RESISTANCE TO CEREAL CYST NEMATODE (*HETERODERA AVENAE* WOLL.) IN BARLEY

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ABSTRACT

Resistance in barley to the Australian pathotype of *Heterodera avenae* Woll. was studied under growth room conditions.

If a sufficient number of barley cultivars were tested to infection with *H. avenae* the reaction showed a continuous range from highly susceptible to highly resistant. At the level of ten females per plant, the cultivars could be separated into susceptible and resistant groups. The group of cultivars with less than ten females per plant included Athenais, CI 8147, Marocaine, Nile, Morocco and Orge Martin. In the first four cultivars a single major gene is known to control the development of the females and this has been confirmed. In Morocco and Orge Martin there were two major genes conditioning the resistance.

Genetic analysis showed that the resistance in Athenais, Nile and Morocco is controlled by different genes. The resistance in CI 8147 and Morocco was also controlled by different genes. In Athenais and Marocaine the gene conditioning the resistance is not the same but it is probably closely linked.

There was an association between head type and resistance to *H. avenae* in Athenais, Marocaine and Nile but not in Morocco and Orge Martin.

Results on the response of host to infection with *H. avenae* showed that the resistance in Morocco reduced both the numbers of larvae established and females developed in the roots, while in Galleon it reduced the number of females but not established larvae. The type of roots available for nematodes infection also had a significant effect on the number of females produced on a host.

Populations of *H. avenae* were cultured repeatedly on barley cultivars with and without resistance genes over three generations in pots under glasshouse

environment. On the resistant cultivar Galleon there was no change in virulence in the *H. avenae* populations.

The growth and yield of barley cultivars at different initial population densities of *H. avenae* under a glasshouse environment indicated that at high initial densities the cultivars differed in response to initial density and this may be due to tolerance.

STATEMENT

This thesis contains no material which has been accepted for the award of any other 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.

LITA SOETOPO

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CHAPTER 1

INTRODUCTION

The cereal cyst nematode (*Heterodera avenae* Woll.) is an important pathogen of wheat, barley and oats in most temperate areas of the world. A native of the Middle-East, it was probably carried to Europe with the early movement of cereals and was first recognized as a pathogen of cereals in 1874 in East-Germany (Kuhn, 1874), but is now recorded in continental Europe, the British Isles, USSR, U.S.A, Canada, North Africa, Israel, Peru, India, Japan and New Zealand (Ritzema Bos, 1891; Hansen, 1897; Theobald, 1908; Filipjev, 1925; Putnam & Chapman, 1935; Cameron, 1946; Delanoe, 1953; Mezetti, 1953; ıchinohe, 1954; Minz, 1956; Krusberg & Hirschmann, 1958; Prasad *et al.*, 1959; Hirschmann *et al.*, 1966; Jensen *et al.*, 1975; Grandison & Halliwell, 1975). In Australia, it has been present since the early nineteen hundreds (Davidson, 1930) and may have been transported from Europe or Britain with the introduction of cereals. It is largely confined to South Australia and Victoria (Meagher, 1972), although it has been recorded in Western Australia (Parkin & Goss, 1968) and New South Wales (McLeod, 1969).

H. avenae has been known for many years as the cause of yield loss in cereal crops (Gair, 1965; Kort, 1972); but it is only in the last 15 years that serious economic losses due to this pathogen have been recognized (Ritter, 1982). In the United Kingdom, the average annual loss caused by *H. avenae,* is estimated to exceed £ 2 million (Cotten, 1970a). In Australia, with more extensive areas infected, losses in yield of up to 30% have been recorded in barley (Sparrow & Dube, 1981) and in wheat from 0.39 to one t/ha (Rovira & Simon, 1982). With more than two million hectares in Victoria (Meagher, 1968) and South Australia (Rovira, ~~1978; Rovira *et al.*,~~ 1981) infested by *H. avenae,* the estimated annual loss in wheat production is 479,000 tonnes (Brown, 1981a) or a value of U.S. $ 70 million (Brown, 1984).

Control of this pathogen is difficult because it occurs across such large areas and it has, as host, the three important cereals - wheat, barley and oats - in the South Australian agricultural system. The use of non-host crops, such as natural pasture or legumes in crop rotation markedly reduced nematode populations (Millikan, 1938). Most farmers in the southern wheat belt derive their income from the production of both sheep and cereal grains, but at the present time rotations that include a long pasture phase are unpopular with growers who want to intensify their cereal cropping (Brown, 1982; 1984). The economic pressures of modern agriculture, which require three cereal crops in four years, have meant that rotational crops no longer give adequate control.

The introduction of nematicides (Gurner *et al.*, 1980) while currently satisfactory and economic, can only be regarded as an interim measure. Increasing costs of the chemicals and their application and decreasing prices for cereal grains in the world market probably means that there is a limited life to the use of chemicals in the agricultural system. The use of these toxic chemicals over an extended period even at low concentration, must be viewed with apprehension.

The use of resistance to control the nematode is a much more desirable approach particularly in the long term in Australia where only one pathotype has been recorded (Brown, 1969; 1974; 1982; O'Brien & Fisher, 1979). Two resistant cultivars have been released recently, one - the barley Galleon in South Australia, has become a popular and successful cultivar (Sparrow & Dube, 1981); the other - the wheat Katyil, has been unsuccessful in Victoria (Brown, 1984). Galleon was released in 1981; although it is not suitable for malting its yield potential and disease resistance made its release as a feed cultivar imperative, and it was expected to become an important component in the integrated control of *H. avenae* (Sparrow & Dube, 1981). By 1985, it rose to occupy about 40% of the South Australian barley area or 0.47 million hectares (Sparrow pers. comm.). Katyil bred specifically for resistance to *H. avenae* (Brown, 1984) has been unpopular because of its low yield potential.

Apart from their ability to control populations of *H. avenae* , resistant cultivars must also have the desired agronomic qualities and the potential to outyield current cultivars, otherwise growers will have little incentive to grow them. To avoid such failure, it is necessary to know and understand as much as possible about all aspects of the resistance to *H. avenae*. As resistant cultivars become more important in the integrated control programs of *H. avenae,* it is essential to understand the genetic basis of resistance because for an efficient breeding program, a knowledge of the inheritance of resistance is necessary.

CHAPTER 2

LITERATURE REVIEW

2.1 The host-parasite relationships of plant-parasitic nematodes

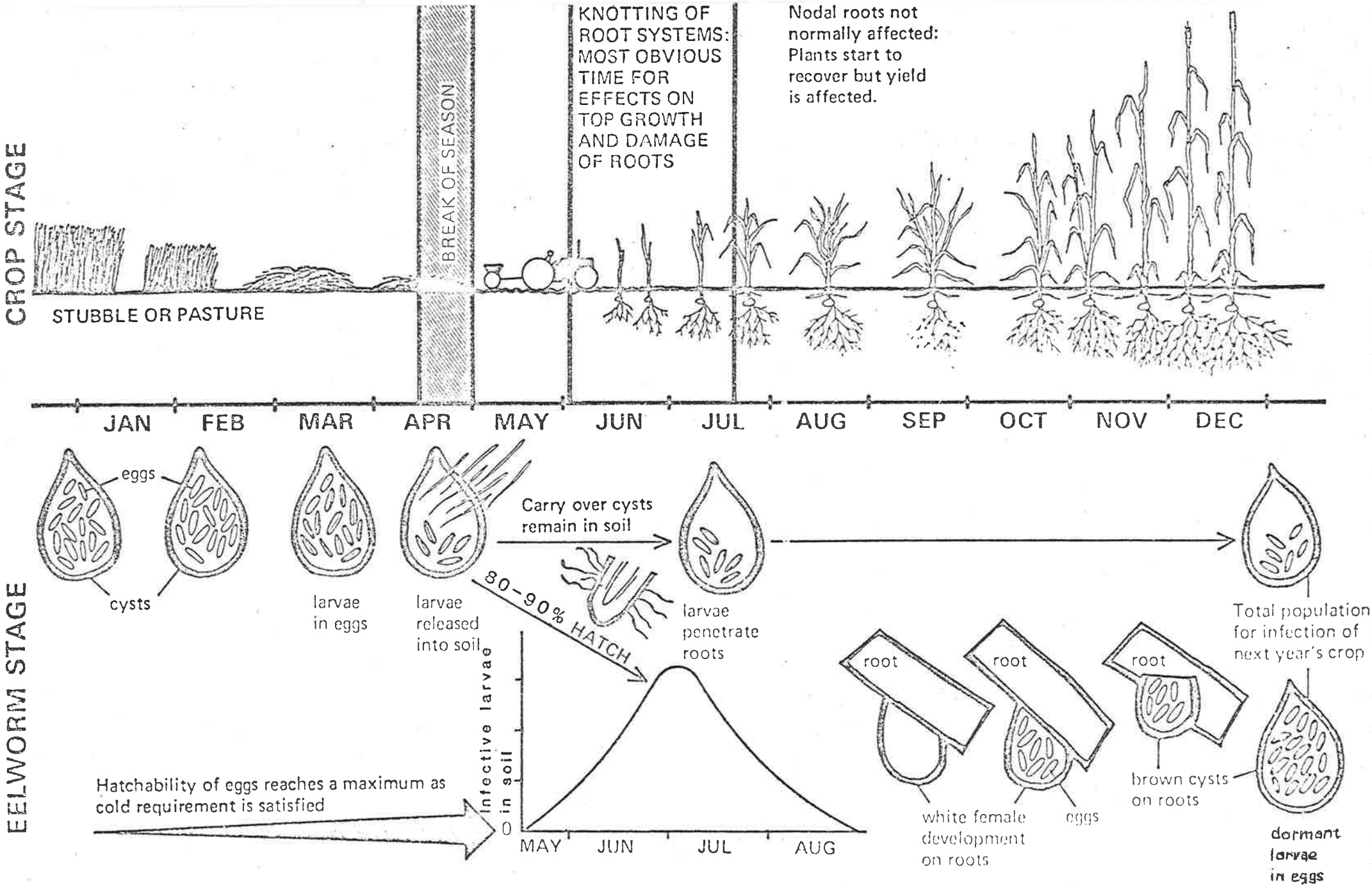
The invasion of host tissue by parasites initiates a complex and variable developmental pattern between the host and the parasite. The interaction between host and parasite is determined by several factors. Differences in the physiological responses, morphological structures and nutritional status of host genotypes could affect their susceptibility or resistance to invasion; similar variation in parasites could influence their growth rates and virulence. Physical environment, timing and level of infestation could also affect the interaction between host and parasite (Webster, 1969; Loomis & Adams, 1983).

A pre-parasitic or pre-infectional relationship with the host occurs in some plant parasitic nematodes. The eggs of some species of *Heterodera* and *Globodera* are stimulated to hatch by exudates from the roots of host plants (Wallace, 1965), e.g. hatching of *H. goettingiana* is stimulated in soil by the presence of host roots (Jenkins & Taylor, 1967), and potato root exudates stimulate a rapid hatching in *G. rostochiensis* (Webster, 1969). While in the main, hatching is stimulated by exudates from host plants there are exceptions. Pea root exudate stimulates the larval emergence of *H. schactii*, but these nematodes do not feed or develop on pea roots (Webster, 1969). *G. rostochiensis* is also stimulated to hatch by some non-host clones of *Solanum tuberosum* ssp. *andigena* (Williams, 1978). Some populations of *H. avenae* and *H. glycines* are not known to respond to any root diffusates (Jenkins & Taylor, 1967) but Williams & Beane (1979) showed that some populations of these nematodes do respond to root diffusate. Some other root exudates have a nematicide action, i.e. exudates from raspberry cane are toxic to *Longidorus elongatus* and decrease the soil population of

this nematode (Taylor & Murrant, 1967). *Tagetes* sp. (Oostenbrink *et al.*, 1957) and *Asparagus officinalis* (Rohde & Jenkins, 1958) also contain compounds that are toxic to nematodes.

The simplest relationship between nematodes and their hosts probably is that of the migratory ectoparasites e.g. *Paratylenchus* spp. and *Tylenchorhynchus* spp. (Jenkins & Taylor, 1967). These nematodes move freely about the root surface, feed on root hairs and epidermal cells near the root tip; except for overall stunting of the root system, they cause little effect on host-plant growth through nutritional competition, although they may decrease water absorption in young seedlings (Kirkpatrick *et al.*, 1964). Much more complex are the host-parasite relationships of the sedentary endoparasites such as *Heterodera* spp. and *Meloidogyne* spp. The hatched infective second stage larvae of these genera enter the host root at the root tip (Johnson & Fushtey, 1966), assume a feeding site and become sedentary; all the subsequent development takes place within the roots of the host plant (Jenkins & Taylor, 1967). *H. avenae* spends the greater part of its life cycle within the root tissue of the host plant and completes one generation a year. It carries over from one season to another as a brown, egg-containing cyst. The cyst is tough and resistant to desiccation and high temperature (Dube *et al.*, 1979). Each cyst usually contains 200-250 eggs, although a very full cyst contains over 600 eggs (Andersen, 1961; Banyer & Fisher, 1976). The adult male of *H. avenae* is vermiform and free living, whereas the adult female is sub-spherical and immobile. Following fertilization, the female lays embryoned eggs; when the female dies, its body wall hardens to form a lemon-shaped, brown cyst (Shepherd, 1965). Its life cycle in Australia is adapted to the cereal growing season (Fig. 1), with hatching coinciding with the sowing of the cereal crop (Banyer & Fisher, 1971a; 1972). Eggs of *H. avenae* start to hatch after the break of season in April-May, maximum rate of hatching is reached at the end of May and hatching is complete by early July. Approximately 85% of eggs hatch each year (Fisher, pers. comm.), while the remainder stay dormant in the cyst and carry over into the following year. Heavily infested root

Figure 1. Life cycle of cereal cyst nematode (*Heterodera avenae*) in relation to time of year and current farming practices in Australia (Dube *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
80-90% HATCH
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
Infective larvae in soil
0
MAY
JUN
JUL
AUG

systems are deformed, short, abnormally branched and shallow (Andersen, 1961; Goss, 1967). These malfunctioning root systems cause typical above ground symptoms of stunting, chlorosis, reduced tillering and reduction in yield (Jenkins & Taylor, 1967).

When the nematode larvae reach the host they usually penetrate near the growing point of the root (Johnson & Fushtey, 1966). They penetrate the superficial root cells by means of the mechanical thrusting action of the stylet (Jenkins & Taylor, 1967). The presence of the parasite may induce the host to produce compounds which activate enzymes of the parasite which in turn break down host compounds leading to the production of an environment suitable for parasite growth (Rohde, 1965). When cyst (*Heterodera* spp) and root-knot (*Meloidogyne* spp) nematodes establish permanent feeding sites in host roots they induce the formation of enlarged symplastic structures called syncytia and giant cells, respectively; these structures are essential for a successful host-parasite relationship of these species (Barron, 1940; Christie, 1949; Peacock, 1959; Dropkin & Nelson, 1960; Webster, 1969). The giant cells and syncytia are produced by the break down of cell walls (Endo, 1962), incorporation of cytoplasm and enlargement of nuclei (Krusberg, 1963) to form a large, multinucleate transfer cell that supplies nutrients or metabolites essential for the normal development of the nematode (Giebel, 1982). Giant cells are initiated by *M. incognita* larvae within a few days in tomato roots (Bird, 1961). Giant cells induced by the root-knot nematodes, *Meloidogyne* spp, usually occur in the form of a cluster of multinucleate cells near the lip region of the nematode. Hyperplasia and hypertrophy often surround the region of infection and usually cause galls to be formed terminally or subterminally on the infected root (Endo, 1971). Syncytia induced by the cyst nematodes, *Heterodera* spp, usually are elongate with the ends merging with normal tissue and each syncytium is generally associated with only one larva (Endo, 1971). Host reaction is necessary for continued development of the parasite. Failure of the transfer cell (syncytium) to develop or function as a source of food for the nematode will result in its death (Seinhorst, 1961).

Common antigenic properties appear to be a feature of compatible host-pathogen interaction between *M. hapla* and cotton and soybean (McClure; Misaghi & Nigh, 1973). Jones *et al.*, (1981) suggested that interaction between *Globodera* spp and potato depends on the compatibility of the nematode saliva and host-cell cytoplasm. Both host and parasite have genetic mechanisms for recognition and it is this mechanism that determines the interaction (Nelson, 1977). Most published work on the genetics of host-parasite interaction is concerned with fungal pathogens. Relatively little work has been done on the genetics of other plant parasites, such as nematodes, but what is known about them suggests that in their interaction with their hosts, they are remarkably like fungi (Day, 1974). According to Ellingboe (1976) genetic interaction between hosts and parasites follows a quadratic check pattern. When a host and a parasite interact, the presence or absence of the host gene combined with the presence or absence of the parasite gene gives four possible interactions, only one of which leads to a unique susceptible phenotype. If more than one pair of host-parasite genes are involved, the interactions follow the pattern described by Flor (1942,1955,1971; Schwarzbach, 1981) that for each gene that conditions resistance or susceptibility in the host there is a corresponding gene conditioning virulence or avirulence in the pathogen. Thus a host with a particular resistance gene is susceptible only to a parasite that has the complementary gene for virulence, regardless of other virulence genes present (Schwarzbach, 1981).

Jones (1974) suggested that a gene-for-gene relationship might exist between potato cyst nematodes and resistant potatoes, i.e. between *Globodera rostochiensis* (Rol) and *Solanum tuberosum* ssp. *andigena* with resistance gene H1, and between *G. pallida* (Pa1) and *S. tuberosum* ssp. *multidissectum* with the resistance gene H2. Males of all genetic constitutions (NN, Nn, nn) can mature in the roots of resistant plants, but only recessive females (nn) can mature (Jones *et al.*, 1981). There is no information available on the barley - *H. avenae* systems. Cook *et al.*, (1978) observed that a mixed population of British pathotypes 3 and Ha12 was able to produce cysts

Sabarlis (Ha2), but their progenies failed to develop on Sabarlis the following year. It has been suggested that the ability to overcome resistant gene Ha2 ~~are~~ is not stable in mixed populations and this may be due to hybridization, with British pathotype 3 characters recessive or F1 progenies not viable. British pathotype 3 was originally recognised by Cotten (1967) as an *H. avenae* pathotype, but Cook (1975) has found it to be morphologically distinct.

2.2 Breeding and resistance to cereal cyst nematode (*Heterodera avenae*) in barley

The CCN (cereal cyst nematode) problem in temperate cereal areas is sometimes not obvious to the casual observer and may tend to be overlooked or be given a different explanation (Cotten, 1970a). As with other nematodes (Mai, 1977), it may be present in a particular area for sometime before it becomes a serious problem. From the first introduction of the nematode to an area, it may take twenty years to develop to a level causing economic damage (Mai, 1977); dispersal to neighboring areas may also be slow unless aided by wind (Meagher, 1968; 1972; 1977; 1982). In Australia, CCN has become widely distributed throughout the southern cereal belt, largely as the result of movement of cysts by wind during the turbulent dust storms that occur periodically in this region (Meagher, 1968; 1972; 1977; 1982). Other important factors that regulate the rate of increase of the nematodes include, host, crop-rotation, seasonal condition, soil type and fertility (Jones & Kempton, 1978). The effect of CCN on crop yield will depend on the host, population in the soil, type of soil, weediness and rainfall (Dube *et al.*, 1979). If susceptible cultivars are to be grown, the control measures must include resistant rotation crops, resistant cereals and fallow (Sparrow & Dube, 1981). The more resistant cereals that are available, the less dependence there is on alternative means of control.

Rohde (1960) noted that resistance is any characteristic of a plant or any interaction between host and parasite which retards or prevents the occurrence of a parasitic relationship between the plant parasitic nematode and its host. Resistance to nematodes may be due to the production of toxic root exudates, lack of nematode larval attractant or egg hatching stimulation in the exudates, a barrier to penetration or a failure of nematode to develop within plant tissue (Jenkins & Taylor, 1967; Webster, 1969). The roots of cereal plants do not seem to have characteristic anatomical or chemical barriers to entry by larvae of *H. avenae*. Similar numbers of *H. avenae* larvae can invade roots of resistant plants as readily as those of susceptible plants, and development continues normally at least until the fourth moult, when in resistant plants female development breaks down (Cotten & Hayes, 1969; Williams, 1970; O'Brien & Fisher, 1978; Empson & Gair, 1982). In the resistant barley cultivar 'Sabarlis' development of larvae stops at the third stage, while a few individuals, mostly males, continue to reach maturity (Rivoal, 1976). Histological study of the cultivar 'Sabarlis' showed that invading larvae of *H. avenae* stimulated the initiation of giant cell feeding sites, but subsequently the cytoplasm of these cells became sparse and vacuolated (Cook, 1974), and failed as a source of food for the developing nematode. The lack of suitable food could disturb nematode ontogeny and increase the ratio of males to females (Christie, 1959; Dropkin & Nelson, 1960). A high ratio of males to females seems also to be a characteristic of *H. avenae* in resistant wheat (Brown, 1974). Since there is no published data on sex reversal in *H. avenae* (O'Brien, 1972), it can only be assumed that at times of nematode competition for space and food, female larval development is restricted and the larvae may die, resulting in a sex-dependent death rate during development which alters the sex ratio (Trudgill & Parrott, 1969; Ross & Trudgill, 1969).

Resistance to *H. avenae* does not prevent invasion of the root system, but few or no cysts are produced (Empson & Gair, 1982). The production of few cysts on resistant plants has been used by plant breeders to select resistant cultivars in which the

nematode has a reduced capacity to reproduce (Sparrow & Dube, 1981). The development of resistant cultivars may involve ten to twelve years of work and screening of several thousand breeding lines if resistance has to be transferred initially from an unadapted or wild source (Howard & Cotten, 1978). Genetic studies have indicated that resistance to *H. avenae* is usually simply inherited (Howard & Cotten, 1978); and in barley resistance appears to be controlled by single major genes (Cotten & Hayes, 1969). At present, there are at least three genes known for resistance in barley (Andersen & Andersen, 1970); Ha1 present in cultivars Fero and Drost (Cook & York, 1982), Ha2 in barley No.191 (Cotten & Hayes, 1969; Andersen & Andersen, 1973) and Ha3 in cultivar Morocco (Cook & York, 1982). Barley cultivars developed with resistance to CCN are now becoming available to growers. These include the European spring barleys Ansgar, Sabarlis and Tyra which have resistance to pathotypes 1 and 2 derived from No. 191 (Howard & Cotten, 1978) and Galleon resistant to the Australian pathotype which presumably inherited its resistance from the Egyptian cultivar CI 3576 (Sparrow, 1979).

Commenting on work with *Meloidogyne* species, Fassuliotis (1979) observed that there were probably as many techniques as workers, and this is also probably true for *Heterodera avenae* testing (Cook & York, 1982). There are several different assays for testing for resistance to *H. avenae* used in different laboratories. While the optimal conditions for selecting for resistance may not vary, there are other practical constraints and choices influencing selection techniques (Cook & York, 1982). Infested soil with the level of infestation between eight to ten eggs per gram of soil, in clay pipes, plastic bags or glass tubes (Andersen, 1961; 1963; Andersen & Andersen, 1982) or in pots, with initial nematode density around 50 eggs per gram of soil (Cotten, 1967; Cotten & Hayes, 1969; Hayes & Cotten, 1970; Cook & York, 1982) give reasonably good results; but the number of females counted varies considerably from experiment to experiment (Andersen, 1963). Other workers have adopted agar growing media to study the resistance to *H. avenae* (Brown, 1974; Rivoal *et al.*, 1978). Test tube method with mono-axenic culture and agar medium using a single cyst as inoculum offers a relatively

simple way of screening for resistance and studying of nematodes (Brown, 1974; 1977). But since density of nematodes in inocula has been a major source of variation (O'Brien & Fisher, 1974) and hatching from cysts can be unpredictable, it would seem preferable to use hatched larvae as inoculum (O'Brien, 1976). By using polyvinyl chloride (p.v.c) tubes to grow the plants in a controlled enviroment and a standard number of freshly hatched larvae as inoculum, O'Brien & Fisher (1977) were able to reduce the variation in reactions within the test cultivars and they obtained large differences in the number of females on the roots of susceptible and resistant cultivars.

Methods for counting the final number of females developed on the test plants also varies. Cotten (1967), Brown (1974) and O'Brien & Fisher (1977) counted the total number of females developed on a plant after washing the whole root system free from soil; the females were collected on a fine sieve and transfered to a counting dish. Andersen (1963) counted the number of females through the glass wall on plants grown in tubes and for plants grown in plastic bags the number of females was counted on the surface of the root ball. Different laboratories use different method to classify plant reaction into resistant or susceptible. Cotten & Hayes (1969) computed the 95% confidence limits of the square root transformation of reactions of the susceptible parent, and the resistant parent combined with that of the F1 population to objectively allocate the reactions of F2 plants to resistant and susceptible classes. Andersen & Andersen (1970) used Sun II oats and Herta barley as control cultivars. A plant with numbers of females less than 5% of the number of females on the control cultivars was considered as resistant. O'Brien & Fisher (1974) used a resistance rating modified from Brown & Meagher (1970) based on the number of females produced per plant as follows :

Females per root system :	Rating :	Reaction :
0	1	Resistant
1-10	2	Moderately resistant
11-50	3	Susceptible
> 50	4	Very susceptible

Backcrossing is the classic system of introducing a single gene from a donor cultivar to improve a locally adapted cultivar which is used as the recurrent parent in a series of backcrosses (Brown & Ellis, 1976; Brown, 1977; Sparrow, 1979). The system depends upon identification of F1's which are heterozygous for the desired gene, in this case of resistance to *H. avenae*, which is dominant over susceptibility (Sparrow, 1979).

With the method developed by O'Brien & Fisher (1977) it is possible to screen for resistance before anthesis (Sparrow, 1979). The plants are grown in a controlled enviroment and are assessed two months after the last inoculation (Fisher, 1982b), resistant F1 plants can be grown on and further backcrossed to the recurrent parent. Selection of homozygous resistant lines was made after the second and third backcross to reduce the chance of losing modifier and other useful genes from the donor parent (Sparrow & Dube, 1981). An arbitrary demarcation of resistance was set at five white females per plant (Sparrow, 1979).

There is evidence that resistance to *H. avenae* could be affected by some environmental factors. Temperature has been reported to affect the levels of resistance and susceptibility. Person (Dedryver & Doussinault (1979) suggested that the resistance of barley P. 3122 to the French pathotype 2 may be overcome at temperatures of 23°-25°C. In Australian tests, the wheat Aus 10894 had 0.7 cyst/plant in the field at mean soil temperature 14°C but 10.7 cysts/plant in glasshouse test at an air temperature of 17°-22°C (Cook & McLeod, 1980). However, Rovira (1982) found that *H. avenae* caused a severe root stunting on wheat grown at a soil temperature of 10°C but not at 15°C.

In Australia, the presence of only one pathotype of *H. avenae* (Brown, 1969; O'Brien & Fisher, 1979) has made breeding for resistance less complicated than in Europe, the United Kingdom or India, where several pathotypes are present, and often occur in mixed populations (Swarup *et al.*, 1979; Andersen & Andersen, 1982; Cook & York, 1982); in these countries it may be necessary to use the resistance from several sources to provide a more effective control of this pathogen. Incorporation of genes for resistance to more than one pathotype within a species, is possible. Resistance to two

pathotypes of *H. avenae* has been incorporated into barley cultivars at several European plant breeding centres (Cook, 1974). It may also be necessary to find and develop alternative sources of resistance to prevent the development of resistance-breaking pathotypes when growing resistant cultivars extensively. However, the spread of a soil-born pathogen like *H. avenae* is generally slow, and provided wind dispersal is not a serious way of dissemination, new pathotypes would not be disseminated rapidly compared with the spread of diseases involving air-borne fungi, and therefore, resistant cultivars should remain effective for relatively long periods (Cook, 1974; Russell, 1978; Lamberti & Taylor, 1983).

2.3 The inheritance of resistance to *Heterodera avenae* in barley

2.3.1 Heterogeneity in *H. avenae* populations

The existence of pathotypes in *H. avenae* was first demonstrated by Andersen (1959; 1961); further work in Britain (Cotten, 1963; 1967; Fiddian & Kimber, 1964; Saynor, 1975), Netherlands (Kort *et al.*, 1964), Germany (Lucke, 1976), Sweden (Walstedt, 1967), Norway (Stoen, 1971) and France (Rivoal, 1977) has confirmed the wide spread occurrence of pathotypes of *H. avenae* differing in pathogenicity. Andersen (1959) demonstrated that the Danish populations consisted of two pathotypes of *H. avenae*. In the Netherlands, Kort *et al.*, (1964) isolated four Dutch pathotypes. In Australia, only one pathotype is known and it differs from the European pathotypes (Brown, 1969; Brown, 1974; O'Brien & Fisher, 1979; Andersen & Andersen, 1982; Brown, 1982). In France, Rivoal (1977) reported the presence of four French pathotypes. In India, Mathur *et al.*, (1974) found five populations of *H. avenae* differing in their virulence, and a later investigations by Swarup *et al.*, (1979) indicated the presence of even more pathotypes.

Essentially, populations of *H. avenae* can be divided into three groups (Andersen & Andersen, 1982) :

1. Those to which a number of West European cultivars, including Fero, Drost, Ortolan and Alfa are resistant, have been designated pathotype 1.

2. Populations able to break the resistance of Fero, Drost, Ortolan and Alfa, but are unable to reproduce on No. 191 and Siri have been designated pathotype 2.

3. Populations able to break the resistance of No. 191 and Siri, but unable to reproduce on Morocco have been designated pathotype 3; these populations possibly consist of more than one type.

Andersen & Andersen (1982) suggested a two figure system to distinguish different pathotypes. The first figure identifies pathotypes within a group and the second figure the group as designated above. Thus Ha11 is the first pathotype in group one avirulent on the gene Ha1.

Pathotype study has made it possible to identify different sources of resistance in barley and their interrelationships. However, one of the problems has been to relate the different pathotypes in different countries i.e. the five in Netherlands, four in France, three in the United Kingdom, two in Denmark, two in Sweden, one in Australia and the possible five in India. In countries such as Norway and Germany, where the nematodes occur, the pathotype position is not known. Andersen & Andersen (1982) have attempted to relate the pathotypes in different countries on the basis of their relation to the barley genes Ha1 and Ha2 from Drost and No.191, respectively (Table 1).

Person-Dedryver & Doussinault (1984) reported that Fr4 is similar to Andersen's pathotype Ha12, but the Fr2 and Fr4 pathotypes differ from each other as do Fr3 and Ha11. A group of pathotypes, including the Australian pathotype are virulent on both the Ha1 and Ha2 genes and therefore differ from most European pathotypes. This group is avirulent against the gene Ha3 from Morocco. Within these groups, further separations are possible using genes from different sources. There are insufficient genes for resistance in wheat to attempt to separate pathotypes in relation to this host and in

Table 1. Reaction of barley cultivars to pathotypes of *H. avenae* (modified after Andersen & Andersen, 1982).

	Pathotypes									
Group of pathotypes				1			2		3	
Pathotype no.	Ha11	Ha21	Ha31	Ha41	Ha51	Ha61	Ha12	Ha13	Ha23	Ha33
Classification :										
Dutch	A	D			B	E	C			
French	Fr3			Fr1			Fr2-Fr4			
British	B1						B2		B3	
Danish	D1						D2			
Sweden	S1						S2	Aust		
Cultivars :										
Varde/Emir	S	S	-	S	S	S	S	S	S	S
Drost/Ortolan(Ha1)	R	R	R	R	R	R	S	S	S	S
KVL 191/Siri(Ha2)	R	R	R	S	S	S	R	S	S	S
Morocco (Ha3)	R	R	R	R	R	R	R	R	(R)	R
Marocaine 079	R	R	-	-	R	S	R	R	(R)	-
Bajo Aragon 1-1	R	-	-	R	R	R	R	S	S	R
Herta	S	S	R	-	R	S	S	S	-	-
Martin 403-2	R	-	-	R	-	R	R	R	S	S
Dalmatische	(R)	-	-	S	-	R	S	S	(R)	S
La Estanzuela	R	-	-	-	Rp	-	Rp	S	R	-
Harlan 43	R	R	-	-	-	-	R/Rp	-	(R)	S
Athenais	R	-	-	-	-	-	S	R	-	-
CI 3726	R	-	-	-	-	-	S	-	-	-
CI 3780	R	-	-	-	-	-	R	-	-	-
CI 4226	R	-	-	-	-	-	S	-	-	-
CI 8147	R	-	-	-	-	-	S	R	-	-
Nile	R	-	-	-	-	-	R	R	-	-
Orge Martin	R	-	-	-	-	-	R	-	-	-
Rika cb 545	-	-	-	-	-	-	-	-	S	-
Fero cb 917	R	R	-	-	R	-	S	-	-	-
Quinn CI 1024	R	S	-	-	S	-	R	-	-	-
CI 3515	S	S	-	-	R	-	S	-	-	-
IBDN 61 No. 14	R	S	-	-	R	-	S	-	-	-
L 62	S	-	-	-	S	S	S	S	-	-
Osiris	R	-	-	-	R	R	S	S	-	-
Ogalitsu	R	-	-	-	R	R	S	S	-	-
Rabat	R	-	-	-	R	S	R	-	-	-

Note : S = susceptible, R = resistant, (R) = moderate resistant, Rp = partial resistant, - = no observation, Aust = Australian pathotype.

oats the situation is still confused (Cook, 1975). More information is needed for the five or more pathotypes in India to compare them with those in the rest of the world. Much of the resistance to *H. avenae* has been found in barleys from North Africa (Morocco, Tunisia, Egypt), and so study of the variation in nematode populations in that area would be worthwhile.

