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A STUDY OF RESISTANCE TO CEREAL CYST NEMATODE (*HETERODERA AVENAE* WOLL.) LOCATED IN THE RYE GENOME OF TRITICALE.

by

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ABSTRACT

The cereal cyst nematode, *Heterodera avenae* Woll., is a serious pest of cereals in Australia and many other parts of the world. The available literature on the distribution and economic importance of *H. avenae* and its control, including the use of genetic resistance and tolerance, is reviewed. Despite extensive screening, only one wheat line (AUS 10894) is known to have a useful level of resistance to the Australian pathotype of the nematode.

This study describes the transfer of *H. avenae* resistance identified in a triticale (*X Triticosecale* Witt.) line (T701-4-6) into a better genetic background in triticale, the location of the chromosome with the gene for resistance, and the initiation of the transfer of the resistance gene into wheat. The resistance of T701-4-6 was shown to be stronger than that of AUS 10894 and probably similar to that of South Australian Rye. Inheritance of the resistance was also shown to be monogenic and, to a large extent, dominant.

Nine homozygous resistant triticale lines were produced by crossing T701-4-6 as the non-recurrent parent to two susceptible, but high-yielding, triticale lines (T1006 and T1116). One of the nine lines, which was found to be resistant to wheat stem rust strain 34-2,12, has been submitted to the National Rust Control Programme, Castle Hill, for use in a triticale backcrossing programme.

The gene for resistance to the nematode has been located on rye chromosome 6 (6R) of T701-4-6 through backcrossing of the line to wheat and correlating the presence of particular rye chromosomes in the backcross progenies with the reactions of these plants to the nematode. Rye chromosomes were identified by C-banding, supported by isozyme analysis in the cases of 4R and 6R and by a morphological marker in the case of 5R. Preliminary evidence suggests that the gene may be on the long arm of 6R.

A disomic substitution of 6R for chromosome 6D of wheat was selected and crossed to Sears' mutant line _ph1b ph1b_ to induce homoeologous pairing and recombination between 6R and 6D or other wheat chromosomes. Four F_2 progeny from that cross were shown to be _ph1b_ homozygotes through observations on the relative frequencies of chromosome configurations at metaphase I in those plants and their F_1 hybrids with _Aegilops variabilis_. Three of the four F_2 plants were double monosomic for 6R and 6D and 1220 F_3 seeds are available from them for screening for wheat-rye recombinants. A programme has been initiated for production of disomic 6R(-6A) and 6R(-6B) substitutions for additional crosses to the _ph1b ph1b_ mutant.

The efficiency of the nematode assay dropped seriously in the course of the project leading to some results which are only tentative.

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CHAPTER ONE

GENERAL INTRODUCTION

The cereal cyst nematode (*Heterodera avenae* Wollenweber) is a serious pest of many cereals in the world (Lamberti and Taylor, 1983). It has been recorded in, at least, thirty-one countries (Meagher, 1977) where it attacks mainly oats, wheat, barley, rye and triticale in addition to some grasses (Duggan, 1961; Wiese, 1977; Cook, 1982).

It was first reported in South Australia by Davidson in 1930 who suggested that it had been known in this state since 1906. It was correctly identified in 1922 (Davidson, 1930) and is likely to have been introduced to Australia about 1860. Europe, probably Germany, is a likely origin of the Australian population and spread within Australia has been attributed mainly to dust-storms (Meagher, 1972).

In Australia *H. avenae* is a serious pest mainly in South Australia and the Wimmera and Mallee districts of Victoria (Millikan, 1938; Meagher, 1972, 1977; King *et al*, 1982; Brown, 1983a) infesting over two million hectares (Brown, *loc. cit.*). It has also been recorded in the Geraldton area of Western Australia (MacNish, 1964; Parkin and Goss, 1968), in friable soils near Koraleigh in New South Wales (McLeod, 1968) and in northern New South Wales (Southwell and McLeod, 1981).

Field infestation by _H. avenae_ is usually observed as patches of stunted plants with yellowish leaves (Wallace, 1965). Infected plants have shallow, multibranched, and matted-looking root systems with swellings. In mature plants, white gravid females of the nematode can be found attached to the roots. Plants suffer reduced tillering (Davies, 1961), premature ear formation (Duggan, 1961) and small or poorly-filled heads. O'Brien (1976) recorded slow early seedling growth, reduced number of fertile spikelets, grains per inflorescence and hence yield of wheat and barley.

Field population levels recorded include 20-60 eggs/gm. of soil in South Australia (Fisher, pers. comm.) 20 or more in Victoria (Brown, 1983a) and 100-200 in South-east England (Graham and Stone, 1975).

The methods of control that are available are rotation (Millikan, 1938), resistance (O'Brien and Fisher, 1974; Brown, _loc. cit._), nematicides (Gurner, _et al_, 1980) and tolerance (Fisher, _et al_, 1981). Of these the least desirable, from all points of view, is the use of chemicals and a combination of resistance and rotation should be sufficient for control. However, despite extensive screening only one gene for resistance has been identified in wheat. This source of resistance will sustain a minimum of 3-5 eggs/gm. of soil of the Australian pathotype (Fisher, 1982). From the results of Meagher and Brown (1974), Fisher (_loc. cit._) estimated that a yield loss of 15-30% will be suffered by an intolerant cultivar like Olympic at that

level of infestation. Before this, essentially partial, resistance in wheat against the Australian pathotype was reported, Brown and Meagher (1970) suggested the introgression of resistance from a genus related to wheat - possibly rye (*Secale cereale*) cultivar 'South Australian'. Modern techniques for pest/disease resistance gene transfer like protoplast fusion, somaclonal variation and molecular manipulations have shown some potential for success. However the success of traditional methods like alien chromosome substitutions, induced translocations and induced homoeologous recombination is well documented (Knott and Dvořák, 1976; Sears, 1972, 1981).

The aims of the current studies have been to introduce a resistance gene in Triticale against the Australian pathotype of *H. avenae* into a better genetic background in triticale; to isolate and identify the chromosomal location of this gene; and to initiate its transfer into wheat.

CHAPTER 2

LITERATURE REVIEW

2.1 LIFE CYCLE AND DISTRIBUTION.

In Australia there is only one generation of *H. avenae* per year (Wallace, 1965; Banyer, 1966; Meagher, 1977). There is no spring hatch as occurs in North-West Europe (Sparrow and Dube, 1981).

Banyer and Fisher (1971a) categorize hatching of Australian populations into a larval development phase and an eclosion phase with 10°C and 20°C as optimum temperatures for each phase respectively. There is a marked rise in hatching rate with the onset of low soil temperatures in late autumn - winter in South Australia (Banyer and Fisher, 1971b). Whereas Winslow (1955), Hesling (1957) and Banyer and Fisher (1971a) could find no influence of host root exudates on hatching, Williams and Beane (1972, 1979) reported such an influence at 10-15°C and Kerry and Jenkinson also (1976) at soil temperatures during the European spring.

The embryo develops into a vermiform larva, moults to a second-stage larva and then breaks out of the egg shell into the soil from where it may invade a host root. Within the root it feeds near its head from an induced giant cell which is formed from the breakdown of the walls between adjacent cells (Wallace, 1965).

After three further moults, the nematode becomes either an elongate male or a lemon-shaped female. Control of sex determination in the family Heteroderidae is a controversial issue (Triantaphyllou, 1973). The female remains sedentary as a protrusion from the root whereas the male moves into the soil from where it may reach and fertilise the female. The female produces a mass of eggs and later tans into a cyst which can remain in the soil for many months.

Temperature, soil moisture and soil structure probably all limit the distribution of the nematode despite earlier reports (Wallace, 1965; Meagher, 1972) that temperature was not limiting. The specialised temperature requirements for hatching (Banyer and Fisher, 1971a) suggest that the nematode requires a period below 20°C for hatching. This requirement would exclude tropical countries as a habitat. The presence of soil moisture is essential for all nematodes and soil structure interacts with soil moisture in regulating nematode activity (Wallace, 1963). The optimum particle size for motility of second stage juveniles is a diameter of 130 micrometres (Wallace, loc. cit.) i.e. fine sand. Hence fine sands would be optimal but not exclusive and in South Australia damage is experienced in heavier soils such as red-brown earths (Banyer, 1966) - a situation that occurs in other countries (Davies, 1961; Graham and Stone, 1975; Wiese, 1977). Only in Victoria is infestation limited to light-textured soils (Meagher, 1968) though Brown (1983a, 1984b) does not agree with this.

2.2 PATHOTYPES

Based on an International Test Collection of cereals at least ten different pathotypes of *H. avenae* have been differentiated world-wide (Andersen and Andersen, 1982).

Three, and possibly five, pathotypes have been suggested for India (Mathur *et al*, 1974) some of which may be identical to European pathotypes (Swarup *et al*, 1979). Three pathotypes have been recorded in Britain (Saynor, 1975), two in Denmark (Andersen, 1959), six in Germany (Jones, 1972; cited by Sidhu and Webster, 1981) four in The Netherlands (Kort *et al*, 1964), two in Sweden (Cook and Williams, 1972) and four in France (Rivoal, 1977; cited by Andersen and Andersen, 1982). Some of the French pathotypes may be identical to North European ones (Andersen and Andersen, *loc. cit.*).

A single unique pathotype, Ha13 (Andersen and Andersen, *loc. cit.*), has been recorded in Australia (McLeod, 1976; O'Brien and Fisher, 1979; Brown, 1974, 1982) contrary to some earlier suggestions (O'Brien and Fisher, 1974; Ellis and Brown, 1976).

Meagher and Brown (1974) and Meagher (1982) suggested that comparable population densities of *H. avenae* cause more severe yield loss in Australia than in Europe. This may have led Brown (1983b) to suggest that the Australian pathotype is

the most aggressive in the world. Fisher (1982) and Meagher (1972, 1982) emphasized the differences between Europe and Australia in seasonal cropping patterns _vis a vis_ hatching of the nematode and in soil fertility and climate. In England, 25 per cent of eggs hatch in Autumn (Kerry and Jenkinson, 1976) effectively removing this proportion from invasion of Spring cereals and so reducing the damage to the cereals. Moreover, Brown (1984a, 1984b) has misinterpreted Meagher's (1970) hatching data. Most rapid hatching occurred in May and hatching was almost complete by early July, a hatching cycle that coincides with cereal sowing in May/June in Australia. In fact, spring-sown cereals in Europe, grown under conditions favouring heavy nematode invasion, suffer severe damage (Graham and Stone, 1975) even though, with rising spring temperatures, they have an advantage over similar seedlings in Australia (Meagher, 1982).

2.3 ECONOMIC IMPORTANCE.

The assessment of economic injury to cereals by *H. avenae* is difficult and variable. In wheat, at 2 eggs/gm. of soil either no loss in grain yield is obtained (Rovira and Simon, 1982) or there is a 20% loss (Meagher and Brown, 1974). Meagher and Brown's estimate is based on a single soil sample and so is untrustworthy. These estimates probably vary with the tolerance of the cereal cultivar (Fisher *et al*, 1981), the level of nutrition and the agronomic factors like rotation so that the amount of loss is very variable. The estimate of 20% yield loss (Meagher, 1982) costing A$40-80 million (Rovira and Simon, *loc. cit*; Brown, 1983b, 1984a, 1984b) is simply a guess and may show little relation to the actual figure.

The introduction of chemical control to the Australian agricultural system (Gurner *et al*, 1980) has given some indication of responses to nematicides and it is not uncommon to obtain a 20 per cent increase in yield of barley, a 30 per cent increase in wheat and a 50 per cent increase in oats. This probably reflects the generally held belief that barley is more tolerant than wheat which is more tolerant than oats. This situation is probably related to the number of seminal roots produced by each cereal genus (Fisher, 1982). However, for all these cereals, if three susceptible crops are grown in succession, then the third is a complete failure. This is similar to the situation in Canada (Fushtey and Johnson,

1966) where a 50% loss in yield is common and total oat crop failures are not unknown. Heavy infestations in Europe may lead to a 30 per cent or more yield loss and in United Kingdom alone, annual loss due to the nematode is estimated at £ 2 million (Cotten, 1970). Losses in the United Kingdom are probably lower because of biological control by the parasitic fungus, Nematophthora gynophila (Kerry et al, 1980). In Australia parasites contribute little to control of the nematode (Stirling and Kerry, 1983).

In south-eastern Australia, the cereal cyst nematode is known to occur together with a number of root-pathogenic fungi. The most important association in the field is between H. avenae and Gaumannomyces graminis (causal organism of take-all) (Patel, 1983). While from pot tests, the synergistic effect of H. avenae and Rhizoctonia solani (Meagher and Chambers, 1971; Meagher et al, 1978) has been claimed as the major cause of yield loss in cereals (Meagher 1982; Brown, 1983a, 1984a, 1984b) this did not prove to be the case in field studies (Patel, loc. cit.).

2.4 CONTROL

2.4.1 Crop Rotation.

For many years crop rotations, including fallows and legumes, have been used to combat the cereal cyst nematode (Garrett, 1934; Wallace, 1965; Meagher and Rooney, 1966). A single season's fallow reduced the soil population of the nematode by 56 to 75 per cent (Meagher and Rooney, *loc. cit.*). However, rotations may be of limited value on small farms (Brown, 1973).

2.4.2 Resowing.

In South Australia, resowing an infested field after a catch crop had been sown earlier in the season has been proposed (Robinson, 1961; Banyer, 1966). Success, however, depends on growing conditions for the late-sown crop and the number of infective larvae still in the soil.

2.4.3 Early Sowing.

Early sowing of cereal crops in Australia (April-May) has been suggested as a method of control (Millikan, 1938; Robinson, 1961; Banyer, 1966; Dube *et al*, 1979; Meagher, 1972) based on the idea that the plant would be well established before the nematodes

hatch. Brown and Pye (1981) and Brown (1983b, 1984a) also recommend early sowing but this recommendation is based on an erroneous interpretation of hatching. With adequate seasonal rainfall hatching is most rapid in May, a time when Brown and Pye (*loc. cit.*) recommend sowing to avoid damage. With a high initial larval density, damage will occur in early sowings.

2.4.4 Weed Control.

Control of susceptible weeds like wild oats and Wimmera ryegrass prevents a build-up of the nematode in the soil (Meagher, 1972; Dube *et al*, 1979).

2.4.5 Biological Control.

Andersson (1982) has suggested the use of fungi like *Nematophthora gynophila* and *Verticillium chlamydosporium* as part of an integrated control programme. The decline of *H. avenae* populations under cereal monoculture in Europe (Gair *et al*, 1969; Cotten, 1970; Graham and Stone, 1975; Empson and Gair, 1982) has been attributed to the activities of such fungi (Kerry *et al*, 1980, 1982). However, *N. gynophila* is not present in Australian soils and *V. chlamydosporium* is not sufficiently efficient to effect significant reductions in Australian populations (Stirling and Kerry, 1983).

2.4.6 Tillage Practices.

Higher grain yields of wheat and fewer *H. avenae* females on the roots were obtained after direct drilling compared to conventional cultivation in Australia (Rovira and Simon, 1982; Roget and Rovira, 1983). In India, Mathur *et al* (1983) reported increased wheat yields and decreased *H. avenae* densities after two or three deep summer ploughings, at weekly intervals, prior to the wheat season.

2.4.7 Chemical Control.

Using a low-volume nematicide applicator Gurner *et al* (1980) demonstrated that economic chemical control of *H. avenae* in South Australia is feasible. Brown (1983c) made similar findings in Victoria. Nevertheless, the cost of nematicides relative to wheat prices, the extra work at seeding and the environmental implications of using such chemicals make this a rather unpopular choice. Chemical control is uneconomic in Europe probably because of the relatively less damage by the nematode there, the climatic conditions and the cultural practices (Andersson, 1982).

2.4.8 Host Resistance.

Resistance is explained as host inefficiency (Meagher, 1972) for the growth and reproduction of the nematode (Parlevliet, 1981). This is distinct from 'tolerance' which refers to the ability of the host plant to grow and yield well despite infection by the nematode (Schafer, 1971; Parlevliet, *loc. cit.*).

