tolerance mechanism might also control the proliferation of lateral roots from galls. If so, a count of lateral roots growing from galls would provide a simple tolerance assay.

CHAPTER VII

GENERAL DISCUSSION AND FUTURE WORK

7.1 Mechanism of tolerance in wheat to *H. avenae*

Suggestions which have been proposed for the mechanism of tolerance to plant nematodes are: (i) a capacity for compensatory growth for damaged roots (Trudgill & Cotes, 1980); (ii) an excess of roots to enable the plant to tolerate a certain amount of loss without affecting top growth (Seinhorst, 1961), and (iii) reduced sensitivity of roots to nematode attack (Trudgill & Cotes, 1980). The first of these possibilities for cereals is inconsistent with the findings in this thesis. Tests have shown that compensatory root growth did not occur following root damage. Although tolerant cultivars suffered significant loss in primary seminal root length (Table 5.1), this did not induce compensatory seminal lateral root growth and these two characters were not correlated significantly (Table 5.3). The difference between my results and those of Trudgill and Cotes (1980, 1981, 1982, 1983b) and Evans (1982b) (who found that more tolerant potato cultivars had larger root systems when grown in soil heavily infested with *G. rostochiensis* than when lightly infested) may lie in the nematode density or time of sampling. Trudgill and Cotes (1983b) used 10,000 larvae per pot and Evans (1982b) used up to 105 eggs/g. If in Chapter 6.2, only the nematode densities 0 and 700 larvae per tube had been used, the same observations might have been made. By using small nematode densities, my tests showed that the intolerant cultivar Condor behaved in the same way as RAC311 but at a much lower density. Time of sampling was another major factor. Other workers measured plants at $6\frac{1}{2}$-9 weeks after sowing while laboratory trials at 10°C in this thesis ceased at about 7 weeks after sowing with most measurements taken before that time. It is quite possible that the effects of nematode attack could be expressed

in different ways throughout growth.

The second mechanism seems feasible and is supported by the 4 weeks reserve found in plants in Chapter 4.1.3. However, this does not explain why the tolerant cultivar, RAC311, did not suffer significant root loss even at 700 nematodes per plant (Chapter 6.2.2).

The third proposition has more support than the other two. For example, Chew (1979 in Cook & York, 1982) found an association between the tolerance of partially resistant oats to *H. avenae* and reduced necrotic response to nematode feeding. Evans (1982a) found that disturbance to potato plant physiology by *G. rostochiensis*, measured by nematode-induced abscisic acid production, was reduced in more tolerant cultivars. This may be the case for other growth substances such as auxins (Viglierchio & Yu, 1968), ethylene (Orion & Minz, 1969), cytokinins (Brueske & Bergeson, 1972), proline (Meon *et al.*, 1978), proteins (Melakeberhan *et al.*, 1982) and other amino acids (Bleve-Zacheo & Melillo, 1982; Krauthausen & Wyss, 1982), nucleic acids, enzymes and growth regulators activity. More specifically, cytokinins (Kochba & Samish, 1972) and auxins (Kochba & Samish, 1971) have been associated with the production of giant cells and galls of *Meloidogyne javanica* and may also be associated with that of *H. avenae*. Unfortunately, explanation of tolerance using the hormone theory would be complicated by the interaction of the growth substance with its receptor site which itself may be a limiting factor (Trewevas, 1982). However, my work in this thesis has shown that the tolerant RAC311 was much less sensitive than the intolerant Condor. A much higher nematode density was required to alter the physiology of RAC311 plants even to produce a significant quantity of gall tissue (Table 6.2.2) and smaller galls were produced on RAC311 than on Condor with the same inoculum density (Table 6.3.1). Production of smaller galls at a given nematode density may reduce the 'metabolic sink' effect thereby maintaining supply to the plant of many growth substances such as glucose (Betka & Wyss, 1982) and nutrient ions (Barth *et al.*, 1982). The

production of a smaller amount of gall tissue may be the reason why resistant potato cultivars tended to appear more tolerant to *G. rostochiensis* than susceptible cultivars (Evans, 1982c; Trudgill & Cotes, 1983a). Although most damage is caused by invading juveniles (and this is not affected by resistance) some damage is also associated with the development of females and their syncytia (Seinhorst & Den Ouden, 1971). If tolerance of cultivars, which are not equally resistant or susceptible, is assessed on the basis of the effect of initial nematode density only, then damage caused by development of galls and larvae is ignored so that resistant cultivars will appear tolerant.