In potato cyst nematode (*G. rostochiensis)* populations, Jones *et al.*, (1967), by using computer simulation techniques, predicted changes that could be expected under the growing of resistant potato genotypes. He suggested that the useful life of monogenic resistant varieties would be prolonged by alternating resistant with susceptible cultivars. *G. rostochiensis* in England exists in a mixed population. The resistant genotypes have selected the aggressive individuals from the population after a few generations (Fisher, pers. comm.).

The situation with *H. avenae* differs in some important aspects from *G. rostochiensis*. Virulence in *H. avenae* appears to be dominant (Andersen, 1963), the nematode does not require a hatching factor but it is carried on other hosts common in rotations. Consequently predictions and experience obtained regarding the long-term effect of growing nematode resistant potatoes will not necessarily apply to the effect of growing resistant barley on infested soil. However, in the short-term, results on cereals show that not only do the resistant genotypes outyield comparable susceptible genotypes in heavily infested soil (Cotten, 1970b), but that they also decrease nematode populations in the soil and consequently have a beneficial effect on the yield of susceptible crops grown in the following season.

2.3.2 Inheritance of resistance to *H. avenae*

Study of the inheritance of resistance of plants to nematodes could lead to a better understanding of the nematodes, their host plants, or the relationships between the

hosts and the nematodes. Resistance of plants to nematodes can be far better utilized if the nature of inheritance of this resistance is known. Unlike wheat, there are several sources of resistance in barley to *H. avenae*. The first evidence of resistance was recorded in 1920, when Nilsson-Ehle found that cysts failed to develop on the roots of a number of Swedish barley cultivars (*Hordeum vulgare* L. : Primus, Svanhals and Chevalier) (Andersen, 1959). Resistance in the cross Chevalier x Gull was dominant in the F1 generation, but due to technical difficulties, the nature of the genetic mechanism of the resistance in the F2 and F3 generations could not be determined (Nilsson-Ehle, 1920).

Before the occurrence of pathotypes in *H. avenae* was recognized, incomplete resistance or moderate susceptibility in some barley and oat cultivars had been reported. A barley cultivar can be resistant to one pathotype but susceptible to another. Some cultivars are completely resistant with no cysts on their roots, while other resistant cultivars allow the development of some cysts (Andersen & Andersen, 1970). Resistance to pathotype 1 was found in many barley cultivars, e.g. in Drost; resistance to both pathotypes 1 and 2 was found in cultivars Nos. 14 and 191. Drost and No.191 were susceptible to the four Dutch pathotypes but Morocco and Marocaine were resistant. Resistance to the Australian pathotype was found in the cultivars Athenais, CI 8147, Marocaine 079, Morocco, and Nile (O'Brien *et al.*, 1979), but Drost, Ortolan, No 191 and Siri were susceptible (Brown & Meagher, 1970).

The recommendations for nomenclature of genes for resistance in barley is Ha1, for the gene for reaction to *H. avenae*, locus 1 (Anon., 1981). Where alleles are identified, a capital initial letter represents a dominant, and a lower case a recessive allele (Cook & York, 1982).

Unlike in wheat (Cook& York, 1982) resistance to *H. avenae* has been found in several barley cultivars. Genes at a minimum of three loci (Ha1, Ha2 and Ha3 according to Andersen & Andersen, 1970) have been identified in barley (Cotten & Hayes, 1969); these genes are inherited as monogenic dominant.

The resistance genes Ha2 (Cotten & Hayes, 1969; Andersen & Andersen, 1973) and Ha3 are located on the long arm of barley chromosome 2, closely linked with each other, and may even be alleles at the same locus (Andersen, 1976), but both Ha2 and Ha3 are not linked with Ha1 (Cotten & Hayes, 1969; Andersen & Andersen, 1970). According to Andersen & Andersen (1982) the gene Ha2 is epistatic to Ha1. The location of Ha1 has not yet been determined, though there is an indication that it may be on the short arm of barley chromosome 2 (Cook & York, 1982).

The origin of the gene Ha1 is not clear, but it is found in a number of Danish and German barleys (Cook & York, 1982). This resistance was first recognized by Nilsson-Ehle (1920) in cultivars Chevalier, Hanchen and Primus. Pathotype Ha1-virulent populations are common throughout north-western Europe (Cook & York, 1982). This gene is useful in Scandinavia and Germany where pure pathotype Ha11-populations occurs, but of little use in Britain where pathotype Ha12, virulent on Ha1, predominates (Saynor, 1975). The resistance gene Ha2 introduced by S. Andersen from the barley cultivar No.191 of unknown origin has been widely used in resistance breeding in Europe (Cook & York, 1982). The resistance gene Ha3 is present in the cultivar Morocco of North African origin and is effective against pathotypes Ha11, Ha51, Ha61, Ha12 and Ha13 (O'Brien *et al.*, 1979; Cook & York, 1982).

There are other sources of resistance in barley which are yet to be confirmed, like the HaEmir type, the resistance in cultivar La Estanzuela (Cook & York, 1982) and Harlan 43 (Cook & York, 1982). La Estanzuela and Harlan 43 are resistant to British pathotype 3, but both are partially resistant to pathotypes Ha11 and Ha12 (Cook &York, 1982; Cook, 1975; 1982). It has been suggested that the partial resistance in Harlan 43 is controlled by a single recessive gene (Hayes & Cotten, 1971). The HaEmir type resistant to pathotype Ha51 occurs in a number of north European cultivars, but there is no information on the genetic control of this resistance gene. Pure HaEmir-avirulent *H. avenae* populations are found in Norway, Netherlands and India, but have not been found in Britain (Cook & York, 1982).

Complementary genes ~~has~~ have been reported responsible for the resistance in Siri , Ortolan, P 31-322-1 and Vogue to the four French pathotypes, and ~~that~~ these genes differed from the Ha1 and Ha2 genes described by Andersen & Andersen (1982) (Person-Dedryver & Doussinault, 1984).

2.4 The relation between nematode population density and plant growth and yield

According to Barker & Olthof (1976) the fundamental quantitative relationships between plant parasitic nematodes and growth and yield of annual crops are primarily a function of pre-plant densities. The initial density of nematodes required to cause significant plant damage and yield loss varies with nematode species. For those nematodes which complete several generations a year, have a large potential for increase and may multiply more than a thousand times within a growing season (Jones & Kempton, 1978) low initial numbers can often cause severe damage on the host plant e.g. *Meloidogyne* spp on tobacco (Ferris, 1972; Rickard, 1973) or tomato (Barker *et al.*, 1976). For those nematodes which multiply only once in a growing season e.g. *Heterodera avenae* , build-up of a population may occur following the growing of susceptible cultivars, and severe damage can be expected on the third successive crop (Fisher, pers. comm.). Andersson (1982) reported that the average multiplication rate of *H. avenae* in oats at initial densities of about one egg/g soil is generally well below ten times, and only rarely has about 20 times multiplication been found. In barley, under Danish conditions, the equilibrium densities vary between two to 40 eggs/g soil (Andersen, 1980).

The relation between CCN and the amount of damage to the three major cereals - oats, wheat and barley - has been established by a number of workers (Hesling, 1957; Duggan, 1961). Oats are generally regarded as being damaged more than wheat which is

damaged more than barley and these differences have been related to the different numbers of seminal roots produced by each of these genera (Fisher, 1982a). Duggan (1961) recorded that, on average, populations causing a reduction of 20% in barley, caused a 34% loss in yield of wheat and a complete failure in oats in Ireland. Dixon (1969) reported that for every ten eggs/g of soil before cropping there was an approximate loss of 376 kg/ha in oats, 188 kg/ha in wheat and 75 kg/ha in barley in England. In the Netherlands, the same situation applies but barley suffers little damage (Kort, 1972). In India, the situation may be different in that barley suffers more damage than wheat or oats (Handa *et al.*, 1985). In Australia, in Victoria, wheat suffers more than oats or barley (Millikan, 1938), but in South Australia, the situation is similar to that in England and Ireland and barley may suffer as much as 30% loss in yield (Sparrow & Dube, 1981).

All of these comparisons are of a general nature and in most instances are the results of comparison of a limited number of cultivars. The generality, that oats produces fewer seminal roots than wheat which produces fewer than barley and by so doing changes the density of nematodes within the roots (Fisher, 1982a) explains the differences between the genera. But the demonstration that within *Triticum*, wide variation in tolerance exists (Fisher *et al.*, 1981) suggests that other mechanisms are also operating. The reduced damage in the wheat selection RAC 311 (now the commercial cultivar Bayonet) is due to a difference in host : pathogen relations in that smaller galls are produced (Stanton, 1983), this cultivar is as tolerant as barley under South Australians conditions and the oat variety, N.Z Cape is more tolerant than barley (Dube & Fisher, pers. comm).

In Australia, most work has been done on wheat because it is the major cereal in the agricultural system. Meagher & Brown (1974) found that a population level of two eggs/gram of soil reduced the yield of wheat by 20% but Rovira (1982) found that this density did not affect wheat yield. Although these estimates of losses are little more than guesses, they are an indication of losses in yield and dollars to the Australian cereal

industry (Rovira *et al.*, 1981; Brown, 1981a; 1981b; 1984). Although it has been claimed that damage from CCN is greater in Australia than in Europe (Meagher, 1972; Brown, 1972; Meagher & Brown, 1974; Meagher *et al.*, 1978) ~~but~~ there is little good evidence for this.

In Australia some authors have recommended early sowing in May as a means of avoiding damage (Brown & Pye, 1981; Brown, 1984) but this stems from a misinterpretation of hatching data. For example, Meagher (1970) suggested that the amount of hatching is related to early seasonal rainfall rather than the initial density of eggs, but little evidence was presented here. Brown (1984) redrew Meagher's data to fit his interpretation claiming that maximum hatching occurs in early July. The maximum rate of hatching occurs at the point of inflection of the optimal curve of hatching (see Fig. 1) which occurs in May if seasonal rains start early enough. Banyer & Fisher (1971a; 1971b) showed that eggs of the Australian populations of *H. avenae* become hatchable when soil temperature drops below 20°C, with maximum rate occuring at about 10°C and the minimum slightly below 5°C, but that eggs do not hatch until free water is available. The data of Williams & Beane (1979) support this hypothesis for British populations and in France, different pathotypes have different optima and different ranges (Rivoal, 1978). The hatching of eggs of the British pathotypes in autumn (Kerry & Jenkinson, 1976), the cessation of hatching in the cold winter months and the recommencement in spring support the Banyer & Fisher (1971a; 1971b) hypothesis. Early sowing, which is dependent on early rain, is not a method that can be recomended to avoid damage. Delayed sowing will produce lower yields (French & Schultz, 1984) whether infested or not so that it is advisable to sow early and control the nematode in other ways. In areas with favourable spring rainfall, spring sowing will avoid damage because few infective larvae remain in the soil (Davies & Fisher, 1976; Dube *et al.*, 1979) after mid-August but loss in yield will result from delayed sowing.

AIM OF EXPERIMENTAL WORK

The aim of the present study was to examine the inheritance of resistance in barley to the cereal cyst nematode (*Heterodera avenae* Woll.). Although part of this problem has been examined before (O'Brien, 1976) there are numerous aspects that still remain to be investigated. The resistance in the six barley cultivars reported by O'Brien (1976) need to be confirmed and studied further particularly with respect to the relationship between the genes. Whether tolerance has any importance in barley is also examined. Particular emphasis is placed on the variation in numbers of females in the assays. Experiments are described which indicate variation in reaction in barley to *H. avenae* , the effect of initial density of *H. avenae* on barley growth and yield, the mechanism of resistance in barley and the possibility of change in the virulence in the South Australian populations of *H. avenae* .

CHAPTER 3

VARIATION IN THE REACTION OF BARLEY CULTIVARS TO THE AUSTRALIAN PATHOTYPE OF *Heterodera avenae* Woll

The interactions between host, nematode and environment could determine the number of females produced on a plant and thus the multiplication rate of the nematode. The resistance/susceptibility and tolerance/intolerance of the host are important factors in regulating multiplication rate. The effect of host resistance can best be seen at low initial densities where it reduces the rate of multiplication markedly (Jones & Kempton, 1978). As most known resistance in barley is related to a single major gene, the effect of resistance on multiplication rate should be dramatic, but in wheat the same single major genes in different genetic backgrounds may have slightly different reactions (O'Brien *et al.*, 1979) so that it has been suggested that modifiers may contribute to variation in numbers of females. In addition, the number of females that are produced on resistant cultivars can be affected by initial density (O'Brien & Fisher, 1974) and temperature (Person-Dedryver & Doussinault, 1979; Cook & McLeod, 1980) while the heterozygous condition of the cultivars can also lead to variation in number of females (Cotten & Hayes, 1969; O'Brien *et al.*, 1979). However, the variation caused by these factors tends to be rather small though it may be significant.

The tolerance of a host can also affect numbers of females produced. The amount of damage that is caused by the juveniles may in turn affect the number that can develop (Jones & Kempton, 1978). Normally, this effect occurs at relatively high densities but the density at which it occurs may interact with nutrition. There is no information available that relates numbers of juveniles used in assays for resistance to levels that may be damaging so that this effect is largely unknown. In addition, the different assays may promote different effects; those assays using juveniles as inoculum may produce a more concentrated local infection, which may lead to more damage, than

assays using eggs or cysts in soil in which hatching may regulate the numbers of juveniles available for invasion at any one time. Numbers invading from eggs may vary depending on initial density and cold treatment (Banyer & Fisher, 1971) so variation may still be expected. Numbers of females produced on a host may also depend on the approach to assessment. Some workers count all females (Cotten, 1967; Brown, 1974; O'Brien & Fisher, 1977) while others simply record presence or absence of females after an inspection of the surface of the root-ball (Andersen, 1963). The latter method is more likely to produce recordings of zero females.

In this study the reaction of barley cultivars from all parts of the world to inoculation with a standard number of freshly hatched juveniles of the Australian pathotype of *H. avenae* is examined.

3.1 Materials and Methods

Thirty two barley cultivars from the Waite Agricultural Research Institute collection (Appendix 1) were tested for their reaction to the Australian pathotype of *Heterodera avenae* Woll., in two separate experiments. These experiments were conducted in a growth room at 15°C constant temperature under 10 hours of continuous fluorescent light in each 24 hour period.

The method employed was as follows. Barley plants were grown in open ended polyvinyl chloride (p.v.c) tubes 2.5 cm internal diameter and 13 cm long, filled with sandy loam containing John Innes nutrients at half strength and no peat. The tubes were placed at random in a wire-grid on a basal 3 cm layer of potting soil. Seeds were placed on damp filter paper for 48 hours at 4°C and then for 24 hours at 16°C to initiate germination. Seedlings with the first one to four seminal roots of about 1 cm were selected. One seedling per tube was sown approximately 2.5 cm deep into the soil. For inoculation the appropriate volume of a suspension of a standard number of freshly hatched juveniles of *H. avenae* per ml of water was prepared. A constant stream of air

was blown through the suspension for even distribution of the juveniles while 1 ml aliquots were drawn with a pipette and released on the soil surface close to each seedling. The seedlings were inoculated at planting and 3, 6, 9, and 15 days later.

One hundred juveniles per inoculation were used in the first experiment and 125 juveniles in the second experiment. A fully randomized design with six plants per cultivar was used in these experiments.

Nine weeks after the last inoculation, the plants were harvested. The tubes were removed from the base tray, placed in glass jars and soaked in water. The plants were removed from the tubes, placed on a set of sieves (apertures : 1.40 mm, 0.710 mm and 0.250 mm), and the roots were washed with a strong jet of water. The white females were collected on the smallest sieve, transferred to a counting dish and counted under 10x magnification. Assessment of the reaction of the cultivars evaluated was based on the number and range of the white females produced per plant.

3.2 Results

The reaction of each cultivar as measured by the mean number of white females developed on a plant showed a continuous range from 42.67 on Clipper and Indian Dwarf to less than one on Orge Martin and Morocco (Table 2). On the susceptible cultivar Clipper in experiment 2, inoculation with nematode initial density of 5x125 juveniles, gave a higher mean number of white females produced per plant than the initial density of 5x100 juveniles in experiment 1; on the resistant cultivar CPI 18197 the different nematode initial densities did not affect the number of white females produced.

In the first experiment (Fig. 2a), the mean number of females and the range of variation showed that the cultivars fell readily into two groups, which could be separated at the level of 10 females per plant. But in the second experiment (Fig. 3a) the separation was less clear, it being particularly difficult to classify the cultivars Prior D/A, 2 EBYT 16 and Orge Prophete.

Table 2. The number of white females per plant on barley cultivars following inoculation with *H. avenae*.

Cultivars		White females (no./plant) Untransformed Mean	DMRT	Range	Square root transformation Mean	DMRT	Range
Experiment 1 (initial density 5 x 100 juveniles) :							
Golden Promise	(GP)	39.83		28.00-46.00	6.29		5.29-6.78
Freja	(F)	39.67		28.00-67.00	6.23		5.29-8.19
Clipper	(C)	37.33		29.00-52.00	6.08		5.39-7.21
Weeah	(W)	33.17		26.00-41.00	5.74		5.10-6.40
Shannon	(Sh)	31.67		16.00-49.00	5.56		4.00-7.00
Mink	(Mi)	31.17		19.00-39.00	5.55		4.36-6.25
Resibee	(R)	30.83		22.00-37.00	5.54		4.69-6.08
WI 2231 D	(WI)	28.17		20.00-51.00	5.23		4.47-7.14
Betzes	(B)	25.33		14.00-34.00	4.98		3.74-5.83
Suifu	(S)	23.33		21.00-27.00	4.83		4.58-5.20
CPI 18197	(CP)	7.17		3.00-10.00	2.64		1.73-3.16
CI 8147	(C8)	5.17		4.00- 6.00	2.26		2.00-2.45
Chevron	(Ch)	4.17		1.00- 9.00	1.85		1.00-3.00
Athenais	(At)	3.33		0.00- 9.00	1.40		0.00-3.00
Nile	(Ni)	1.50		0.00- 5.00	0.94		0.00-2.24
Morocco	(Mo)	0.17		0.00- 1.00	0.17		0.00-1.00
Experiment 2 (initial density 5 x 125 juveniles) :							
Indian Dwarf	(ID)	42.67		34.00-48.00	6.52		5.83-6.93
Clipper	(C)	42.67		30.00-56.00	6.48		5.48-7.48
Beecher	(Bc)	41.67		25.00-58.00	6.40		5.00-7.62
Zephyr	(Z)	35.17		26.00-50.00	5.89		5.10-7.07
Bayardi	(By)	33.50		23.00-44.00	5.75		4.80-6.63
Mazurka	(Mz)	28.67		20.00-43.00	5.31		4.47-6.56
Corvett	(Co)	28.00		15.00-37.00	5.24		3.87-6.08
Arivat	(Ar)	22.33		15.00-28.00	4.70		3.87-5.29
Prior D/A	(P)	17.33		10.00-32.00	4.09		3.16-5.66
2 EBYT 16	(2E)	16.17		11.00-26.00	3.97		3.32-5.10
Orge Prophete	(OP)	11.50		3.00-25.00	3.22		1.73-5.00
Tintern	(T)	8.17		5.00-12.00	2.82		2.24-3.46
CI 3576	(C3)	7.67		3.00-12.00	2.71		1.73-3.46
CPI 18197	(CP)	6.33		3.00-12.00	2.51		2.24-2.83
La Mesita	(LM)	6.33		3.00-12.00	2.46		1.73-3.46
Galleon	(G)	4.83		0.00-11.00	1.89		0.00-3.32
Marocaine	(Ma)	1.33		0.00- 3.00	1.02		0.00-1.73
Orge Martin	(OM)	0.83		0.00- 3.00	0.52		0.00-1.73

Note : mean values connected by the same line are not significantly different at p=0.05.
DMRT = Duncan's multiple range test.

Figure 2. The distribution of white females per plant on barley cultivars, inoculated with 5x100 juveniles of *H. avenae*.

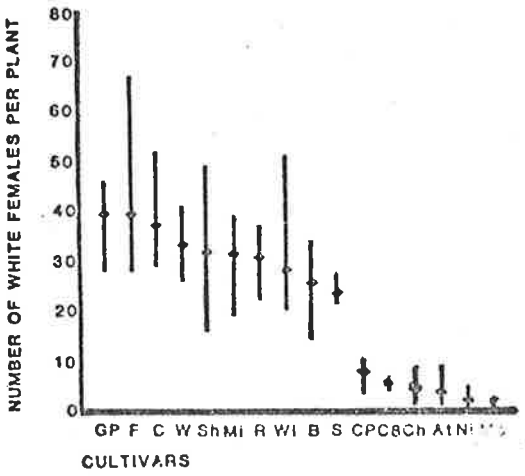
a. Untransformed.

b. Square root transformation.

Figure 3. The distribution of white females per plant on barley cultivars, inoculated with 5 x 125 juveniles of *H. avenae*.

a. Untransformed.

b. Square root transformation.


NUMBER OF WHITE FEMALES PER PLANT
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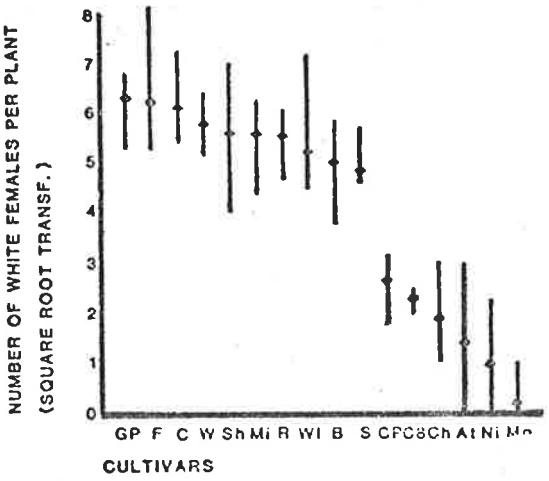
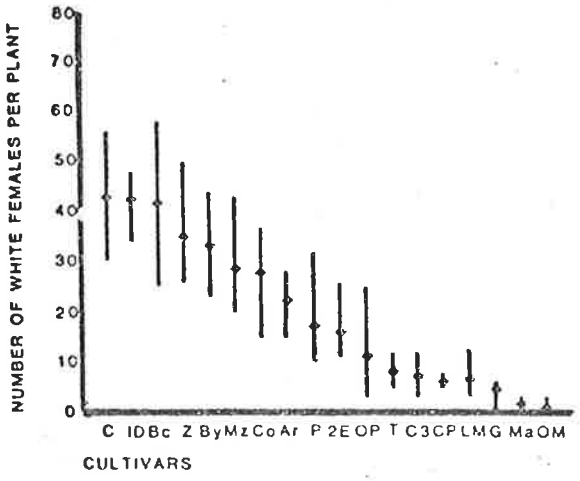
70
60
50
40
30
20
10
0
GP F C W Sh Mi R Wi B S CPC8Ch At Ni
CULTIVARS

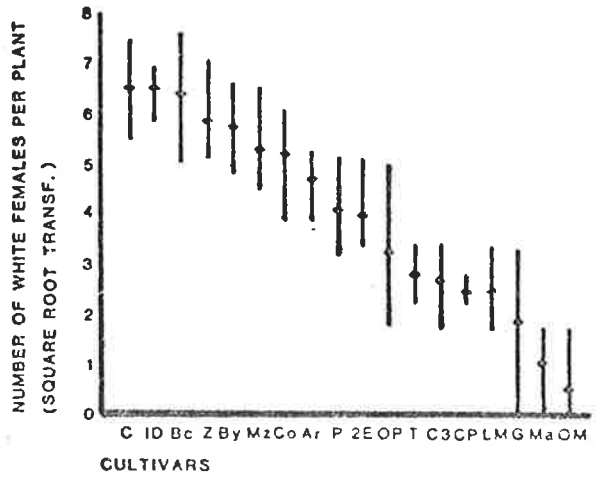
2.a


NUMBER OF WHITE FEMALES PER PLANT
(SQUARE ROOT TRANSF.)
8
7
6
5
4
3
2
1
0
GP F C W Sh Mi R Wi B S CPC8Ch At Ni Ma
CULTIVARS

2.b


NUMBER OF WHITE FEMALES PER PLANT
80
70
60
50
40
30
20
10
0
C ID Bc Z By Mz Co Ar P 2E OP T C3CP LMG Ma OM
CULTIVARS

3.a


NUMBER OF WHITE FEMALES PER PLANT
(SQUARE ROOT TRANSF.)
8
7
6
5
4
3
2
1
0
C ID Bc Z By Mz Co Ar P 2E OP T C3CP LMG Ma OM
CULTIVARS

3.b

The variation in the range of white females on cultivars with means above 10 females per plant was generally greater than on cultivars with a mean below 10 females per plant (Figs. 2a and 3a). Square root transformation of the data made the variation within those groups more uniform (Figs. 2b and 3b). However, Orge Prophete with a mean of 11.50 females per plant was still difficult to classify, and its range of variation overlapped with the range of cultivars with a mean below 10 females per plant.

Analysis of variance (Appendix 2) and Duncan's multiple range test (Table 2) were made on both untransformed and square root transformed data. Although the results show that many cultivars differed significantly, this may not have any practical value; the statistical difference may only be due to the size of the means and may not necessarily reflect or explain the nature of the difference between those cultivars.

3.3 Discussion

The results here showed that if sufficient barley cultivars are tested against *H. avenae*, there is a continuous range of reaction from highly susceptible to almost completely resistant. The different nematode initial densities (5x100 juveniles in experiment 1 and 5x125 juveniles in experiment 2) did not affect the number of white females produced on the resistant cultivar CPI 18197, but on the susceptible cultivar Clipper, inoculation with initial density of 5x125 juveniles increase the number of white females produced.

In the first experiment, at the level of 10 females per plant the cultivars could be separated into susceptible and resistant groups. The group of cultivars with less than 10 females per plant include some in which a single major gene is known to control the development of the females (O'Brien *et al.*, 1979). Most of these cultivars originated in North Africa, Greece or Turkey. Tintern is a resistant cultivar released in Wales (Anon, 1982), Orge Martin is resistant to pathotype Ha11 and Ha12 (Cook, 1977) but there is little information on CPI 18197, La Mesita and CI 3576.

In the second experiment, if the single cultivar Orge Prophete was removed, then variation in reaction to *H. avenae* would fall readily into the same two groups which could be classified as susceptible or resistant. Such classification would suggest a genetic basis for an initial separation but would leave the wide variation in the means of the susceptible group unexplained.

There are a number of possible explanations for this variation. The commercial cultivars from several countries are probably rather homogeneous genetically compared to cultivars such as Orge Prophete which may well represent one of the old 'land races'. If some cultivars are in fact more heterogeneous than others, then it is reasonable to expect more variation in them. On untransformed data the most variable reactions were shown by Beecher, Freja, Shannon and WI 2231D, the first three of which are commercial cultivars and the last is a rather recent single plant selection from the Waite Agricultural Research Institute. With transformed data the most variable reactions were shown by Beecher, Orge Prophete, Freja, Shannon, WI 2231D, Galleon, Athenais and Nile. It seems unlikely that the variation within a cultivar can be explained on the basis of genetic heterogeneity.

Another possible explanation is that different degrees of tolerance can affect the number of females produced (Seinhorst, 1967). Such a factor could contribute to variation if the initial density of the inoculum is sufficiently high. Nothing is known of the tolerance of these cultivars. As well, the small containers used for the growth of the plants, may have a significant effect on root growth, particularly during the early stages of the growth, infection and development of the host parasite relationship. Such an effect might contribute to increased variation particularly that between cultivars.

An alternative hypothesis that would explain continuous variation in numbers of females on different cultivars is that the system of inheritance is under polygenic control. There is no evidence yet that any of the resistance to *H. avenae* in cereals is under polygenic control; all resistance examined so far is controlled by major genes (Andersen, Andersen 1968; Cotten & Hayes, 1969; O'Brien *et al.*, 1979).

Lastly, there may be a different genetic control of variation in this susceptible group of cultivars consisting of modifiers that may produce different means and variation. Cultivars with less than 10 females per plant have shown a rather narrow range of variation and this may represent an expression of the resistance gene on the development of females, which suppresses the effect of any modifiers.

CHAPTER 4

THE INHERITANCE OF RESISTANCE TO THE AUSTRALIAN PATHOTYPE OF *H. avenae* Woll. IN BARLEY

After the report by Nilsson-Ehle (1920) on the resistance of some Swedish barley cultivars to *H. avenae* , there were no significant studies made on the inheritance of resistance to this nematode, until the isolation of two pathotypes in Denmark by Andersen (1959). At first, the resistance was associated with a single dominant gene (Andersen, 1961), then two genes which were at two different loci (Andersen & Andersen, 1968) and subsequently four genes have been reported (Cotten & Hayes, 1969). Based on the gene-for-gene hypothesis, at least six genes have been predicted for resistance in barley to *H. avenae* (Hayes & Cotten, 1970). Recently, it has been reported that complementary genes were responsible for the resistance in barley to the French pathotypes (Person-Dedryver & Doussinault, 1984).

Most resistance that has been investigated (Andersen, 1961; Cook & York, 1982) is controlled by single major genes. Such a system of inheritance allows two classes of plant reaction - susceptible or resistant, or three classes if the heterozygous condition should vary from the homozygous. These classes should be easily and clearly separable on the basis of number of females produced in an assay. Often, however, a wide range in the number of cysts occurs on genetically homozygous susceptible genotypes even where plants are grown under uniform conditions. As a result there can be a problem in separating certain plants into resistant or susceptible classes (Cotten & Hayes, 1969). In addition, intermediate degrees of resistance have been observed. One of the problems in determining the precise nature of this type of resistance is that the assay needs to be sufficiently precise to be able to separate with ease and confidence a susceptible reaction from a resistant reaction.

A preliminary examination of resistance in barley to the Australian pathotype

showed that some genes for resistance were present and this resistance was controlled by single dominant genes (O'Brien, 1976). However, the assay for resistance was not sufficiently accurate to be certain and there was a suggestion that more genes may be available.

This chapter seeks information on genes that are involved in the resistance in barley to *H. avenae* , and examines the interrelationships of these genes for resistance, using modifications of the assay used by O'Brien *et al.*, (1979).

4.1 Materials and Methods

The methods used, unless otherwise stated, were similar to the methods described in section 3.1. Initial density used throughout the experiments was 5x125 juveniles of *H. avenae.*

Barley cultivars, Athenais (At), CI 8147 (C8), Nile (Ni) and Morocco (Mo), resistant to a South Australian population of *H. avenae* (O'Brien & Fisher, 1977), Marocaine 079 (Ma) resistant in Victoria as well (Ellis & Brown, 1976) and Orge Martin (OM) a resistant cultivar from Algeria, all of a six-row type, except for CI 8147 which is a two-row type, were used in various crosses to the susceptible two-row cultivar Clipper (C) from Australia. Clipper was chosen because it is widely grown in South Australia and in previous experiments it supported the development of large numbers of females of *H. avenae* (Table 2, section 3.2). Betzes (CI 6398) is a two-row barley cultivar originating in Germany; it was introduced to U.S.A. from Poland in 1938, and released to growers in Montana in 1957 (Wiebe & Reid, 1961) but now it is grown in Canada (Sparrow, pers. comm.). Betzes$^{(B)}$ was included because in a previous experiment (Table 2, section 3.2) it allowed the development of an intermediate number of females.