Since the discovery of some resistant barley (Nilsson-Ehle, 1920), wheat (Nielsen, 1966) and oats (Millikan, 1938; Cotten, 1963) breeding for resistance to *H. avenae* has become increasingly important as an economical means to provide material for controlling the pest.

Against the Australian pathotype resistance in wheat has been reported in Festiguay, Dural and Duramba (McLeod, 1976); Loros (AUS 11577) (Brown, 1974); Portugal 131, Loros (AUS 90248) and Spring Wheat (AUS 10894) (O'Brien and Fisher, 1974); Psathias (AUS 881) (Brown, 1974).

Resistance sources in oats include *Avena sterilis* (Cc4658), *Avena strigosa*, Avon and Cooba (Brown, 1974, 1982, 1983a).

In barley, Athinias, CI 8147, Marocaine 079 (C.I. 8334), Martin 403-2, Morocco (CI 3902) and Nile have good resistance to the Australian pathotype (O'Brien and Fisher, 1974, 1977, 1979; McLeod, 1976; Brown, 1974, 1982, 1983a).

Weethalle and South Australian are ryes resistant to the nematode (Cook, 1974; Brown, 1974; McLeod, 1976; Brown, 1982, 1983a). Some genotypes of Aegilops spp. have strong resistance to H. avenae (Brown, 1974; Dosba, et al, 1978). Brouwer and Castleman (1981) reported moderate resistance and tolerance in a triticale cultivar Towan.

Galleon was released in 1981 as the first commercial barley cultivar bred for cereal cyst nematode resistance in Australia (Sparrow and Dube, 1981). Similarly a wheat cultivar, Katyil, was released in Victoria in 1982 (Brown and Young, 1982) with resistance from spring wheat (AUS 10894). The danger of relying on major gene (race-specific) resistance is appreciated (O'Brien and Fisher, 1979). However the relatively slow spread expected of any resistance-breaking pathotypes means resistance could be comparatively long-lasting (Russell, 1978; Lamberti and Taylor, 1983; Cook, 1974; Cook and York, 1981). Nevertheless, Russell (loc. cit.) suggests the accumulation of an array of host resistance genes as a 'polygenic resistance block' (Andersson,

1982) against the pest. Meanwhile, mixtures of susceptible and resistant cultivars or alternate cropping of the two have been proposed (Andersen, 1980 - cited by Andersson, 1982) even though partially resistant pure cultivars may be more useful.

2.5 INHERITANCE OF RESISTANCE.

Inheritance of resistance to *H. avenae* includes both dominant and recessive expressions of the genes and one to several resistance genes in a host have been reported.

2.5.1 Wheat.

Using monosomic analysis Slootmaker *et al* (1974) located the dominant resistance gene in Loros wheat (Nielsen, 1966) on chromosome 2B. Spring wheat (AUS 10894) has a dominant resistance gene at the same or a closely linked locus but may have additional modifier genes (O'Brien *et al*, 1980).

2.5.2 Oats.

Resistance from *Avena sativa* L. (C.I. 3444) and *A. sterilis* (I. 376), incorporated by backcrossing into Sun II, have been used in breeding (Cook and York, 1982). Cultivar Nelson was derived from C.I. 3444 and has a single dominant resistance gene on monosome XV and modifier genes on monosomes VIII and X (Chew *et al*, 1981). According to Andersen and Andersen (1970) (cited by Cook and York, 1982), three dominant loci control resistance in I. 376. Cotten and Hayes (1972), however, suggested only two complementary dominant genes.

Cultivar Panema, bred from I.376, has only one dominant gene which is on the same chromosome as the gene in Nelson (Chew *et al*, 1981). The two genes are probably allelic or closely linked to each other (Cook and York, 1982).

In *Avena byzantina* (P.I. 17502) resistance is dominant, monogenic and probably at a locus distinct from those of the other two *Avena spp*. (Cotten and Hayes, 1972).

Cook and York (1982) found a single recessive gene in oat cultivar Silva independent of the C.I. 3444 gene in Nelson. Another cultivar, Mortgage Lifter, has two independent recessive genes for resistance (Cotten and Hayes, 1972).

2.5.3 Barley.

At least six resistance genes in barley against *H. avenae* have been predicted on the basis of the gene-for-gene hypothesis (Hayes and Cotten, 1971). However, virulence in *H. avenae* is dominant contrary to the normal pattern for gene-for-gene relationships (Andersen, 1965).

Rha 1 is a dominant resistance gene probably on the short arm of chromosome 2 and present in cultivars Drost, Brage and Fero (Cook and York, 1982). *Rha* 2, also a dominant gene, is located on the long arm of chromosome 2 (Cotten and Hayes, 1969; Andersen and Andersen, 1973). Linkages between this locus and six-row and liguleless loci respectively were established by Cotten and Hayes (*loc. cit.*).

Athinias and Marocaine 079 have the same single dominant resistance gene (O'Brien *et al*, 1979) independent of the *Rha* 1 locus (Cook and York, 1982). Nile and Athinias have different single dominant genes both of which differ from the gene(s) in Morocco (O'Brien, *et al*, 1979).

2.5.4 *Rye*.

A specific mode of inheritance is yet to be reported in rye (Sidhu and Webster, 1981).

2.6 MECHANISMS OF RESISTANCE AND TOLERANCE.

2.6.1 Resistance.

Dropkin and Webb (1967) reported less invasion of the roots of resistant tomato seedlings compared with susceptible ones by larvae of *Meloidogyne hapla*. On the contrary, numerous reports indicate that root invasion by larvae is not influenced by host resistance to *H. avenae* (Wålstedt and Bingefors, 1963; Williams, 1970; O'Brien and Fisher, 1977), *Globodera* spp. (Howard, 1959; Trudgill and Parrot, 1969; Trudgill and Cotes, 1983) or *Meloidogyne* spp. (Brodie *et al*, 1960; Reynolds *et al*, 1970; McClure *et al*, 1974a).

Dropkin (1969) and Dropkin and Webb (1967) observed necrosis in roots of tomato seedlings in response to invasion by larvae of *Meloidogyne incognita acrita*.

Larval emigration from roots (Reynolds *et al*, 1970; O'Brien, 1976), retarded development of larvae (McClure *et al*, 1974b; Brodie *et al*, 1960; Fassuliotis, 1970), death of larvae in roots (Shepherd, 1959), inadequate syncytia (giant cell) development (Fassuliotis, 1970) and early degeneration of syncytia (Endo, 1965) have all been associated with resistance. Some biochemical mechanisms of plant resistance to nematodes have been suggested (Giebel, 1974; 1982). For instance,

β-galactosidase activity has been implicated in the reaction of potato to Globodera rostochiensis (Giebel, 1974).

While resistance to H. avenae has been associated with a high male to female ratio of the nematode (Brown, 1974) this may not mean sex reversal or environmental determination of sex. It may reflect a differential development rate or death of one of the two sexes on resistant hosts.

Dropkin (1969) noted a reduction in resistance of some tomato seedlings to Meloidogyne spp. with increase in temperature. Environmental influence on resistance to H. avenae pathotypes was reported by Giebel (1982).

2.6.2 Tolerance.

Generally the decreasing order of tolerance of the major cereals to H. avenae attack is considered to be barley, wheat and oats (Duggan, 1961; Davies, 1961; Meagher, 1972; Graham and Stone, 1975). Fisher (1982) associated the relative tolerance of the cereals to their number of seminal roots (mean of 3 for oats, 5 for wheat and 7 for barley), since the initial invasion by larvae is the most damaging (Duthoit, 1964; O'Brien and Fisher, 1981; Simon and Rovira, 1982; Seinhorst, 1961). However, Cook (1974) explained the higher tolerance of barley relative to oats in terms of their differential response to larval invasion.

Intra-specific variation in tolerance may depend on a relative capacity for compensatory growth for damaged roots. Tolerant potato cultivars tend to grow larger root systems in soil heavily infested with *Globodera rostochiensis* than in lightly infested or nematicide treated soil (Trudgill and Cotes, 1982, 1983).

From laboratory experiments Volkmar (1983) reported greater ability to withstand water stress in tolerant oat cultivars as compared to intolerant ones. Reduced sensitivity of roots to nematode attack, for instance in the amount of gall tissue produced, has been noted in tolerant plants (Trudgill and Cotes, 1980; Stanton, 1983). Stanton (*loc. cit.*) proposed late recovery of growth as observed for wheat cultivar Cook (Fisher *et al*, 1981) as a possible tolerance mechanism.

2.6.3 Resistance and Tolerance Estimates.

Fox and Spasoff (1976) reported that resistance and tolerance of tobacco to *Heterodera solanacearum* are under separate genetic controls. Jones (1956) suggested a similar situation for sugar beet and *Heterodera schactii*.

Resistance to *H. avenae* is assessed by the number of gravid females on the roots of the host plant.

Tolerance, however, is difficult to assess or select for in segregating populations (Cook and York, 1982). It has been evaluated by yield loss differences between crops from infested and non-infested paddocks (Fisher _et al_, 1981) or from nematicide-treated and untreated plots on infested sites (Seinhorst, 1961; Wilson _et al_, 1983). As a difference between two yield estimates, yield loss harbours the error variances of both estimates thus reducing precision (Parlevliet, 1981). Stanton (1983) criticised the use of percentage yield loss as a measure of tolerance as being influenced by the relative yield potentials of the cultivars involved.

Furthermore, yield loss may reflect the combined effects of resistance and tolerance (Parlevliet, 1981). Schafer (1971) advises the elimination of resistance as a confounding factor in tolerance estimates. The influence of resistance may be through its effect on the number of establishment sites induced by the nematode larvae (O'Brien and Fisher, 1977).

On the other hand, tolerance could determine the maximum number of gravid females a host plant will sustain because the state of the root system and general plant growth after heavy nematode invasion depends on host tolerance (Graham and Stone, 1975).

While some workers (Graham and Stone, loc. cit.; Cotten, 1970) found consistently higher yields in resistant lines than susceptible ones in infested fields others (Trudgill and Cotes, 1983; Fisher, et al, 1981) found no such advantage. Since host plants suffer less damage from the development of the females and their syncytia than from the initial juvenile invasion the apparent conflict in the reports may be due to differences in nature of resistance among the various cultivars tested. Resistance seems to be an advantage only after larval invasion.

Fisher (1982) suggested 12 and 3 females per plant as the levels of 'practical' resistance for highly tolerant and highly intolerant cultivars respectively. He further advises that resistance should be incorporated into tolerant cultivars - a view supported by Andersson (1982).

2.7 Alien germ plasm as a source of disease/pest resistance in common wheat (*Triticum aestivum* (L.) em.Thell.)

With increasing uniformity of modern cultivars and the loss of many traditional land races alien germ plasm is increasing in importance as a source of genes for wheat improvement. Introgression of genes from relatives of wheat which have one or more genomes in common with wheat is relatively easy because synapsis and crossing-over occurs between homologous chromosomes in the hybrids.

Species of *Aegilops* L., *Agropyron* Gaertn., *Haynaldia* Schur. and *Secale* L. are considered relatives of common wheat (Sears, 1981). While many of them have been successfully crossed to wheat, gene transfer from them to wheat is difficult. Common wheat comprises three genomes - A, B and D. *Triticum monococcum* and *Aegilops squarrosa* L. [*Triticum tauschii* (Coss.) Schmal.] have been recognised as donors of the A and D genomes, respectively, to hexaploid wheat. The donor of the B genome is controversial (Kimber, 1974). Following their discovery of the absence of chromosome 4 of diploid wheat (*d4*) from hexaploid wheat, Wazuddin and Driscoll (1986) have proposed an alternative hypothesis of the evolution of polyploid wheats. This involves the loss of *d4* and the retention of two versions of chromosome 4B at the early tetraploid stage and therefore implies that the A,B and D genome progenitors donated six, eight and seven chromosomes, respectively, to hexaploid wheat. On the basis

of resemblance between different nullisomics and from a study of nullisomic-tetrasomic combinations the twenty-one pairs of wheat chromosomes have been placed in seven homoeologous groups of three (Sears, 1952).

Chromosomes of many of the other members of the subtribe Triticinae fit into the homoeologous groups defined in wheat (Gupta, 1972; Quinn and Driscoll, 1967). Those of rye were placed in the groups by Sears (1968), Gupta (1971) and Koller and Zeller (1976). However, for a particular locus on a chromosome, homoeologous relationships may be complicated by translocations during evolution (Appels, 1982). Translocations in, at least, three chromosomes differentiate cultivated rye, _Secale cereale_, from the wild species _S. montanum_ Guss. and _S. vavilovii_ Grossh. This may explain why mixed homoeologies were assigned to chromosomes 4R, 7R and 6R of Imperial Rye (Koller and Zeller, _loc. cit._).

2.7.1 Barriers to Introgression of Alien Genes into Wheat.

Factors that have frustrated alien germ plasm exploitation so far include lack of crossability, differences in ploidy level, embryo abortion, sterility of hybrids, linkages between useful and deleterious genes and lack of synapsis and crossing-over between wheat and alien chromosomes (Knott and Dvořák, 1976).

Reciprocal crosses and several genotypes are often used to circumvent the non-crossability and sterility barriers. For instance, Riley and Chapman (1967a) reported genes in Chinese Spring Wheat which facilitate crossing to rye.

To overcome differences in ploidy level genetic bridges and doubling of the chromosome number of the wild species have been useful. Embryo rescue techniques have almost become standard practice in interspecific hybridization. While unfavourable linkages on alien chromosome segments may be broken with enough backcrosses and large populations, sometimes special cytogenetic procedures are necessary.

Chromosome pairing in wheat is restricted to homologues by a diploidization mechanism controlled mainly by a gene(s) on the long arm of chromosome 5B (Okamoto, 1957; Riley and Chapman, 1958a). Hence there is normally no synapsis between wheat and alien chromosomes (Khush, 1973). Minor suppressors of allosyndesis have been located on wheat chromosomes 3Dβ (Mello-Sampayo, 1968; Driscoll, 1972, 1973a), 3A (Driscoll, 1972) and 4D (Driscoll, 1973b).

On the contrary, pairing promoting factors have been reported to exist on chromosome 3Dα (Driscoll, 1972), on the long arms of wheat chromosomes 5A and 5D

and on the short arm of chromosome 5B (Feldman, 1966; Riley and Chapman, 1967b). Many researchers therefore agree that chromosome pairing in wheat reflects a balance between genes that promote and those which suppress homoeologous pairing (Riley et al, 1973; Jouve, et al, 1980; Fedak and Armstrong, 1981). What is controversial is the mode of action of the main pairing suppressor gene(s) on 5BL. Driscoll et al (1972) disagreed with Feldman's (loc. cit.) hypothesis that the gene(s) affects premeiotic association of chromosomes - an event Feldman considered as a prerequisite for synapsis. Driscoll et al (loc. cit.) suggested that the gene(s) seems to act after completion of homoeologous association by controlling which of the associated chromosomes can proceed to synapsis.

In the absence of the suppressor gene on chromosome 5BL there is formation of multivalents involving homoeologues and synapsis of chromosomes from related genera (Riley and Kempana, 1963; Riley et al, 1981). Driscoll and Quinn (1970) demonstrated genetic variation among cultivars of Triticum aestivum which influences the level of chromosome pairing in intergeneric hybrids.

Knowledge about the control of pairing in wheat has enabled many workers to succeed in introducing alien chromosome segments into it through induced recombination. Others have used ionising irradiation.

2.7.2 Deleterious Effects of Alien Chromatin.

Triticum aestivum L. and its relatives have evolved their current genetic constitutions over many years. Any significant loss of chromatin or its gain from an alien source is likely to cause imbalance resulting from undesirable duplications, deletions or gene linkages.

Interaction between rye genes and the wheat genotype has been blamed for the low fertility of rye addition lines (Riley and Chapman, 1958b). The abnormal cytological behaviour, aberrant endosperm development and poor kernel characteristics of wheat-rye hybrids have been attributed to rye heterochromatin (Bennett, 1977; Bennett *et al*, 1977; Gustafson and Bennett, 1982). However, Varghese and Lelley (1983) have questioned this suggested influence of heterochromatin.