Although this thesis contains only preliminary work and an explanation of a tolerance mechanism can only be speculative at this stage, that of host sensitivity to nematode invasion appears to be the most useful hypothesis at this time. However, some cultivars may possess different tolerance mechanisms. For example, Cook may be intolerant but still yield well in the presence of *H. avenae* (Fisher *et al.*, 1981) because it recovers or escapes from damage (Chapter 2.3.3).

7.2 Tolerance assay

One of the aims of this thesis was to provide plant breeders with a simple, non-destructive method of assessing tolerance. Unfortunately, the only parameter of top growth which was found to be correlated with tolerance was the increase in shoot dry weight 52 days after inoculation at sowing and, to measure this, one must destroy the plant. However, it is probably consistent with the early growth rating of plants grown in infested soil (Fisher *et al.*, 1981) which may be as useful as shoot dry weight increase in assessing tolerance. Other parameters which were significantly correlated with tolerance (root dry weight of control plants and primary

seminal root length change at 29 days after sowing) involved measurement of roots would only be useful for assessment of genetically homogenous lines.

It is possible that the effect of inoculation on length of leaf 1 or on height would provide a suitable assay for tolerance. The inoculum density in my tests was probably not high enough to allow consistent expression of these, and perhaps, other characters but there is some indication that they may be useful (Table 4.3.3; 4.3.4). Refinement of the method, by altering inoculum density, may reveal other characters for assessment of tolerance.

Another possibility, if a correlation with tolerance is found, is to measure hormone levels in control plants or the changes in these levels with inoculation. Gall weight and lateral root proliferation from galls might also be correlated with tolerance and provide suitable assays.

Until another method is found, the early growth rating of inoculated plants (Fisher *et al.*, 1981) remains the most suitable technique available for assessing tolerance in heterogeneous plant populations.

7.3 Relationship between tolerance and nematode populations

Other aspects of tolerance which deserve consideration, especially if tolerance is to be used as a control method, are the effects of tolerance on multiplication rate of the nematode and of resistance on tolerance.

This thesis has shown that tolerance decreased damage to roots so that roots of tolerant plants were not shortened as much by inoculation as were intolerant plants (Chapter 4.3; Chapter 5). The amount of damage to the plant, i.e. reduction of root growth, is a determining factor of the number of nematodes developing on the plant (Jones & Perry, 1978). Thus, a tolerant plant will effectively support a larger nematode population than an intolerant plant (Andersen, 1961; Andersson, 1982; Gair, 1965; Cook & York, 1982; Seinhorst, 1961). Therefore, by definition, tolerance decreases

the resistance of a plant (Andersson, 1982), resulting in a greater final population density which may well overcome tolerance in the following year and devastate an intolerant crop. In fact, it may be possible to assess tolerance on the basis of the ceiling level, i.e. the maximum population which the plant will support.

Fisher (1982) has suggested that the suitable level of resistance in a highly tolerant plant is less than 12 females per plant and in a highly intolerant cultivar is less than 3 females per plant. Just as it is important to use tolerance in a resistant cultivar to reduce damage by the nematode, it is also important to incorporate resistance in tolerant cultivars, at least to the levels suggested above, to maintain reduced populations so that tolerance will not be overcome.