The reactions of populations from the following crosses were assessed : F1's and F2's of single crosses AtxC, C8xC, MaxC, NixC, MoxC and OMxC; F1's of first backcrosses (AtxB)xB, (AtxC)xC, (C8xC)xC, (MaxC)xC, (NixC)xC, (MoxC)xC and

(OMxC)xC; F1's of three way crosses (AtxMa)xB, (AtxMa)xC, (AtxMo)xC, (AtxNi)xC, (C8xMo)xC and (NixMo)xC; F3's of single plant selections from the F2's of single crosses AtxC and C8xC.

The six single crosses were made in winter, July-September 1981; the F1 plants were grown for the first 6 weeks in a growth room to induce tillering before being transferred into the glass-house, and grown through summer 1982, until maturity. Seven backcrosses and six three way crosses were made in the winter, July-September 1982.

The F1 and F2 segregating populations were tested for reaction to *H. avenae* using the tube assay in the growth room throughout 1982. Tests of F1's of the first backcrosses and the three way crosses and single plant selections from the F2's of single crosses of AtxC and C8xC were carried out in 1983.

To test whether there was an association between resistance to *H. avenae* and head type in barley, observation was made on the F2 populations of the single crosses AtxC, MaxC, NixC, MoxC and OMxC. After assessment for resistant and susceptible reaction to *H. avenae*, the F2 plants of the single crosses were grown in wooden boxes in the glasshouse until heading and their head type recorded.

The number of plants tested for resistance was as follows. Twenty plants from each parent. For the single crosses, 20 plants from each of the F1 populations, 100 plants from each of the F2 populations. For backcrosses, 20 plants from each of the F1 first backcross populations with the exception of 10 plants for the (AtxB)xB population. For three way crosses, 75 plants from each of the F1's of (C8 xMo)xC, (NixMo)xC, and (AtxMo)xC populations, 25 plants from the F1 of the (AtxNi)xC population, 20 plants from the F1 of the (AtxMa)xC population were used in each of two successive tests and 16 plants from the F1 of the (AtxMa)xB population. Plants nos.16, 24, 34, 45, 48 and 100 of the F2 single cross of AtxC which in the F2 population test produced 11, 24, 13, 14, 17 and 16 white females per plant, respectively, and plants nos.12, 31 and 64 of the F2 single cross of C8xC which produced 20, 22 and 23 white females per plant, respectively, were selected for the F3 test.

Resistance was determined by the number of females on plant roots (Andersen, 1961), and a bimodal distribution was determined for resistant and susceptible reactions of the plants. The range of number of females for the resistant cultivars was 0 - 12 per plant and for the susceptible cultivar Clipper was 27 - 67 per plant. Classification into resistant and susceptible reactions in the F2's of single crosses, F1's of first backcrosses and F1's of three way crosses was based on the reaction observed in the parents and F1's of single crosses. A chi-square test was used to test the goodness of fit of the data to the expected segregation ratios. For one degree of freedom the ' Yates correction term' was added to the chi-square formula (Strickberger, 1985).

4.2 Results

The number of white females produced per plant on the susceptible parent, Clipper, was consistently high throughout the tests and the distribution showed a clear separation from the resistant parents i.e. Athenais, CI 8147, Marocaine 079, Nile, Morocco and Orge Martin. The lowest number of white females per plant on Clipper was 27 and the highest was 67; on the resistant parents, the lowest number of white females per plant was nil and the highest was 12 (Tables 3 and 5).

Distribution of the F1 plants of the single crosses of AtxC, C8xC, MaxC, NixC, MoxC, and OMxC was skewed towards that of the resistant parents (Figs. 4 - 9), but the range of number of white females per plant was always greater than for the resistant parents (Table 3). The F2 populations of the single crosses of AtxC, C8xC, MaxC and NixC, segregated into a 3 : 1 ratio indicating a single dominant gene for resistance in Athenais (Fig. 4), CI 8147 (Fig. 5), Marocaine (Fig. 6), and Nile (Fig.7), but those of MoxC and OMxC segregated into a 15 : 1 ratio, suggesting there were two genes responsible for the resistance in Morocco (Fig. 8) and Orge Martin (Fig.9). The chi-square values for the expected segregation ratios are given in Table 4.

In the F2 populations of the single crosses, six plants from the F2's of AtxC and five plants from the F2's of C8xC although classified as susceptible were found to be intermediate between the resistant and susceptible parents (Figs. 4 and 5). These plants either belonged to the resistant group, but produced more females, or they belonged to the susceptible group, but produced fewer females. An F3 test of these single plant selections showed that some of the F3 populations segregated for resistance and susceptibility .

In the F3 test of the single plant selections from AtxC, the progeny of plants nos. 45 and 48 gave all resistant reactions, showing them to be homozygous for resistance; plant nos. 16 and 100 were probably homozygous, but the progeny of plants nos. 24 and 34 produced more white females than expected, although their range of distribution was still below the range of distribution on the susceptible parent Clipper (Table 6a). The range of distribution of white females of the progeny of plant no. 34 was skewed towards that of the resistant parent, so it was probably homozygous for resistance. Plant no. 24 was probably heterozygous but the size of the population tested was too small to show the segregation (Fig. 10).

In the F3 test of the selections from C8xC, the progeny of plant nos.12 and 31 segregated into resistant and susceptible reactions and were therefore heterozygous but plant no. 64 was homozygous resistant (Table 6b; Fig 11).

The F1 plants of the backcrosses of (AtxB)xB, (AtxC)xC, (C8xC)xC, (MaxC)xC and (NixC)xC segregated into a 1 : 1 ratio, but those of (MoxC)xC and (OMxC)xC segregated into a 3 : 1 ratio (Table 7). These results showed that a single gene was responsible for resistance in Athenais, CI 8147, Marocaine and Nile (Fig. 12) but in Morocco and Orge Martin (Fig. 13) there were two genes responsible for the resistance.

Table 3. The number of white females per plant on parent cultivars and their F1's following inoculation with *H. avenae*.

Parents	No.of plants tested	White females (no/plant) Mean	Range	F1 populations	No.of plants tested	White females (no/plant) Mean	Range
Clipper	80	39.95	28 - 67				
Athenais	20	1.70	0 - 6	At x C	20	2.95	0 - 14
CI 8147	20	5.00	0 -12	C8 x C	20	6.70	0 - 14
Marocaine	20	0.65	0 - 2	Ma x C	20	4.30	0 - 12
Nile	20	1.85	0 - 5	Ni x C	20	4.70	1 - 13
Morocco	20	0.05	0 - 1	Mo x C	20	1.80	0 - 5
O/Martin	20	0.40	0 - 2	OM x C	20	0.75	0 - 3

Table 4. Heredity of resistance in the F2 populations of single crosses of six barley cultivars.

F2 populations	No.of plants tested	White females (no/plant) Mean	Range	Proposed segregation ratio	Number of plants Expected R : S	Found R : S	Chi square value	(df=1)
AtxC	100	12.61	0-70	3 : 1	75 : 25	72 : 28	0.33	P> 0.50
C8xC	100	9.54	0-52	3 : 1	75 : 25	78 : 22	0.33	P> 0.50
MaxC	100	13.90	0.48	3 : 1	75 : 25	71 : 29	0.65	P> 0.25
NixC	100	11.96	0-42	3 : 1	75 : 25	73 : 27	0.11	P> 0.50
MoxC	100	5.12	0-35	15 : 1	93.75 : 6.25	93 : 7	0.01	P> 0.90
OMxC	100	2.33	0.32	15 : 1	93.75 : 6.25	95 : 5	0.09	P> 0.75

Note : R = resistant ; S = susceptible.

Figure 4. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single cross Athenais x Clipper.

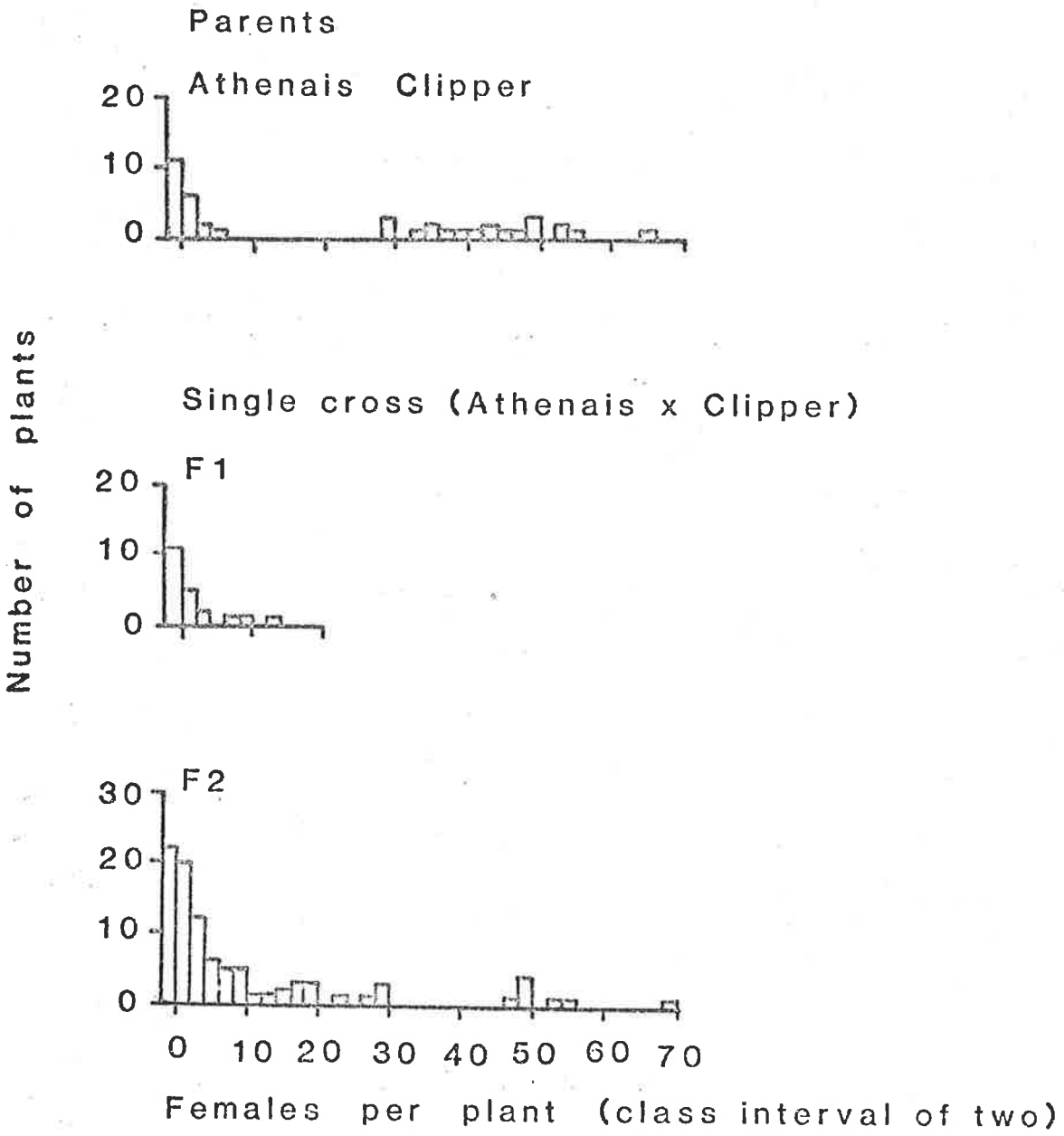

Parents
Athenais Clipper
20
10
0
Single cross (Athenais x Clipper)
F1
20
10
0
F2
30
20
10
0
0 10 20 30 40 50 60 70
Number of plants
Females per plant (class interval of two)

Figure 5. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single cross CI 8147 x Clipper.

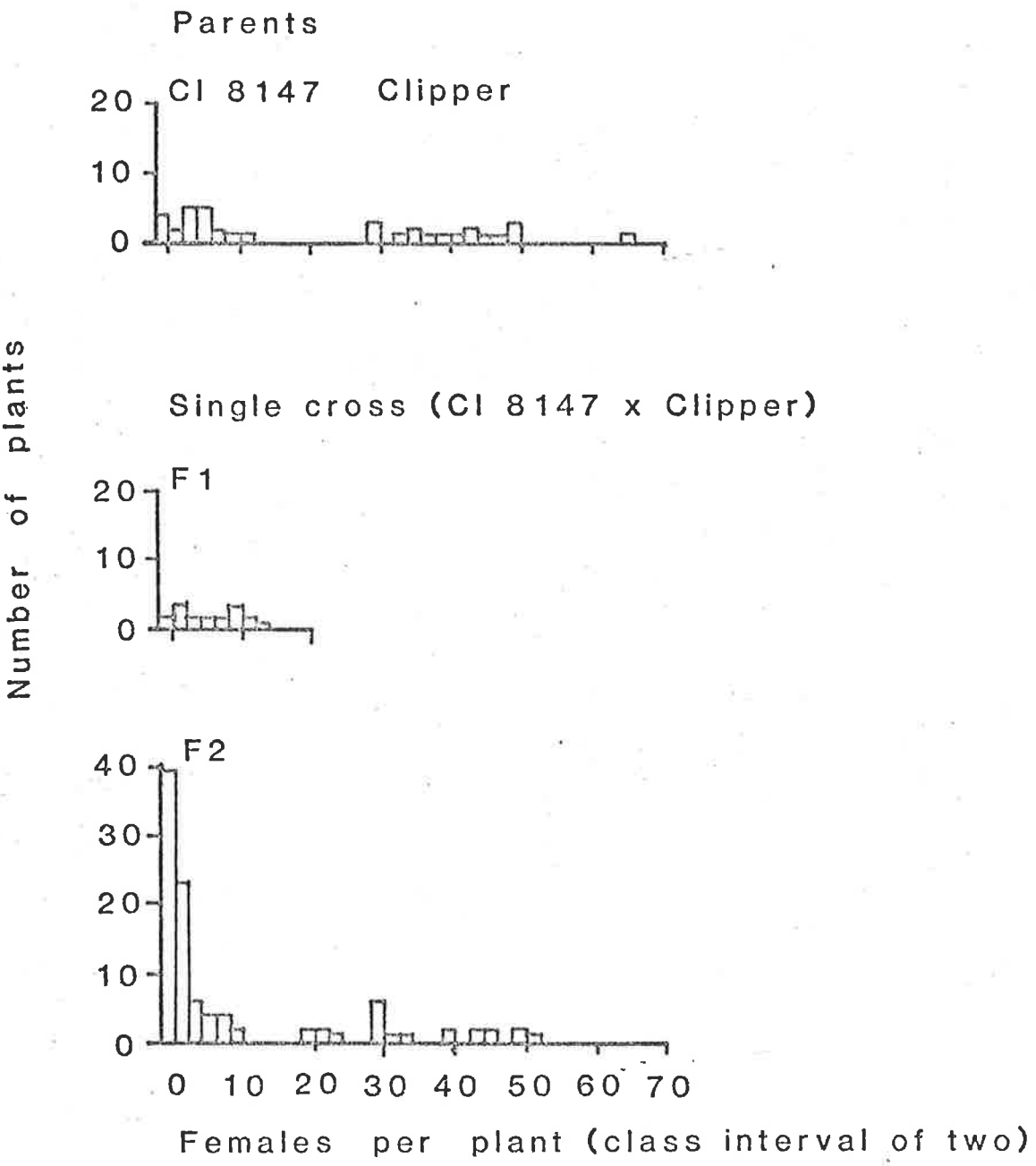

Parents
CI 8147 Clipper
20
10
0
Single cross (CI 8147 x Clipper)
F1
20
10
0
F2
40
30
20
10
0
0 10 20 30 40 50 60 70
Females per plant (class interval of two)
Number of plants

Figure 6. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single cross Marocaine x Clipper.

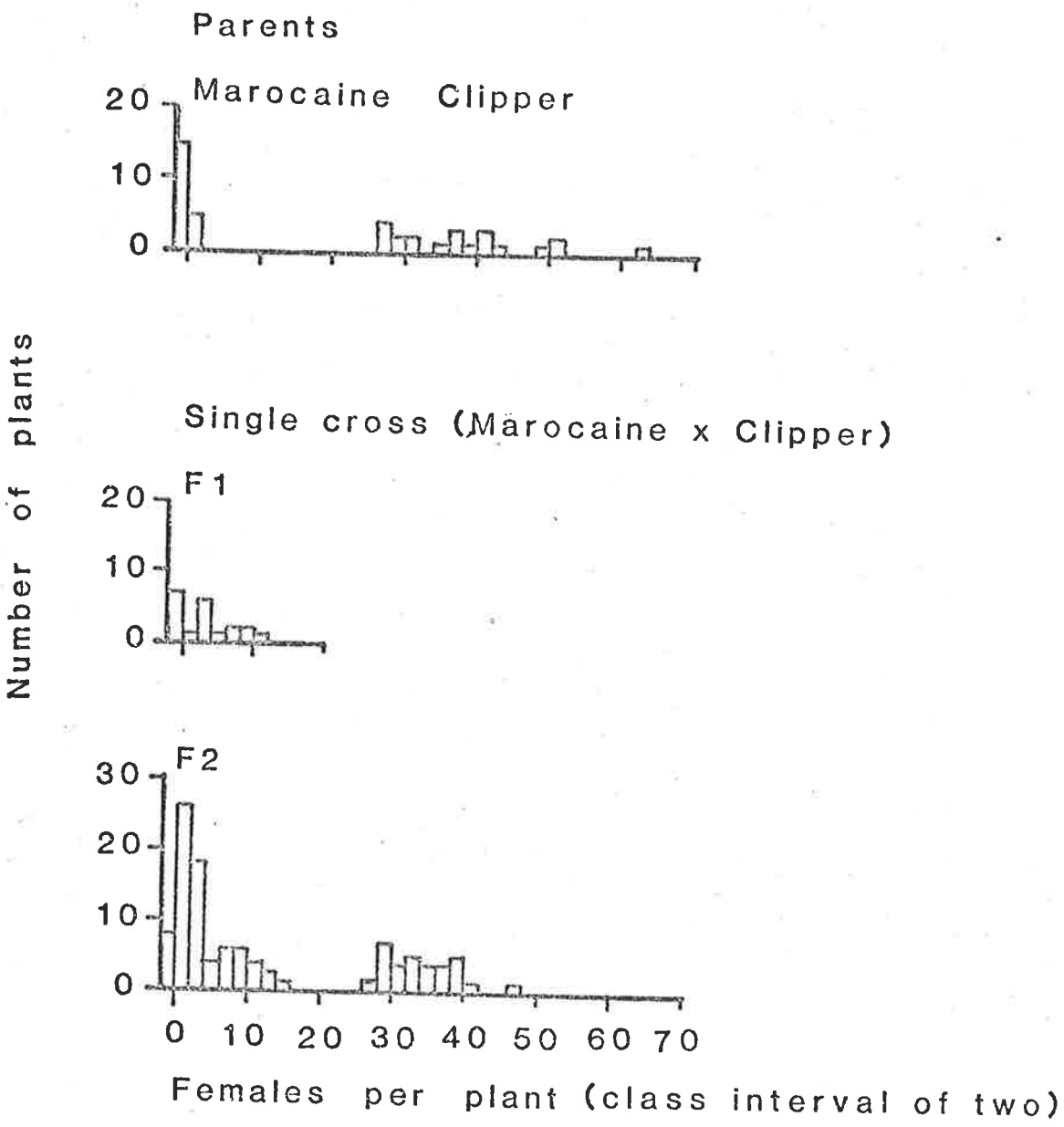

Parents
Marocaine
Clipper
20
10
0
Single cross (Marocaine x Clipper)
F1
20
10
0
F2
30
20
10
0
0 10 20 30 40 50 60 70
Number of plants
Females per plant (class interval of two)

Figure 7. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single cross Nile x Clipper.

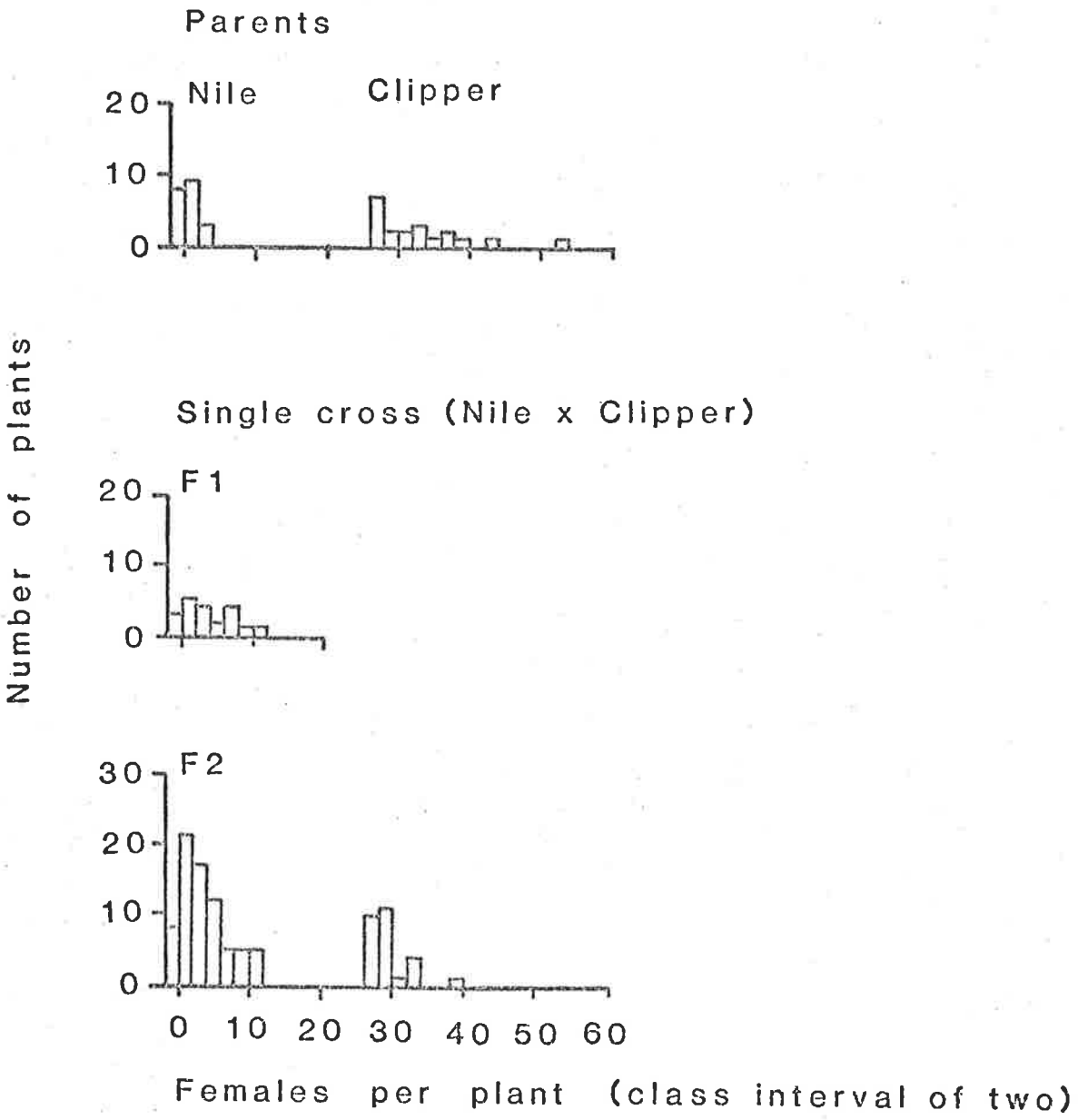

Parents
Nile
Clipper
20
10
0
Number of plants
Single cross (Nile x Clipper)
F1
20
10
0
F2
30
20
10
0
0 10 20 30 40 50 60
Females per plant (class interval of two)

Figure 8. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single cross Morocco x Clipper.

Figure 9. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single cross Orge Martin x Clipper.

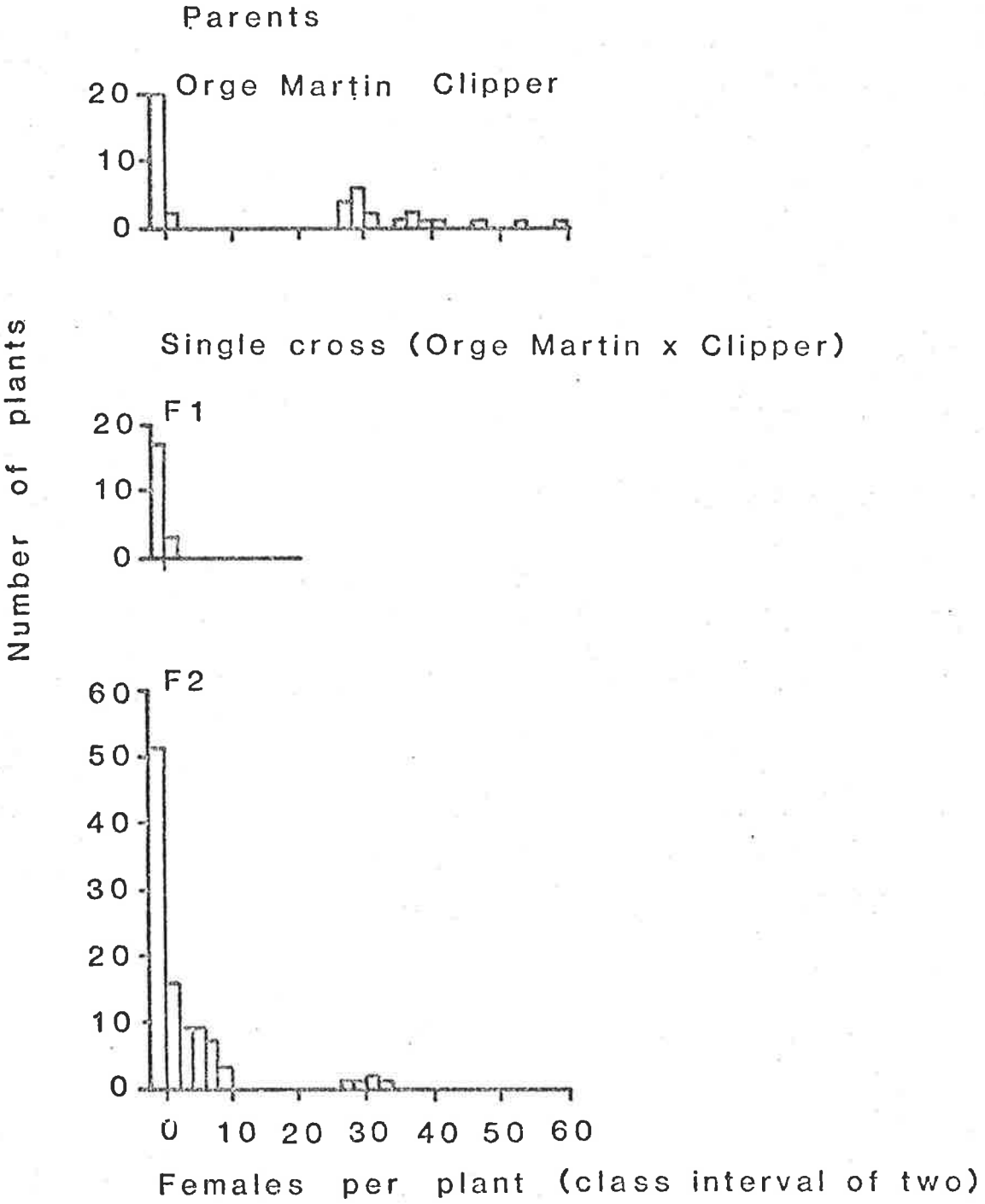

Parents
Orge Martin Clipper
20
10
0
Single cross (Orge Martin x Clipper)
F1
20
10
0
F2
60
50
40
30
20
10
0
0 10 20 30 40 50 60
Number of plants
Females per plant (class interval of two)

Table 5. The number of white females per plant on parent cultivars used in the backcrosses and three way crosses.

Parents	No. of plants tested	White females (no/plant) Mean	Range
Clipper	70	35.21	27 - 57
Betzes	10	28.00	13 - 42
Athenais	20	2.15	0 - 9
CI 8147	10	5.20	1 - 12
Marocaine	10	1.00	0 - 4
Nile	20	1.50	0 - 5
Morocco	25	0.20	0 - 2
O/Martin	10	0.20	0 - 1

The F1 plants of three way crosses of (AtxMa)xC and (AtxNi)xC segregated into a 3 : 1 ratio (Table 8), and their distributions resembled those for the F2's of the single crosses (Figs. 14 and 15) which suggested that the resistances in Athenais and Marocaine, and Athenais and Nile were not the same.

O'Brien (1976) found that the distribution for the three way cross of (AtxMa)xC resembled an F1 of the single cross and suggested that the resistance in Athenais and Marocaine was the same. As the results here conflicted with those of O'Brien (1976), a second test of this cross was carried out with the same result, a 3 : 1 ratio. The similar three way cross using Betzes instead of Clipper as the susceptible parent also indicated that different genes were involved.

Table 6a. The number of white females per plant on the F3 population of single plant selections from the F2's of single cross of At x C.

	No.of plants tested	White females (no/plant) Mean	Range	Number of plants found R : S	Genotype
Parents:					
Clipper	10	29.70	27 - 40		
Athenais	10	0.00	0 - 4		
		Mean females/plant :			
At x C :		F2 plants	F3 popls		
Plant no. 16	10	11	4.00	0 - 13	10 : 0	Homozygous
Plant no. 24	10	24	13.50	6 - 20	10 : 0	Heterozygous
Plant no. 34	10	13	4.80	0 - 18	10 : 0	Homozygous
Plant no. 45	10	14	0.30	0 - 1	10 : 0	Homozygous
Plant no. 48	10	17	3.50	1 - 5	10 : 0	Homozygous
Plant no.100	10	16	5.70	1 - 14	10 : 0	Homozygous

Note : R = resistant; S = susceptible.

Table 6b. The number of white females per plant on the F3 population of single plant selections from the F2's of single cross of C8 x C.

	No.of plants tested	White females (no/plant) Mean	Range	Proposed segregation ratio	Number of plants Expected R : S	Found R : S	Chi square value*	Genotype
Parents :								
Clipper	10	37.80	30 - 57					
CI 8147	10	3.40	0 - 7					
		Mean females/plant :						
		F2	F3					
C8 x C :		plants	popls					
Plant no. 12	15	20	8.53	0 - 30	3 : 1	11.25 : 3.75	14 : 1	2.69	Hetero
Plant no. 31	15	22	8.73	0 - 40	3 : 1	11.25 : 3.75	14 : 1	2.69	Hetero
Plant no. 64	15	23	4.80	0 - 10	-	- -	15 : 0	-	Homo

Note : R = resistant

S = susceptible

* = chi square value > at P = 0.10

Hetero = heterozygous

Homo = homozygous

Figure 10. Frequency distribution of number of white females of *H. avenae* per plant of parents and F3 population of single cross Athenais x Clipper.