Alien chromosome segments in wheat have also been associated with depression in yield (Weinhues, 1973), inferior baking quality (Knott, 1971) and yellow flour colour (Sharma and Knott, 1966; Dvořák, 1975).

2.7.3 Addition and Substitution of Alien Chromosomes.

Fertile amphiploids have been produced by doubling the chromosome number of hybrids between wheat and some related genera. Such amphiploids usually exhibit low

fertility, undesirable genes of the wild species and genetic antagonism between the parental genotypes (Riley and Chapman, 1958b). Not surprisingly, only the wheat-rye amphiploid, Triticale, has found a place in commercial agriculture (Driscoll, 1981).

To reduce the undesirable characteristics of the amphiploids, O'Mara (1940) suggested a procedure for the addition of single pairs of alien chromosomes to the full complement of wheat chromosomes. These alien addition lines provide useful information about the genes on the particular alien chromosomes and their interaction with the genotype of the recipient species. However, alien addition lines are unstable and return to the euploid condition through pollen selection. Many of them show low fertility and adverse effects on agronomic characteristics of the recipient species. None of them has been of direct commercial value but they have been used in irradiation-induced translocations to incorporate alien chromatin into wheat (Sears, 1956, 1967; Knott, 1961; Driscoll and Jensen, 1964; Sharma and Knott, 1966; Wienhues, 1966). Alien addition lines have also been used to generate disomic substitutions of the alien chromosomes for some of the chromosomes of wheat (Unrau et al, 1956).

Disomic alien substitution lines show more cytological stability than disomic alien addition lines

but, in addition to the influence of alien chromatin, the former may have problems caused by the wheat nullisomic condition. Some alien chromosome substitutions are inviable owing to severe genetic imbalance and Sears (1972) proposed the substitution of critical alien chromosome arms instead of whole chromosomes to reduce the imbalance. According to Khush (1973) rye chromosomes do not compensate as well for wheat chromosomes as those of Agropyron spp. and Aegilops spp. do. While homoeologous substitutions have been particularly successful (Knott, 1964) only a few of the many available substitutions (Driscoll, 1983) have found direct use in commercial agriculture. Several East European wheat cultivars have chromosome 1 of rye with mildew and stripe rust resistance genes substituted for 1B of wheat (Mettin et al, 1973; Zeller, 1973). A West German wheat cultivar, Weique, which has resistance to stem, leaf and stripe rusts was reported to comprise two sub-lines: a 1R (-1B) substitution and a translocation involving the replacement of the short arm of 1B, or part of it, by a 1R segment (Zeller and Sastrosumarjo, 1972). Substitution lines are also useful for genetic analysis (Kuspira and Unrau, 1957; Law, 1966, 1968; Sasaki et al, 1968).

2.7.4 Translocations.

Spontaneous translocations between wheat and alien chromosomes have been reported (Weinhues, 1973) especially where Triticales have been crossed to wheat (May and Appels, 1982; Lukaszewski and Gustafson, 1983). Most of these may have resulted from the misdivision of wheat and alien chromosomes in the hemizygous condition and the fusion of non-homologous chromatids (Lukaszewski and Gustafson *loc. cit.*).

Sears (1956) developed a procedure for using ionising irradiation to induce translocations between non-homologous chromosomes. This led to the release of 'Transfer' which has a reciprocal translocation involving wheat chromosome 6B and a chromosome from *Aegilops umbellulata* giving resistance to wheat leaf-rust. Other examples of irradiation-induced translocations include the transfer of stem rust resistance from *Agropyron elongatum* to wheat chromosome 6A (Knott, 1961); leaf-rust resistance from *Agropyron* to 7D of wheat (Sharma and Knott, 1966) and leaf-rust resistance from *Agropyron intermedium* to a wheat chromosome (Weinhues, 1966).

Induced translocations involve many deleterious exchanges even though translocations between homoeologues are reported to predominate (Knott, 1964, 1968; Weinhues, 1973). Driscoll and Jensen (1963) and, later, Sharma and Knott (1966) detected successful translocations by observing segregation for the desired characteristic in progeny of the irradiated lines. Consequently Driscoll and Jensen (loc. cit.) produced 'Transec' - a wheat-rye translocation stock carrying resistance genes to both leaf rust and powdery mildew from rye. Even when the genetic detection of translocations is used, confirmation is necessary through cytological observations on F_1 hybrids from crossing with euploid wheat (Driscoll and Jensen, loc. cit.). The volume of cytological work involved in the use of irradiation-induced translocations is a major disadvantage.

Target alien genes near the end of an alien chromosome lend themselves more easily to successful transfer by translocation. Others may necessitate transfer of long alien segments with the attendant deleterious effects (Sears, 1972).

Commercial utilization of products of induced translocation is hampered by their yield disadvantage when homozygous (Wienhues, 1973; Driscoll, 1981; Zeller and Hsam, 1983) and depressed quality levels (Sharma and Knott, 1966; Knott, 1971; Koebner et al, 1984).

2.7.5 Induced Recombination.

Rye is one of the relatives of common wheat known to have genes which suppress the activity of the pairing regulator gene (Ph) on chromosome 5BL of wheat (Bernard and Bernard, 1978; Fedak and Armstrong, 1981; Naranjo and Palla, 1982). Lelley (1976) proposed more than two alleles in rye probably acting additively to promote homoeologous pairing in wheat while Dvořák (1977) reported a polygenic system. Riley, Chapman and Miller (1973) suggested that the short arm of chromosome 5R of rye may carry the pairing promoter(s). Naranjo and Palla (1982) reported the probable influence of 5R on homoeologous pairing of wheat chromosomes. From Triticale x Rye hybrids, Jouve et al (1980) attributed the high homoeologous pairing observed to the inhibition of a single dose of the Ph allele of 5B by two doses of 5R.

Bielig and Driscoll (1970a) demonstrated that the long arm of chromosome 5R lacks the pairing regulator gene(s) of chromosome 5BL. From studies on substitutions of 5R for its three wheat homoeologues Bielig and Driscoll (1970b) concluded a null effect on homoeologous pairing by 5R. Perhaps it could be argued that the strong influence of the absence of the Ph gene on 5B in all the lines they compared might have confounded any influence of the rye chromosome or misdivision products.

Despite earlier skepticism (Nakajima, 1952; Riley and Kimber, 1966) there have been numerous reports of wheat-rye chromosome pairing, albeit at low frequencies (Lacadena, 1967; Bielig and Driscoll, 1970a, 1970b; Jouve *et al*, 1980; Naranjo and Lacadena, 1980; Fedak and Armstrong, 1981; Naranjo, 1982). Such pairing has occurred in situations where the homoeologous pairing inhibitor gene (*Ph*) was absent or had been suppressed. However there may be variability among the rye chromosomes in their affinity with wheat chromosomes. Naranjo (1982) reported a high frequency of wheat-rye meiotic pairing between chromosomes of homoeologous group one. On the contrary, Riley and Kimber (1966) could not find any pairing between the long arm of chromosome 6R and any wheat chromosome even though the activity of the *Ph* gene had been suppressed. Jouve *et al* (1980) mentioned the probable increase in wheat-rye chromosome pairing by the presence of chromosome 3D but admitted the general influence of the new interaction of pairing systems.

Certain strains of *Aegilops speltoides* Tausch. [= *Triticum speltoides* (Tausch.) Gren. ex Ritcher], *Aegilops mutica* Boiss. [= *Triticum tripsacoides* (Jaub. et Spach.) Bowden] and *Aegilops longissima* Schweinf. et Muschl. in Muschl. [= *Triticum longissimum* (Schweinf. et Muschl. in Muschl.) Bowden] inhibit the diploidization mechanism in common wheat (Riley and Law, 1965; Riley

and Kimber, 1966). To promote pairing between chromosomes of wheat and those of an alien species a hybrid between wheat and one of those *Aegilops* spp. could be crossed with the alien species. However it is difficult to pick up the desired transfer chromosome because of the ability of the *Aegilops* chromosomes to pair with those of wheat and the alien species. Despite this limitation Riley *et al* (1968) used a modification of this method to transfer stripe rust resistance from *Aegilops comosa* to chromosome 2D of wheat to produce the wheat line 'Compair'.

Crossing wheat monosomic 5B or nullisomic 5B tetrasomic 5A/5D to an alien species or a disomic alien addition or substitution line produces some F_2 progeny which are nullisomic for 5B and hence without the *Ph* gene. Alternatively the mutant wheat line *ph1b ph1b* (Sears, 1977) could be used in place of monosomic 5B and nullisomic 5B tetrasomic 5A/5D. These methods are expected to replace wheat chromatin with more or less homoeologous alien chromatin (Sears, 1981). The low fertility of 5B- and *Ph 1-* deficient hybrids restricts the opportunities for transfers. To reduce sterility problems Sears (1981) suggested the use of less effective pairing mutants like *ph1a* (Wall *et al*, 1971) or *ph2* on 3DS (Sears, 1977) but added that the decreased frequency of allosyndesis may negate any advantage of increased fertility.

For greater precision of alien chromosome segment transfer it is better to eliminate or suppress *Ph1* activity in a monosomic alien substitution line. Transfers are more likely to involve a particular, predetermined wheat chromosome. For instance, seventeen out of twenty transfers Sears (1972) obtained from the offspring of a mono-3Ag., mono-3D, nulli 5B, tri-5D line involved chromosome 3D. Using a telosome of the critical arm of the alien chromosome instead of the full chromosome facilitates the cytological determination of homoeologous pairing frequency and the identification of transfer chromosomes (Sears, *loc. cit.*).

Even using induced allosyndesis it is difficult to introduce only a short interstitial alien chromosome segment into wheat. Unless the critical gene is close to the telomere two crossovers close to each other are required and this is rare even between homologues (Sears, 1981). Sears (*loc. cit.*) proposed the crossing of two transfer chromosomes with exchanges proximal and distal, respectively, to the critical gene in order to produce a wheat chromosome with an intercalated alien segment. In the absence of exchanges on both sides of the critical gene the transfer chromosome with its exchange closest to the gene may be combined with a homoeologous wheat chromosome in a *Ph1*-deficient background.

Despite the many reports of wheat-rye pairing only Joshi and Singh (1978) and Koebner and Shepherd (1985) have claimed successful gene transfer from cereal rye to wheat through induced recombination. It is not certain whether the transfers reported by Joshi and Singh (*loc. cit.*) resulted from homoeologous recombination or centric fusion of wheat and rye telocentrics. Koebner and Shepherd (*loc. cit.*) used the *ph1b* mutant (Sears, 1977) to induce recombination between the long arm of chromosome 1R of cereal rye and wheat chromosomes. They observed a maximum recombination frequency of 2.87 per cent between a rye glutelin locus tightly linked to the centromere and the heterochromatic telomere on 1RL.

2.7.6 Alien Gene Expression in Common Wheat.

To be useful in wheat breeding alien genes must be epistatic to those of wheat or interact with them to produce the desired effect (Riley and Chapman, 1958b; Riley, 1960). Riley and Chapman (*loc. cit.*) attributed most of the modifications of the wheat phenotype in their wheat-rye addition lines to interaction between wheat and rye genes.

Expression of resistance to pests and diseases is often modified when resistance genes are introduced into a new genetic background (Knott and Dvořák, 1976).

Incomplete epistasis was blamed for the wheat mildew resistance in the wheat-rye amphiploid of Riley and Chapman (*loc. cit.*) as compared to the immunity of the rye parent. Riley and Bell (1958) reported that the high resistance to yellow rust in *Aegilops comosa* and *Ae. ovata* was not expressed in amphiploids involving these species and wheat. Stem rust resistance genes in tetraploid wheats are reported to have been suppressed by genes on chromosome 7DL of common wheat (hexaploid) (Kerber and Green, 1980). Some triticales expressed only the low leaf rust resistance of their wheat parents but not the strong resistance of their rye parents (Quinones *et al*, 1972).

Contrary to the foregoing examples, however, there are many examples of successful utilization of alien genes for wheat improvement as mentioned in previous sections.

CHAPTER THREE.

MATERIALS AND METHODS.

3.1 SEEDS - SOURCES and HISTORY.

T701-4-6.

This is a hexaploid triticale line received from Professor C.J. Driscoll, Agronomy Department, Waite Agricultural Research Institute who obtained it from Dr. E. Matheson of the University of New England. Originally bred by CYMMIT, it has the parentage: KOALA X TCL MAYA II - ARM "S". The only rye named in its pedigree was "Merced". Preliminary evidence suggested that it may have resistance to *H. avenae* (Fisher, pers. comm.) and *Septoria nodorum* (Driscoll, pers. comm.). It has good tolerance to attack by *Heterodera avenae* (Dube, unpublished data) but a poor seed finish.

T1006 and T1116.

These are high-yielding hexaploid triticale lines bred by CYMMIT and supplied by Professor C.J. Driscoll. T1006 has the parentage Maya II-Arm "s" and the pedigree X2802-38N-5M-6N-6M-1Y-1M-0Y. Corresponding information for T1116 are Inia - Arm "s" and X1648-8N-2M-0Y-3M-0Y. Both lines are susceptible to *H. avenae* (Fisher, pers. comm.) but moderately tolerant (*sensu* Schafer, 1971) to its attack (Dube, unpublished data).

COORONG.

Seeds of this commercial triticale cultivar, which was bred in South Australia, were supplied by Professor C.J. Driscoll. It is susceptible to the cereal cyst nematode (Dube, unpublished data).

AROONA and HALBERD.

These are commercial hexaploid wheat cultivars that were bred in South Australia and received from Dr. A.J. Rathjen, Agronomy Department, Waite Institute. They are known to be susceptible to *H. avenae* (O'Brien and Fisher, 1974; Fisher pers. comm.).

SOUTH AUSTRALIAN RYE and CHINESE SPRING WHEAT.

Seeds of rye cultivar South Australian which has high resistance to *H. avenae* (Brown, 1974) and those of euploid Chinese Spring Wheat were also supplied by Professor C.J. Driscoll.

CHINESE SPRING MONOSOMICS AND *AEGILOPS VARIABILIS*.

Dr. K.W. Shepherd, Agronomy Department, Waite Institute, provided seeds of *Aegilops variabilis*, Chinese Spring monosomic 6A, and Chinese Spring monosomic 6B. Seeds of Chinese Spring double monosomics 5B,6B and 5B,6A were obtained from Dr. R. McIntosh, Plant Breeding Institute, Castle Hill, Australia.

SPRING WHEAT (AUS 10894).

Seeds of this *H. avenae* resistant line (O'Brien and Fisher, 1974) were provided by Dr. J.M. Fisher, Plant Pathology Department, Waite Institute.

3.2 METHODS.

3.2.1 Crossing.

All crosses were made between plants growing in a glasshouse at approximately 20°C and under natural light.

3.2.2 Examination of Somatic Chromosomes.

Seeds were germinated on moist filter paper in petri dishes at 27°C until the seminal roots were approximately 1.5cm. long. One root was cut off and pre-fixed in cold water (*circa* 2°C) for 26 hours and then fixed in a 3:1 mixture of ethanol (98%) and glacial acetic acid for, at least, ten minutes. Meristematic cells were teased onto a glass micro slide in a drop of forty-five per cent acetic acid. After placing a cover glass (18x18mm.) on the mass of cells the slide was warmed gently over a flame and then pressed between layers of filter paper to spread the cells. The cover slip was removed by the liquid nitrogen freezing method. The slide was then dried on a hot-plate for about ten minutes and kept in a desiccator at least overnight.