7.4 Comparison of tolerance to nematodes with tolerance to drought and other stresses

It has been suggested that damage caused to plant growth by nematodes is the result of water stress (O'Brien & Fisher, 1981) and nutrient deficiency (Gair, 1965) due to root damage. The implication of this is that drought tolerance is the mechanism of tolerance to nematodes and that by selecting for drought tolerance cultivars will be more nematode tolerant.

Much work has been done on changes in hormone and amino acid levels of plants subjected to environmental stresses but little of it is understood. It appears, though, that drought tolerance is reliant on large changes in levels of growth substances, such as proline (Aspinall, 1980; Singh *et al.*, 1973) and abscisic acid (Larqué-Saavedra & Wain, 1976), in plants suffering water stress. Tolerance to nematodes, however, is associated with reduced disruption to normal plant physiology. For example, *Globodera rostochiensis* induces a smaller increase in abscisic acid levels in more tolerant than in

intolerant potato cultivars (Evans, 1982a). Furthermore, more tolerant potato cultivars have higher concentrations of abscisic acid when uninfested. These observations are consistent with those in this thesis. Roots of more tolerant wheat cultivars grew more slowly in early growth (germination) when uninoculated (Tables 4.3.1; 5.3) and this could have been due to inhibition by high abscisic acid levels (Russell, 1977). Although tolerant and intolerant cultivars reacted similarly to *H. avenae* the difference between them lies in the extent of the reaction. More tolerant cultivars were altered less by inoculation with *H. avenae* and this is consistent with smaller nematode-induced increases in ABA levels in tolerant potato cultivars as observed by Evans.

Work in this thesis has found that shoot apex development was unaffected by nematodes (Table 3.1) but water stress retards development of the apex (Angus & Moncur, 1977). Nitrogen deficiency (Brouwer, 1966; Drew *et al.*, 1973) and water stress (Brouwer, 1966) reduce the shoot : root ratio by reducing shoot growth. In the former case root extension was increased (Brouwer, 1966; Tennant, 1976) but root growth was relatively insensitive to water stress (Brouwer, 1966). In my work, roots were more severely affected by *H. avenae* than were shoots and this took the form of reducing root extension as shown by primary seminal root lengths.

Another important consideration in comparing nematode and drought tolerance is that of time of response. Plants responded to nematode attack within 17 days of inoculation (Chapter 6.2.2). Most of this time would have been used for penetration, establishment and initiation of galls. It is unlikely that this initial reaction could be related to drought or nutrient deficiency after such a short time. Furthermore, difference in initial root growth rate (which is related to tolerance) was detected very early, i.e. about 3-5 days after germination (Fig. 6.1.1).

It seems, therefore, that drought tolerance and nematode tolerance, and possibly tolerance to other forms of stress, are not equivalent. In order to evaluate the situation clearly, the relationship between hormonal and morphological changes in plants following water stress and nematode attack, on one hand, and drought and nematode tolerance, on the other, should be determined.

7.5 Future work

Very little work has been done on tolerance so there are many avenues of investigation yet to be covered. This thesis establishes the existence of tolerance under field conditions but this should also be demonstrated in a controlled environment. This would involve comparison of yield of plants of two or more cultivars inoculated with a range of initial nematode densities as in Chapter 3. Tolerance in oats to *H. avenae* has recently been established in pots (Cook & Chew, 1982) by comparing yields over a range of initial nematode densities.

There is sufficient evidence in this thesis to suggest that more tolerant cultivars respond less to the nematode although this should be confirmed (Chapter 6.3.3). At present the most profitable line of investigation of this mechanism seems to be an examination of the physiology of tolerance. The first step requires the determination of the source of difference between galls of RAC311 and Condor, i.e. syncytia or surrounding tissue. Growth substances involved in initial root growth rate, gall formation, etc. and their relationship with tolerance and changes following inoculation should be determined. This, however, will be complicated by the involvement of the physiological control over movement of assimilates from the endosperm and shoot to the roots.

Other important areas for future work are the development of a more suitable tolerance assay for use by plant breeders and also the establishment of the relationship between resistance and tolerance, especially if tolerance is to be used as a method of control.

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