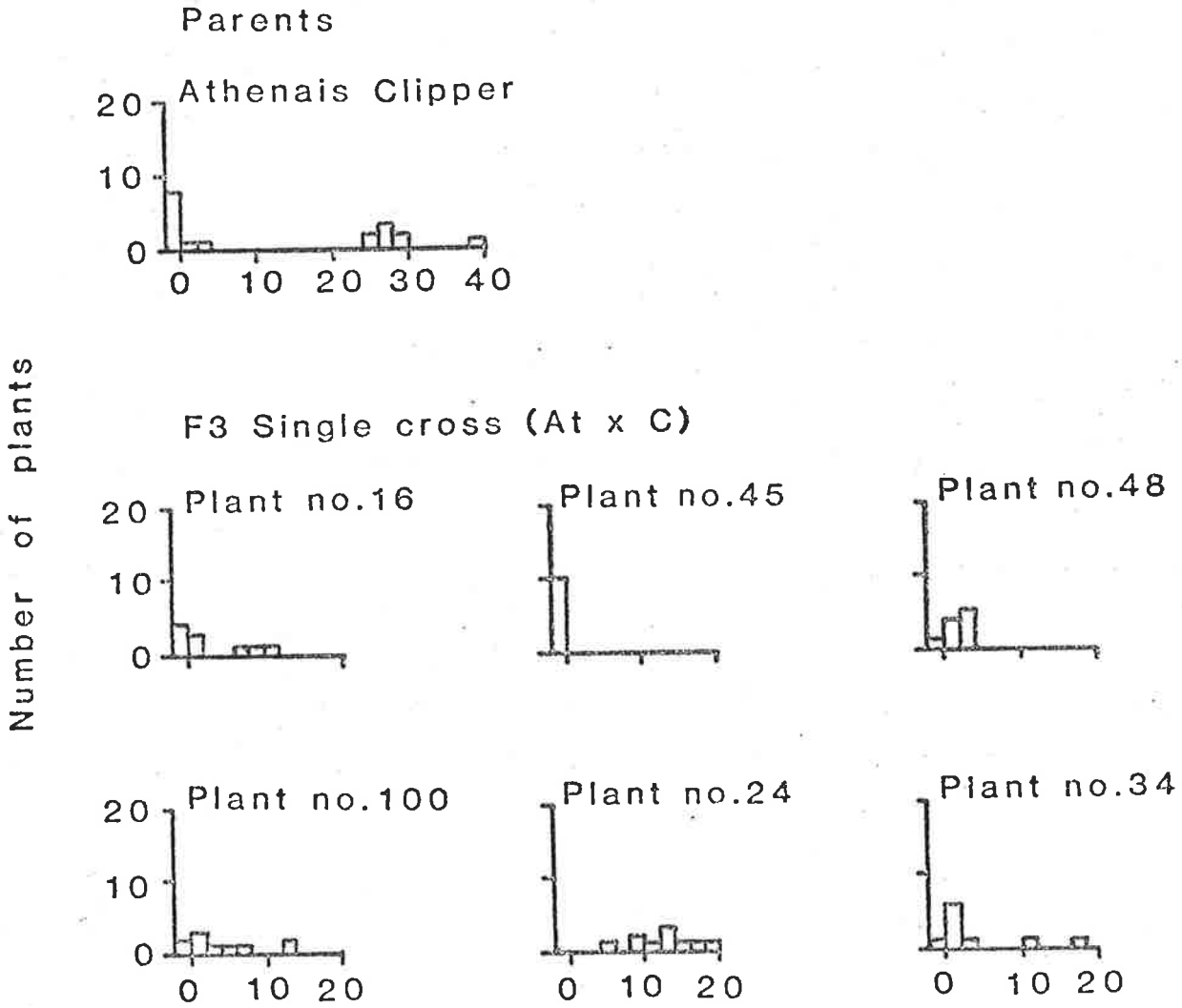

Parents
Athenais Clipper
20
10
0
0 10 20 30 40
F3 Single cross (At x C)
Plant no.16
Plant no.45
Plant no.48
Plant no.100
Plant no.24
Plant no.34
0 10 20
Number of plants
Females per plant (class interval of two)

Figure 11. Frequency distribution of number of white females of *H. avenae* per plant of parents and F3 population of single cross CI 8147 x Clipper.

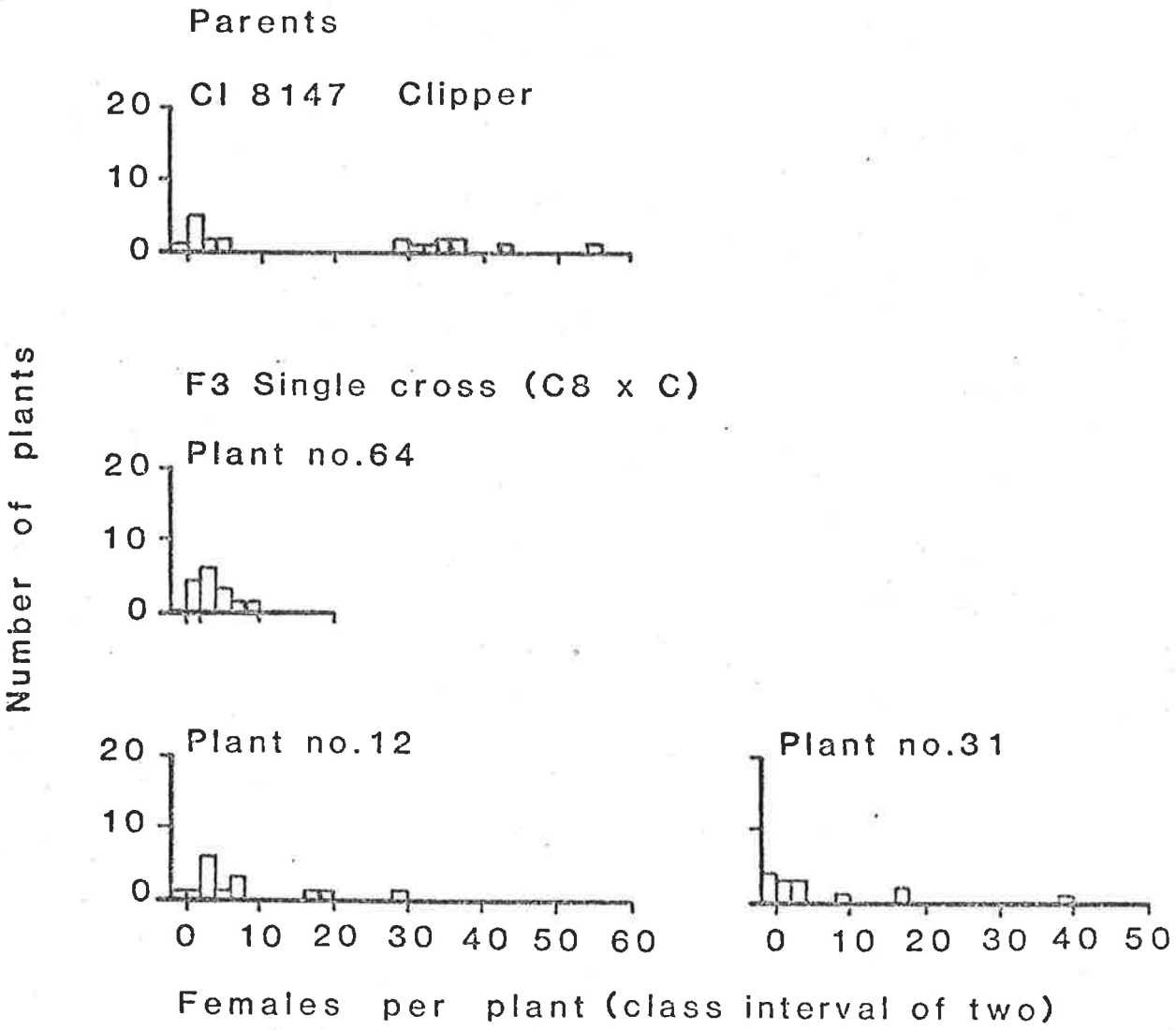

Parents
CI 8147 Clipper
20
10
0
F3 Single cross (C8 x C)
Plant no.64
Plant no.12
Plant no.31
Number of plants
0 10 20 30 40 50 60
0 10 20 30 40 50
Females per plant (class interval of two)

Table 7. Heredity of resistance in the F1 backcrosses populations of six barley cultivars.

F1 backcrosses populations	No.of plants tested	White females (no/plant) Mean	Range	Proposed segregation ratio	Number of plants Expected R : S	Found R : S	Chi square value	(df=1)
(AtxB)xB	10	18.20	0-35	1 : 1	5 : 5	6 : 4	0.10	P> 0.75
(AtxC)xC	20	19.50	3-50	1 : 1	10:10	10:10	0	P = 1
(C8xC)xC	20	16.85	4-42	1 : 1	10:10	12 : 8	0.45	P> 0.50
(MaxC)xC	20	20.65	0-46	1 : 1	10:10	10:10	0	P = 1
(NixC)xC	20	18.40	3-50	1 : 1	10:10	11: 9	0.05	P> 0.90
(MoxC)xC	20	14.60	0-40	3 : 1	15 : 5	13 : 7	0.60	P> 0.50
(OMxC)xC	20	14.55	0-39	3 : 1	15 : 5	13 : 7	0.60	P> 0.50

Note : R = resistant ; S = susceptible.

Table 8. Heredity of resistance in the F1 of three way crosses populations of six barley cultivars.

Three way crosses populations	No.of plants tested	White females (no/plant) Mean	Range	Proposed segregation ratio	Number of plants Expected R : S	Found R : S	Chi square value	(df=1)
(AtxMa)xB	16	13.00	0-40	3 : 1	12 : 4	12 : 4	0	P = 1
(AtxMa)xC	40*	8.58	0-35	3 : 1	30 : 10	33 : 7	0.83	P> 0.25
(AtxNi)xC	25	15.04	2-40	3 : 1	18.75 : 6.25	20 : 5	0.12	P> 0.50
(AtxMo)xC	75	7.25	0-40	7 : 1	65.62 : 9.38	66 : 9	0.002	P> 0.90
(C8xMo)xC	75	3.76	0-30	7 : 1	65.62 : 9.38	72 : 3	4.20	P> 0.01
(NixMo)xC	75	1.48	0-30	7 : 1	65.62 : 9.38	73 : 2	5.76	P> 0.01

Note : R = resistant ; S = susceptible; * = two tests each of 20 plants.

Figure 12. Frequency distribution of number of white females of *H. avenae* per plant of parents and F1 populations of backcrosses of (At x C) x C, (At x B) x B, (C8 x C) x C, (Ma x C) x C and (Ni x C) x C.

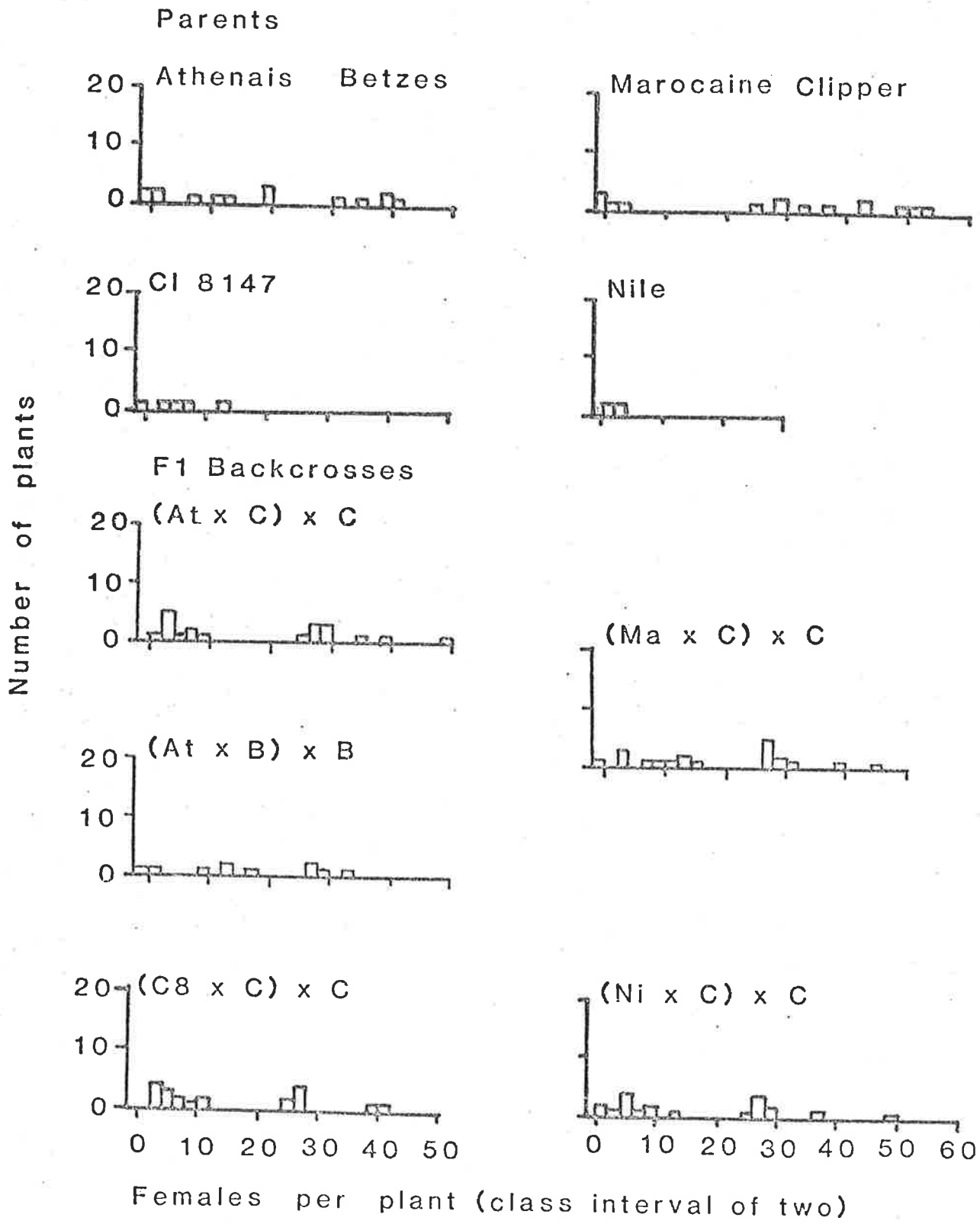

Parents
Athenais
Betzes
Marocaine
Clipper
CI 8147
Nile
F1 Backcrosses
(At x C) x C
(Ma x C) x C
(At x B) x B
(C8 x C) x C
(Ni x C) x C
Number of plants
20
10
0
0 10 20 30 40 50
0 10 20 30 40 50 60
Females per plant (class interval of two)

Figure 13. Frequency distribution of number of white females of *H. avenae* per plant of parents and F1 populations of backcrosses of (Mo x C) x C and (OM x C) x C.

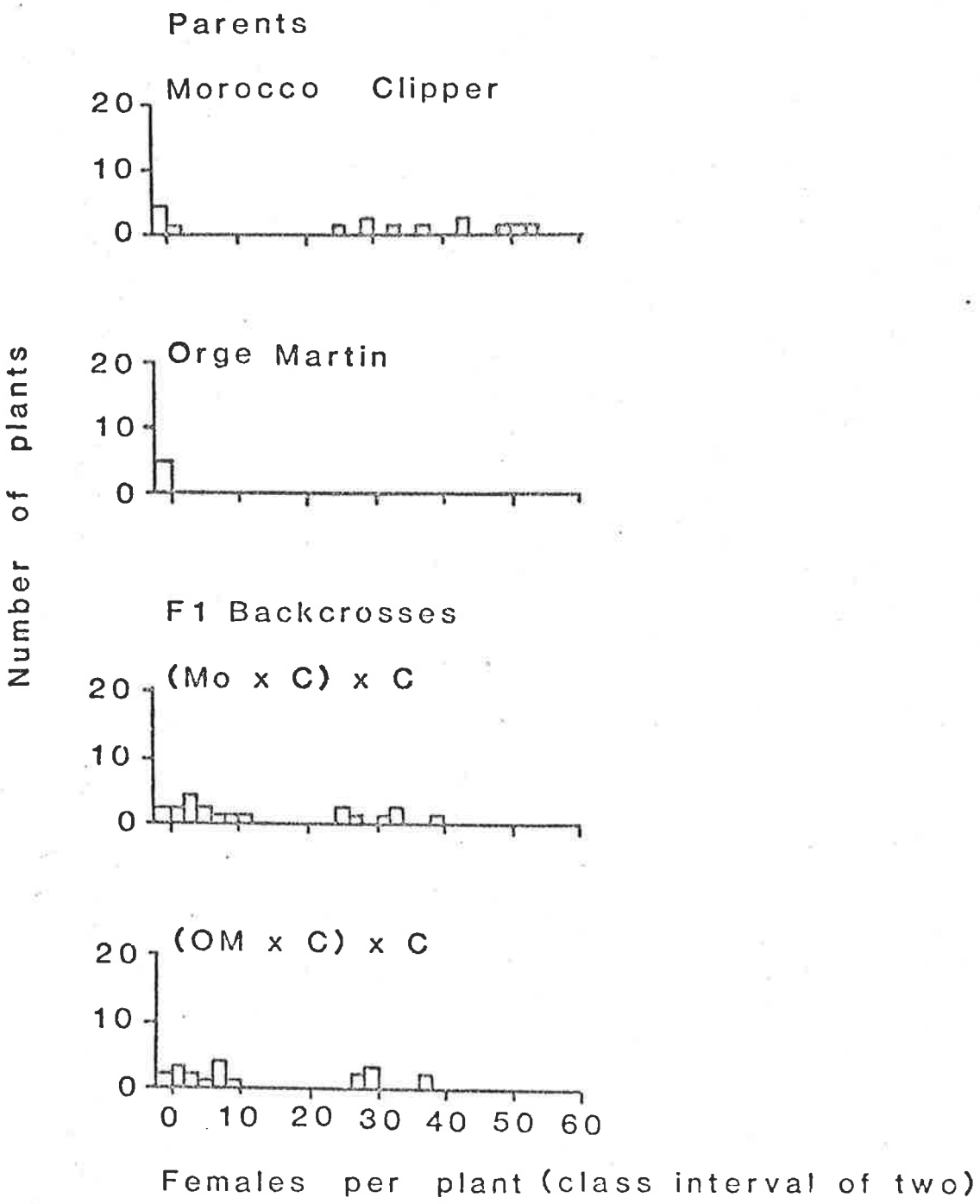

Parents
Morocco
Clipper
Orge Martin
F1 Backcrosses
(Mo x C) x C
(OM x C) x C
Number of plants
20
10
0
0 10 20 30 40 50 60
Females per plant (class interval of two)

Figure 14. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single crosses of At x C and Ma x C and F1 populations of three way crosses of 1st and 2nd test of (At x Ma) x C and (At x Ma) x B.

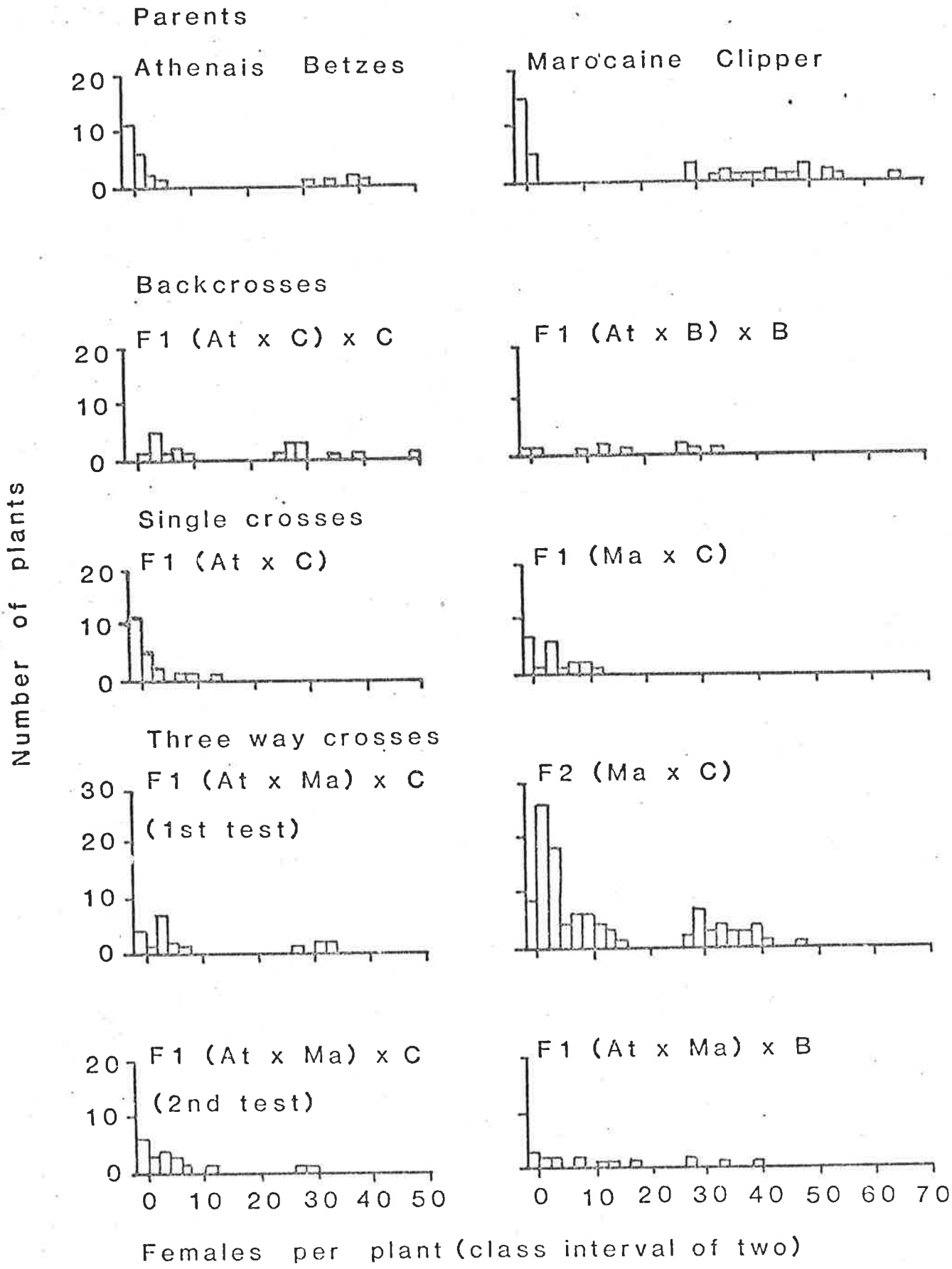

Parents
Athenais Betzes
Marocaine Clipper
Backcrosses
F1 (At x C) x C
F1 (At x B) x B
Single crosses
F1 (At x C)
F1 (Ma x C)
Three way crosses
F1 (At x Ma) x C
(1st test)
F2 (Ma x C)
F1 (At x Ma) x C
(2nd test)
F1 (At x Ma) x B
Number of plants
Females per plant (class interval of two)

Table 9. Test for segregation of head type in the F2 single cross populations of six barley cultivars.

F2 populations	No.of plants tested	Observed F2 plants two-row : six-row	Chi-square value for the expected segregation ratio 3 : 1 (df=1)		10 : 6 (df=1)	
AtxC	100	75 : 25	0	P=1	6.14	P>0.01
MaxC	100	76 : 24	0.01	P>0.90	7.21	P<0.01
NixC	100	66 : 34	3.85	P>0.025	0.38	P>0.50
MoxC	100	70 : 30	1.08	P>0.25	2.08	P>0.10
OMxC	100	64 : 36	5.88	P>0.01	0.04	P>0.75

The F1 populations of three way crosses of (AtxMo)xC, ~~(C8xMo)xC and (NixMo)xC~~ segregated into a 7 : 1 ratio for three independent genes for the resistance reaction (Table 8), their distributions resembled those for the F2 populations (Figs. 16 and 17) which suggested that the resistances in Athenais, ~~CI 8147 and Nile~~ was ~~were~~ not the same as the resistance in Morocco.

These results (Table 8) indicated that there was a high probability of difference between the genes for resistance in Athenais and Marocaine (Fig. 14), Athenais and Nile (Fig. 15) and Athenais and Morocco (Fig. 16), but a lower probability in CI 8147 and Morocco (Fig. 16) and Nile and Morocco (Fig. 17).

Observation on the head type in the F2 populations of the single crosses of At x C, Ma x C, Ni x C, Mo x C and OM x C showed that the F2's segregated into two-row resistant, six-row resistant, two-row susceptible and six-row susceptible plants. Head type is known to be under the control of two genes Vv and Ii. If the alleles of the latter are similar in both parents a 3 : 1 segregation ratio (two-row : six-row) occurs in the F2 of a

Table 10. Investigation on the relationship between resistance to *H. avenae* and head type in five barley cultivars.

F2 populations	No. of plants tested	Observed F2 plants RV: Rv: rV: rv	Chi-square value from contingency table	(df=1)
At x C	100	50 : 22 : 25 : 3	3.24	P>0.05
Ma x C	100	48 : 28 : 23 : 1	7.94	P<0.01
Ni x C	100	41 : 32 : 25 : 2	10.08	P<0.01
Mo x C	100	65 : 28 : 5 : 2	0.11	P>0.50
OM x C	100	60 : 35 : 4 : 1	0.08	P>0.75

Note : RV (genotype) = two-row resistant plant (phenotype)
Rv (genotype) = six-row resistant plant (phenotype)
rV (genotype) = two-row susceptible plant (phenotype)
rv (genotype) = six-row susceptible plant (phenotype)

cross between cultivars with different head types, but if the alleles are different the segregation ratio is 10 : 6. Commercial two-row cultivars contain the recessive i gene. The five crosses involving six-row parents were tested for these ratios (Table 9). The results indicated that Athenais, Marocaine and Morocco contain the same recessive i gene as Clipper but that Nile and Orge Martin probably have the dominant I gene.

On the association between resistance to *H. avenae* and head type, the results showed (Table 10) that in the crosses involving Athenais, Marocaine and Nile there was a high probability of linkage between the characters, but not in Morocco and Orge Martin.

Figure 15. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single crosses of (At x C) and (Ni x C) and F1 population of three way crosses of (Atx Ni)xC.

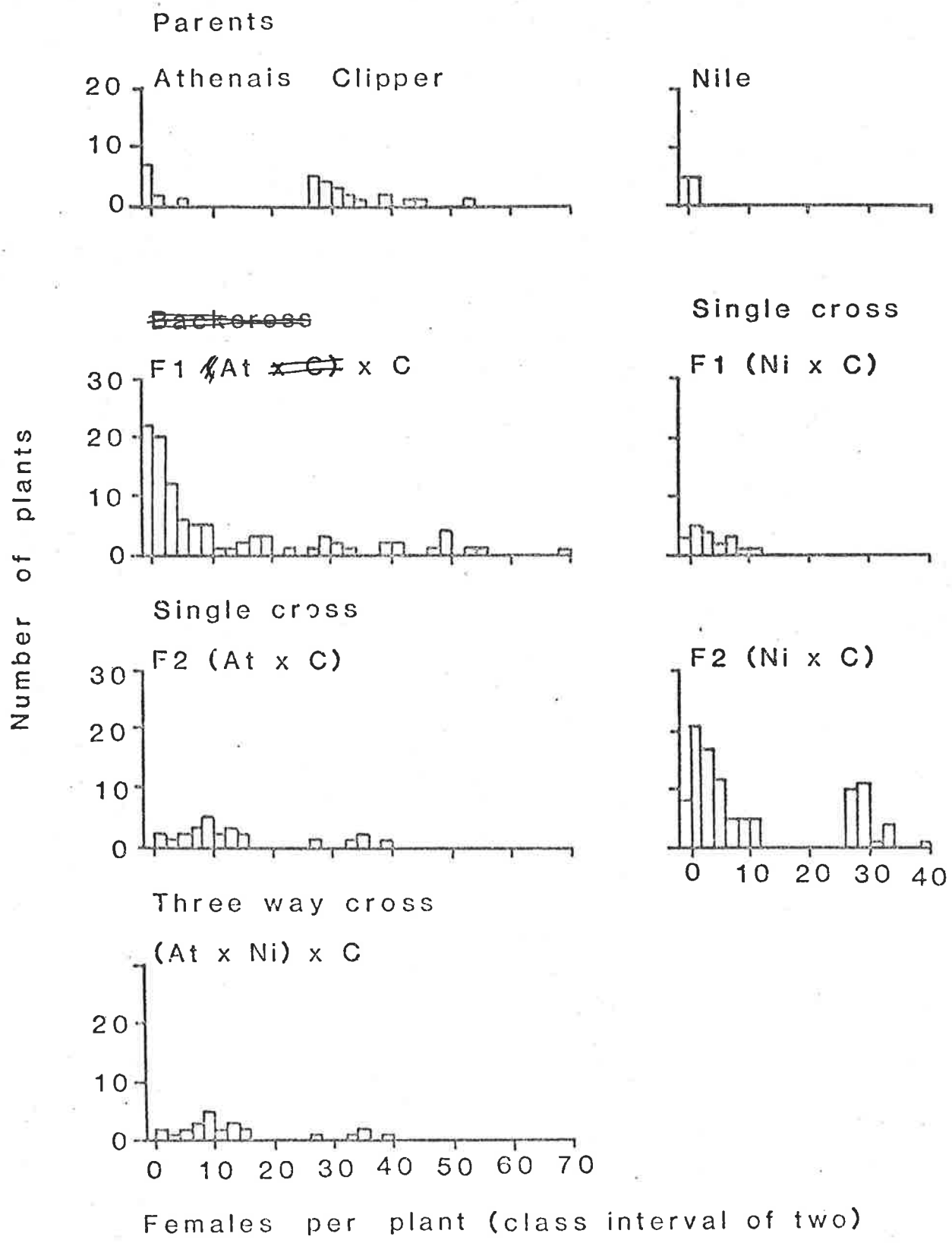

Parents
Athenais Clipper
Nile
20
10
0
Backcross
F1 (At x C) x C
Single cross
F1 (Ni x C)
30
20
10
0
Single cross
F2 (At x C)
F2 (Ni x C)
0 10 20 30 40
Three way cross
(At x Ni) x C
0 10 20 30 40 50 60 70
Number of plants
Females per plant (class interval of two)

Figure 16. Frequency distribution of number of white females of *H. avenae* per plant of parents, F1 and F2 populations of single crosses of (C8 x C) and (Mo x C) and F1 populations of three way crosses of (At x Mo) x C and (C8 x Mo) x C.