For C-banding a batch of slides was placed in a saturated solution of barium hydroxide for five minutes

at $60^{o}C$, washed, and then transferred into a solution of Saline Sodium Citrate (SSC) (7.0g. Sodium Chloride plus 3.53g. tri-sodium citrate per 400ml. of water) at $60^{o}C$ for thirty minutes. The SSC solution was agitated a few times to prevent the formation of bubbles on the surface of the slides. After the SSC treatment the slides were rinsed with water and left in Giemsa staining solution for ten minutes, washed and then dried rapidly with a hair dryer. The Giemsa solution was made up of eighty parts of distilled water; six parts of monobasic buffer (7.8g. Sodium dihydrogen orthophosphate per 100ml. water); six parts of dibasic buffer (7.1g. disodium hydrogen orthophosphate per 100ml. water) and eight parts of Giemsa stain (Gurrs Improved R66). For long term storage slides were mounted in Euparol.

3.2.3 Cytological Analysis of Meiosis.

An immature inflorescence was removed and placed in a small amount of water in a petri dish. Starting in the middle of the spike one anther of a primary or secondary floret was removed and placed in a drop of one per cent acetocarmine on a glass micro slide. Pollen mother cells were teased from the anther and examined under the microscope for the stage of cell division. If they were not at the Metaphase 1 stage anthers of florets above or below the previous ones were checked. When an anther at the right stage was found the

remaining two anthers from the same floret were placed in a 3:1 mixture of ethanol and glacial acetic acid for at least ten minutes. Pollen mother cells from the fixed anthers were teased onto a glass micro slide in a drop of either acetocarmine (one per cent) or forty-five per cent acetic acid. Acetocarmine was used when the slides were not intended for C-banding. The C-banding procedure was similar to the one described previously except that treatment in barium hydroxide solution lasted only four minutes.

3.2.4 Embryo Culture.

In a laminar flow cabinet, an immature seed was dipped in ninety-eight per cent ethanol for a few seconds and then left in a three per cent solution of Sodium hypochlorite for three minutes. After washing the seed in sterile distilled water the embryo was excised onto the surface of an autoclaved artificial growth medium (Cooper and Driscoll, 1985) in a culture bottle. The capped bottles were stored at 20°C in the dark until regeneration of seedlings began. Bottles with seedlings were transferred to artificial light until they were big enough for transfer into potting soil.

3.2.5 Electrophoresis.

Alcohol Dehydrogenase (E.C. 1.1.1.1.).

The brush end of the endosperm of each pre-germinated seed was cut off and mashed in a plastic centrifuge vial containing 30 μl. of pH 7.5 Carlson's buffer (Carlson, 1972) plus 2-Mercaptoethanol. All vials were centrifuged for three minutes at 10,000 rpm. Approximately 3 μl. of the supernatant in each tube was withdrawn into grooves made on cellulose acetate paper (Cellogel 250). The paper had been soaked in water for fifteen minutes followed by equilibration in electrode buffer for twenty minutes prior to making of the grooves.

The grooves were equidistant, approximately equal in width and length, parallel to the edge of the paper and 2 cm. from the edge on the cathode side of the apparatus. Wicks of chromatography paper linked the cellogel to a pH 8.9 Tris-glycine electrode buffer [14.0 g/l. Tris(hydroxymethyl) aminomethane plus 22.6 g/l. glycine]. Electrophoresis was conducted at room temperature using a current of 0.8 to 1.0 mA/cm. width of cellogel for not more than one and a half hours. The paper was then stained in the following mixture until the isozyme bands were sharp : 1 ml. of 0.1M Tris Hcl buffer (pH 8.0) plus 0.2 ml. each of fifty per cent

ethanol, 0.3 M Magnesium chloride, 10 mg/ml.

β-Nicotinamide adenine dinucleotide, 0.5 mg/ml. Phenazine methosulphate and 2 mg/ml. 3 - (4,5 - Dimethylthiazol-2-yl) - 2,5-diphenyl tetrazolium bromide. The stain recipe was modified after Tanksley (1979).

Glutamate Oxaloacetate Transaminase (E.C. 2.6.1.1.).

The procedure was adapted from that of Hart (1975). The separating gels were two vertical polyacrylamide slabs made by mixing the following solutions and polymerizing them between glass plates on two sides of a perspex block : 10 ml. of a 36.6 g/100 ml. Tris-Hcl buffer (pH.8.9), 20 ml. of a 28.735 g/100 ml. Acrylamide and Bis-acrylamide mixture (38:1), 50 ml. of water, 0.55 ml. of Ammonium persulphate and 25 μl. of N,N,N^1,N^1 - Tetramethylethylenediamine (Temed). The gels were usually 8 cm. high and 16 cm. wide. Stacking gels, 3.5 cm. high, were polymerised on top of them from the following mixture: 2.5 ml. of a 5.0g/100ml. Tris-Hcl buffer (pH. 6.9), 5.0 ml. of a 12.5 g/100ml. mixture of acrylamide and bis-acrylamide (4:1); 12.5 ml. of water, 60 μl. of ammonium persulphate and 24 μl. of N,N,N^1,N^1 -Tetramethylethylenediamine. Before setting of the stacking gels combs were partly immersed in the mixture to provide, at least, twenty equidistant slots about 1.2 cm. deep. Leaf tissue was taken from seedlings being

screened for their reaction to H. avenae. Approximately half of the second leaf from the top of four-leaf stage seedlings was macerated in plastic centrifuge vials containing 60 μl. of Carlson's buffer plus four per cent sucrose and 6 μl/ml. of 2-Mercaptoethanol. The samples were centrifuged for two minutes at 10,000 rpm. At least 20 μl. of the super-natant in each tube was transferred into a corresponding slot in the stacking gel. A pH 8.57 Davis electrode buffer [2.88 g/l. glycine and 0.5 g/l. Tris (hydroxymethyl) aminomethane] was used in both the upper and lower chambers of the apparatus. Electrophoresis was done at 40 mA constant current per slab for the first three hours and at 50 mA per slab for another hour by which time the front of green leaf pigment had moved into the buffer in the lower chamber. The set-up was kept at 2-4°C throughout the electrophoresis. Using freshly prepared stain of the following recipe, gels were stained in the dark for about ten minutes: 85 ml. of 0.2M Tris - Hcl buffer, 75 mg. pyridoxal - 5' - phosphate, 300 mg. aspartic acid, 150 mg. α - ketoglutaric acid (sodium salt), and 225 mg. Fast Blue BB.

3.3 Screening for Resistance to *H. avenae*.

The assay reported by Fisher (1982) was used with minor modifications. Seeds of each cereal line or cultivar were surface-sterilised with sodium hypochlorite and pre-germinated in petri-dishes until the first three seminal roots were each about 1 cm. long. If root tips were required for cytological preparations one seminal root of each seedling was excised before planting. The seedlings were planted in a sand-loam mixture in opaque polyvinyl chloride tubes 2.5 cm. internal diameter and 13 cm. long which had been arranged in an appropriate experimental design on a base of potting soil (fig. 1). Each seedling was inoculated with 100 second-stage larvae of *H. avenae* per 1 ml. of water at planting and on four further occasions at three-day intervals.

Plants were grown at 15°C constant temperature under ten hours daylength (flourescent light) and were harvested 75 days after sowing.

At harvest the roots of each plant were washed over a set of three sieves (1.68 mm., 710 μm. and 250 μm. mesh apertures, respectively). Any white females collected on the bottom sieve (250 μm.) were counted under 10 x magnification. All selected resistant plants were potted for further growth in a glasshouse.

Fig. 1. Experimental set-up of *H. avenae* assay.

CHAPTER FOUR

COMPARATIVE RESISTANCE OF T701-4-6.

4.1 Introduction.

Only one gene has been identified in wheat for resistance to *H. avenae* (Nielsen, 1966; Slootmaker *et al*, 1974; O'Brien *et al*, 1980). In Australia this gene is not very effective against the prevailing pathotype. Rye cultivar South Australian has strong resistance to the nematode while Weethalle rye and Towan triticale have moderate resistance.

This chapter compares the resistant triticale line, T701-4-6 with the most resistant wheat line in Australia, Spring Wheat (AUS 10894). South Australian rye and Halberd Wheat were included as standard resistant and susceptible cultivars respectively.

4.2 Materials and Methods.

Twenty seeds each of T701-4-6 and AUS 10894, five seeds of South Australian rye and six of Halberd were assayed by the procedure detailed in section 3.3. A completely randomized design was used.

4.3 Results.

Halberd, the standard susceptible cultivar, allowed the production of 40.66 females per plant (table 1) while the three resistant lines restricted reproduction of the nematode. T701-4-6 allowed the production of fewer females than AUS 10894. Variation in numbers of females per plant was greatest with Halberd and least for T701-4-6 (table 1 and figure 2). The variation in numbers of females on AUS 10894 (2-16 females per plant) was considerable.

4.4 Discussion.

The relatively high number of females per plant on Halberd and the variation from 30 to 60 females per plant suggest that this was a good assay, similar to those of O'Brien *et al* (1980). However, more females were produced on AUS 10894 than they obtained. The increased number of females on AUS 10894 could have been caused by a number of factors such as genetic differences between seed lots, variation in the genetic constitution of the larvae, and better nutritional status and/or growing conditions of the test plants. The assay used by O'Brien *et al* (1980) was modified by Fisher (1982) to improve the nutritional status of the plants and reduce the stress of invading larvae on them. It is likely that the increased numbers are a result of these modifications. Nevertheless, there is little doubt that AUS 10894 is resistant.

TABLE 1

COMPARISON OF T701-4-6 TO STANDARD RESISTANT AND SUSCEPTIBLE LINES.

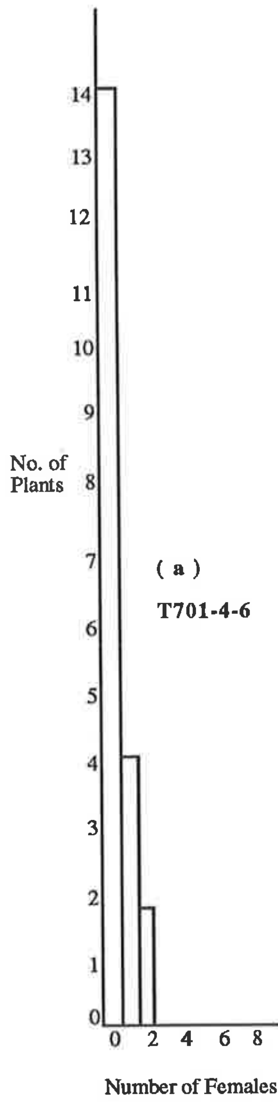
Cultivar/Line	Number of *H. avenae* females per plant Mean ± s.e.m.*	Number of Seedlings tested
T701-4-6	0.40 ± 0.66	20
South Australian Rye	2.40 ± 1.98	5
AUS 10894	8.15 ± 3.96	20
Halberd	40.66 ± 9.86	6

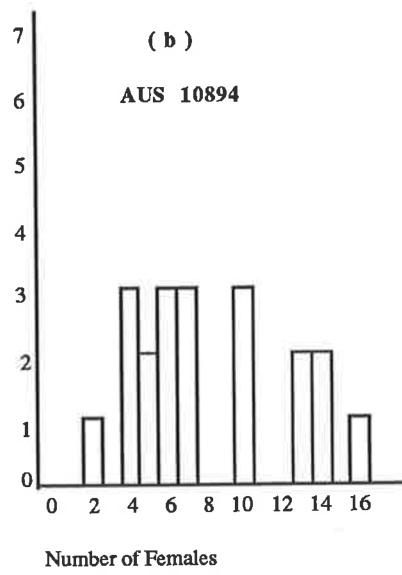
Least significant difference (Lsd) (0.01 level) for comparing T701-4-6 with AUS 10894 = 3.78.

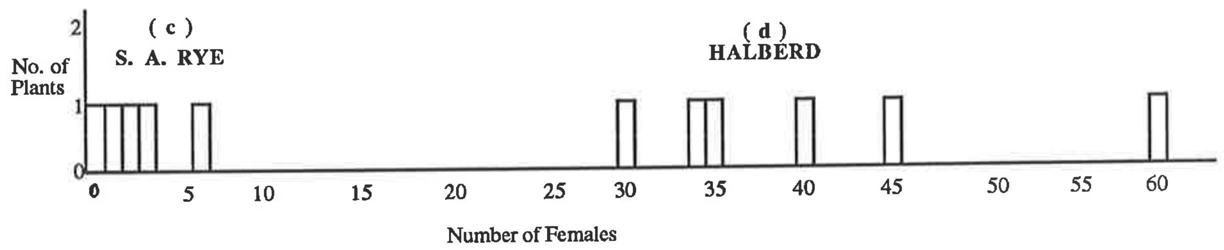
Lsd (0.05 level) for comparing T701-4-6 with South Australian rye = 4.47.

*s.e.m. : standard error of the mean.

Fig. 2. Frequency distribution of females per plant on the roots of T701-4-6, AUS 10894, S.A. rye and Halberd.


No. of Plants
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
(a)
T701-4-6
0 2 4 6 8
Number of Females


(b)
AUS 10894
7
6
5
4
3
2
1
0
0 2 4 6 8 10 12 14 16
Number of Females


No. of Plants
2
1
0
(c)
S. A. RYE
(d)
HALBERD
0 5 10 15 20 25 30 35 40 45 50 55 60
Number of Females

The resistance of T701-4-6 was superior to that of AUS 10894 and comparable to that of South Australian rye although the small sample of the rye necessitates caution in any close comparison.

Genetic control of resistance in T701-4-6 could be from the wheat genome, the rye genome or both. The strength of the resistance may even be a reflection of interaction between the two genomes. Investigation into this will be reported in later chapters.

CHAPTER FIVE

INHERITANCE STUDIES AND TRANSFER OF RESISTANCE TO *H. AVENAE* AND WHEAT STEM RUST FROM T701-4-6 INTO BETTER-PERFORMING TRITICALES.

5.1 Transfer of Resistance to *H. avenae* into better-performing Triticales.

5.1.1 Introduction.

T701-4-6 has strong resistance to the cereal cyst nematode but its grain yield and seed quality are relatively poor. To make use of the resistance in a triticale cultivar it would be necessary to transfer the gene(s) for resistance to a better genetic background.

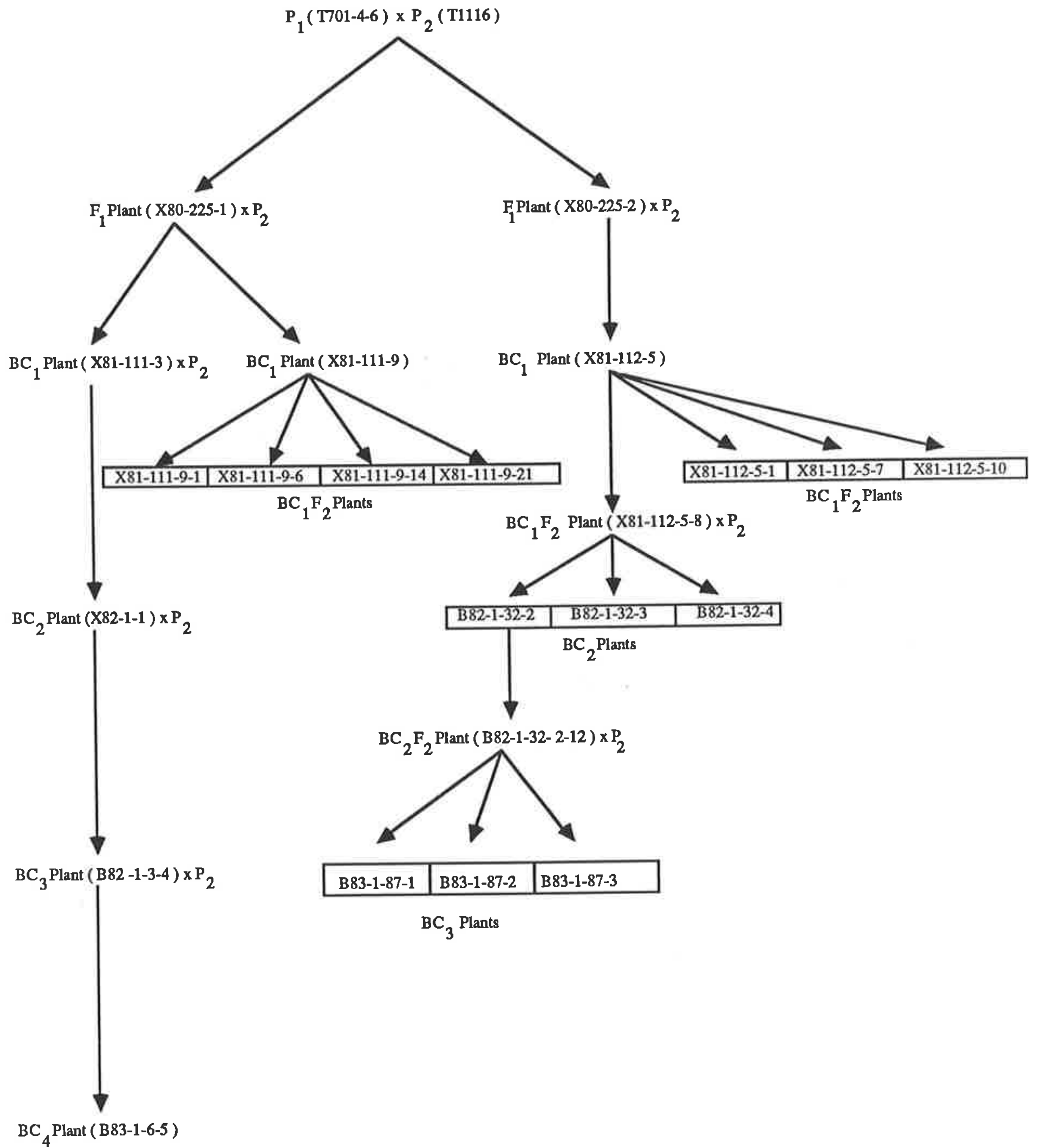
Since Harlan and Pope (1922) pointed out the value of back-crosses in small grain breeding, many workers have reported successful use of the method, especially for the transfer of simply-inherited characters in self-pollinated crops (Briggs, 1938; Briggs and Allard, 1953; Lawrence and Frey, 1975). Owing to the greater opportunity of cross-overs, backcrossing is more effective in obtaining recombination between linked alleles (Briggs and Allard, *loc. cit.*). The probability of recovery of the recurrent parent type is higher with backcrossing than with selfing. Generally six back-crosses, with selection for the required trait, are

suggested in order to recover the recurrent parent type (Briggs and Allard, *loc. cit*; Stam and Zeven, 1981). However, Lawrence and Frey (*loc. cit.*) reported that F_2 lines of BC_1 to BC_4 generations seemed to be the best for selecting high-yielding transgressive segregates from crosses between *Avena sativa* and *Avena sterilis*. Leininger and Frey (1962) also found considerable heterosis for yield among BC_1 to BC_4 families in oats. A BC_2F_4 spring rape line, TB19, yielded consistently higher than its higher-yielding parent in a backcross programme where low selection pressure was applied (Thurling, 1982). Frey and Browning (1971) reported both desirable and undesirable associations among genes for resistance to crown rust and those for grain yield in oats.

5.1.2 Materials and Methods.

A backcross programme initiated by Professor C.J. Driscoll to transfer the gene(s) for resistance to *H. avenae* from T701-4-6 into two high-yielding triticale lines with good seed quality, T1116 and T1006, was continued from the BC_1 stage until the BC_4 generation (figure 3). In a series of experiments seedlings from different generations of crosses involving either T1116 or T1006 were assayed for resistance to *H. avenae* as detailed in section 3.3. A total of 399 BC_1F_3 seedlings from 13 parent plants,

Fig. 3. A section of the Triticale Backcross Programme.

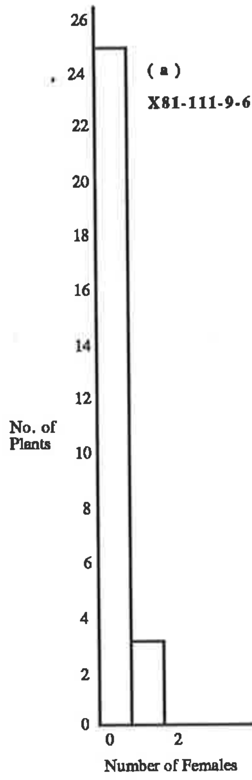

P1 (T701-4-6) x P2 (T1116)
F1 Plant (X80-225-1) x P2
F1 Plant (X80-225-2) x P2
BC1 Plant (X81-111-3) x P2
BC1 Plant (X81-111-9)
BC1 Plant (X81-112-5)
X81-111-9-1
X81-111-9-6
X81-111-9-14
X81-111-9-21
BC1F2 Plants
X81-112-5-1
X81-112-5-7
X81-112-5-10
BC1F2 Plants
BC1F2 Plant (X81-112-5-8) x P2
BC2 Plant (X82-1-1) x P2
B82-1-32-2
B82-1-32-3
B82-1-32-4
BC2 Plants
BC2F2 Plant (B82-1-32- 2-12) x P2
BC3 Plant (B82 -1-3-4) x P2
B83-1-87-1
B83-1-87-2
B83-1-87-3
BC3 Plants
BC4 Plant (B83-1-6-5)

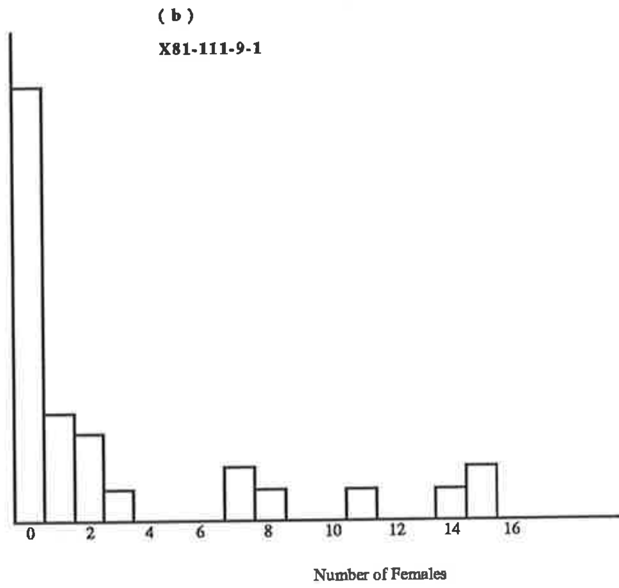
252 BC_2 seedlings from 68 female parents, 986 BC_2F_2 seedlings from 98 plants and 637 BC_2F_3 seedlings from 32 plants were assayed. In addition, 243 BC_3 seedlings from 108 female parents, 301 BC_3F_2 seedlings from 32 plants, 19 BC_4 seedlings from two female parents and 77 BC_4F_2 seedlings from 11 plants were assayed. Usually only plants classified as resistant were backcrossed to the respective recurrent parents or allowed to self. However, a few plants were allowed to self even though they did not show clear resistant reactions in order to investigate their true reaction to the nematode through progeny tests. F_2 lines were classified as homozygous resistant if all their selfed progenies were found to be resistant provided the total number of progeny assayed exceeded 25.

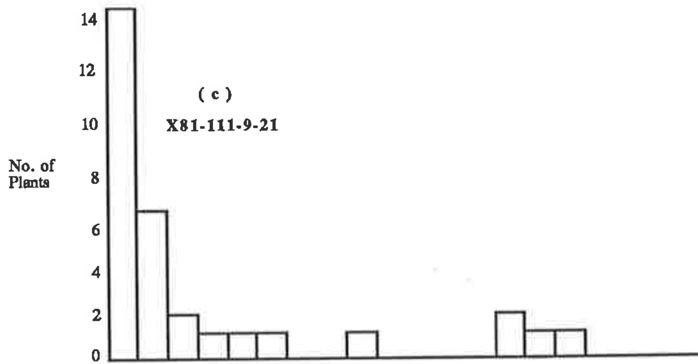
5.1.3 Results.

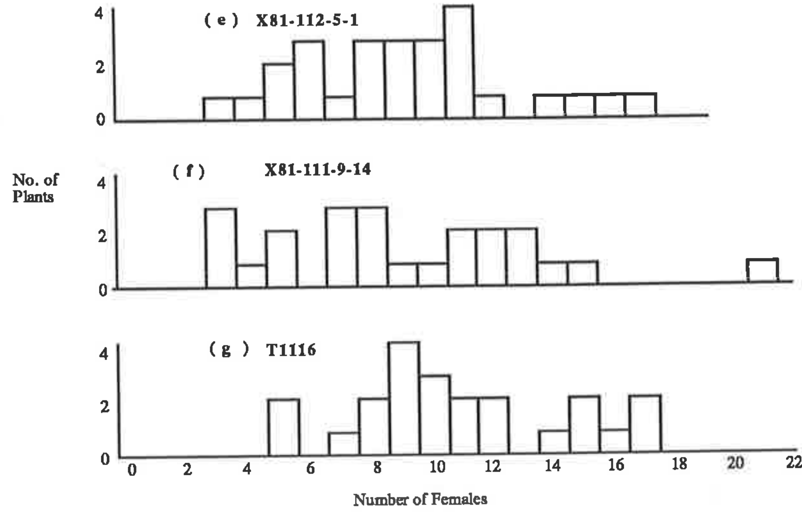
Two BC_1F_2 lines and six BC_2F_2 lines which were derived from T1116 as the recurrent parent were selected as homozygous resistant to the nematode. One BC_1F_2 line involving T1006 as the recurrent parent was also selected as homozygous resistant. The reactions of selfed progenies of five BC_1F_2 plants presented in figure 4 provide examples of progeny test results used to select homozygous resistant lines. X81-111-9-6 was considered a probable homozygous

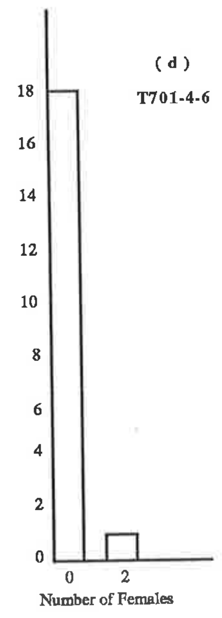
Fig. 4. Frequency distribution of number of *H. avenae* females per plant on parents and selfed progenies of BC_1F_2 plants.


(a)
X81-111-9-6
No. of Plants
Number of Females


(b)
X81-111-9-1
Number of Females


(c)
X81-111-9-21
No. of Plants


(e) X81-112-5-1
(f) X81-111-9-14
(g) T1116
No. of Plants
Number of Females


(d)
T701-4-6
Number of Females

resistant line whereas X81-111-9-1 and X81-111-9-21 were heterozygous. Occasionally, plants which were probably mis-classified as resistant produced selfed progenies which reacted similarly to the progenies of X81-112-5-1 and X81-111-9-14 (fig. 4).

5.2 Wheat Stem Rust Resistance in T701-4-6.

5.2.1 Introduction.

On many occasions since wheat production started in Australia wheat stem rust (*Puccinia graminis tritici*) has caused serious yield losses (Mayfield, 1980). The best method of control so far has been the use of resistant cultivars. Strain 34-2,12, a mutation from 34-2, is pathogenic on triticale cultivars with gene *Sr27* and is widespread in the eastern wheat belt of Australia. When the backcross programme for nematode resistance (section 5.1) had already advanced to the BC_3 generation it was decided to include selection for resistance to wheat stem rust strain 34-2,12 with the aim of producing lines which were homozygous resistant to both rust and the nematode.

5.2.2 Materials and Methods.

Twenty-five seeds of each of the three parents used in the backcross programme (section 5.1) were sent to the Plant Breeding Institute, Sydney University, for testing against wheat stem rust strain 34-2,12. Subsequently, 490 selfed seeds from 23 BC_1F_2 plants, 39 F_1 seeds from crosses of 8 BC_1 plants to their recurrent parents, 237 selfed seeds from 17 BC_2 plants and 40 seeds from two BC_2F_2 plants were also sent for

testing against the strain. Finally, 17 F_1 seeds from crosses of 4 BC_2 plants to their recurrent parents and 205 selfed seeds from BC_3 plants were submitted for screening by the Plant Breeding Institute. Further selfing and/or crossing to the recurrent parents were concentrated on nematode-resistant lines which produced some progeny that were resistant to the rust strain.

Occasionally selfed seeds of plants classified as resistant to the wheat stem rust strain were assayed for nematode resistance. When a mutant strain of the rust, 34-2,12,13, was recorded by the Plant Breeding Institute seeds of the non-recurrent parent, T701-4-6, were sent for testing against it.

5.2.3 Results.

All the seedlings of T701-4-6 proved resistant (25p;)* to wheat stem rust strain 34-2,12 whereas all those of T1116 were susceptible (25p3+). Seedlings of T1006 showed some variation in their reactions (1p;3p2=20p3+).

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* The number preceding `p' refers to the number of seedlings.
P - Seedling.
; - hypersensitive flecks with no uredia development.
2= - Small uredia in green islands.
3+ - Very large pustules; no distinguishable chlorotic effect. Susceptible interaction (Luig, 1983).

From two BC_1F_2 plants, X81-112-5-7 and X81-112-5-8 (fig. 3), 23 and 19 selfed progenies, respectively, proved resistant to the rust strain. Progeny of another BC_1F_2 plant, X81-112-5-10 (fig. 3) segregated 22 resistant: 5 susceptible. All these three BC_1F_2 plants were derived from crosses involving T1116. All the twenty selfed progeny of X81-111-5-7 which were assayed for *H. avenae* resistance were found to be resistant. They supported a mean of 0.25 females per plant (range: 0 to 1) as compared to the means of 0.16 (range: 0 to 1) and 22.6 (range: 10 to 37) females per plant supported by the resistant (T701-4-6) and susceptible (T1116) parents, respectively. On the other hand, two out of twenty selfed progeny of X81-112-5-8 were susceptible to *H. avenae* while the rest were resistant.

Thirteen BC_2 seedlings resulting from crosses of four BC_1 plants to their recurrent parent (T1116) were resistant to the rust strain whereas 26 BC_2 seedlings from crosses of four other BC_1 plants to T1116 were all susceptible. Eight BC_2F_2 seedlings from two BC_2 plants segregated (4 resistant: 4 susceptible) in their reactions to the rust strain but 229 BC_2F_2 seedlings from 15 other BC_2 plants were all susceptible.

All four BC_3 progeny from a cross between one BC_2 plant and T1006 were resistant to the rust strain while three such progeny involving another BC_2 plant and T1116 segregated (2 resistant : 1 susceptible). Ten BC_3 seedlings from two other BC_2 plants were all susceptible to the rust strain. All the 190 selfed progenies from eight BC_3 plants (involving T1116 as recurrent parent) were susceptible to the rust. However, 15 selfed progeny from one BC_3 plant (also derived from backcrossing to T1116) segregated, 11 resistant: 4 susceptible, to the rust. A progeny test indicated that this BC_3 plant was susceptible to the nematode.

The total numbers of seedlings tested from individual lines which segregated in their reaction to the rust strain were too few for any accurate inheritance pattern to be worked out.

The new rust strain (34-2,12,13) was pathogenic on T701-4-6 so further rust screening was suspended. However, one BC_2F_2 line, B82-1-32-2-12 (fig. 3), was given the accession number T5624 and submitted to the National Rust Control Programme, Castle Hill, for use as a parent in a triticale backcross programme. This line has resistance to both _H. avenae_ and wheat stem rust strain 34-2,12 and was derived from backcrossing to T1116.

5.3 Inheritance of Resistance to *H. avenae* in T701-4-6.

5.3.1 Introduction.

For effective breeding of plants resistant to pests and diseases knowledge of the mode of inheritance of resistance is very useful. Resistance to *H. avenae* in Loros wheat has been attributed to a single dominant gene (Nielsen, 1966; Slootmaker *et al*, 1974) which may be identical to the one in AUS 10894 and AUS 90248 (O'Brien *et al*, 1980). Resistance in some barley lines have been attributed to single dominant genes (O'Brien *et al*, 1979) while recessive genes have been associated with the resistance in some oat lines (Cotten and Hayes, 1972; Cook and York, 1982). Data on the inheritance of resistance to *H. avenae* in T701-4-6 were extracted from the backcross programme outlined in section 5.1.