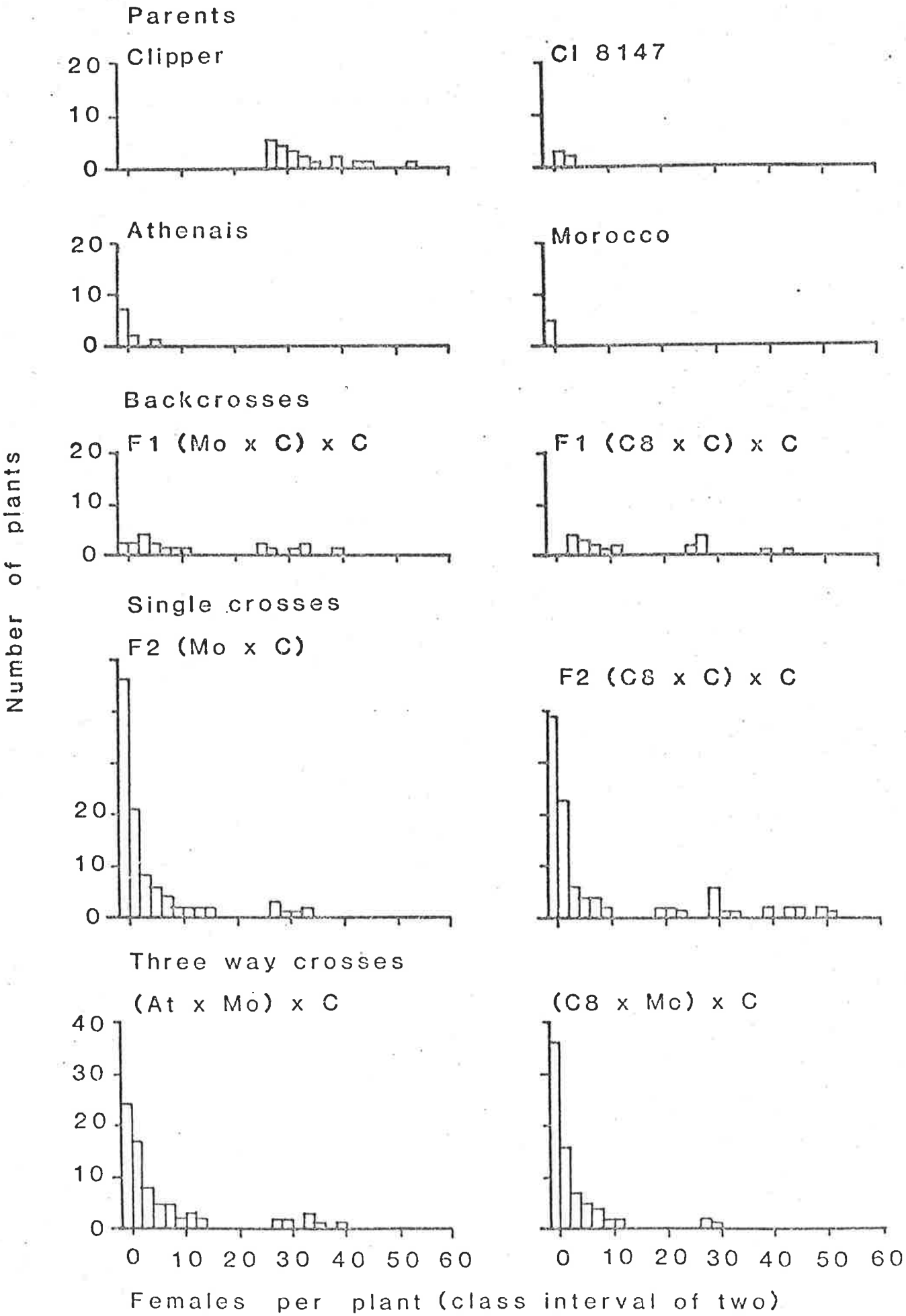
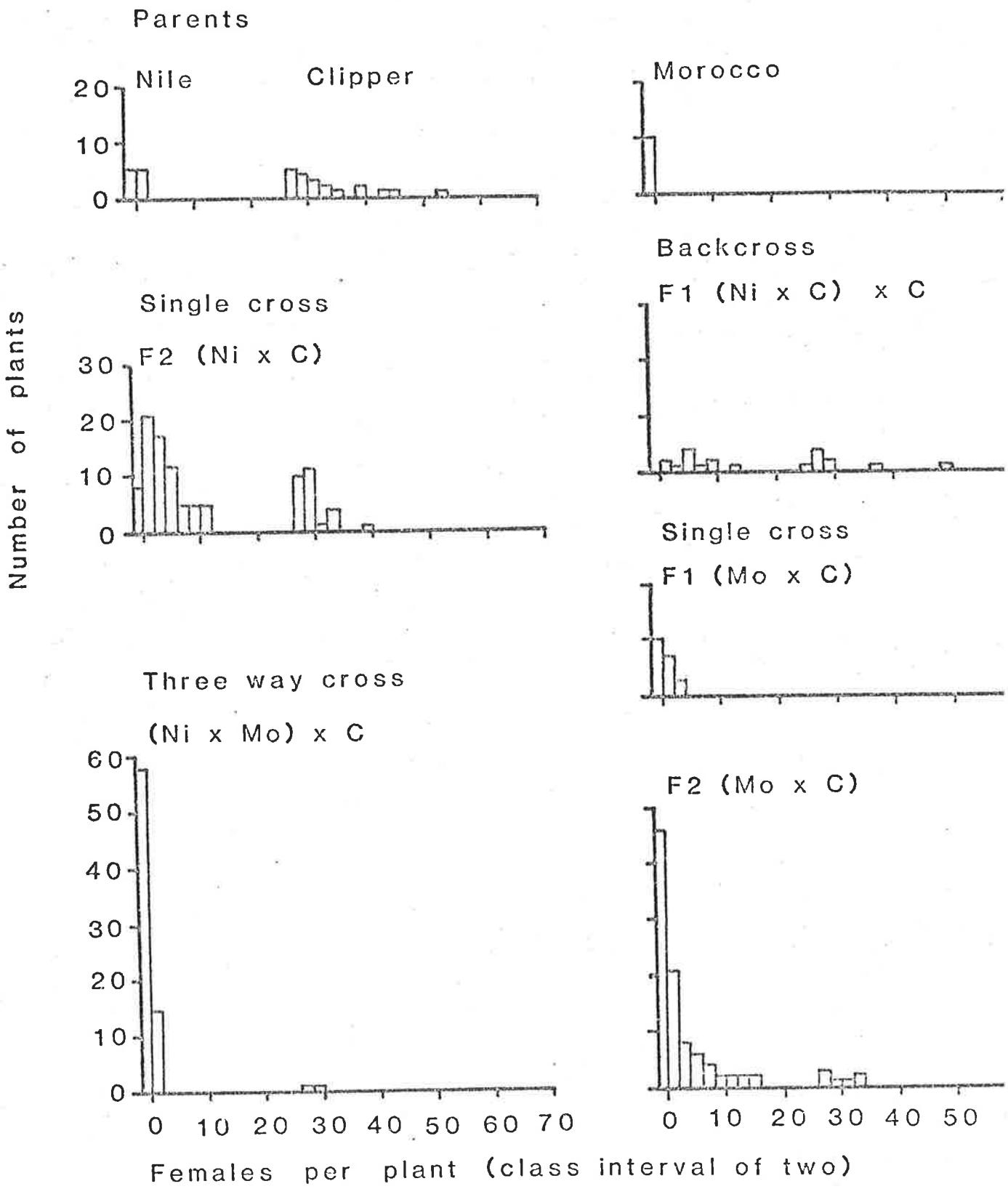

Parents
Clipper
CI 8147
Athenais
Morocco
Backcrosses
F1 (Mo x C) x C
F1 (C8 x C) x C
Single crosses
F2 (Mo x C)
F2 (C8 x C) x C
Three way crosses
(At x Mo) x C
(C8 x Mc) x C
Number of plants
Females per plant (class interval of two)

Figure 17. Frequency distribution of number of white females of *H. avenae* per plant of parents, F2 populations of single crosses and F1 populations of backcrosses of (Ni x C) and (Mo x C) and F1 population of three way crosses of (Ni x Mo) x C.


Parents
Nile
Clipper
Morocco
Backcross
F1 (Ni x C) x C
Single cross
F2 (Ni x C)
Single cross
F1 (Mo x C)
Three way cross
(Ni x Mo) x C
F2 (Mo x C)
Number of plants
0
10
20
30
40
50
60
70
Females per plant (class interval of two)

4.3 Discussion

With the modified assay system, the variation in reaction within cultivars was minimized. For most crosses there was clear separation of plants in the F2 populations into a susceptible or a resistant reaction. There was some drift in the reaction of heterozygous plants, and a few were classified as susceptible e.g. in the F2 populations of single crosses Athenais x Clipper, and CI 8147 x Clipper (Figs. 3 and 4). Of approximately 1500 plants tested only 16 could not be classified with ease. From these 16 plants (11 from Athenais x Clipper and five from CI 8147 x Clipper) nine plants (six from the cross Athenais x Clipper and three from the cross CI 8147 x Clipper) were tested in F3; most were homozygous for resistance and a few were heterozygous. This indicates that some heterozygous resistant plants can allow the production of more females than an acceptable number for resistance.

There were no aberrant susceptible plants in other crosses with Clipper. Nevertheless within the susceptible class there was variation in number of females produced. This could be due to external factors affecting gene expression, the genetic background of the plants or the heterozygous condition of the resistant plants.

Temperature has been reported affecting the expression of resistance to *H. avenae* in barley (Person-Dedryver & Doussinault, 1979) and in wheat (Cook & McLeod, 1980), although the report on wheat must be accepted with caution. Cook & Williams (1972) obtained evidence of 'background' resistance, and an example is found in the cultivar Sabarlis and KVL 191 (Ellis & Brown, 1976). The number of females on these cultivars was considerably fewer than on the control, but it was three times as high on KVL 191 as on Sabarlis; this was unexpected, as Sabarlis contains the resistance gene Ha2 from KVL 191 (Cook, 1975). Heterozygous plants have seemed particularly liable to 'erosion' of their resistance (Cook & York, 1982). There is no evidence yet that the resistance in barley to the Australian pathotype of *H. avenae* is affected by temperature.

Another source of variation in number and difficulty in interpretation was seen when Betzes was used as the susceptible parent. It was classified as having an intermediate reaction to *H. avenae*, with a lower number of females than Clipper (Table 2, section 3.2). Nevertheless, in comparison with Clipper, Betzes behaved in much the same way so that it had to be considered as a susceptible host. This suggests that the smaller number of females was due to an effect of environment or genetic background. Betzes was not a satisfactory susceptible parent for use in genetic analysis, because the minimum number of females produced on it tended to overlap with the range of heterozygous resistant plants. This suggests that for examining the inheritance of resistance it is advisable to choose as the susceptible parent a host cultivar which allows the development of the highest number of females in order to avoid overlap in the range of distribution of females on resistant and susceptible progeny.

The results from F1 populations of first backcrosses of (AtxC)xC and (C8xC)xC confirmed that there is one dominant gene conditioning the resistance in these cultivars. According to Cook & York (1981) the resistance gene in Athenais and CI 8147 is free from the Ha1 type of resistance of Drost/Ortolan (Table 11).

A single dominant gene is also responsible for the resistance in cultivars Nile and Marocaine 079. Results from the F2 and first backcross populations showed clear segregation for one dominant gene in each of the cultivars. In a cross between Nile x Siri (resistance type Ha2), Cook *et al.*, (1979) found there were no susceptible segregants in 100 F2 plants, and concluded that the same or closely linked loci are involved in these two cultivars.

The segregation in the F2 and first backcross populations of the crosses involving cultivars Morocco and Orge Martin showed that more than one gene was responsible for the resistance in these cultivars. O'Brien *et al.*, (1979) detected two genes in Morocco effective against the Australian pathotype of *H. avenae* and in Britain the differential F2 reaction of the cross Pallas 4 x Morocco line has confirmed this (Cook & York, 1981).

When Morocco and Orge Martin were the resistant parents the mean and range of white females per plant in the F1's were smaller than those of the F1's involving Athenais, CI 8147, Nile and Marocaine 079. Perhaps this expresses the number of genes involved in the resistance in cultivars Morocco and Orge Martin.

The gene for resistance in Athenais and Marocaine 079 may be the same (O'Brien *et al.*, 1979). However, in the F1 populations of the three way cross of (AtxMa)xC, five susceptible plants carried 29, 32, 33, 34, and 35 white females respectively. Another test was conducted to confirm this result; in this test, two susceptible plants were found, carrying 30 and 35 white females respectively; while in the cross of (AtxMa)xB, four susceptible plants were found, carrying 28, 29, 35 and 40 white females respectively. These results provide some evidence that the resistance in Athenais and Marocaine 079 may not be the same but that they could be closely linked to each other.

Results from the F1 populations of the three way crosses involving cultivars Athenais, Nile and Morocco gave evidence that the genes for resistance in Athenais, Nile, and Morocco are different. In the F1 progeny of the three way cross of (NixMo)xC and (C8xMo)xC fewer susceptible plants were recovered than in (AtxMo)xC. It is possible that expression of resistance genes is stronger in the crosses Nile x Morocco and CI 8147 x Morocco than in Athenais x Morocco.

The possibility that the resistances in Athenais and Morocco; Athenais and Nile; CI 8147 and Morocco; and Nile and Morocco were controlled by different genes (O'Brien *et al.*, 1979) is confirmed in this experiment. In the six barley cultivars evaluated here, at least four different genes conferring resistance to the Australian pathotype of *H. avenae* are clearly present with the possibility of two more in Morocco and Orge Martin. ~~Although~~ the two genes in Orge Martin were not tested in a three way crosses and may carry similar genes to some of the other cultivars.

A summary of the genes for resistance to the Australian pathotype of *H. avenae* in six barley cultivars and their interrelationship is given in Table 11.

Table 11. A summary of the resistance genes in six barley cultivars to the Australian pathotype of *H. avenae.*

Cultivars	No. plants tested	Females *H. avenae* produced (no/plant) Mean	Range	Nature of inheritance: Mode	Gene interrelationships	Comparisons with others
Athenais	50	1.70	0 - 9	Mono(D)	different from Ma different from Ni different from Mo	not Ha1[a)]
CI 8147	40	4.65	0 - 12	Mono(D)	different from Mo	not Ha1[b)]
Marocaine	30	0.77	0 - 4	Mono(D)	different from At	?
Nile	40	1.68	0 - 5	Mono(D)	different from At different from Mo	same or closely linked to Ha2[a)]
Morocco	45	0.13	0 - 2	Di(D)	different from At different from C8 different from Ni	Ha3[c)]
O/Martin	30	0.33	0 - 2	Di(D)	-	?

Note : Mono = monogenic
Di = digenic
(D) = dominant
reference :

a) Cook *et al.*, (1979)
b) Cook *et al.*, (1977)
c) Andersen & Andersen (1970; 1982)

Evidence was found which indicated that the resistance conferred by the Ha2 gene of barley No.191 (cb 824) is closely linked with Vv gene (two-row, six-row) on chromosome 2 (Cotten, 1966). Based on the two years' data the recalculated recombination percentage between Haha and Vv was 13.2 ± 1.96 (Cotten, 1967). Cotten & Hayes (1969) reported that the Ha2 gene of barley No.191 (cb 824) which is on the long arm of chromosome 2 is located between v and li genes, 10.2 ± 2.65 units from v (gene for head type) and 27.5±4.47 units from li (gene for ligules). Andersen & Andersen (1973) found that the recombination value between Ha2 gene of Siri and Vv were 19.2 ± 1.7 and 11.5 ± 1.4. They concluded that there were obviously two values for the genetic distance between Ha and Vv i.e. approximately 12 and approximately 18 units and both have a rather high probability. In Athenais the gene for resistance was not linked to head type, but the F2 populations of 50 plants were probably too small to detect the Ha-Vv linkage (Cook *et al.*, 1979).

The association between head type and resistance to *H. avenae* in five barley cultivars was tested for 'independence' by means of 2 x 2 contingency tables. The results (Table 10) indicated that there was a high probability of linkage between these characters in the crosses involving Athenais, Marocaine and Nile. This is in agreement with the reports in the previous paragraph which concluded that genes controlling these characters were located on the same chromosome. The results for crosses involving Morocco and Orge Martin in which two resistance genes have been identified did not show an association with head type but this could be due to interference between genes.

The sources of resistance to *H. avenae* examined are, all except one, six-row. In Australia there is a preference for two-row commercial cultivars. A close linkage between resistance and six-row type could make the task of transferring that resistance to acceptable local cultivars difficult and would necessitate selection within large populations in order to obtain the desired recombinants. Clearly more detailed study with larger segregating populations than were used here would be needed to determine the various linkages with accuracy.

CHAPTER 5

EFFECT OF TIME OF INOCULATION WITH *H. avenae* ON NUMBER OF LARVAE AND ~~NUMBER OF~~ WHITE FEMALES IN BARLEY

In response to nematode infection, host genotypes will express resistance by either limiting the penetration of nematode larvae or preventing normal growth and development of female nematodes. Resistance in barley to *H. avenae* had no effect on larval penetration (Cotten, 1967) and similar numbers of *H. avenae* invaded roots of resistant plants as readily as those of susceptible plants (O'Brien & Fisher, 1978). But host effects on penetration occurred with *H. schachtii,* in which the reduced nematode numbers were due to a higher death rate (Shepherd, 1959). In *Meloiaogyne incognita acrita,* all larvae migrated out of the roots of resistant alfalfa plants after successful penetration, because there was no host response to infection (Reynolds *et al.,* 1970). Cytological changes occurred in the roots of corn (*Zea mays* L. cv Pride 5) when infected with *H. avenae* and these changes inhibited mating and reduced egg production (Johnson & Fushtey, 1966).

Very little is known of the response of barley resistant to *H. avenae* infection. This study is aimed at a better understanding of this response.

5.1 Effect of time of inoculation with *H. avenae* on number of larvae and number of white females in barley

5.1.1 Materials and Methods

Four barley cultivars used in this experiment were Morocco a resistant cultivar from North Africa, and three cultivars from South Australia, Galleon resistant to the Australian pathotype of *H. avenae,* Clipper susceptible and Schooner which is still

under investigation; these cultivars were tested for their response to infection with the Australian pathotype of *H. avenae* .

Seeds were pregerminated (see section 3.1) and planted in pots of diameter 10 cm and 10 cm height filled with sandy loam containing John Innes nutrients at half strength and no peat. The experiment was laid out in a split-plot factorial design and conducted in a growth room at a constant temperature of 15°C with 10 hours of continuous fluorescent light in each 24 hour period. The seedlings were inoculated with 200 juveniles of *H. avenae* at planting or 10 or 20 days after planting. Twelve seedlings of each cultivar were inoculated at each time, six plants were harvested 20 days after each inoculation for assessment of number of larvae and six plants were harvested 70 days after the last inoculation for assessment of number of females. For assessment of number of larvae, the roots were washed free of soil particles and then stained using lactophenol cotton blue (Goodey, 1937; Goodey, 1957), before counting under 10x magnification. Assessment for number of white females was as described in section 3.1.

Analysis of variance was done on all the variables measured and the difference between the means was tested using the least significant difference test.

5.1.2 Results

For all the variables measured there was no interaction between cultivars and time of inoculation (Appendices 3a and 3b). In all cultivars, the highest number of larvae establishing in the roots was found in plants inoculated 10 days after planting, and the lowest number of larvae in plants inoculated at planting (Table 12). Overall, Clipper had the highest number of larvae establishing in the roots, with Morocco the lowest, Schooner and Galleon had a similar number of larvae in their roots (Table 13). These results showed that resistance did not prevent larvae establishing in barley roots, although fewer were found in Morocco and Galleon. When inoculated at time of planting, Morocco had the lowest number of larvae established in the roots, followed

Table 12. The number of larvae established and number of white females produced in barley roots, after inoculation at different times and harvested 20 days after each inoculation.

Time inoculation (days)	Larvae established (mean no/plant)	White female produced (mean no/plant)
Planting	32.50 **a**	3.92
10	62.50 **c**	3.00
20	50.60 **b**	2.67
LSD (P=0.05)	6.84	**n.s**

Note : mean values followed by different letters are significantly different at P=0.05

by Schooner, and then Galleon and Clipper. When inoculated at 10 days and 20 days after planting, Morocco had fewer larvae established compared to Galleon, Schooner and Clipper. On average, Morocco significantly had the lowest number of larvae established with Clipper the highest, but there was no significant difference between Galleon and Schooner (Table 13).

In all cultivars, different times of inoculation did not significantly affect the number of females produced; although fewer were found when the plants were inoculated at 20 days after planting, with the highest when inoculated at planting (Table 14).

Clipper produced the highest number of white females per plant followed by Schooner and Galleon, the differences were significant (Table 14). In Morocco, no

Table 13. The number of larvae established in four barley cultivars after inoculation at different times and harvested 20 days after each inoculation.

	Larvae established (mean no/plant)			
	Time inoculation (days)			
Cultivars	Planting	10	20	Mean no.larvae/plant
Morocco	22.80	41.20	28.20	30.70 **a**
Galleon	41.20	64.20	53.00	52.80 **b**
Schooner	31.70	70.70	53.00	51.80 **b**
Clipper	46.20	73.80	68.30	62.76 **c**
LSD (P=0.05)				7.88

Note : mean values followed by different letters are significantly different at P=0.05

Table 14. The number of white females produced in four barley cultivars.

	White females produced (mean no/plant)			
	Time inoculation (days)			
Cultivars	Planting	10	20	Mean no.females/pl
Morocco	0	0	0	0
Galleon	0	0.17	0.67	0.28 **a**
Schooner	6.67	5.00	2.83	4.83 **b**
Clipper	9.00	6.83	7.17	7.67 **c**
LSD (P=0.05)				1.49

Note : mean values followed by different letters are significantly different at P=0.05.

white females developed at any time of inoculation. In Galleon, no white female developed when inoculated at time of planting and similar, but negligible numbers of white females were produced when inoculated at 10 and 20 days after planting (Table 14).

5.1.3 Discussion

In all cultivars, when inoculated at time of planting and 20 days after planting, fewer larvae were in the roots than when inoculated at 10 days after planting, yet numbers of females in susceptible hosts may have been greater when inoculated at planting. This result can probably be explained by the distribution of larvae within the root system. At planting, about three root tips were available for the nematodes to penetrate and a little later some root tips of lateral branches would become available. By 10 days, probably five seminal root-tips would be available and many more root-tips of lateral branches. By 20 days, the root-tips of the main axes of the seminal roots would have been at the bottom of the pot, together with the root-tips of some lateral branches. This growth habit may explain, to some extent, the differences in numbers of larvae in the root systems at the various times. As more root-tips would be present at 10 days, it would be expected that more larvae would penetrate and establish as the results showed. This pattern was similar whether resistance genes were present or not though fewer larvae were found in the roots of Morocco compared to Galleon, Schooner and Clipper.

The resistance of Morocco severely reduced the number of larvae which established. In susceptible and resistant roots of wheat larvae of *H. avenae* had established within 12 hours (O'Brien, 1976). So sampling twenty days after inoculation should allow time for the larvae to establish within the root. It suggested that in Morocco, the larvae could not find establishment sites and left the roots. The resistance in Galleon reduced numbers established but not to a great extent. In fact, numbers in Galleon and Schooner ~~did not differ.~~ were similar The resistance in Morocco ~~then~~ probably differs

from that in Galleon and as Morocco has two genes for resistance (section 4.2) it might be suggested that one of these affects larval establishment and the other development of female nematodes. The result in this experiment showed that in Galleon, only the second type of resistance is present. Less damage is likely to result with resistance to larval establishment as probably 'syncytia'would not form.

Despite the fact that in all cultivars more larvae established when inoculated 10 days after planting, at least in susceptible hosts more females appeared to result from inoculation at planting. This could result if females were produced mainly on the primary axes of the root system and not in the lateral branches. The proportion of larvae established in the primary axes and lateral branches needs to be determined.

5.2 Effect of ~~different~~ time of inoculation with *H. avenae* on number and distribution of larvae in the root system of barley

This section investigated the distribution of *H. avenae* larvae in barley root systems and whether different times of inoculation had an effect on the number of larvae established and the number of white females developed.

5.2.1 Materials and Methods

Materials and methods used were similar to those in section 5.1.1. Twenty four seedlings of each of the barley cultivars Schooner and Clipper were inoculated at each of three times of inoculation; twelve plants were harvested 20 days after each inoculation for assessment of numbers of larvae and twelve plants were harvested 70 days after the last inoculation for assessment of number of females. Before planting the germinated seeds were observed for number and length of seminal roots and sampling of plants was

taken at 10 and 20 days later for similar observations. Numbers of larvae were counted separately in primary axes (seminal) and secondary (lateral) roots. Methods for larval and female assessment were as described in section 5.1.1.

5.2.2 Results

There was no interaction between cultivars and time of inoculation (Appendices 3c to 3f). As in the results of the previous experiment (section 5.1.2) the highest number of larvae established was found when the plants were inoculated at 10 days after planting. There was no significant difference in number of larvae when the plants were inoculated at planting or 20 days later (Table 15). However, when the number of larvae established in the root were assessed separately in primary axes and lateral branches there were fewer larvae in the primary axes when older plants were inoculated. But in the lateral branches more larvae were found when inoculation was delayed with the highest being at 10 days after planting. Numbers of larvae in the primary axes and lateral branches were about equal when inoculated at time of planting. This number decreased in the primary axes but increased in the lateral branches when inoculation was applied at 10 and 20 days after planting (Table 15).

At time of planting seedlings had one to three primary axes in the barley cultivar Clipper with root length ranging from 0.1 to 0.8 cm; and in the cultivar Schooner there were one to four primary axes with root length ranging from 0.3 to 1.0 cm. Ten days later the plants in both cultivars had five to six primary axes and lateral branches with the seminal root length ranging from 11.5 to 18.0 cm. Twenty days after planting the plants had five to six primary axes and lateral branches, with the primary axes length ranging from 14.5 to 22 cm. In both cultivars the highest number of females produced was found when the plants were inoculated at planting and the number was reduced significantly by delayed inoculation (Table 16). There were more larvae establishing in the root system in Clipper compared to Schooner and the difference was significant

Table 15. The distribution of *H. avenae* larvae in the root systems of barley, inoculated at different times and harvested 20 days after each inoculation.

Time of inoculation (days)	Mean number larvae established in the root system		
	Primary axes	Lateral branches	Total
Planting	30.13 **c**	37.20 **a**	67.30 **a**
10	8.83 **b**	69.90 **b**	78.80 **b**
20	5.50 **a**	64.30 **b**	69.90 **a**
LSD (P=0.05)	2.28	7.15	7.93

Note: mean values followed by different letters are significantly different at P=0.05.

Table 16. Effect of different times inoculation on the number of white females produced in barley, harvested at 90 days after the first inoculation.

Time inoculation (days)	Mean number white females per plant	
Planting	20.04 **c**	
10	9.71 **b**	
20	5.58 **a**	LSD (P=0.05) =2.78

Note: mean values followed by different letters are significantly different at P=0.05.

Table 17. The distribution of *H. avenae* larvae in the root systems of barley cultivars Schooner and Clipper.

Cultivars	Mean number of larvae established in the root system		
	Primary axes	Lateral branches	Total
Schooner	13.64 **a**	53.50 **a**	67.20 **a**
Clipper	16.00 **b**	60.80 **b**	76.80 **b**
LSD (P=0.05)	1.86	5.83	6.87

Note: mean values followed by different letters are significantly different at P=0.05.

Table 18. The number of white females in barley cultivars Schooner and Clipper .

Cultivars	Mean number white females per plant	
Schooner	10.25 **a**	
Clipper	13.31 **b**	LSD (P=0.05) =2.27

Note: mean values followed by different letters are significantly different at P=0.05.

(Table 17) and similar results were obtained for the number of females produced per plant (Table 18).

5.2.3 Discussion

Fewer larvae established and fewer females developed in Schooner compared to Clipper. There is no information available on the reaction of Schooner to *H. avenae,* but this result indicated that it has no resistance to this nematode, although fewer females were produced than on Clipper.

There was an effect of delaying the time of inoculation on the establishment and development of nematodes. When inoculated at time of planting, the larvae penetrated the main axes and only later when they became available did they penetrate the lateral branches. Fewer larvae were able to penetrate the primary axes at 10 and 20 days after planting because the root tips had grown out of range, but more larvae were found in the lateral branches. The earlier the inoculation, the greater the proportion (and number) of larvae in the main axes and the greater the number of females produced. This result showed that the type of root available is important in the production of females in barley.

The development of *H. avenae* larvae in the roots of cereals is dependent on the formation of feeding sites or syncytia (Giebel, 1982). It is possible the nematodes that do develop in the lateral roots were mainly males; and that lateral branches being thinner than the seminal roots did not have sufficient nutrients necessary for female development and can therefore support fewer females. More study is needed to investigate the relationship between the sex of the nematode and the position in barley root system where the nematodes invaded and developed.

CHAPTER 6

INVESTIGATION ON THE POSSIBILITY OF CHANGES IN VIRULENCE IN THE *H. avenae* POPULATIONS IN SOUTH AUSTRALIA

A danger in breeding resistant cultivars is the possibility of developing resistance-breaking pathotypes (Williams, 1970). It is likely that the growing of genotypes resistant to specific nematode races will lead to an increase in the frequency of nematode individuals with virulence genes able to overcome the resistance genes in the host. A plant gene that will eliminate almost all members of a nematode population applies great selection pressures in favour of surviving individuals with uncommon genes (Jones *et al.*, 1981). The rate of increase of such individuals will depend on the frequency of the virulence genes in the initial population, their dominance relationship, their mutation rate, the carry-over of unhatched larvae from one season to the next, and the frequency of cropping with resistant and susceptible crops (Hayes & Cotten, 1970).

H. avenae populations in Australia have been introduced either from Europe or England some 100 years ago. It seems that they came from a single cyst, since they behave as a pure, uniform population with so far no change in virulence. Results from testing wheat resistance showed no change in the number of females produced after six generations (Fisher, pers. comm.).

The objective of this experiment was to investigate the possibility of changes in virulence in the *H. avenae* populations in South Australia. Whether the reproductive capacity, i.e. production of females and therefore of eggs, was increased, maintained or decreased, when populations of *H. avenae* were cultured repeatedly on barley with and without resistance genes.

6.1 Materials and Methods

Four barley cultivars were used in this experiment, Clipper as the susceptible standard, Galleon as the commercial resistant cultivar, WI 2231D a susceptible sister line of Galleon, and Prior regarded as having an intermediate reaction (see section 3.2). Cysts of *H. avenae* were collected in late summer 1982 from infested barley roots of the respective cultivars grown in the field. Cysts from Prior were collected from Charlick Exp.Sta, cysts from Galleon, WI 2231D and Clipper were collected from Pinery and Charlick Exp. Sta. After the cysts were removed from the roots and soil, they were stored at 20° C for 8 weeks and then at 10° C for 4 weeks.

The first experiment in pots produced the second generation of *H. avenae*. The experiment was conducted in the glasshouse in early winter 1982. The plants were grown in pots of diameter 15 cm and 14 cm height, filled with a sandy loam containing John Innes nutrients at half strength and no peat. Twenty plants with one plant per pot were grown for each cultivar. Inoculation was applied as follows : two cysts were placed inside a terylene bag which was placed approximately 1,5 to 2 cm deep in the soil, and the pre-germinated barley seedling was planted above the bag. The plants were grown until maturity. After the plants had matured they were removed from the pots and the cysts were collected from the roots and soil as described in section 3.1. The total number of cysts per plant was counted. For number of eggs per cyst, samples were taken from the total number of cysts after leaving some cysts to be used as inoculum in the next generation in pots. Cysts for inoculum were stored at 5° C until needed for the third generation in the following winter 1983. Before using these cysts for inoculum, cysts were taken from 5° C and stored at 20° C for 8 weeks and then at 10° for 4 weeks to stimulate hatching. The fourth generation in pots was conducted in winter 1984.

6.2 Results

In all cultivars, in the second generation there was an increase in number of cysts per plant compared to the number of cysts per plant collected from the field (Table 19). Prior produced the highest number of cysts with an average of 39.65 cysts, WI 2231D 32.00 cysts, Galleon 4.65 cysts and Clipper 17.83 cysts per plant.

The third generation also showed an increase in number of cysts on WI 2231D (75%), Prior (153.21%) and Clipper (30.80%) compared to the second generation. But there was about 45% reduction in number of cysts produced on Galleon.

The fourth generation in pots showed a slight decline in number of cysts on WI 2231D (21.80%), Prior (19.87%) and Clipper (15.95%), and on Galleon about 17% reduction compared to the third generation.

Because number of cysts on the susceptible hosts varied from year to year, the number of cysts on Galleon the most resistant cultivar was expressed as a percentage of the number of cysts on Prior the most susceptible in this experiment. For the second, third and fourth generations, the number of cysts on Galleon as a percentage of those on Prior were 11.73%, 2.46% and 2.55% respectively.

The number of eggs per cyst on Galleon was in most generations significantly fewer than on the other three cultivars (Table 20).

In the second generation on Clipper there were ten plants which produced low numbers of cysts ranging from zero to seven cysts per plant. It was suspected that the cysts collected on Clipper from the field and used as inoculum were contaminated with fungi which caused poor hatching in pots or the seeds were genetically not pure. On Galleon there were five plants which produced a high number of cysts ranging from 10 to 20. The possible reasons for this result on Galleon were : it could be due to more aggressive nematode individuals or it was simply normal variation. To test the first possibility, the cysts from four Galleon plants which had more than ten cysts per plant

Table 19. The number of cysts per plant in four barley cultivars.

	H. avenae generation (number cysts/plant)							
	Field	Pot						
	1981(**1st**)	1982 (**2nd**)		1983 (**3rd**)		1984 (**4th**)		
Cultivars	Mean	Mean	Range	Mean	Range	Mean	Range	
Galleon	0.37	4.65	0 - 20	2.47	0 - 12	2.05	0 - 10	
WI 2231D	2.28	32.00	0 - 75	56.20	13 - 98	43.95	18 - 80	
Prior	2.67	39.65	6 - 134	100.40	31 - 194	80.45	22 - 137	
Clipper	3.20	17.83	0 - 51	72.75	12 - 139	61.15	10 - 98	

Table 20. The number of eggs per cyst in four barley cultivars.

	H. avenae generation (mean number eggs/cyst)			
	Field	Pot		
Cultivars	1981(**1st**)	1982 (**2nd**)	1983 (**3rd**)	1984 (**4th**)
Galleon	150.00 **a**	140.00 **a**	145.56 **a**	142.35 **a**
WI 2231 D	154.50 **a**	155.42 **b**	206.84 **b**	186.50 **b**
Prior	190.00 **b**	149.06 **b**	212.47 **b**	208.74 **c**
Clipper	220.75 **c**	156.20 **b**	214.59 **b**	201.12 **bc**

t value (P=0.05) : 2.306.

Note : mean values followed by different letters are significantly different at P=0.05.

Table 21. Number of white females produced on Clipper in the tube-test.

1982-2nd generation in pots			1983-seed homogeneity test in tubes	
Plant no.	Mean no. cysts/plant	No. plants tested	Mean no. females/plant	Range
Control	-	20	41.30	34 - 60
1	1	10	39.33	33 - 42
2	4	10	37.66	33 - 45
5	0	10	35.33	33 - 38
8	4	10	37.16	31 - 46
9	2	10	37.33	33 - 39
11	2	10	36.16	30 - 41
15	7	10	40.83	33 - 53
17	0	10	33.83	31 - 40
18	4	10	36.00	34 - 38
19	6	10	37.33	35 - 41

Table 22. Number of white females and number of cysts produced on Galleon.