5.3.2 Materials and Methods.

For additional data on the inheritance of resistance to *H. avenae* in T701-4-6 it was crossed to a triticale cultivar, Coorong, which is susceptible to the nematode. Ten seeds of each standard susceptible line, T1116 and Aroona; 20 seeds each of T701-4-6 and Coorong; 34 of the F_1 progeny; 40 of BC_1 and 200 of F_2 were put through the nematode assay described in section 3.3.

The data from the backcross programme (section 5.1) were based on 10 plants each of the parents, F_1 progenies and a standard susceptible cultivar, Halberd; 73 BC_1 plants and 85 BC_1F_2 plants.

5.3.3 Results.

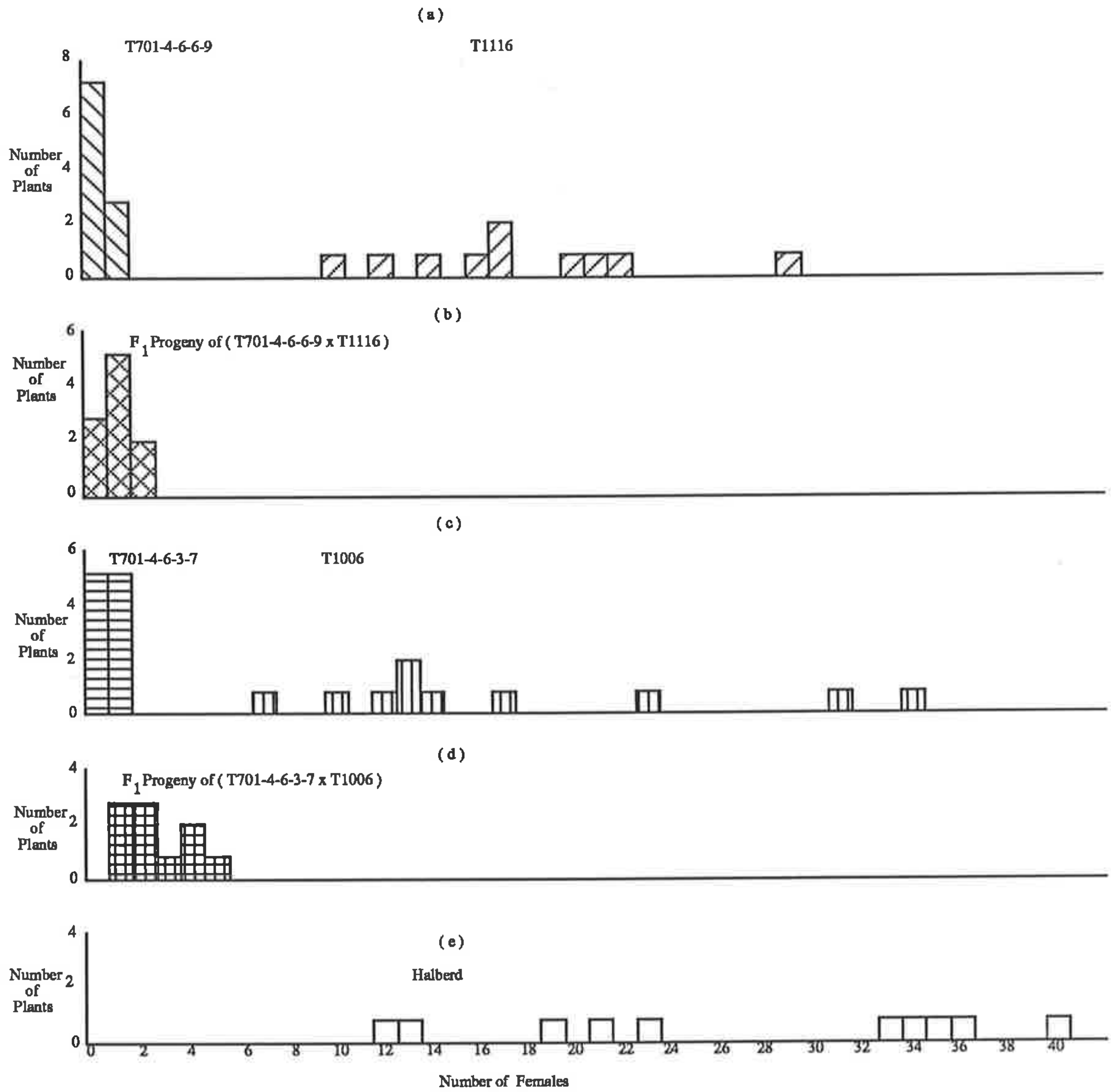
The experiment set up to provide additional data had to be abandoned because initial samples of 25 plants of Coorong and 10 each of T1116 and Aroona supported unacceptably low numbers of H. avenae females. The mean number of females per plant which developed on Coorong, T1116 and Aroona were 3.6 (range 1-6), 3.0 (1-7) and 5.5 (2-9), respectively.

In the backcross programme the two triticale lines used as resistant parents, T701-4-6-3-7 and T701-4-6-6-9, were both derived from selfing of T701-4-6. The mean number of H. avenae females per plant (± standard error) developed on T701-4-6-6-9 and T701-4-6-3-7 were 0.3 ± 0.15 and 0.5 ± 0.16, respectively. Corresponding results for the susceptible standard cultivar, Halberd, and the susceptible parents T1116 and T1006 were 26.6 ± 3.22, 17.8 ± 1.74 and 17.4 ± 2.86, respectively. The F_1 progeny of the cross between T701-4-6-6-9 and T1116 supported a mean (± standard error) of 0.9 ± 0.23 females per plant. The F_1 progeny of the cross between T701-4-6-3-7 and T1006

allowed the development of a mean of 2.5 $\pm$ 0.45 females per plant (fig. 5).

There was no clear bi-modal distribution of reactions of the BC_1 segregants and some of them could not be classified as either resistant or susceptible (Fig. 6.). Using the raw data, their square root or log transformations, the ranges within which 95 per cent of the parents and F_1 populations could be expected to fall were calculated (Cotten and Hayes, 1969). Classifying the BC_1 segregants on the basis of these ranges still left some plants unclassified. The maximum number of females which developed on the homozygous resistant parents and the F_1 hybrids were, therefore, used as the approximate demarcation line between resistance and susceptibility (Cotten and Hayes, loc. cit.). The observed frequencies of resistant and susceptible plants of the BC_1 progeny were 14:15 for the crosses invloving T1116 and 11:33 for those involving T1006. The former ratio conformed to a 1:1 ratio for a single dominant gene hypothesis ($0.75<P<0.90$) whereas the latter deviated significantly ($P<0.005$).

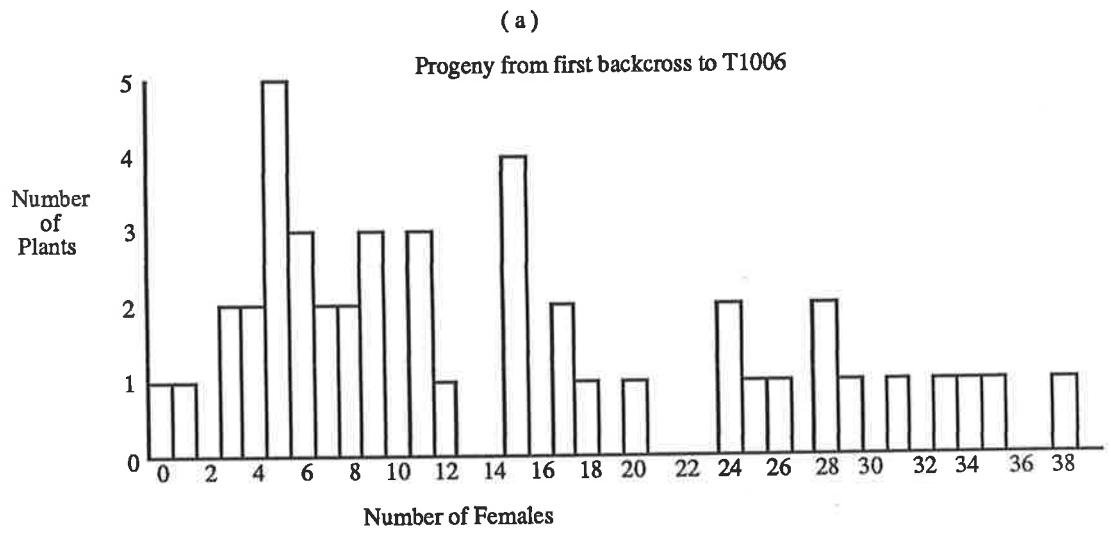
Fig. 5. Frequency distribution of *H. avenae* females per plant on parents, F_1 progenies and Halberd wheat.



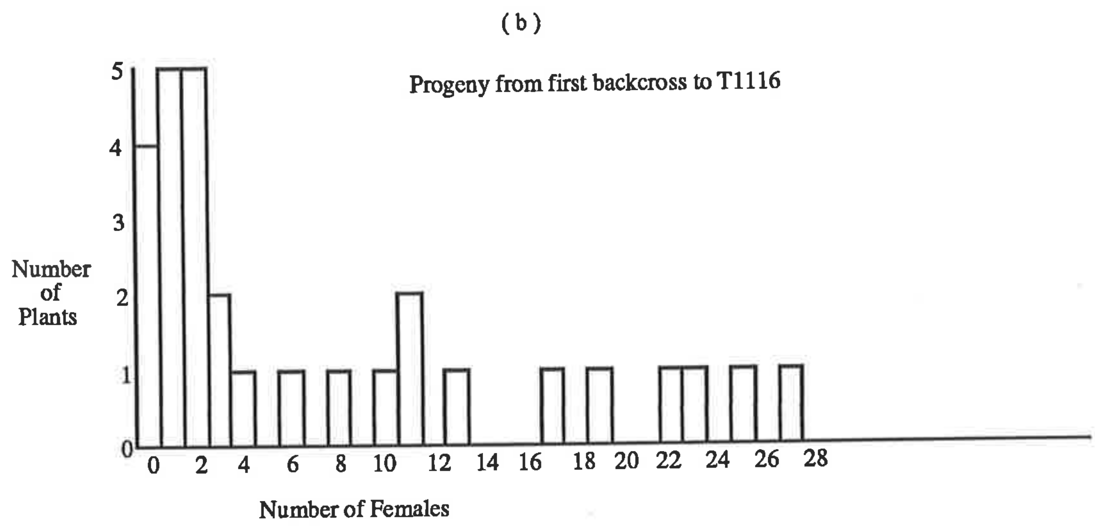
(a)
T701-4-6-6-9
T1116
Number of Plants
(b)
F1 Progeny of (T701-4-6-6-9 x T1116)
Number of Plants
(c)
T701-4-6-3-7
T1006
Number of Plants
(d)
F1 Progeny of (T701-4-6-3-7 x T1006)
Number of Plants
(e)
Halberd
Number of Plants
Number of Females

Fig. 6. Frequency distribution of *H. avenae* females per plant on BC_1 progenies.

(a)



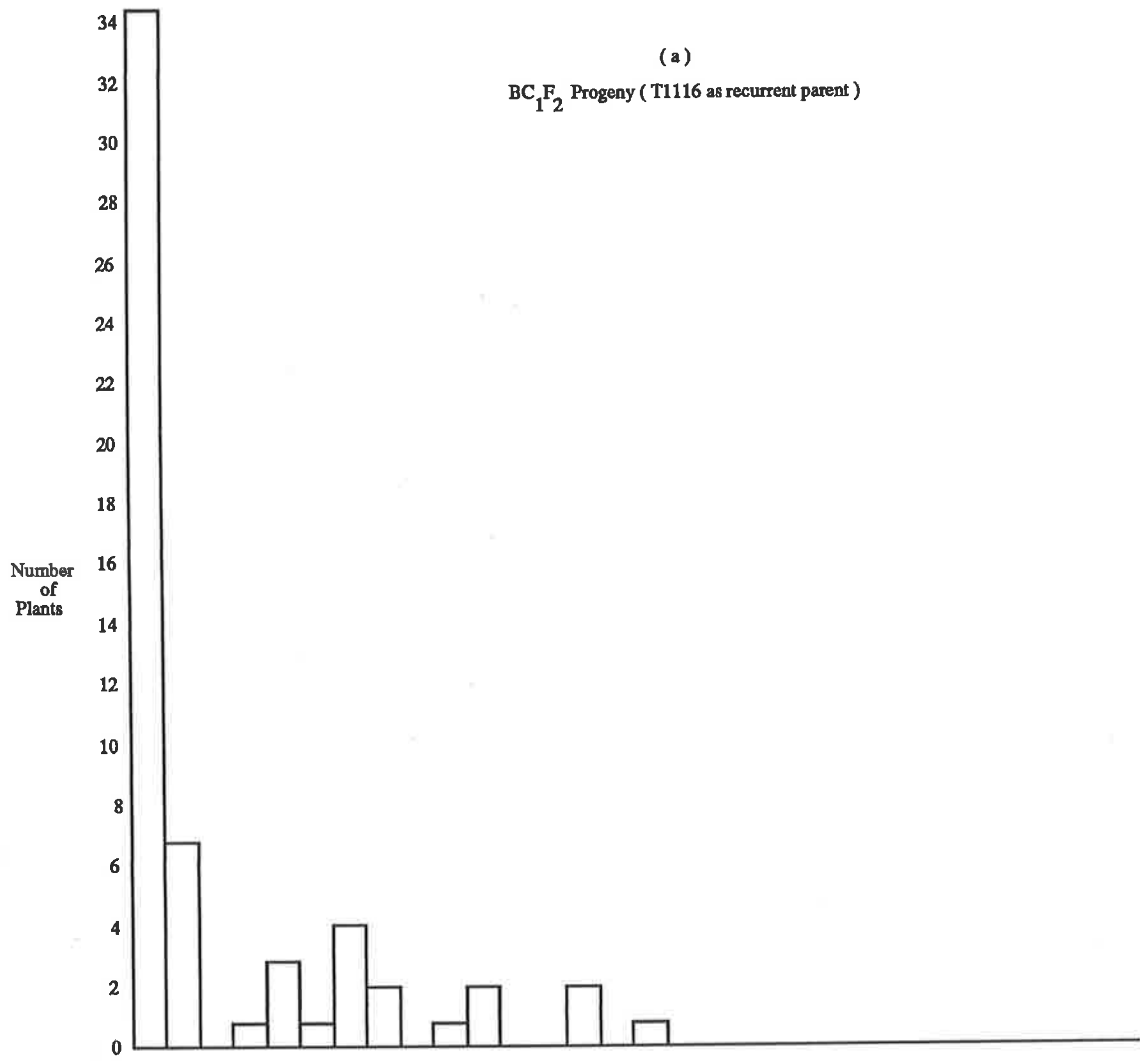
(b)

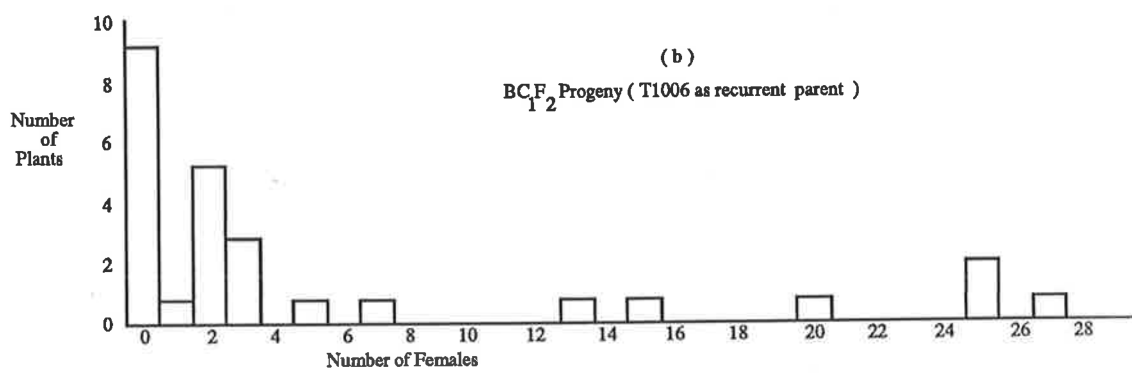


The absence of a clear bi-modal distribution was again evident for the segregation of BC_1F_2 plants (Fig. 7). Using the same line of demarcation as for the BC_1 plants the observed segregation ratios were 41:17 and 19:8, respectively, for crosses to T1116 and T1006 both of which conformed to a 3:1 ratio for a single dominant gene hypothesis ($0.25<P<0.50$ for the former and $0.50<P<0.75$ for the latter).