1982-2nd generation in pot			1983-seed homogeneity test in tube		1983-3rd generation in pots		
Plant no.	Mean cysts/pl	No.plants tested	Mean females/pl	Range	No.plants tested	Mean cysts/pl	Range
Control	-	20	3.0	0 - 10			
2	10	10	2.4	0 - 8	4	7.00	1 - 12
5	20	10	3.6	0 - 12	9	1.20	0 - 3
9	12	10	2.8	0 - 9	4	5.25	1 - 10
16	15	10	2.9	0 - 12	6	2.16	0 - 6

were selected for inoculum in the third generation. To test the second possibility, Galleon plants which produced more than ten cysts and similarly for Clipper which had less than seven cysts per plants were selected for further test using p.v.c tubes in the growth room (see section 3.1). The assumptions were as follows :

1. If in the next generation there is an increase in the total number of cysts produced per plant on Galleon, it means a more aggressive nematode individual would be selected out from the *H. avenae* population.

2. Normal variation would show, if in the next generation the range in the number of cysts produced per plant on Galleon is similar to the previous generation.

3. If the seeds are not genetically pure, there would be a decrease in the total number of females produced per plant on Galleon and Clipper in the tube test.

The tube test result on Clipper showed that the seeds used were genetically pure as all gave a susceptible reaction to infection with *H. avenae* (Table 21). On Galleon the results also showed that the seeds were genetically pure as all gave resistant reaction to

infection with *H. avenae* (Table 22). Comparisons with results from the third generation in pots indicated that it was unlikely that a more aggressive nematode individual was present (Table 22).It is possible that the high number of cysts per plant on Galleon in the second generation in pots was due to normal variation.

6.3 Discussion

Over three generations in pots, there was no detectable change in proportion of cysts produced on Galleon. The resistance in Galleon did affect or reduce number of eggs per cysts as well as reducing the number of cysts per plant.

The number of cysts produced on susceptible hosts varied from generation to generation and so as an adequate expression of increase on resistant hosts, percentage results were used. These results suggested that there was either no change in number or the change was too small to detect. Testing over a longer period is necessary, but the results suggest that if a change occurs, it will not rapidly affect the population. The results also suggest that the few females which were produced on the resistant hosts are not of a different genetic constitution so they must result from variation in the resistant reaction and their presence does not suggest a rapid build up of an aggressive population.

Results in this experiment in pots under a glasshouse environment indicated that there was no rapid change in virulence in the *H. avenae* populations in South Australia, but testing over a longer period is essential both under controlled environment and in the field.

CHAPTER 7

RELATION BETWEEN INITIAL POPULATION DENSITY OF *H. avenae* Woll. AND GROWTH AND YIELD OF BARLEY

Most of the information on CCN and amount of damage on cereals in Australia relates to wheat (Meagher & Brown, 1974; Simon & Rovira, 1982). The amount of damage suffered by different wheat cultivars varies (Fisher *et al.*, 1981) but barley has not been examined.

The relation between the initial populations of *H. avenae*, growth and yield of a number of barley cultivars and final populations of nematode are investigated in this chapter.

7.1 General Materials and Methods

Barley cultivars (Appendix 4) with different degrees of reaction to *H. avenae* were grown in pots. Seeds were pregerminated as described in section 3.1. One seedling per pot was sown approximately 2.5 cm deep into the soil. A suspension of juveniles of *H. avenae* was used to produce different initial densities. A dilution series was made up to give the required initial density. Inoculation was applied immediately following planting. Plants were examined regularly for pests and diseases. Pyrethrum and Bayrusil were used to control green aphid and powdery mildew respectively, which occurred on infrequent occasions. Watering was applied once a week, or when the soil surface in the pot was dry. The experiments were laid out in a split-plot factorial design with five replicates. After the plants had matured, the pots were allowed to dry out. Plants were harvested by cutting at soil level. Cysts were recovered by washing the roots on a set of sieves (see section 3.1), and the number of cysts collected from the root and soil were counted.

The following characters were recorded : the number of days to awning, the number of days to heading, plant height, plant dry weight, root dry weight, total number of tillers (infertile and fertile tillers), number of fertile tillers (heads), grain yield, 100-seed weight, number of seeds per plant, number of cysts per plant and number of eggs per cyst.

The number of days to awning was assessed at the time the awns started to emerge from the boot. The number of days to heading was assessed when the flag-leaf had opened and showed half the length of the spike. Plant height measured from the base of the plant to the base of the head, without straightening out any natural bending, at the time of harvest. Plant dry weight was measured as the air-dried weight of the plant without roots, at the time of harvest. Root dry weight was measured as the air-dried weight of the roots, after collection of cysts. At harvest, the number of tillers was measured as the total number of tillers per plant and the number of heads indicated the total number of fertile tillers per plant. The yield was the total clean grain weight per plant. The total number of matured brown cysts per plant and the number of eggs per cyst were recorded.

Analysis of variance was calculated for each of the characters measured (where the results were significant the table of analysis of variance is given in appendices 6 - 9); the difference between treatments was calculated using the least significant difference test. Transformation log (x+1) was applied on data of number of cysts. Regression analysis was also done on all the characters measured.

Meteorological records at the Waite Agricultural Research Institute, on soil temperature, air temperature and rainfall during the experimental seasons from 1982 to 1984, are given in Appendix 5.

7.1.1 1982-Field pot experiment

7.1.2 Materials and Methods

To obtain information on the performance of selected barley cultivars grown under a range of *H. avenae* initial densities in pots under field conditions, plants were grown in pots, 25 cm internal diameter and 25 cm height, containing two kg sandy loam soil without organic matter. Pots were submerged in the soil in the field, leaving approximately 3 cm of edge above soil level; the experiment was arranged in a split-plot factorial design, with five replicates.

Barley cultivars used were : Clipper, the susceptible standard; Galleon, a resistant cultivar released in 1981, and WI 2231D, a sister line of Galleon which was classified as having an intermediate type of reaction to *H. avenae* (see section 3.2).

Initial population densities of *H. avenae* applied were as follows : 0 (control), 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 and 2048 juveniles per pot.

7.1.3 Results

Plant characters :

Number of days to awning and heading

In all cultivars, the number of days to awning and heading was slightly but not significantly increased as nematode population density increased (Table 23). In the absence of nematodes Galleon was first to expose awns followed 2 days later by WI 2231D and Clipper, but the difference between cultivars was not significant (Table 24).

Plant height.

Nematode initial density did not affect plant height (Table 23), but there was a difference between cultivars (Table 24). Galleon and WI 2231D were of similar height, but Clipper was taller.

Table 23. Effect of nematode initial density on growth and yield of barley (mean values over three cultivars).

Nematode initial density (juv/pot)	Awning (no.days)	Heading (no.days)	Plant height (cm)	Plant dry wt (g/pl)	Total tillers (no/pl)	Fertile tillers (no/pl)	Grain yield (g/pl)	100-seed wt (g/pl)	Seeds (no/pl)
0	87.47	99.33	66.36	78.5	42.60	39.40	32.57	4.66	700
1	87.47	99.33	65.85	67.3	39.80	36.67	30.03	4.58	657
2	87.47	99.33	65.46	65.6	39.60	36.53	29.05	4.56	643
4	87.80	99.67	64.97	64.6	39.27	36.00	28.10	4.61	601
8	88.13	99.93	64.63	62.5	38.27	33.67	27.75	4.58	601
16	88.13	99.93	64.71	61.7	38.27	34.73	28.09	4.60	600
32	88.53	100.20	64.36	56.6	31.73	28.53	26.10	4.56	553
64	88.53	100.20	64.33	55.0	33.33	28.53	26.26	4.62	563
128	88.53	100.20	63.60	52.4	30.80	28.27	24.21	4.49	540
256	90.13	101.67	63.98	50.4	29.67	26.73	23.95	4.35	525
512	91.13	102.67	63.26	48.2	28.73	25.53	23.24	4.45	516
1024	91.67	103.07	63.01	46.6	27.20	25.20	22.12	4.36	502
2048	91.67	102.87	62.49	46.8	26.33	24.73	22.09	4.37	492
LSD (P=0.05)	n.s	n.s	n.s	18.56	9.82	9.35	n.s	n.s	n.s

Table 24. Effect of nematode initial density on growth and yield of barley cultivars (mean values over all densities).

Cultivars	Awning (no.days)	Heading (no.days)	Plant height (cm)	Plant dry wt (g/pl)	Total tillers (no/pl)	Fertile tillers (no/pl)	Grain yield (g/pl)	100-seed wt (g/pl)	Seeds (no/pl)
Galleon	87.85	99.57	61.54^{a}	67.90^{b}	39.86^{b}	37.06^{b}	30.74^{c}	4.67^{b}	657^{b}
WI 2231D	89.86	101.46	62.67^{b}	62.20^{b}	39.60^{b}	34.71^{b}	26.58^{b}	4.58^{b}	571^{ab}
Clipper	89.22	100.91	68.94^{c}	44.40^{a}	23.37^{a}	21.58^{a}	21.96^{a}	4.30^{a}	501^{a}
LSD (P=0.05)	n.s	n.s	0.88	7.98	4.94	4.17	3.05	0.22	133

Note : mean values followed by different letters are significantly different at P=0.05.

Plant dry weight

In all cultivars, plant dry weight decreased as the nematode initial density increased (Table 23). The difference in plant dry weight when compared to control was significant at initial densities above 16 juveniles/pot. Galleon and WI 2231D had similar plant dry weights and both differed significantly from Clipper (Table 24).

Number of total and fertile tillers

Numbers of total and fertile tillers were affected by inoculation (Table 23); they decreased as the nematode initial density increased. When compared to the control, the differences in numbers of total and fertile tillers were significant from initial densities 32 to 2048 juveniles/pot; at initial density below 16 juveniles/pot they did not differ significantly from the control. Galleon and WI 2231D had a similar number of total and fertile tillers and both differed significantly from Clipper (Table 24).

Table 25. Effect of nematode initial density on the number of cysts per plant.

Nematode initial density (juv/pot)	Mean number of cysts per plant {log (x + 1)}		
	Galleon	WI 2231D	Clipper
0	0	0	0
1	0	0	0
2	0	0	0
4	0	0	0
8	0	0	0.23
16	0	0	0.23
32	0	0	0.69
64	0	0.96	1.27
128	0.60	2.09	2.33
256	0.90	2.72	2.99
512	1.46	3.01	3.22
1024	2.05	3.48	3.94
2048	1.53	3.73	4.30

LSD (P=0.05) : cultivar x nematode initial density : 0.62.

Grain yield

In all cultivars, grain yield was reduced as the nematode initial density increased, but this reduction was not significant (Table 23). Between cultivars the difference was significant (Table 24) with Galleon producing the highest yield followed by WI 2231D and Clipper the lowest.

100-seed-weight

In all cultivars, inoculation with different nematode initial densities did not have a significant effect on 100-seed-weight (Table 23). Galleon had the heaviest seed weight, followed by WI 2231D and Clipper (Table 24), the difference was significant between Galleon and Clipper but WI 2231D was not differed significantly from Galleon.

Number of seeds

Galleon produced more seeds per plant compared to WI 2231D and Clipper (Table 24). The difference betweeen Galleon and Clipper was significant with WI 2231D intermediate. Although the number of seeds per plant on all cultivars decreased as the nematode intial density increased, but the difference was not significant (Table 23).

Nematode character :

Number of cysts

The number of cysts produced on all three cultivars increased as initial density increased but more cysts were produced on Clipper than on WI 2231D on which more were produced than on Galleon (Table 25).

7.1.4 Discussion

In the absence of nematodes, the growth of the three cultivars differed; Clipper was distinct from the other two cultivars, which as sister lines, could be expected to be more closely related. Clipper was a taller cultivar, with fewer total and fertile tillers, so it is not surprising that the plant weight differed.

There was, however, no significant difference in the time of maturing (number of days to awning and heading) between the three cultivars. Although Galleon was first to expose awns followed about two days later by Clipper and WI 2231D. The difference between Galleon, WI 2231D and Clipper in plant height was due to the inherent character of these cultivars. Plant dry weight decreased as the nematode initial density increased.

Initial density of nematodes affected the ~~number of~~ total numbers of tillers and fertile tillers and presumably because of this affected ~~by~~ shoot weight. Again, Galleon and WI 2231D behaved in a similar way to the increasing density of nematodes with WI 2231D usually recording bigger losses than Galleon at the higher initial densities. Clipper was more affected by increasing density of nematodes than were Galleon and WI 2231D, producing fewer total number of tillers and fertile tillers and fewer seeds per plant in response to inoculation with different nematode initial density. It was surprising that significant differences in grain yield could not be shown. Reduction in grain yield due to nematode initial density, although not significantly different, was consistently greater with WI 2231D and Clipper than with Galleon and presumably this was due to the resistance of Galleon.

In the absence of nematodes Clipper produced the heaviest shoots, but at higher densities both Clipper and WI 2231D produced fewer shoots than Galleon, with Clipper more affected by inoculation. Galleon and WI 2231D produced similar number of tillers and heads (Table 24), and they showed a similar pattern as nematode initial density increased. Clipper produced fewer tillers and heads, and it was more affected by inoculation than were Galleon and WI 2231D. Compared to WI 2231D and Clipper, Galleon produced the highest grain yield, this reflected as more seeds produced per plant by Galleon. The lack of differences in yield probably resulted from excessive variation. As a result of inoculation, yield declined by about 30%, but such a massive decline was not significant even though a statistical analysis on the cultivars as a separate variable has been applied it did not produce a significant result. It seems likely that differences in

yield did occur but variation prevented the demonstration of this.

There was a significant interaction between cultivars and inoculation with different nematode initial density (Table 25). On Galleon there was no effect of nematode initial density on final number of cyst produced, but on WI 2231D and Clipper the final number of cysts produced were increased as the nematode initial density increased, with Clipper producing more cysts than WI 2231D .

Because of the absence of differences in yield under different nematode initial density in this experiment, tolerance of the three cultivars could not be examined. The experiment was repeated in the following year with attempts to control variation.

7.2 1983-Field pot and glasshouse experiments

To confirm the previous year's results on the effect of *H. avenae* initial population density on barley, a similar experiment with a few modifications was conducted both in the field and in the glasshouse. For the experiment conducted in the field, border plants were grown in an attempt to reduce variation caused by environment. Nematode initial density of 1 and 2048 juveniles were omitted from these experiments, instead intial densities of 96 and 192 were added and the highest initial density was 1024 juveniles per pot.

7.2.1 1983-Field pot experiment

7.2.1.1 Material and Methods

Materials and methods were similar to those in section 7.1.1, except in this experiment the soil used was sandy loam containing John Innes nutrients at half strength with no peat, and to minimize the effect of environment Galleon was grown in pots and

arranged as border plants between blocks and surrounding the experimental site. *H. avenae* initial population densities applied were as follows : 0 (control), 2, 4, 8, 16, 32, 64, 96, 128, 192, 256, 512 and 1024 juveniles per pot.

7.2.1.2 Results

Plant characters :

Number of days to awning and heading

In all cultivars, the number of days to awning and heading slightly increased as the nematode initial density increased. At the highest nematode density awning was delayed by 2 days in Clipper and WI 2231D, but this difference was not significant (Table 26) and there was no difference between cultivars (Table 27).

Plant height

Nematode initial density did not significantly affect plant height (Table 26). But the difference between cultivars was significant (Table 27), with Clipper being the tallest cultivar followed by Galleon and WI 2231D.

Plant dry weight

In all cultivars, plant dry weight decreased as the nematode initial density increased (Table 26). When compared to control (in the absence of nematodes), in all cultivars plant dry weight was significantly reduced from nematode initial density 96 to 1024 juveniles/pot. But there was no significant difference in plant dry weight between cultivars (Table 27).

Numbers of total and fertile tillers

The numbers of total and fertile tillers between cultivars differed significantly (Table 27). Galleon and WI 2231D had similar numbers but Clipper fewer than these two cultivars. The reduction in numbers of total and fertile tillers (Table 26) due to increasing density of nematodes was not significant but even so were quite substantial

Table 26. Effect of nematode initial density on growth and yield of barley (mean values over three cultivars).

Nematode initial density (juv/pot)	Awning (no.days)	Heading (no.days)	Plant height (cm)	Plant dry weight (g/pl)	Total tillers (no/pl)	Fertile tillers (no/pl)	Grain yield (g/pl)
0	88.87	99.87	59.60	42.00	27.13	24.93	21.05
2	89.27	100.27	59.30	38.20	26.53	24.53	19.85
4	89.00	100.27	57.51	37.60	25.20	23.00	19.18
8	89.40	100.40	57.49	37.10	24.80	22.47	19.19
16	89.40	100.40	56.93	34.80	24.47	21.73	19.04
32	90.00	100.60	56.77	35.70	24.07	21.07	18.29
64	90.00	100.60	57.01	35.30	23.80	20.60	18.25
96	90.00	100.60	57.90	33.80	23.40	20.40	18.08
128	90.40	100.93	57.10	30.10	22.87	19.87	16.81
192	91.07	101.53	53.21	30.30	22.47	19.53	16.46
256	91.07	101.53	57.40	28.90	21.47	17.87	15.73
512	91.47	101.93	55.73	24.60	18.93	14.67	14.11
1024	90.87	102.00	52.53	20.80	16.07	12.07	12.17
LSD (P=0.05)	n.s	n.s	n.s	11.76	n.s	n.s	n.s

Table 27. Effect of nematode initial density on growth and yield of barley cultivars (mean values over all densities).

Cultivars	Awning (no.days)	Heading (no.days)	Plant height (cm)	Plant dry weight (g/pl)	Total tillers (no/pl)	Fertile tillers (no/pl)	Grain yield (g/pl)
Galleon	89.11	100.31	57.04 **b**	37.00	27.17 **b**	23.17 **b**	20.26
WI 2231D	90.68	101.08	53.00 **a**	31.90	23.71 **b**	20.69 **b**	17.80
Clipper	90.40	101.14	60.38 **c**	30.20	18.63 **a**	16.23 **a**	14.60
LSD (P=0.05)	ns	n.s	2.97	n.s	4.45	4.31	n.s

Note : mean values followed by different letters are significantly different at P=0.05.

particularly in relation to number of fertile tillers in which a 50% reduction was obtained.

Grain yield

Neither nematodes nor cultivar caused significant differences in grain yield, although reduction in yield at the highest nematode intial density when compared to control was greater than 40 % (Table 26).

Nematode character :

Number of cysts

The number of cysts produced on Clipper and WI 2231D increased as initial density increased (Table 28) but there was no increase on Galleon. At the highest initial density, more cysts were produced on Clipper than on WI 2231D.

Table 28. Effect of nematode initial density on number of cysts.

Nematode initial density (juv/pot)	Mean number cysts per plant [log (x+1)]		
	Galleon	WI 2231D	Clipper
0	0.00	0.00	0.00
2	0.00	0.00	0.00
4	0.00	0.00	0.14
8	0.00	0.00	0.14
16	0.00	0.00	0.28
32	0.00	0.00	0.56
64	0.00	0.60	1.37
96	0.14	0.64	1.70
128	0.42	1.57	1.93
192	0.50	2.18	1.97
256	0.58	2.32	2.50
512	0.96	2.51	2.63
1024	1.32	3.12	3.49

LSD (0.05) : cultivar x nematode initial density : 0.50.

7.2.1.3 Discussion

Essentially this was a repetition of the first experiment with modification aimed at reducing variation. But the modifications either did not work or were overcome by a different environment in the second year, because similar results were obtained. Inoculation with different nematode initial densities had a significant effect only on plant dry weight but not on number of days to awning, number of days to heading, number of tillers, number of heads and grain yield. Between cultivars there were differences in plant height and number of total and fertile tillers, these differences may be due to the inherent character of the cultivars. Clipper was still the tallest cultivar, producing fewer infertile and fertile tillers, but the difference in dry shoot weight could not be demonstrated. Although different numbers of heads were produced by the cultivars, no difference in grain yield could be demonstrated. Density of nematodes had no significant effect on numbers of total and fertile tillers or grain yield despite differences of over 50% in some instances suggesting that variation was still excessive and the modification adopted did not have a significant effect.

The nematodes multiplied in much the same way in each experiment. There was interaction between cultivars and inoculation with different nematode intial densities. Galleon showed its resistance and limited multiplication while both WI 2231D and Clipper were susceptible and the number of cysts produced increased as the nematode initial density increased. In the second experiment, there was an indication that multiplication at the highest density was greater on Clipper than on WI 2231D suggesting that these cultivars were behaving in the different ways suggested by Jones & Kempton (1978) for cyst-forming nematodes. The overall results were a little dissappointing and further modification should be attempted.

7.2.2 1983-Glasshouse experiment

In the experiments in the field over two years, control of variation was not achieved. Increasing density of nematodes failed to affect growth and yield of barley. It was decided that under glasshouse conditions variation might be reduced and a similar experiment was set up.

7.2.2.1 Materials and Methods

The glasshouse had a glass roof only, the sides being made of wire mesh so that the environment was not completely different from that in the field. The plants were grown in pots of 10 cm internal diameter and 10 cm height; other materials and methods were as in section 7.2.1.1.

7.2.2.2 Results

Plant characters :

Number of days to awning and heading

Although in all cultivars the number of days to awning and heading increased as the nematode initial density increased, density did not have a significant effect on these characters (Table 29). There were differences between cultivars in number of days to heading but not in the number of days to awning (Table 30), with Clipper being approximately one day earlier.

Plant height

Nematode initial density did not affect plant height (Table 29). Galleon and WI 2231D were of similar height, with Clipper being the tallest cultivar and the difference was significant (Table 30).

Table 29. Effect of nematode initial density on growth and yield of barley (mean values over three cultivars).

Nematode initial density (juv/pot)	Awning (no.days)	Heading (no.days)	Plant height (cm)	Plant dry weight (g/pl)	Total tillers (no/pl)	Fertile tillers (no/pl)	Grain yield (g/pl)
0	78.40	92.07	42.87	2.39	3.67	2.07	0.93
2	78.40	92.07	42.78	2.33	3.60	2.07	0.91
4	78.40	92.07	42.37	2.28	3.60	2.07	0.90
8	78.40	92.07	42.35	2.27	3.53	2.00	0.89
16	78.47	92.07	42.25	2.25	3.53	2.00	0.89
32	78.73	92.20	42.15	2.25	3.47	1.93	0.88
64	78.73	92.33	42.14	2.24	3.40	1.93	0.87
96	79.00	92.40	39.24	2.24	3.47	2.00	0.87
128	79.00	92.40	41.75	2.23	3.47	1.93	0.87
192	79.00	92.40	41.31	2.20	3.20	1.93	0.86
256	79.40	92.80	40.87	2.09	3.20	2.00	0.85
512	79.60	93.00	40.31	2.05	3.20	1.87	0.82
1024	79.60	93.00	37.97	2.01	3.06	1.73	0.79
LSD (P=0.05)	n.s	n.s	n.s	n.s	n.s	n.s	n.s

Table 30. Effect of nematode initial density on growth and yield of barley cultivars (mean values over all densities).

Cultivars	Awning (no.days)	Heading (no.days)	Plant height (cm)	Plant dry weight (g/pl)	Total tillers (no/pl)	Fertile tillers (no/pl)	Grain yield (g/pl)
Galleon	78.52	92.42 **ab**	38.71 **a**	2.23	3.72 **b**	2.29 **b**	0.96 **b**
WI 2231D	78.88	92.78 **b**	38.99 **a**	2.15	3.45 **b**	2.08 **b**	0.88 **ab**
Clipper	79.17	91.92 **a**	47.67 **b**	2.28	3.08 **a**	1.52 **a**	0.78 **a**
LSD (p=0.05)	n.s	0.68	1.63	n.s	0.35	0.24	0.09

Note : mean values followed by different letters are significantly different at P=0.05.

Plant dry weight

In all cultivars, plant dry weight was slightly decreased as the result of inoculation, but the reduction was not significant (Table 29) and there was no difference between cultivars (Table 30).

Numbers of total and fertile tillers

In all cultivars, nematode intial density did not significantly affect numbers of total and fertile tillers (Table 29). There were significant differences in numbers of total and fertile tillers produced by the cultivars (Table 30). Galleon and WI 2231D produced similar numbers of total and fertile tillers but Clipper produced fewer than these two

Table 30. Effect of nematode initial density number of cysts.

Nematode initial density (juv/pot)	Mean number cysts per plant [log(x + 1)]		
	Galleon	WI 223 D	Clipper
0	0.00	0.00	0.00
2	0.00	0.14	0.28
4	0.00	0.28	0.36
8	0.00	0.77	0.94
16	0.00	0.82	1.19
32	0.00	1.51	1.71
64	0.00	1.95	2.35
96	0.28	2.17	2.98
128	0.50	2.54	2.92
192	0.50	2.77	3.16
256	0.69	3.25	3.61
512	1.36	3.72	4.10
1024	1.56	4.07	4.50

LSD (0.05) : cultivar x inoculation : 0.50

Grain yield

Nematode initial density did not affect grain yield (Table 29), although in all cultivars grain yield was reduced insignificantly as the nematode initial density increased. When compared to control (in the absence of nematodes) yield at the highest initial density was reduced by approximately 15 %. Clipper significantly produced less grains than Galleon but did not significantly differ from WI 2231D and WI 2231D did not significantly differ from Galleon (Table 30)

Nematode character :

Number of cysts

The final number of cysts produced on WI 2231D and Clipper increased as the nematode initial density increased, but the increase on Galleon was marginal though significant. Clipper allowed the production of significantly more cysts than WI 2231D which allowed significantly more than Galleon (Table 31).

7.2.2.3 Discussion

The pattern of growth of the plants under these conditions was different from those grown in large pots in the field. In the glasshouse in small pots, the plants did not grow as well, producing a smaller dry weight, fewer tillers and heads and less grain. This was probably a reflection of the small pot size reducing root growth and restricting water, thus supressing the growth variables. Nevertheless, the variation was also considerably reduced and the plants were much more uniform as considered by the lower least significant differences for most variables. This inhibition of growth by pot size, although it reduced variation, also removed any effect that density of nematodes had on growth variables such as plant dry weight and number of total and fertile tillers. However, it did show some differences in varietal characters that were not evident in the field pots e.g. Clipper yielded less than Galleon suggesting that Clipper reacted less

favourably to growth inhibition than Galleon or WI 2231D.

The numbers of cysts produced in pots varied in the two field trials and the glasshouse trial was similar to one of these. One remarkable change was that at the lower initial densities, cysts were produced on the susceptible cultivars in the smaller pots and this did not happen in the large pots in either experiment in the field. This suggests that the juvenile nematodes had a greater chance of finding roots in the smaller volume of soil and so a greater percentage penetration could be expected. It is thus even more surprising that no effect of nematodes could be found on the growth variables in the small pots.

7.3 1984-Glasshouse experiment

Although the results from 1983 field pot and glasshouse experiments were not satisfactory, there were strong indications of the modifications necessary to relate loss in yield to *H. avenae* initial population density. Small pots in the glasshouse reduced variation but the densities of nematodes were not high enough to cause damage, so the range of initial densities should be increased. As the low densities did not show any significant reduction in yield, these could safely be excluded. In addition to the cultivars used in previous experiments, Prior and Schooner were included for comparison. Prior is of the Chevalier strain imported from England, grown in South Australia, New South Wales, Victoria and Western Australia. It is a cultivar best suited to low-fertility, low-yielding conditions (Sparrow & Doolette, 1975) and was regarded as having an intermediate degree of resistance to *H. avenae* (see Section 3.2). Schooner is a malting type barley cultivar released recently in South Australia and its reaction to *H. avenae* is still under investigation.

7.3.1 Materials and Methods

H. avenae initial population density applied were as follows : 0, 15, 30, 45, 60, 120, 240, 480, 960, 1920, 3840, 7680 and 15360 juveniles per pot. The plants were grown in pots of 12.5 cm internal diameter and 12.5 cm height. Each pot was filled with 750 grams of sandy loam containing John Innes nutrients at half strength with no peat; pots were placed on trays to allow a regular watering from the base. The experiment was laid out in a split-plot factorial design with four replicates and set up in the same glasshouse as those described in section 7.2.2.1. Approximately eight weeks after inoculation, the base trays were filled with water containing a 1/4 strength Hoagland's solution, to maintain soil moisture and to add nutrients to the plants during the vegetative period.

Analysis of variance (Appendix 9) and regression analysis (Appendix 10) were done on the data of all characters measured in this experiment.

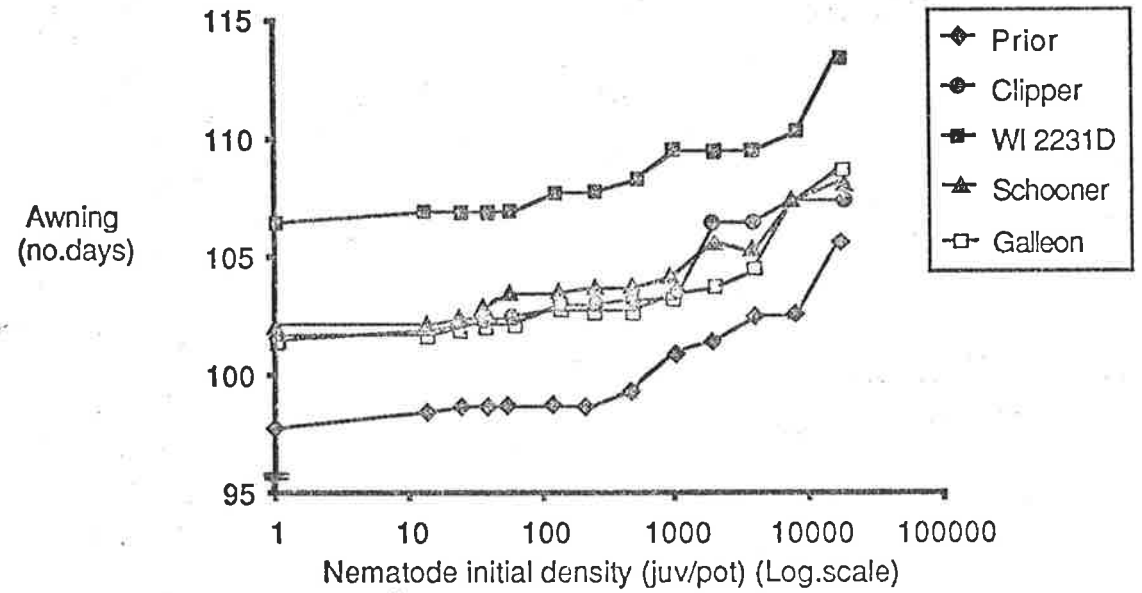
7.3.2 Results

Plant characters :

Number of days to awning and heading

The number of days to awning and heading increased significantly as the nematode initial density increased (Table 32). In the absence of the nematodes, Prior was the first cultivar to expose awns, reaching this stage in about 97 days; Clipper, Schooner and Galleon reached the awning stage at approximately the same time, 102 days after sowing and WI 2231D was four days later (Fig. 18). In each cultivar there was a delay in reaching maturity due to inoculation with nematodes and the length of delay increased with increasing density of nematodes, being about one week in all cultivars at the highest density (Table 32). Inoculation with different nematode initial densities delayed

Figure 18. The number of days to awning of five barley cultivars grown under different *H. avenae* initial population densities.


115
110
105
100
95
Awning
(no.days)
1
10
100
1000
10000
100000
Nematode initial density (juv/pot) (Log.scale)
Prior
Clipper
WI 2231D
Schooner
Galleon

heading, and the delay increased with increasing density of nematodes but at its maximum was about five days (Table 32). Regression analysis on number of days to awning and heading data showed that all cultivars had the same slope but with different intercept.

Plant height

In all cultivars, nematode initial density did not significantly affect plant height (Table 32). The difference between cultivars was significant, with Prior the tallest cultivar followed by Clipper, but there was no significant difference between WI 2231D and Galleon (Table 33).

Plant dry weight and root dry weight

In all cultivars, plant dry weight and root dry weight decreased as the nematode initial density increased (Table 32). At the highest nematode initial density, plant dry weight of WI 2231D was reduced more than that of Galleon. Prior, WI 2231D and Galleon produced the heaviest shoots with Clipper and Schooner the lightest (Table 33). Clipper also produced the lightest root weight followed by WI 2231D, Galleon and Prior, but Schooner produced the heaviest root weight (Table 33). Regression analysis on plant dry weight showed that there was a different slope and different intercept for each cultivar. But for root dry weight, all cultivars had the same slope with the same intercept.