Results of a progeny test of five BC_1F_2 plants, some of which were difficult to classify as resistant or susceptible to <u>H. avenae</u>, are presented in fig. 4. Plant X81-111-9-6 supported three females in an earlier test but all 28 of its selfed progeny allowed the development of a maximum of one female per plant and a mean of 0.1 females per plant. Plants X81-111-9-1 and X81-111-9-21 supported one female and no female, respectively, but their selfed progenies segregated 24:7 (resistant:susceptible) using the same line of demarcation between resistant and susceptible individuals set for the BC_1 and BC_1F_2 plants discussed previously. This fits a 3:1 ratio for a single dominant gene hypothesis ($0.75<P<0.90$). The other two plants, X81-112-5-1 and X81-111-9-14 supported 7 and 5 females, respectively, and their progenies may all be classified as susceptible with respective mean numbers of females of 9.27 and 9.08 per plant. The mean for the susceptible parent (T1116) was 10.86 females per plant.

Fig. 7. Frequency distribution of <u>H. avenae</u> females per plant on BC_1F_2 progenies.


(a)
BC1F2 Progeny (T1116 as recurrent parent)
Number of Plants
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0


(b)
BC1F2 Progeny (T1006 as recurrent parent)
Number of Plants
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
Number of Females

5.4 Discussion.

Selection of lines that were homozygous resistant to *H. avenae* was hampered by serious problems with the screening procedure. Low numbers of females supported by the standard susceptible cultivar and susceptible parents necessitated the repetition of a number of trials. Poor recovery of some of the selected plants transplanted after the screening often led to poor seed production. The inclusion of selection for resistance to wheat stem rust strain 34-2,12 further slowed progress. Emphasis had to be shifted to nematode-resistant plants that produced rust-resistant progeny. Before strain 34-2,12,13 was reported efforts were being made to screen simultaneously for resistance to *H. avenae* and wheat stem rust on the same sets of seedlings. The original aim of selecting many nematode-resistant lines in order to increase the probability of transgressive segregation with respect to agronomic performance was not achieved as a result of the foregoing impediments. Although the new rust strain is pathogenic on the non-recurrent parent the few resistant lines produced will be useful parental stocks for breeding programmes such as the one started by the National Rust Control Programme. The BC_2F_2 line already submitted to that programme (B82-1-32-2-12) would be of better agronomic type than its resistant parent (T701-4-6). Its recurrent parent (T1116) ranked sixth and fourth, respectively, in yield in the first and second Interstate Triticale Yield Trials (Driscoll and McLean, unpublished). The line could lead to a high-yielding line with resistance to both the nematode and the rust.

The inheritance analysis was based on a rather small number of plants. The mean of 26.6 females per plant supported by the standard susceptible cultivar, Halberd, was less than expected but two particular seedlings which allowed development of only 12 and 13 females, respectively, contributed a lot to the low mean obtained. The mean number of females (± standard error) that developed on the two susceptible parents - 17.4 ± 2.86 for T1006 and 17.8 ± 1.74 for T1116 - were clearly higher than those on the resistant parents : 0.5 ± 0.23 for T701-4-6-3-7 and 0.3 ± 0.15 for T701-4-6-6-9. (Standard error of the difference between means was 2.526). However, the difference between the upper limit of the range of reactions of the resistant parents (2) and lower limit of the range of reactions of the susceptible parents (7) (fig. 5) was not big enough to make crosses between them suitable for inheritance studies.

The reactions of the F_1 hybrids (figure 5) suggested dominant gene action but the ranges of 0 to 3 and 1 to 5 females per plant for F_1 (T701-4-6-6-9 X T1116) and F_1 (T701-4-6-3-7 X T1006), respectively, extended beyond the upper limits of the numbers supported by their respective resistant parents. The apparent drift away from strong resistance may indicate an influence of the new genetic background (Cook, 1974; Ellis and Brown, 1976) and is not unusual. Andersen and Andersen (1982) proposed that the effect of a gene for resistance may depend on the whole gene pool of the cultivar and agreed with Cook and York (1982) on

the probable difference in reaction between homozygous and heterozygous plants. When confronted with more serious drifts away from resistance in F_1 hybrids Cotten and Hayes (1972) and O'Brien et al (1980) concluded that resistance to H. avenae was partially dominant over susceptibility in the lines and cultivars involved. The means of the F_1 progenies significantly exceeded those of their resistant parents in the work reported by O'Brien et al (loc. cit.) contrary to the results presented in this thesis (standard error of the difference between the mean number of females per plant supported by the different cereal lines was 2.526).

Many reports on inheritance of resistance to H. avenae include problems with the classification of F_2 progenies. O'Brien et al (1979) and O'Brien et al (1980) could not produce bi-modal distributions of F_2 progenies even after classifying them on the basis of the 95 per cent probability of their falling into one of the parental classes. Since different researchers have used different methods of assessment of resistance it is difficult to compare the various results obtained. The point of demarcation between resistance and susceptibility in segregating populations has generally been selected arbitrarily. Slootmaker et al (1974) classified resistant plants as those which carried not more than one cyst. One progeny of monosomic 2B which fell unexpectedly into the susceptible class was considered a probable nullisomic. In pot and field trials, O'Brien and Fisher (1974) classified plants with no females as moderately

resistant and all other plants as susceptible. Chew et al (1981) considered F_2 oat plants as susceptible if they supported one or more cysts and yet classified five plants with one cyst each as resistant on the basis that the resistant parent, Nelson, was known to produce a few cysts occasionally. Andersen and Andersen (1982) considered zero to two females per plant as a resistant reaction. Person - Dedryver and Doussinault (1984) considered five per cent of the susceptible F_2 class as mis-classified because five per cent of the seedlings of the susceptible parent showed the resistant reaction of no females or females without eggs. Even the more objective method of placing F_2 plants within 95 per cent confidence limits of the parents and F_1 hybrids left many plants unclassified in the work on oats (Cotten and Hayes, 1972). Some of those plants were considered susceptible on the reasoning that they were more likely to be susceptible plants that had partially escaped infection.

Following the choice of a subjective point of demarcation between resistance and susceptibility in the BC_1 and BC_1F_2 populations reported in this chapter, the most likely inheritance pattern of resistance to H. avenae in T701-4-6 is the action of a single dominant gene. The absence of bi-modal distributions may be attributed mainly to inefficiency of the assay, the partial susceptibility of T1006 and T1116 and the possibility of modifier genes. The deviation of the observed ratio from the expected one of the BC_1 progeny (fig. 6a) involving T1006 may be due to the

greater variation of the reactions of the T1006 seedlings and the F_1 hybrids involving it (Figs. 5c and 5d). The results presented in fig. 4 seem to vindicate the point of demarcation chosen for the classification of BC_1 and BC_1F_2 progenies. Plants which supported five to seven females could be considered as susceptible plants that partially escaped infection. Those that supported three females could be resistant plants in which the resistance gene was not being expressed fully.

The results of the nematode assay and those of the rust test did not suggest any linkage between the genes for resistance to the two organisms.

The tentative conclusion on the inheritance of resistance to H. avenae in T701-4-6 is that a single gene is involved which is, to a large extent, dominant.

CHAPTER SIX

CHROMOSOMAL LOCATION OF RESISTANCE GENE.

6.1 Introduction.

Transfer of genes from one cultivar or line to another within the same species can be achieved through homologous recombination following hybridisation. Interspecific gene transfer often requires cytogenetic manipulations. While some of the methods depend on chance combinations of genetic material, knowledge of the gene's location enables greater control and precision of the transfer thus increasing the probability of success. Alien addition and substitution lines provide valuable information about genetic factors on the alien chromosomes and have been used in genetic analyses. Slootmaker *et al* (1974) and Chew *et al* (1981) used monosomic analysis to locate genes for resistance to *H. avenae* on chromosomes 2B of wheat and XV of oats, respectively, whereas Cotten and Hayes (1969) located the *Rha_2* resistance gene on chromosome 2 of barley using translocation stocks and linkage analysis. Rivoal *et al* (1986) have also located a gene(s) for resistance to *H. avenae* on chromosome $6M^v$ of *Aegilops ventricosa* Tausch.

Either the wheat or the rye genome may be the source of the resistance gene in T701-4-6. While the high level of the resistance may suggest that rye is the probable source

(Fisher - pers. comm.) the possible influence of genomic interaction cannot be ignored. Crosses were made between T701-4-6 and two wheat lines with the view to locating the resistance gene on a particular chromosome.

6.2 Materials and Methods.

Each of two lines, T701-4-6-3-7 and T701-4-6-6-9, generated from selfing of T701-4-6 was used in reciprocal crosses with Aroona and Chinese Spring wheats. Embryos from developing F_1 seeds of crosses in which wheat was the female parent had to be excised and cultured on artificial media (Section 3.2.4) to prevent severe endosperm shrivelling and/or death of the embryos. F_1 seeds from the crosses in which triticale was the female parent, and seeds of the parents, were surface-sterilised and germinated in petri dishes in an incubator until the first three seminal roots were each about 1 cm. long. Single roots excised from ten of the F_1 seedlings from each cross were used for examination of somatic chromosomes as detailed in section 3.2.2. Single root tips were also cut off each of ten seedlings of each of the four parents. All these seedlings were then tested for their reaction to *H. avenae* (section 3.3.) after which the F_1 plants were transplanted for backcrossing to their respective wheat parents. Concurrently,cytological analysis of meiosis (section 3.2.3) was performed on a separate batch of F_1 plants, some of which were also allowed to self. A single root was excised from each freshly germinated BC_1

seedling for cytological examination of somatic chromosomes. About one-third of the brush end of the endosperm of each seedling was cut for electrophoresis for alcohol dehydrogenase (Adh) isozyme analysis. These seedlings were then screened for their reactions to the nematode (section 3.3). During the nematode test leaf samples were taken from the seedlings for electrophoresis in relation to Glutamate oxaloacetate transaminase (GOT) isozyme analysis. All plants were transplanted after the nematode test.

At maturity a hairy peduncle was taken as an indication that the particular plant had, at least, the long arm of rye chromosome 5 (5RL) if not the whole chromosome. Some tillers of those BC_1 plants which were classified as resistant to the nematode were allowed to self while others were backcrossed again to their respective wheat parents. The BC_2 and BC_1F_2 seeds obtained were taken through the same processes as were the BC_1 seeds. Some selected BC_2 plants were allowed to self.

6.3 Results.

A C-banded mitotic metaphase preparation of T701-4-6 is shown in figure 8. Adh and GOT zymogram phenotypes of T701-4-6, Aroona, Chinese Spring and S.A. Rye are shown in figures 9a and 9b.

Fig. 8 A C-banded mitotic metaphase preparation of T701-4-6. The rye chromosomes present are labelled with their homoeologous group numbers.

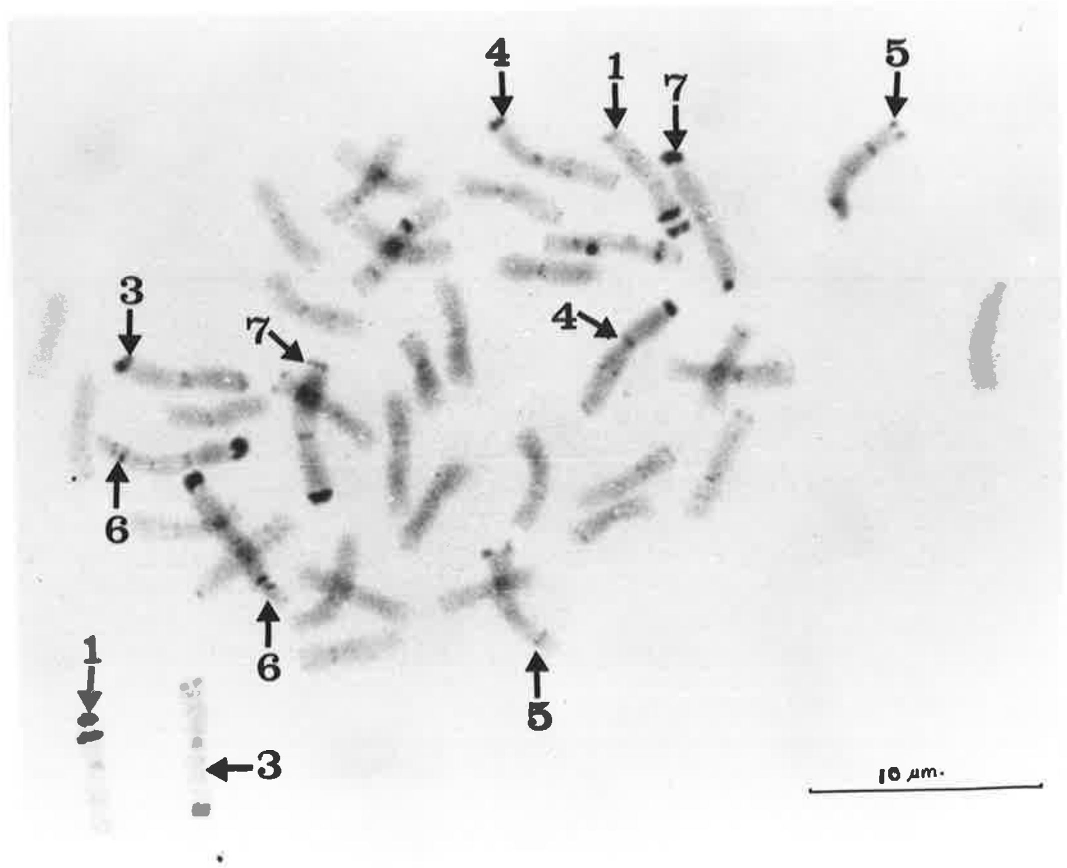

4
1
7
5
3
7
4
6
6
5
1
3
10 µm.

Fig. 9a. Alcohol dehydrogenase zymogram phenotypes.

Fig. 9b. Glutamate oxaloacetate transaminase zymogram phenotypes.

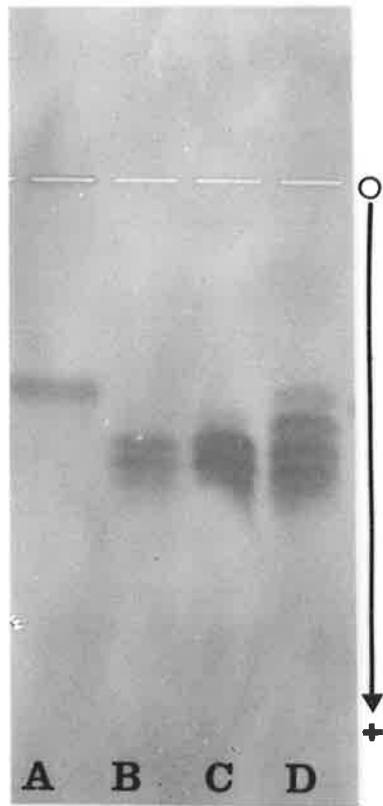
A: S.A. Rye

B: Chinese Spring Wheat

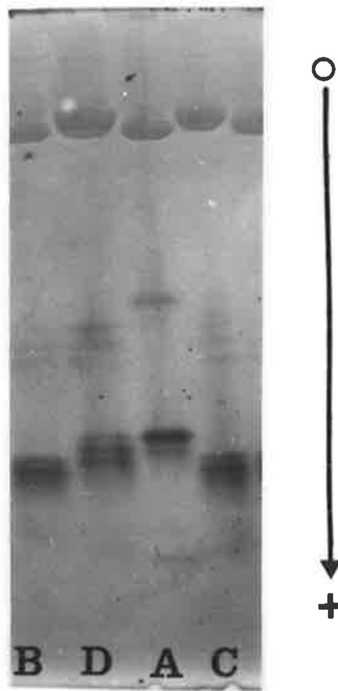
C: Aroona Wheat

D: T701-4-6

The arrows indicate the direction of current flow.



a.



b.