Number of total and fertile tillers

The number of total and fertile tillers decreased as the nematode initial density increased (Table 32). In both variables, Galleon and WI 2231D produced similar numbers of each and significantly more than Prior, Clipper and Schooner, all of which produced similar numbers of total and fertile tillers (Table 33). Regression analysis on both variables showed that there was a different slope with different intercept for all cultivars (Fig. 19; regression lines on number of fertile tillers per plant).

Table 32. Effect of nematode initial density on growth and yield of barley (mean values over five cultivars)

Nematode initial density (juv/pot)	Awning (no.days)	Heading (no.days)	Plant height (cm)	Plant dry wt (g/pl)	Root dry wt (g/pl)	Total tillers (no/pl)	Fertile tillers (no/pl)	Grain Yield (g/pl)
0	101.95	108.80	74.79	15.82	1.89	9.90	9.30	7.04
15	102.30	109.00	74.67	15.61	1.84	9.70	9.15	6.87
30	102.55	109.05	74.57	15.56	1.77	9.60	9.00	6.74
45	102.75	109.25	74.49	15.53	1.71	9.55	8.90	6.76
60	102.80	109.30	74.40	15.35	1.66	9.35	8.80	6.57
120	103.15	109.50	74.39	15.31	1.69	9.40	8.95	6.58
240	103.20	109.50	74.22	15.11	1.65	9.15	8.80	6.44
480	103.50	109.75	74.22	14.78	1.58	8.95	8.65	6.43
960	104.30	110.30	73.98	14.80	1.62	8.65	8.40	6.36
1920	105.40	111.20	73.56	14.19	1.54	8.05	7.70	6.15
3840	105.85	111.70	73.56	13.89	1.50	7.85	7.55	6.02
7680	106.95	112.60	73.45	13.50	1.36	7.50	7.20	5.72
15360	108.70	113.95	72.91	12.47	1.20	6.90	6.75	5.13
LSD (P=0.05)	1.07	0.90	n.s	1.30	0.22	1.23	1.23	0.69

Table 32. Effect of nematode initial density on growth and yield of barley cultivars (mean values over all densities).

Cultivars	Awning (no.days)	Heading (no.days)	Plant height (cm)	Plant dry wt (g/pl)	Root dry wt (g/pl)	Total tillers (no/pl)	Fertile tillers (no/pl)	Grain yield (g/pl)
Galleon	103.62^{b}	109.08^{a}	67.36^{a}	14.86^{b}	1.58^{ab}	10.85^{b}	10.58^{b}	7.28^{b}
WI 2231D	108.50^{c}	112.48^{c}	68.97^{a}	15.72^{bc}	1.52^{a}	10.04^{b}	9.87^{b}	6.74^{b}
Schooner	104.37^{b}	111.25^{b}	70.29^{b}	13.63^{a}	1.83^{b}	7.81^{a}	7.46^{a}	5.64^{a}
Prior	100.19^{a}	108.27^{a}	90.60^{d}	16.00^{c}	1.68^{ab}	7.52^{a}	7.08^{a}	6.37^{a}
Clipper	103.87^{b}	110.42^{b}	73.24^{c}	13.60^{a}	1.47^{a}	7.85^{a}	7.00^{a}	5.81^{a}
LSD (P=0.05)	1.38	1.08	2.74	1.07	0.23	1.15	1.03	0.74

Note : mean values followed by different letters are significantly different at P=0.05.

Grain Yield

In all cultivars, grain yield was reduced as the nematode initial density increased (Table 32). Galleon and WI 2231D significantly yielded more grain than Prior, Clipper and Schooner (Table 33). Regression analysis on grain yield showed that the cultivars had the same slope but different intercepts (Fig. 20).

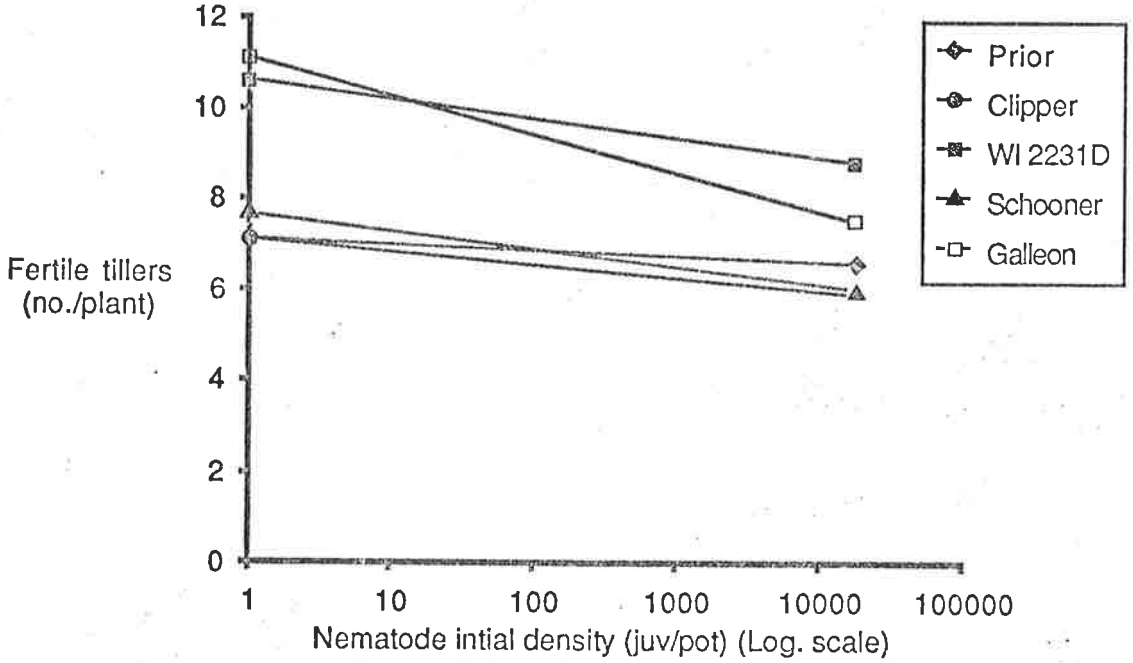
Nematode characters

Number of cysts per plant and number of eggs per cyst

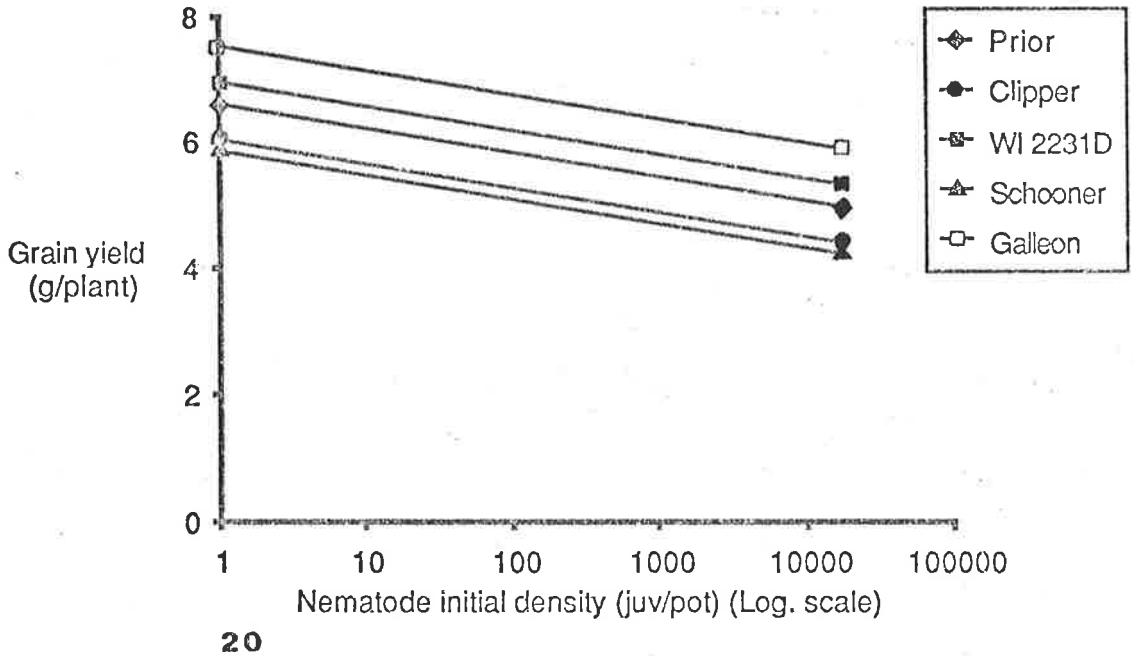
In numbers of cysts and eggs per plant there was a significant interaction between cultivars and nematode initial density. In all cultivars, as nematode initial

Figure 19. Relationship between number of fertile tillers of five barley cultivars and *H. avenae* initial population density.

Figure 20. Relationship between grain yield of five barley cultivars and *H. avenae* initial population density.


12
10
8
6
4
2
0
Fertile tillers
(no./plant)
1
10
100
1000
10000
100000
Nematode intial density (juv/pot) (Log. scale)
Prior
Clipper
WI 2231D
Schooner
Galleon

19


8
6
4
2
0
Grain yield
(g/plant)
1
10
100
1000
10000
100000
Nematode initial density (juv/pot) (Log. scale)
Prior
Clipper
WI 2231D
Schooner
Galleon

20

Table 34. Effect of nematode initial density on number of cysts.

Nematode initial density (juv/pot)	Mean number cysts per plant [log (x+1)]				
	Galleon	WI 2231D	Schooner	Prior	Clipper
0	0	0	0	0	0
15	0	0	0.17	0	0.17
30	0	0.97	0.17	0.72	0.72
45	0	0.92	0.17	0.52	0.82
60	0.17	1.15	0.35	0.97	1.02
120	0.17	1.11	0.45	1.10	1.42
240	0.45	1.51	0.35	1.10	1.53
480	0.69	2.25	0.68	1.82	2.27
960	0.68	2.36	1.29	1.83	2.41
1920	0.80	2.58	1.37	2.25	2.56
3840	0.80	2.91	1.61	2.40	2.91
7680	0.62	2.64	1.22	3.07	3.09
15360	0.62	2.64	1.22	3.97	3.09

LSD (P=0.05) : cultivar x inoculation : 0.28.

Table 35. Effect of nematode initial density on number of eggs.

Nematode initial density (juv/pot)	Mean number eggs per cyst				
	Galleon	WI 2231D	Schooner	Prior	Clipper
0	0	0	0	0	0
15	0	0	88.3	0	131.3
30	0	296.3	89.6	166.9	371.8
45	0	314.2	90.0	229.3	378.8
60	93.8	320.9	164.3	229.4	504.1
120	94.6	323.8	168.3	469.8	481.3
240	176.1	382.9	185.3	476.4	462.9
480	280.8	366.4	176.9	442.9	451.8
960	179.1	328.8	211.2	392.5	425.2
1920	179.0	321.9	212.7	312.5	333.7
3840	171.4	289.4	272.4	308.8	296.3
7680	168.2	248.0	270.3	271.2	264.5
15360	105.6	215.8	175.7	192.7	148.8

LSD (P=0.05) : cultivar x inoculation : 187.18

density increased, the numbers of eggs and cysts produced per plant increased (Table 34). Clipper, Prior and WI 2231D produced similar numbers and more than Schooner which produced more than Galleon. The relation of nematode initial density to cultivar in the production of cysts and eggs differed (Figs. 21 and 22). On Clipper and Prior numbers of cysts produced increased continually with increasing density. On WI 2231D and Schooner, numbers of cysts produced reached a maximum at an initial density of 3840 and then declined while on Galleon the changes in number though significant were suppressed considerably.

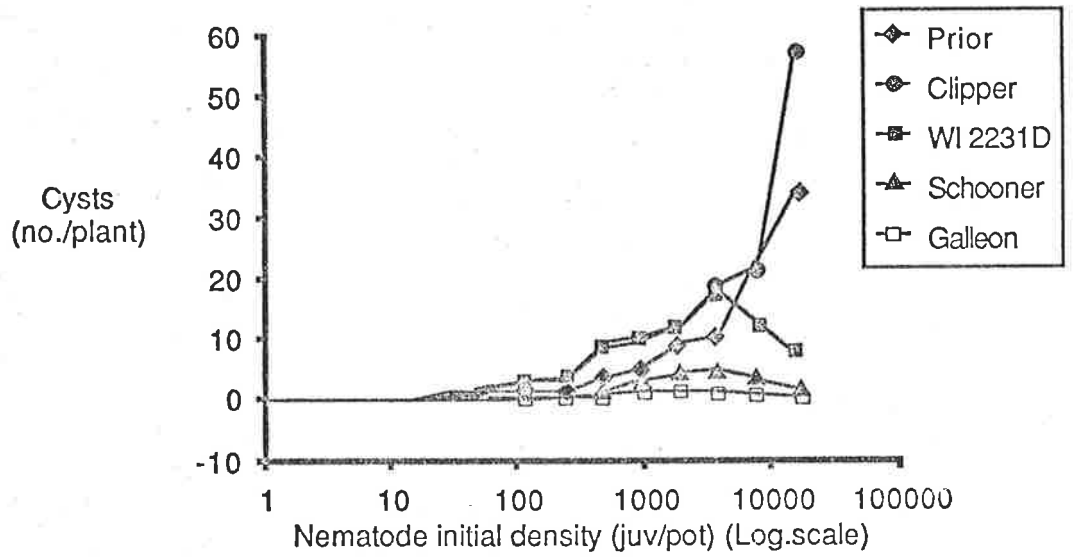
There was also a significant interaction between initial density and the number of eggs per cyst (Table 35). The number of eggs per cyst increased, reached a maximum then decreased as initial density increased. On Galleon and Schooner the number of eggs per cyst was fewer than on WI 2231D, Prior or Clipper.

7.3.3 Discussion

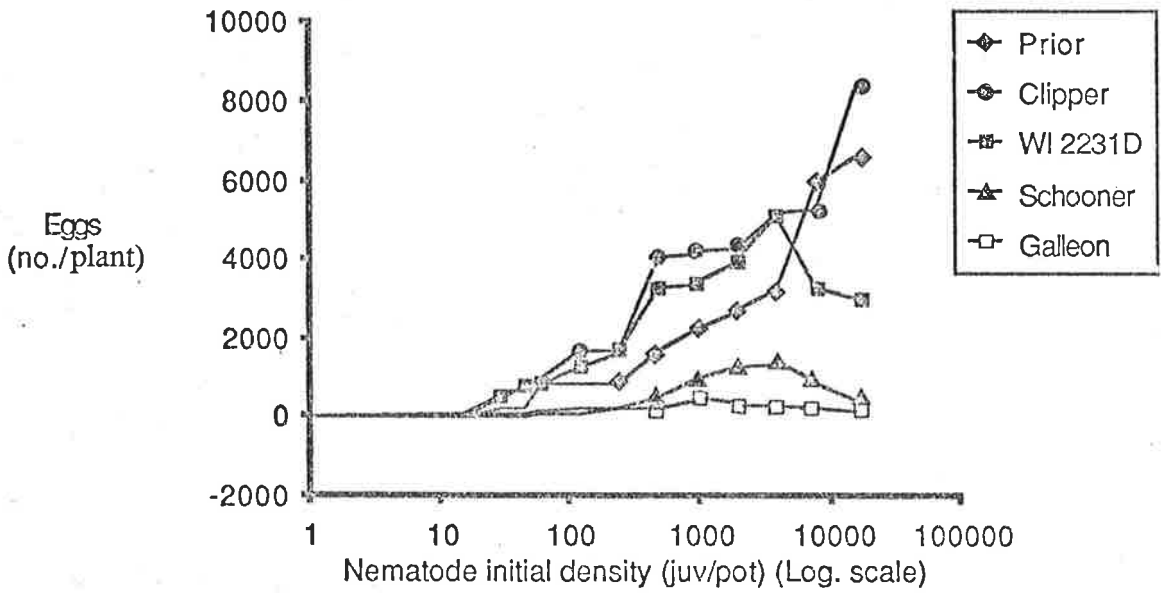
In all cultivars, inoculation with different nematode initial density had a significant effect on all growth characters measured and small differences were sufficient for significance suggesting that variation was controlled sufficiently. Inoculation with nematodes delayed maturity of the plants, increasingly as nematode initial density increased an observation agreeing with Seinhorst (1981) on oats. In South Australia where rainfall at the end of the season is unreliable, such a delay could be critical in affecting yield. The loss in yield in the field could well be greater than that obtained in pots where water was supplied as needed. Inoculation affected early growth of the plants as reflected in shoot dry weight and number of total and fertile tillers. Even in the resistant cultivar, Galleon, early growth was affected suggesting that damage is related to invasion by the nematode and early growth of the nematode so that the type of resistance in Galleon, apparently, does not affect invasion. This reduction in early growth was

Figure 21. The number of cysts per plant on five barley cultivars grown under different *H. avenae* initial population densities.

Figure 22. The number of eggs per plant on five barley cultivars grown under different *H. avenae* initial population densities.


Cysts
(no./plant)
60
50
40
30
20
10
0
-10
1
10
100
1000
10000
100000
Nematode initial density (juv/pot) (Log.scale)
Prior
Clipper
WI 2231D
Schooner
Galleon

21


Eggs
(no./plant)
10000
8000
6000
4000
2000
0
-2000
1
10
100
1000
10000
100000
Nematode initial density (juv/pot) (Log. scale)
Prior
Clipper
WI 2231D
Schooner
Galleon

22

carried through to the final grain yield. The cultivars differed in their potential yield by the same proportion so that the regression lines of yield and nematode initial density were parallel (Fig. 20) showing no difference in tolerance between the cultivars. Nevertheless some characters e.g. plant dry weight, numbers of total and fertile tillers (Fig. 19), showed different responses to inoculation with different nematode initial density and the slopes of the regression lines differed. This suggests that it is probably worthwhile to persist with an examination of tolerance even though time did not permit this on this occasion. The figure relating yield to nematode initial density also suggest that WI 2231D might be the most intolerant cultivar and further control of variation might accomplish a demonstration of differences.

There was an interaction between cultivars and nematode initial density. on cyst numbers. Multiplication rate data showed that a positive correlation between nematode initial population density and the final population density occured on Clipper and Prior. On WI 2231D and Schooner the multiplication rate declined above the initial density of 3840 juveniles. In Galleon, resistance contributes to the decrease of cyst production under high nematode initial density. Reduced yield in Galleon under high nematode initial density could be due to the early damage on the roots by nematodes. Although Prior and WI 2231D showed a better yield potential, under high nematode initial density they suffered a considerable yield loss compared to Schooner and Clipper. Interestingly, the cultivars used in this experiment shared at least one common parent in their pedigree, yet they showed a different performance under different nematode initial density.

7.4. General discussion

The aim of the experiments in this section was to assess the damage due to inoculation with *H. avenae* and to try to determine if differences in tolerance of sufficient magnitude to be of practical use could be found in barley.

In pots in the field variation was too great to permit the demonstration and it would have been necessary to go to large plots to overcome this. It was not possible to obtain sufficient nematodes for inoculation of large plots in the field so the alternative of pots in the glasshouse was used. In the glasshouse, it was possible to show reductions in the growth characters due to increased initial density of *H. avenae*. The cultivars of barley tested differed in growth habit and in potential yield so that the best method for demonstrating differences in tolerance was in a comparison of all slopes of regression lines of a variable against nematode intial density. With some characters, the slopes of these lines differed in different cultivars so there was a suggestion of differences in tolerance but in yield (Fig. 20) no differences could be demonstrated even though yield of WI 2231D appeared to be reduced more than the other cultivars. Persistence in attemps to control variation may well have demonstrated differences in tolerance in relation to yield but absence of time prevented this.

One of the factors which probably contributed to this problem was the soil. Half way through this series of experiments the source of supply of nutrient changed and this was reflected in the number of cysts and eggs that were produced on the plants (Figs. 21 and 22). Despite a repeated search for a better nutrient source, this has not yet been obtained. The soil did not remove the effect of resistance in the host but did affect number of cysts on susceptible hosts. The effect probably operated through nutrition of the hosts and so probably affected growth as well. The possibility of demonstrating differences in tolerance then still remains and is worthy of further examination.

Multiplication of the nematode at high initial densities varied with the cultivar. Galleon, as a resistant cultivar restricted multiplication but Prior, WI 2231D and Clipper, which must all be regarded as susceptible had different effects on multiplication at high initial densities. This effect together with the behaviour of Schooner needs further investigation.

* Many different statistical methods were tried but none gave any more information

CHAPTER 8

GENERAL DISCUSSION

Relating the genetics of the host to the behaviour of *H. avenae* has proved difficult, probably because the assays for number of females have been unreliable and variable causing difficulties in partitioning the susceptible and resistant plants (Hayes & Cotten, 1970). If the initial density is too low, the number of females produced in the susceptible reaction will overlap with the resistant reaction. If inoculum density is too high, competition between nematode individuals will occur (Jones & Kempton, 1978) resulting in overlap in the number of females produced in the susceptible and resistant reaction. In these studies an inoculum ~~density~~ of 625 juveniles of *H. avenae* was used, it has given a satisfactory number of females produced on host roots. Even with this initial density, an almost continuous series of numbers from nil to 40 females per plant was obtained when a series of barley cultivars was tested ~~to~~ by infection ~~to~~ with this nematode. So a constant initial density did not overcome the problem of variation. Similar results have been reported in oats (O'Brien & Fisher, 1974) and in wheat (Fisher, 1982a).

However, with one exception, the resistant cultivars could be separated at the level of ten females per plant. Cultivars which produced less than 30 and more than ten females per plant may possess a different mechanism which reduces the production of females.

To aid determination of the genetic basis of resistance, a cultivar at the extreme end of the susceptible range (Clipper) was used as the susceptible parent to enable clear separation of the susceptible and resistant progeny and in most experiments the separation was simple. Only a few plants could not be definitely categorized. When the progeny of these was tested, they were either homozygous or heterozygous resistant, suggesting a further mechanism of variation, in that some resistant plants under some conditions may support more females than expected in a resistant plant. The reason for

this is unknown. When Betzes, a host which allows production of an intermediate number of females was used as the susceptible parent, more difficulties were encountered but the results suggested that Betzes behaved in a similar way to Clipper i.e. as a susceptible cultivar.

In examining the interrelationships between the genes conditioning the resistance at least four genes were demonstrated with Athenais, CI 8147, Marocaine and Nile each having a single gene (Cook, *et al.*, 1977; 1979); at least two of these genes differed and both differed from the two genes in Morocco. The relation of the two genes in Orge Martin to the two in Morocco still remains to be examined so that there could be up to eight genes available for resistance to *H. avenae*. Where two genes were present in a cultivar, the resistance was more complete and there was a suggestion from the experiment with Morocco that each gene controlled a different aspect of the host parasite relation. Such a difference would also contribute to variation in numbers of females and this, together with the drift in numbers of females that occurred in the heterozygous condition could well account for variations in numbers of females produced on resistant plants.

When juveniles invaded and developed in the main axes of the root system a greater number of females developed than where lateral branches were invaded. It is possible that the size of syncytia is restricted in the narrower lateral branches and the syncytia may not always be able to supply sufficient nutrients for development of females. Thus the distribution of juveniles in the root system is important and this is another source of variation in numbers of females. There is not enough information about the sizes of different parts of the root systems nor about different rates of growth of root systems to show how important a contribution these characters make to the variation.

One of the major contributions to variations in numbers of females, particularly at higher initial densities, is the tolerance of the host (Jones & Kempton, 1978). The experiments reported here suggested differences in tolerance even though this could not

be proved conclusively. From the growth studies, particularly numbers of total and fertile tillers, Galleon appeared to be the least tolerant cultivar. The resistance of Galleon interfered with multiplication of the nematode so that the intolerance of Galleon could not be confirmed from the result on multiplication of the nematode. It needs further experiments with better control on variation to show the tolerance of a cultivar, because the effect of the tolerance of the cultivar on the development of females could make a marked contribution to variation in numbers of females on susceptible hosts in an assay. This factor certainly requires further examination because other possible contributions to variation could come from resistance that expresses itself at high densities or from the genetic background of the cultivar. One factor that does not seem to contribute to variation, at least to the Australian population of *H. avenae,* is the occurence of mixtures of pathotypes or different proportions of aggressive individuals in the population which could relate tolerance/intolerance to multiplication of the nematode.

Three aspects from this study could have practical importance. Firstly, two resistance genes in a cultivar, provided they regulate different aspects of the host/parasite relation, give a greater reduction in numbers of females and hence will give greater population control in the field. Secondly, if it proves correct that one of the genes in Morocco inhibits establishment of juveniles, then this gene could be valuable, not only in reducing nematode populations but also in avoiding damage to the host. Thirdly, tolerant cultivars without resistance will exacerbate the problem in the field because of their ability to multiply the population at high initial densities. Either tolerant cultivars should not be released without resistance or great care should be taken in a rotation to avoid the damage that may result to a following cereal crop.

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Appendix 1. Selected cultivars from the Waite Agricultural Research Institute barley collection and their country of origin.

Cultivars	Abbreviation	Country of origin
CPI 18197	(CP)	Algeria
La Mesita	(LM)	Algeria
Orge Martin	(OM)	Algeria
Orge Prophete	(OP)	Algeria
Clipper	(C)	Australia (S.A)
Galleon	(G)	Australia (S.A)
Prior D/A	(P)	Australia (S.A)
WI 2231D	(WI)	Australia (S.A)
Corvette	(Co)	Australia (S.A/QLD)
Shannon	(Sh)	Australia (Tasmania)
Resibee	(R)	Australia (Victoria)
Weeah	(W)	Australia (Victoria)
CI 3576	(C3)	Egypt
Nile	(Ni)	Egypt
Athenais	(At)	Greece
Indian Dwarf	(ID)	India
Suifu	(S)	Japan
2 EBYT 16	(2E)	Mexico
Marocaine 079	(Ma)	Morocco
Morocco	(Mo)	Morocco
Mazurka	(Mz)	Netherlands
Zephyr	(Z)	Netherlands
Betzes	(B)	Poland/Canada
Freja	(F)	Sweden
Bayardi	(By)	Syria
CI 8147	(C8)	Turkey
Golden Promise	(GP)	United Kingdom
Tintern	(T)	United Kingdom
Mink	(Mi)	United Kingdom
Arivat	(Ar)	U.S.A
Beecher	(Bc)	U.S.A
Chevron	(Ch)	U.S.A

Note : S.A = South Australia; QLD = Queensland.

Appendix 2. Analysis of variance on the number of white females per plant on barley cultivars following inoculation with *H. avenae*, on untransformed and square root transformed data.

2.a Analysis of variance on number of white females (untransformed data) :

Experiment 1 (initial density 5x100 juveniles) :
Variate : White females (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Units Stratum						
Cultivar	15	20141.50	83.91	1342.77	27.82**	1.83
Residual	80	3861.00	16.09	48.26		
Total	95	24002.50	100.00	252.66		
Grand total	95	24002.50	100.00			
Grand mean	21.38					
Total number of observations			96			

Standard errors of differences of means :

Table	Cultivar
Replicate	6
SED	4.01

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	80	6.95	32.5

Appendix 2 cont.

2.b Analysis of variance on number of white females (untransformed data) :

Experiment 2 (initial density 5x125 juveniles) :
Variate : White females (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Units Stratum						
Cultivar	17	22548.71	85.10	1326.39	30.23**	1.75
Residual	90	3948.50	14.90	43.87		
Total	107	26497.21	100.00	247.64		
Grand total	107	26497.21	100.00			
Grand mean	19.73					
Total number of observations			108			

Standard errors of differences of means :

Table	Cultivar
Replicate	6
SED	3.82

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	90	6.62	33.6

Appendix 2 cont.

2.c Analysis of variance on number of white females (square root transformed data):

Experiment 1 (initial density 5x100 juveniles) :
Variate : White females (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Units Stratum						
Cultivar	15	408.83	90.03	27.26	48.15**	1.83
Residual	80	45.29	9.97	0.57		
Total	95	454.12	100.00			
Grand total	95	454.12	100.00			
Grand mean	4.08					
Total number of observations			96			

Standard errors of differences of means :

Table	Cultivar
Replicate	6
SED	0.43

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	80	0.75	18.4

Appendix 2 cont.

2.d Analysis of variance on number of white females (square root transformed data):

Experiment 2 (initial density 5x125 juveniles) :
Variate : White females (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Units Stratum						
Cultivar	17	374.13	87.83	22.01	38.20**	1.75
Residual	90	51.85	12.17	0.58		
Total	107	425.98	100.00	3.98		
Grand total	107	425.98	100.00			
Grand mean	3.97					
Total number of observations			108			

Standard errors of differences of means :

Table	Cultivar
Replicate	6
SED	0.44

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	90	0.76	19.1

Appendix 3. Analysis of variance on the number of larvae and ~~number of~~ white females per plant on barley cultivars following different times inoculation with *H. avenae* .

3. a Analysis of variance on number of larvae :

Variate : Larvae (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square %	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	5	237.6	0.86	47.5		
Rep *Units* Stratum						
Cult	3	9807.0	35.65	3269.0	23.48**	2.78
Treat	2	8792.4	31.96	4396.2	31.57**	3.17
Cult x Treat	6	1012.7	3.68	168.8	1.21^{ns}	2.27
Residual	55	7658.3	27.84	139.2		
Total	66	27270.4	99.14	413.2		
Grand total	71	27508.0	100.00			
Grand mean	49.5					
Total number of observations		72				

Standard errors of differences of means :

Table	Cultivar	Treatment	Cultivar x Treatment
Replicate	18	24	6
SED	3.93	3.41	6.81

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	5	1.99	4.0

Appendix 3 cont.

3.b Analysis of variance on number of white females :

Variate : White females (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square %	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	5	12.28	1.13	2.46		
Rep *Units* Stratum						
Cult	3	745.17	68.28	248.39	50.28**	2.78
Treat	2	20.11	1.84	10.06	2.04^{ns}	3.17
Cult x Treat	6	42.00	3.85	7.00	1.42^{ns}	2.27
Residual	55	271.72	24.90	4.94		
Total	66	1079.00	98.87	16.35		
Grand total	71	1091.28	100.00			
Grand mean	3.19					
Total number of observations		72				

Standard errors of differences of means :

Table	Cultivar	Treatment	Cultivar x Treatment
Replicate	18	24	6
SED	0.74	0.64	1.28

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	5	0.45	14.2

Appendix 3 cont.

3.c Analysis of variance on number of larvae in primary axes

Variate : Larvae in primary axes (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square %	Mean square	Variance ratio	F table (p=0.05)
Units Stratum						
Cult	1	100.35	1.03	100.35	6.45*	3.92
Inoc	2	8566.69	85.35	4283.35	275.29**	3.19
Cult x Inoc	2	2.69	0.03	1.35	0.09^{ns}	3.19
Residual	66	1026.92	10.59	15.56		
Total	71	9696.65	100.00			
Grand total	71	9696.65	100.00			
Grand mean	14.82					
Total number of observations		72				

Standard errors of differences of means :

Table	Cultivar	Treatment	Cultivar x Treatment
Replicate	36	24	12
SED	2.92	3.58	5.06

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	66	12.4	21.7

Appendix 3 cont.

3.d Analysis of variance on number of larvae in lateral branches :

Variate : Larvae in lateral branches (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square %	Mean square	Variance ratio	F table (p=0.05)
Units Stratum						
Cult	1	960.7	3.72	960.7	6.25**	3.92
Inoc	2	14694.2	56.89	7347.1	47.77**	3.19
Cult x Inoc	2	26.0	0.10	13.0	0.09^{ns}	3.19
Residual	66	10150.4	39.29	153.8		
Total	71	25831.3	100.00			
Grand total	71	25831.3	100.00			
Grand mean	57.2					
Total number of observations		72				

Standard errors of differences of means :

Table	Cultivar	Treatment	Cultivar x Treatment
Replicate	36	24	12
SED	0.93	1.14	1.61

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	66	3.95	26.6

Appendix 3 cont.

3.e Analysis of variance on total number of larvae in the root system :

Variate : Total larvae in the root system (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square %	Mean square	Variance ratio	F table (p=0.05)
Units Stratum						
Cult	1	1672.3	10.49	1672.3	8.83**	3.92
Inoc	2	1724.5	10.82	862.3	4.55**	3.19
Cult x Inoc	2	35.9	0.23	17.9	0.10^{ns}	3.19
Residual	66	12504.3	78.46	189.5		
Total	71	15937.0	100.00			
Grand total	71	15937.0	100.00			
Grand mean	72.0					
Total number of observations		72				

Standard errors of differences of means :

Table	Cultivar	Treatment	Cultivar x Treatment
Replicate	36	24	12
SED	3.24	3.97	5.62

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	66	13.76	19.10

Appendix 3 cont.

3.f Analysis of variance on number white females :

Variate : White females (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square %	Mean square	Variance ratio	F table (p=0.05)
Units Stratum						
Cult	1	168.06	3.73	168.06	7.22**	3.92
Inoc	2	2662.69	59.09	1331.35	57.18**	3.19
Cult x Inoc	2	138.86	3.08	69.43	2.98^{ns}	3.19
Residual	66	1536.83	34.10	23.29		
Total	71	4506.44	100.00			
Grand total	71	4506.44	100.00			
Grand mean	11.78					
Total number of observations		72				

Standard errors of differences of means :

Table	Cultivar	Treatment	Cultivar x Treatment
Replicate	36	24	12
SED	1.14	4.83	1.97

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Units	66	4.83	41.0

Appendix 4. The origin of barley cultivars used in Chapter 7 (Fitzsimmons & Wrigley, 1984).

Prior : probably released in 1905, farmer selection.
Parentage was selected from either Archer or Chevalier.

Clipper : registration date 1968.
Parentage : Proctor x Prior A. Proctor was selected from (Kenia x Plumage Archer) and Prior A was selected from either Archer or Chevalier.

WI 2231D : is a non commercial variety.
Parentage : [(Hiproly x Clipper) x WI 2231 (3) and the parentage of WI 2231 was (Proctor x CI 3576).

Galleon : registration date 1981.
Parentage : [(Hiproly x Clipper) x WI 2231 (3)].

Schooner : registration date 1983.
Parentage : WI 2128 x WI 2099. WI 2128 and WI 2029 were selected from (Proctor x Prior) and (Proctor x CI 3576) respectively.

Appendix 5. Meteorological records at the Waite Agricultural Research Institute, June - December, 1982 - 1984.

	Soil temperature at 25 mm °C		Air temperature °C		Rainfall mm	Number days
	Max	Min	Max	Min		
1982						
June	18.8	7.0	14.2	7.3	2.08	17
July	18.4	6.6	13.8	7.3	1.25	14
August	25.6	9.4	18.9	10.3	0.79	8
September	28.3	10.3	17.6	9.6	1.06	10
October	33.7	12.8	20.4	11.3	0.54	13
November	45.6	16.9	20.4	11.3	0.11	2
December	47.8	18.2	27.3	15.5	0.42	3
Mean	31.2	11.6	18.9	10.4	0.89	9.57
1983						
June	19.4	7.6	15.4	8.5	1.13	12
July	17.0	7.1	13.5	7.7	4.12	23
August	21.4	8.9	16.3	9.5	2.92	13
September	24.6	10.3	17.6	10.1	2.56	16
October	33.1	12.3	21.1	11.3	1.83	9
November	39.4	15.6	24.2	13.6	0.35	9
December	45.0	17.9	27.8	16.2	0.65	5
Mean	28.5	11.4	19.4	10.9	1.93	12.42
1984						
June	20.1	7.3	15.8	8.7	16.3	10
July	16.3	6.7	13.1	7.4	3.34	22
August	17.9	8.3	15.1	9.2	4.34	22
September	21.3	8.3	15.5	8.7	2.12	19
October	33.1	12.2	20.6	11.6	0.83	8
November	35.1	15.0	23.2	14.3	1.81	13
December	42.9	17.5	25.9	15.6	0.29	3
Mean	26.6	10.7	18.4	10.7	2.05	13.85

Appendix 6. Analysis of variance on plant height, plant dry weight, number of total and fertile tillers, 100-seed-weight, number of seeds per plant and number of cysts on barley cultivars in 1982-Field pot experiment.

6.a Analysis of variance on plant height

Variate : Plant height (cm)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	2843.67	28.11	710.92		
Rep.Cult. Stratum						
Cultivar	2	2062.44	20.39	1031.22	219.89**	4.46
Residual	8	37.52	0.37	4.69		
Total	10	2099.96	20.76	210.00		
Rep.Cult.Inoc. Stratum						
Inoculation	12	228.40	2.26	19.03	0.56^{ns}	1.75
Cult.inoc	24	77.15	0.76	3.21	0.09^{ns}	1.52
Residual	144	4866.59	48.11	33.80		
Total	180	5172.14	51.13	28.73		
Grand total	194	10115.76	100.00			
Grand mean		64.39				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.38	2.12	3.55
Except when comparing means with same level(s) of :			
cult			3.68

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	4.27	6.60
Rep.cult	8	0.60	0.90
Rep.cult.inoc	144	5.81	9.00

Appendix 6 cont.

6.b Analysis of variance on plant dry weight

Variate : Plant dry weight (g/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	18769.30	11.78	4692.30		
Rep.Cult. Stratum						
Cultivar	2	19589.50	12.29	9794.70	25.21**	4.46
Residual	8	3108.90	1.95	388.60		
Total	10	22698.40	14.24	2269.80		
Rep.Cult.Inoc. Stratum						
Inoculation	12	16415.30	10.30	1367.90	2.03*	1.75
Cult.inoc	24	4570.60	2.87	190.40	0.28^{ns}	1.52
Residual	144	96943.10	60.82	673.20		
Total	180	117929.00	73.98	655.20		
Grand total	194	159396.70	100.00			
Grand mean		58.20				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	3.46	9.47	16.14

Except when comparing means with same level(s) of :

cult 16.41

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	10.97	18.90
Rep.cult	8	5.47	9.4
Rep.cult.inoc	144	25.95	44.60

Appendix 6 cont.

6.c Analysis of variance on number of total tillers

Variate : Tillers (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	3139.40	6.36	784.90		
Rep.Cult. Stratum						
Cultivar	2	11602.60	23.51	5801.30	38.97**	4.46
Residual	8	1191.10	2.41	148.90		
Total	10	12793.60	25.92	1279.40		
Rep.Cult.Inoc. Stratum						
Inoculation	12	5542.00	11.23	461.80	2.45*	1.75
Cult.inoc	24	742.10	1.50	30.90	0.16^{ns}	1.52
Residual	144	27131.90	54.98	188.40		
Total	180	33416.00	67.71	185.60		
Grand total	194	49349.10	100.00			
Grand mean		34.28				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	2.14	5.01	8.61

Except when comparing means with same level(s) of :

cult	8.68

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	4.49	13.1
Rep.cult	8	3.38	9.9
Rep.cult.inoc	144	13.73	40.0

Appendix 6 cont.

6.d Analysis of variance on number of fertile tillers

Variate : Fertile tillers (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	2403.20	5.69	600.80		
Rep.Cult. Stratum						
Cultivar	2	9041.30	21.41	450.70	42.46**	4.46
Residual	8	851.80	2.02	106.50		
Total	10	9893.10	23.42	989.30		
Rep.Cult.Inoc. Stratum						
Inoculation	12	4797.00	11.36	399.70	2.34*	1.75
Cult.inoc	24	556.00	1.32	23.20	0.14^{ns}	1.52
Residual	144	24589.00	58.21	170.80		
Total	180	29942.00	70.89	166.30		
Grand total	194	42238.30	100.00			
Grand mean		31.12				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	1.81	4.77	8.14

Except when comparing means with same level(s) of :

cult 8.27

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	3.93	12.6
Rep.cult	8	2.86	9.2
Rep.cult.inoc	144	13.07	42.0

Appendix 6 cont.

6.e Analysis of variance on 100-seed-weight

Variate : 100-seed-weight (g/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	0.69	2.67	0.17		
Rep.Cult. Stratum						
Cultivar	2	4.15	16.11	2.07	24.62**	4.46
Residual	8	0.67	2.62	0.08		
Total	10	4.82	18.73	0.48		
Rep.Cult.Inoc. Stratum						
Inoculation	12	2.03	7.91	0.17	1.39^{ns}	1.75
Cult.inoc	24	0.64	2.48	0.03	0.22^{ns}	1.52
Residual	144	17.55	68.21	0.12		
Total	180	20.22	78.60	0.11		
Grand total	194	25.73	100.00			
Grand mean		4.52				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.05	0.13	0.22

Except when comparing means with same level(s) of :

cult 0.22

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	0.07	1.50
Rep.cult	8	0.08	1.80
Rep.cult.inoc	144	0.35	7.70

Appendix 6 cont.

6.8 Analysis of variance on number of seeds :

Variate : Seeds (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	812378	7.53	203095		
Rep.Cult. Stratum						
Cultivar	2	787988	7.30	393994	12.65*	4.46
Residual	8	249167	2.31	31146		
Total	10	1037155	9.61	103715		
Rep.Cult.Inoc. Stratum						
Inoculation	12	734620	6.81	61218	1.10^{ns}	1.75
Cult.inoc	24	190334	1.76	7931	0.14^{ns}	1.52
Residual	144	8013535	74.28	55650		
Total	180	8938489	82.86	49658		
Grand total	194	10788022	100.00			
Grand mean			576			
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	31.65	86.1	146.6

Except when comparing means with same level(s) of :

cult 149.2

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	72.20	12.50
Rep.cult	8	48.90	8.50
Rep.cult.inoc	144	235.90	40.90

Appendix 6 cont.

6.g Analysis of variance on number of cysts

Variate : Cysts (no/plant) [log (cyst+1)]

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	0.39	0.17	0.19		
Rep.Cult. Stratum						
Cultivar	2	20.02	8.59	10.01	123.47**	4.46
Residual	8	0.32	0.14	0.08		
Total	10	20.35	8.73	3.39		
Rep.Cult.Inoc. Stratum						
Inoculation	12	180.64	77.55	15.05	102.87**	1.75
Cult.inoc	24	21.03	9.03	0.87	5.99*	1.52
Residual	144	10.53	4.52	0.15		
Total	180	212.20	91.10	1.96		
Grand total	194	232.94	100.00			
Grand mean		1.07				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	39	9	3
SED	0.06	0.18	0.31

Except when comparing means with same level(s) of :

cult 0.31

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	2	0.07	6.60
Rep.cult	4	0.08	7.40
Rep.cult.inoc	72	0.38	35.70

Appendix 7. Analysis of variance on plant height, plant dry weight, number of total and fertile tillers and number of cysts on barley cultivars in 1983-Field pot experiment.

7.a Analysis of variance on plant height

Variate : Plant height (cm)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	1834.61	10.59	458.65		
Rep.Cult. Stratum						
Cultivar	2	1777.41	10.26	888.70	16.46*	4.46
Residual	8	432.01	2.49	54.00		
Total	10	2209.42	12.75	220.94		
Rep.Cult.Inoc. Stratum						
Inoculation	12	735.72	4.25	61.31	0.73^{ns}	1.75
Cult.inoc	24	455.55	2.63	18.98	0.23^{ns}	1.52
Residual	144	12094.56	69.79	83.99		
Total	180	13285.83	76.66			
Grand total	194	17329.86	100.00			
Grand mean		56.81				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	1.29	3.35	5.71
Except when comparing means with same level(s) of :			
cult			5.79

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	3.43	6.00
Rep.cult	8	2.04	3.60
Rep.cult.inoc	144	9.17	16.10

Appendix 7 cont.

7.b Analysis of variance on plant dry weight

Variate : Plant dry weight (g/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	3507.50	6.35	876.90		
Rep.Cult. Stratum						
Cultivar	2	1607.50	2.91	803.70	1.78^{ns}	4.46
Residual	8	3613.10	6.54	451.60		
Total	10	5220.60	9.45	522.10		
Rep.Cult.Inoc. Stratum						
Inoculation	12	6185.90	11.20	515.50	1.91*	1.75
Cult.inoc	24	1384.70	2.51	57.70	0.21^{ns}	1.52
Residual	144	38939.70	70.49	270.40		
Total	180	46510.30	84.20	258.40		
Grand total	194	55238.40	100.00			
Grand mean		33.00				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	3.73	6.00	10.66

Except when comparing means with same level(s) of :

cult 10.40

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	4.74	14.40
Rep.cult	8	5.89	17.80
Rep.cult.inoc	144	16.44	49.80

Appendix 7 cont.

7.c Analysis of variance on number of total tillers

Variate : Tillers (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	1089.60	4.37	272.40		
Rep.Cult. Stratum						
Cultivar	2	2397.70	9.61	1198.80	9.90*	4.46
Residual	8	968.80	3.88	121.10		
Total	10	3366.50	13.50	336.60		
Rep.Cult.Inoc. Stratum						
Inoculation	12	1629.40	6.53	135.80	1.06^{ns}	1.75
Cult.inoc	24	328.30	1.32	13.70	0.11^{ns}	1.52
Residual	144	18527.70	74.28	128.70		
Total	180	20485.40	82.13	113.80		
Grand total	194	24941.40	100.00			
Grand mean		23.17				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	1.93	4.14	7.16

Except when comparing means with same level(s) of :

cult 7.17

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	2.64	11.40
Rep.cult	8	3.05	13.20
Rep.cult.inoc	144	11.34	49.00

Appendix 7 cont.

7.d Analysis of variance on number of fertile tillers

Variate : Fertile tillers (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	805.20	3.46	201.30		
Rep.Cult. Stratum						
Cultivar	2	1839.50	7.91	919.80	8.11*	4.46
Residual	8	907.50	3.90	113.40		
Total	10	2747.00	11.81	274.70		
Rep.Cult.Inoc. Stratum						
Inoculation	12	2403.40	10.34	200.30	1.69^{ns}	1.75
Cult.inoc	24	285.80	1.23	11.90	0.10^{ns}	1.52
Residual	144	17012.90	73.16	118.10		
Total	180	19702.20	84.72	109.50		
Grand total	194	23254.40	100.00			
Grand mean		20.21				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	1.87	3.97	6.86

Except when comparing means with same level(s) of :

cult 6.87

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	2.27	11.20
Rep.cult	8	2.95	14.60
Rep.cult.inoc	144	10.87	53.80

Appendix 7 cont.

7.e Analysis of variance on number of cysts

Variate : Cysts (no/plant) [log (cyst+1)]

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	7.82	3.23	1.95		
Rep.Cult. Stratum						
Cultivar	2	33.20	13.70	16.60	95.29**	4.46
Residual	8	1.39	0.58	0.17		
Total	10	34.59	14.28	3.46		
Rep.Cult.Inoc. Stratum						
Inoculation	12	149.58	61.73	12.46	77.44**	1.75
Cult.inoc	24	27.13	11.20	1.13	7.02*	1.52
Residual	144	23.18	9.57	1.11		
Total	180	199.89	82.50			
Grand total	194	242.31	100.00			
Grand mean		0.86				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.07	0.15	0.25

Except when comparing means with same level(s) of :

cult	0.25

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	0.22	26.1
Rep.cult	8	0.11	13.5
Rep.cult.inoc	144	0.40	46.7

Appendix 8. Analysis of variance on number of days to heading, plant height number of total and fertile tillers, yield and number of cysts on barley cultivars in 1983-Glasshouse experiment.

8.a Analysis of variance on number of days to heading

Variate : Heading (no. days)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	64.13	6.31	16.03		
Rep.Cult. Stratum						
Cultivar	2	24.29	2.39	12.14	4.35*	4.46
Residual	8	22.33	2.20	2.79		
Total	10	46.62	4.59	4.66		
Rep.Cult.Inoc. Stratum						
Inoculation	12	22.07	2.17	1.84	0.30^{ns}	1.75
Cult.inoc	24	10.11	1.00	0.42	0.07^{ns}	1.52
Residual	144	872.74	85.93	6.06		
Total	180	904.92	89.10	5.03		
Grand total	194	1015.67	100.00			
Grand mean		92.37				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.29	0.90	1.52
Except when comparing means with same level(s) of :			
cult			1.56

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	0.64	0.70
Rep.cult	8	0.46	0.50
Rep.cult.inoc	144	2.46	2.70

Appendix 8 cont.

8.b Analysis of variance on plant height

Variate : Plant height (cm)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	163.57	2.94	40.89		
Rep.Cult. Stratum						
Cultivar	2	3376.02	60.76	1688.01	103.81**	4.46
Residual	8	130.09	2.34	16.26		
Total	10	3506.10	63.10	350.61		
Rep.Cult.Inoc. Stratum						
Inoculation	12	137.05	2.47	11.42	0.96^{ns}	1.75
Cult.inoc	24	30.82	0.55	1.28	0.11^{ns}	1.52
Residual	144	1719.08	30.94	11.94		
Total	180	1886.95	33.96	10.48		
Grand total	194	5556.63	100.00			
Grand mean		41.79				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.71	1.26	2.21

Except when comparing means with same level(s) of :

cult	2.18

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	1.02	2.50
Rep.cult	8	1.12	2.70
Rep.cult.inoc	144	3.45	8.30

Appendix 8 cont.

8.c Analysis of variance on number of total tillers

Variate : Tillers (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	2.84	2.92	0.71		
Rep.Cult. Stratum						
Cultivar	2	13.66	14.03	6.83	9.22*	4.46
Residual	8	5.93	6.09	0.74		
Total	10	19.59	20.12	1.96		
Rep.Cult.Inoc. Stratum						
Inoculation	12	6.42	6.60	0.54	1.17^{ns}	1.75
Cult.inoc	24	2.47	2.54	0.10	0.23^{ns}	1.52
Residual	144	66.03	67.83	0.46		
Total	180	74.92	76.96	0.42		
Grand total	194	97.35	100.00			
Grand mean		3.42				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.15	0.25	0.44

Except when comparing means with same level(s) of :

cult 0.43

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	0.14	4.00
Rep.cult	8	0.24	7.00
Rep.cult.inoc	144	0.68	19.80

Appendix 8 cont.

8.d Analysis of variance on number of fertile tillers

Variate : Fertile tillers (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	0.59	0.87	0.15		
Rep.Cult. Stratum						
Cultivar	2	20.47	29.78	10.24	29.68**	4.46
Residual	8	2.76	4.01	0.35		
Total	10	23.23	33.79	2.32		
Rep.Cult.Inoc. Stratum						
Inoculation	12	1.55	2.25	0.13	0.44^{ns}	1.75
Cult.inoc	24	1.13	1.64	0.05	0.16^{ns}	1.52
Residual	144	42.25	61.45	0.29		
Total	180	44.92	65.34	0.25		
Grand total	194	68.75	100.00			
Grand mean		1.96				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.10	0.19	0.35

Except when comparing means with same level(s) of :

cult 0.34

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	0.06	3.10
Rep.cult	8	0.16	8.30
Rep.cult.inoc	144	0.54	27.60

Appendix 8 cont.

8.e Analysis of variance on yield

Variate : Yield (g/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	0.39	5.61	0.09		
Rep.Cult. Stratum						
Cultivar	2	0.98	13.85	0.49	8.34*	4.46
Residual	8	0.47	6.64	0.06		
Total	10	1.44	20.50	0.14		
Rep.Cult.Inoc. Stratum						
Inoculation	12	0.27	3.87	0.02	0.67^{ns}	1.75
Cult.inoc	24	0.04	0.51	0.001	0.04^{ns}	1.52
Residual	144	4.89	69.52	0.03		
Total	180	5.20	73.89	0.02		
Grand total	194	7.04	100.00			
Grand mean		0.87				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.04	0.06	0.12

Except when comparing means with same level(s) of :

cult	0.12

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	0.05	5.80
Rep.cult	8	0.06	7.70
Rep.cult.inoc	144	0.18	21.20

Appendix 8 cont.

8.f Analysis of variance on number of cysts

Variate : Cysts (no/plant) [log (cyst+1)]

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	4	0.78	0.19	0.19		
Rep.Cult. Stratum						
Cultivar	2	117.95	28.55	58.98	200.55**	4.46
Residual	8	2.35	0.57	0.29		
Total	10	120.31	29.11	12.03		
Rep.Cult.Inoc. Stratum						
Inoculation	12	228.06	55.19	19.00	128.35**	1.75
Cult.inoc	24	42.75	10.35	1.78	12.03*	1.52
Residual	144	21.32	5.16	0.15		
Total	180	292.13	70.70	1.62		
Grand total	194	413.22	100.00			
Grand mean		1.46				
Total number of observations			195			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	65	15	5
SED	0.09	0.14	0.25

Except when comparing means with same level(s) of :

cult	0.24

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	4	0.07	4.80
Rep.cult	8	0.15	10.30
Rep.cult.inoc	144	0.38	26.40

Appendix 9. Analysis of variance on number of days to awning & heading, plant height, plant/dry weight and root, number of total and fertile tillers, yield, number of cysts number of eggs per cyst and number of eggs per plant on barley cultivars in 1984 - Glasshouse experiment.

9.a Analysis of variance on number of days to awning

Variate : Awning (no.days)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	44.09	1.23	14.70		
Rep.Cult. Stratum						
Cultivar	4	1819.49	50.81	454.87	43.58**	3.26
Residual	12	125.25	3.50	10.44		
Total	16	1944.74	54.31	121.55		
Rep.Cult.Inoc. Stratum						
Inoculation	12	998.49	27.88	83.21	28.01**	1.75
Cult.inoc	48	59.02	1.65	1.23	0.41^{ns}	1.42
Residual	180	534.65	14.93	2.97		
Total	240	1592.15	44.46	6.63		
Grand total	259	3580.99	100.00			
Grand mean		104.11				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	0.63	0.55	1.33
Except when comparing means with same level(s) of :			
cult			1.22

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	0.48	0.50
Rep.cult	12	0.90	0.90
Rep.cult.inoc	180	1.72	1.70

9.b Analysis of variance on number of days to heading

Variate : Heading (no.days)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	34.91	2.01	11.64		
Rep.Cult. Stratum						
Cultivar	4	587.25	33.78	146.81	22.91**	3.26
Residual	12	76.90	4.42	6.41		
Total	16	664.15	38.20	41.51		
Rep.Cult.Inoc. Stratum						
Inoculation	12	611.40	35.17	50.95	23.93**	1.75
Cult.inoc	48	44.95	2.59	0.94	0.44^{ns}	1.42
Residual	180	383.19	22.04	2.13		
Total	240	1039.54	59.79	4.33		
Grand total	259	1738.60	100.00			
Grand mean		110.30				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	0.50	0.46	1.11

Except when comparing means with same level(s) of :

cult	1.03

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	0.42	0.40
Rep.cult	12	0.70	0.60
Rep.cult.inoc	180	1.46	1.30

9.c Analysis of variance on plant height

Variate : Plant height (cm)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	345.72	1.56	115.24		
Rep.Cult. Stratum						
Cultivar	4	18679.68	84.07	4669.92	113.88**	3.26
Residual	12	492.07	2.21	41.01		
Total	16	19171.76	86.28	1198.23		
Rep.Cult.Inoc. Stratum						
Inoculation	12	76.19	0.34	6.35	0.44^{ns}	1.75
Cult.inoc	48	39.00	0.18	0.81	0.06^{ns}	1.42
Residual	180	2586.54	11.64	14.37		
Total	240	2701.72	12.16	11.26		
Grand total	259	22219.20	100.00			
Grand mean		74.09				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	1.26	1.20	2.87
Except when comparing means with same level(s) of :			
cult			2.68

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	1.33	1.80
Rep.cult	12	1.78	2.40
Rep.cult.inoc	180	3.79	5.10

9.d Analysis of variance on plant dry weight

Variate : Plant dry weight (g/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	42.71	2.90	14.24		
Rep.Cult. Stratum						
Cultivar	4	265.18	17.99	66.29	10.66**	3.26
Residual	12	74.64	5.06	6.22		
Total	16	339.82	23.06	21.24		
Rep.Cult.Inoc. Stratum						
Inoculation	12	235.00	15.94	19.58	4.45**	1.75
Cult.inoc	48	63.76	4.33	1.33	0.30^{ns}	1.42
Residual	180	792.59	53.78	4.40		
Total	240	1091.35	75.05	4.55		
Grand total	259	1473.88	100.00			
Grand mean		14.76				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	0.49	0.66	1.51
Except when comparing means with same level(s) of :			
cult			1.49

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	0.47	3.20
Rep.cult	12	0.69	4.70
Rep.cult.inoc	180	2.10	14.20

9.e Analysis of variance on root dry weight

Variate : Root dry weight (g/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	1.31	3.06	0.44		
Rep.Cult. Stratum						
Cultivar	4	4.23	9.90	1.06	3.66*	3.26
Residual	12	3.47	8.12	0.29		
Total	16	7.71	18.02	0.48		
Rep.Cult.Inoc. Stratum						
Inoculation	12	8.45	19.76	0.70	5.48**	1.75
Cult.inoc	48	2.16	5.06	0.05	0.35^{ns}	1.42
Residual	180	23.14	54.10	0.13		
Total	240	33.75	78.92	0.14		
Grand total	259	42.77	100.00			
Grand mean		1.62				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	0.11	0.11	0.27

Except when comparing means with same level(s) of :

cult 0.25

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	0.08	5.10
Rep.cult	12	0.15	9.20
Rep.cult.inoc	180	0.36	22.20

9.f Analysis of variance on number of total tillers

Variate : Tillers (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	17.06	1.08	5.69		
Rep.Cult. Stratum						
Cultivar	4	481.25	30.50	120.31	16.63**	3.26
Residual	12	86.85	5.50	7.24		
Total	16	568.09	36.01	35.51		
Rep.Cult.Inoc. Stratum						
Inoculation	12	216.32	13.71	18.03	4.54*	1.75
Cult.inoc	48	61.95	3.93	1.29	0.33^{ns}	1.42
Residual	180	714.35	45.28	3.97		
Total	240	992.62	62.91	4.14		
Grand total	259	1577.77	100.00			
Grand mean		8.81				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	0.53	0.63	1.45

Except when comparing means with same level(s) of :

cult	1.41

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	0.30	3.40
Rep.cult	12	0.75	8.50
Rep.cult.inoc	180	1.99	22.60

9.g Analysis of variance on number of fertile tillers

Variate : Fertile tillers (no/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	20.38	1.25	6.79		
Rep.Cult. Stratum						
Cultivar	4	596.83	36.48	149.21	25.90**	3.26
Residual	12	69.14	4.23	5.76		
Total	16	665.97	40.70	41.62		
Rep.Cult.Inoc. Stratum						
Inoculation	12	160.85	9.83	13.40	3.43*	1.75
Cult.inoc	48	85.27	5.21	1.78	0.45^{ns}	1.42
Residual	180	703.73	43.01	3.91		
Total	240	949.85	58.05	3.96		
Grand total	259	1636.20	100.00			
Grand mean		8.40				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	0.47	0.63	1.42
Except when comparing means with same level(s) of :			
cult			1.40

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	0.32	3.90
Rep.cult	12	0.67	7.90
Rep.cult.inoc	180	1.98	23.50

9.h Analysis of variance on grain yield

Variate : Yield (g/plant)

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	7.82	1.77	2.61		
Rep.Cult. Stratum						
Cultivar	4	94.55	21.42	23.64	8.15**	3.26
Residual	12	34.79	7.88	2.90		
Total	16	129.34	29.30	8.08		
Rep.Cult.Inoc. Stratum						
Inoculation	12	63.78	14.45	5.32	4.20*	1.75
Cult.inoc	48	12.68	2.87	0.26	0.21^{ns}	1.42
Residual	180	227.75	51.60	1.27		
Total	240	304.22	68.92	1.27		
Grand total	259	441.38	100.00			
Grand mean		6.37				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	0.33	0.36	0.83
Except when comparing means with same level(s) of :			
cult			0.80

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	0.20	3.10
Rep.cult	12	0.47	7.40
Rep.cult.inoc	180	1.12	17.70

9.i Analysis of variance on number of cysts

Variate : Cysts (no/plant) [log (x+1)]

Source of variation	Degree of freedom	Sum of square	Sum of square (%)	Mean square	Variance ratio	F table (p=0.05)
Rep Stratum	3	0.88	0.28	0.29		
Rep.Cult. Stratum						
Cultivar	4	78.47	24.80	19.62	41.70**	3.26
Residual	12	5.64	1.78	0.47		
Total	16	84.11	26.58	5.26		
Rep.Cult.Inoc. Stratum						
Inoculation	12	153.69	48.58	12.81	64.19**	1.75
Cult.inoc	48	41.80	13.21	0.87	4.36*	1.42
Residual	180	35.92	11.35	0.20		
Total	240	231.41	73.14	0.96		
Grand total	259	316.40	100.00			
Grand mean		1.17				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	0.13	0.14	0.33

Except when comparing means with same level(s) of :

cult	0.32

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	0.07	5.70
Rep.cult	12	0.19	16.20
Rep.cult.inoc	180	0.45	38.00

9.j Analysis of variance on number of eggs per cyst :

Variate : Eggs (no/cyst)

Source of variation (p=0.05)	Degree of freedom	Sum of square	Sum of square (%)	Mean	Variance square	F table ratio
Rep Stratum	3	56855	0.64	18952		
Rep.Cult. Stratum						
Cultivar	4	1642879	18.44	410720	10.54**	3.26
Residual	12	467743	5.25	38979		
Total	16	2110622	23.69	131914		
Rep.Cult.Inoc. Stratum						
Inoculation	12	2614594	29.34	217883	13.19**	1.75
Cult.inoc	48	1156074	12.97	24085	1.46*	1.42
Residual	180	2972519	33.36	16514		
Total	240	6743187	75.68	28097		
Grand total	259	8910664	100.00			
Grand mean		224.70				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	38.72	40.64	95.50
Except when comparing means with same level(s) of :			
cult			90.87

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	17.08	7.60
Rep.cult	12	54.76	24.40
Rep.cult.inoc	180	128.51	57.20

9.k Analysis of variance on number of eggs per plant

Variate : Eggs (no/plant)

Source of variation (p=0.05)	Degree of freedom	Sum of square	Sum of square (%)	Mean	Variance square	F table ratio
Rep Stratum	3	1953	1.61	6510		
Rep.Cult. Stratum						
Cultivar	4	2742	22.58	6854	40.38**	3.26
Residual	12	2037	1.68	1697		
Total	16	2945	24.62	1841		
Rep.Cult.Inoc. Stratum						
Inoculation	12	4105	33.81	3421	29.46**	1.75
Cult.inoc	48	2804	23.10	5842	5.03*	1.42
Residual	180	2091	17.22	1161		
Total	240	9000	74.13	3750		
Grand total	259	1214	100.00			
Grand mean		1529				
Total number of observations			260			

Standard errors of differences of means :

Table	Cult	Inoc	Cult.inoc
Replicate	52	20	4
SED	255.50	340.80	775.50

Except when comparing means with same level(s) of :

cult 762.00

Stratum standard errors and coefficients of variation :

Stratum	DF	SE	CV%
Rep	3	316.50	20.70
Rep.cult	12	361.30	23.60
Rep.cult.inoc	180	1077.70	70.50

Appendix 10. Regression analysis on number of fertile tillers and grain yield of barley cultivars in 1984-Glasshouse experiment.

10.a Regression analysis on number of fertile tillers

Y-Variate = fertile tillers (no/plant)

Regression coefficients

	Estimate	S.E	T
Constant	11.1250	0.292	38.06
X	-0.00023180	0.0000594	-3.90
Cult Schooner	-3.410	0.413	-8.25
Cult W12231D	-0.445	0.413	-1.08
Cult Prior	-3.959	0.413	-9.58
Cult Clipper	-3.939	0.413	-9.53
X.Cult Schooner	0.0001244	0.000084	1.48
X.Cult W12231D	-0.0001128	0.000084	-1.34
X.Cult Prior	0.0001941	0.000084	2.31
X.Cult Clipper	0.0001532	0.000084	1.82

Analysis of variance

	DF	SS	MS
Regressn	9	782.10	86.90
Residual	250	854.10	3.42
Total	259	1636.20	6.32
Change	-9	-782.10	86.91

10.b Regression analysis on yield

Y-Variate = yield (g/plant)

Regression coefficients

	Estimate	S.E	T
Constant	7.533	0.153	49.15
X	-0.0001060	0.0000154	-6.87
Cult Schooner	-1.644	0.210	-7.81
Cult W12231D	-0.541	0.210	-2.57
Cult Prior	-0.909	0.210	-4.32
Cult Clipper	-1.472	0.210	-6.99

Analysis of variance

	DF	SS	MS
Regressn	5	148.90	29.775
Residual	254	292.60	1.152
Total	259	441.50	1.705
Change	4	6.20	1.554