Analysis of variance, followed by Duncan's new multiple range test applied to the nematode reactions of all the parents and F_1 progenies, showed that the triticale parents and F_1 progenies were all resistant; Aroona was susceptible and Chinese Spring was partially resistant (Table 2). Since there were no significant differences in the reactions of the two triticale parents and F_1 progenies involving them, all data of progeny from subsequent crosses involving a particular wheat parent (e.g. all crosses to Aroona) and the two triticale parents were combined.

The range of reactions of the F_1 (T701-4-6 X Aroona) progeny extended beyond that of the triticale parents (Figure 10).

Seed setting on the F_1 plants was 1.01 seeds per spike from a total of 69 spikes.

The combined BC_1 population from all crosses showed no clear bi-modal distribution of resistant plants to susceptible ones (Figure 11). Using the maximum number of females per plant on the F_1 population as the demarcation point between resistance and susceptibility the observed ratio of 68:65 (resistant:susceptible) conformed to the expected 1:1 ratio for a single dominant gene hypothesis ($0.75<P<0.90$).

TABLE 2.

MEAN NUMBER OF *H. AVENAE* FEMALES PER PLANT SUPPORTED BY PARENTS AND F_1 PROGENIES OF TRITICALE X WHEAT CROSSES.

Lines	Ranked Mean Number of Females Per Plant
T701-4-6-6-9	0.3 a *
T701-4-6-3-7	0.4 a
F_1 (T701-4-6-6-9 x Aroona)	0.5 a
F_1 (T701-4-6-3-7 x Chinese Spring)	0.8 a
F_1 (T701-4-6-6-9 x Chinese Spring)	1.3 a
F_1 (T701-4-6-3-7 x Aroona)	1.3 a
Chinese Spring	4.8 b
Aroona	20.5 c

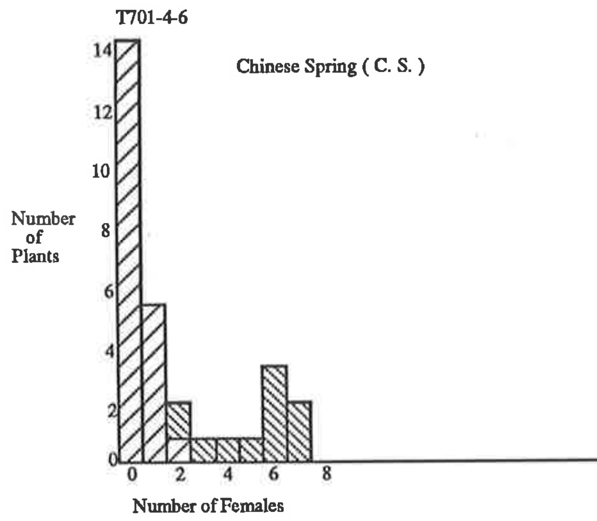
LEAST SIGNIFICANT RANGES (LSR) FOR COMPARISONS BETWEEN ANY PAIR OF RANKED LINE MEANS USING DUNCAN'S NEW MULTIPLE RANGE TEST.

Number of means separating any two line means being compared	0	1	2	3	4	5	6
LSR (5 per cent level)	1.48	1.56	1.62	1.64	1.68	1.70	1.72
LSR (1 per cent level)	1.97	2.06	2.16	2.16	2.18	2.22	2.24

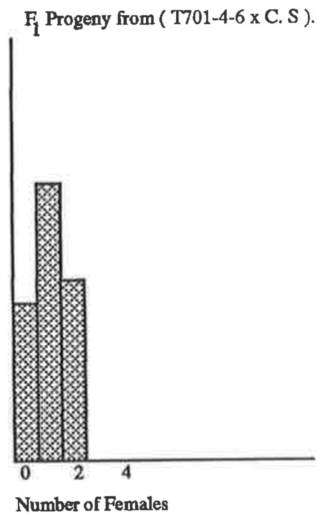
* Any two line means followed by the same letter do not differ significantly.

Fig. 10. Frequency distribution of the number of females per plant supported by the parents and F_1 progenies of Triticale X Wheat crosses.

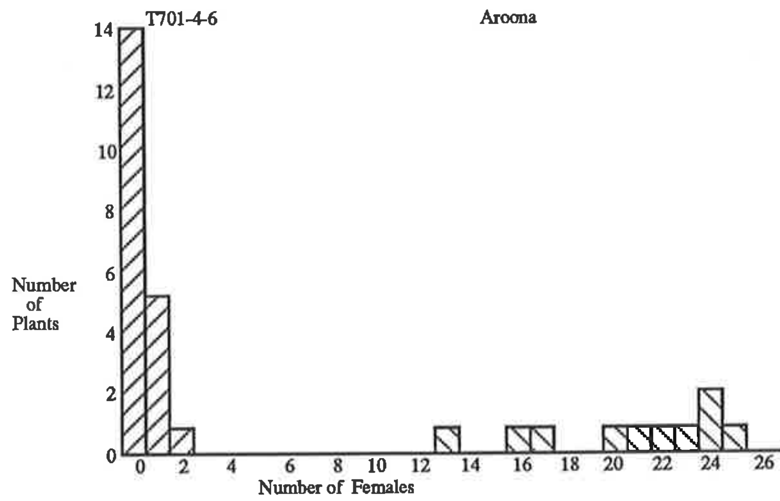
(a)



(b)



(c)



(d)

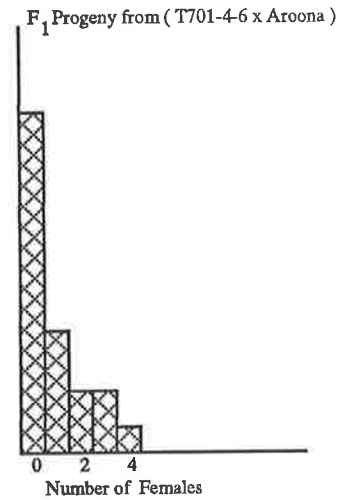
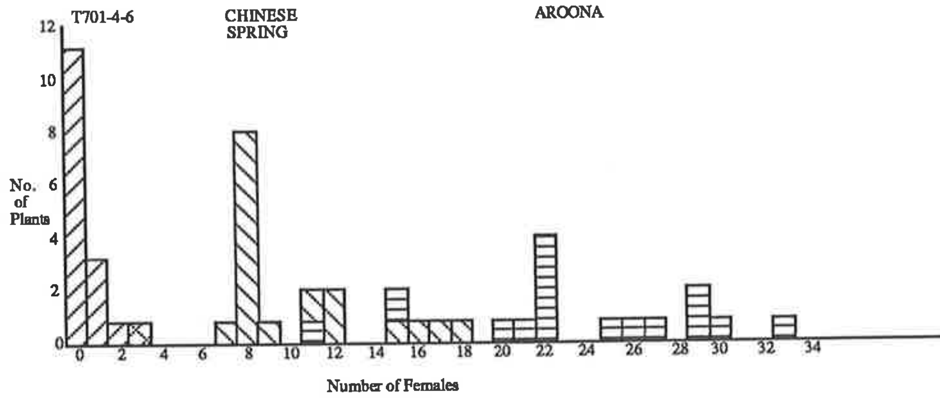
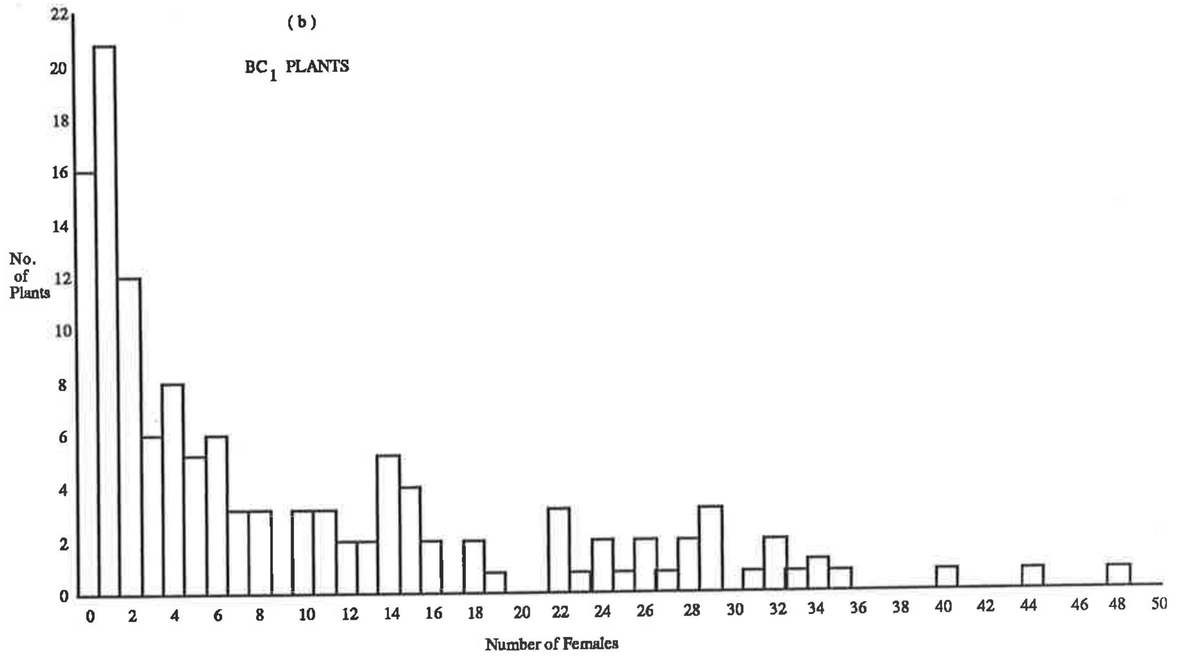


Fig. 11. Frequency distribution of the number of females supported per plant by parents and BC_1 progenies of Triticale X Wheat crosses.


(a)
T701-4-6
CHINESE SPRING
AROONA
No. of Plants
Number of Females


(b)
BC1 PLANTS
No. of Plants
Number of Females

From a correlation of the results of rye chromosome identification and those of the nematode test it was observed that BC_1 plants that had chromosome 6 of rye (6R) supported an average of 1.45 $\pm$ 0.18 females per plant (Table 3). In the absence of 6R, plants containing the other rye chromosomes, irrespective of the number and their combinations, allowed larger numbers of females to develop (means of 15.56 $\pm$ 2.15 in the presence of 5R to 19.44 $\pm$ 2.26 when 4R was present but 6R was absent) (Table 3 and Figure 12). For instance, a plant carrying 22 females had rye chromosomes 1,3,4 and 7 and another one which supported 33 females had chromosomes 1,4 and 5 of rye. However, seven* BC_1 plants in which 6R was not identified restricted development of females to less than the upper limit of numbers found on F_1 progenies. Five of them were from crosses to Chinese Spring. The range of females per plant supported by the BC_1 progeny containing 6R was 0 to 4 with a mean of 1.45 compared to 0 to 3 for the T701-4-6 parents with a mean of 0.50. Some of the BC_1 plants from crosses involving Aroona supported far more females than their susceptible parent.

The nematode resistance screening of BC_2 and BC_1F_2 plants was not very efficient judging from the reaction of susceptible controls. For instance, the overall mean number

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* While fig. 12 suggests that 8 BC_1 plants were involved both 1R and 3R were identified in one of the plants.

TABLE 3.

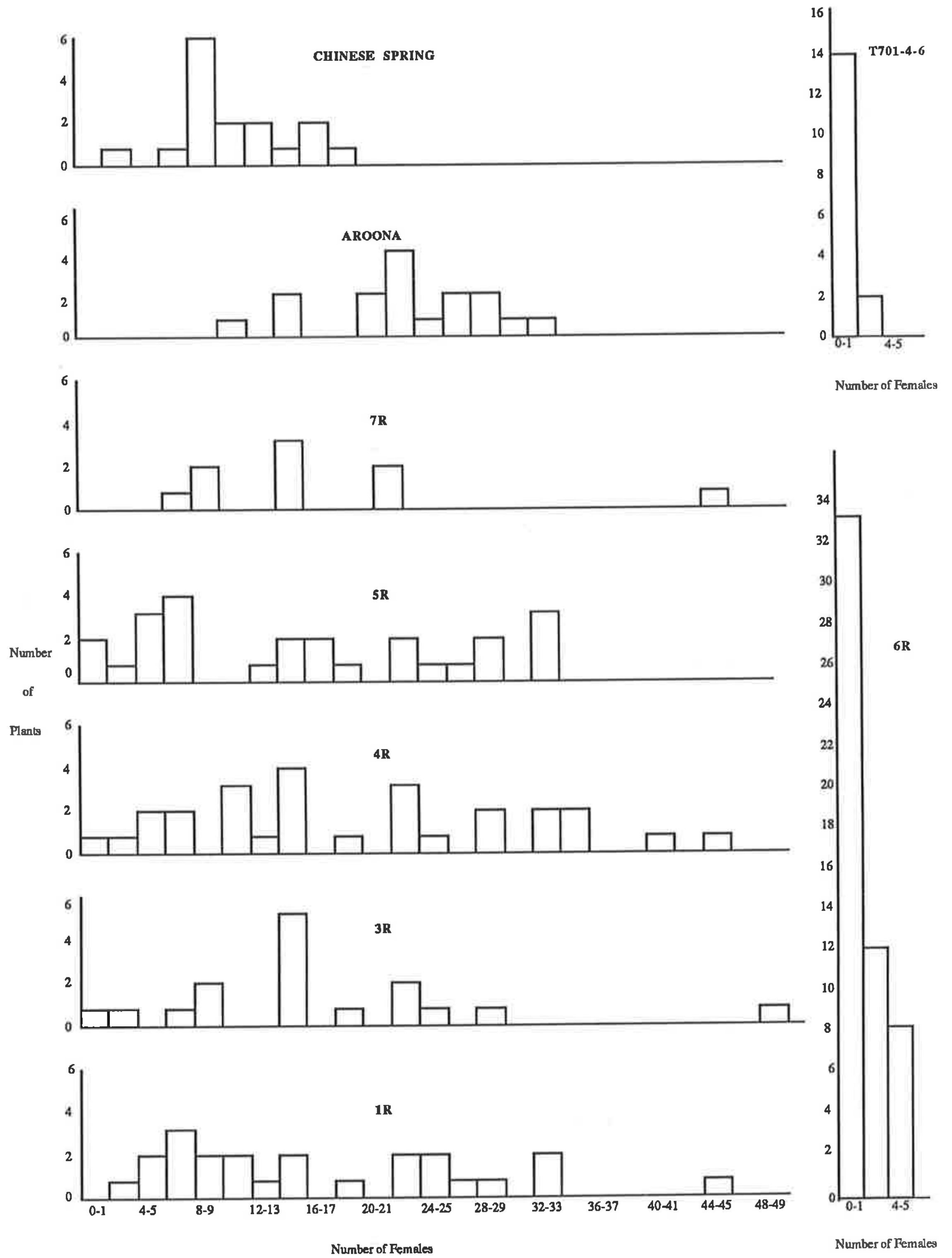
NUMBER OF *H. AVENAE* FEMALES SUPPORTED BY PARENTS AND BC_1 PLANTS IN RELATION TO THE PRESENCE OF RYE CHROMOSOMES.

LINE OR RYE CHROMOSOME PRESENT IN BC_1 PLANTS*	MEAN	STANDARD ERROR	RANGE	NUMBER OF PLANTS
T701-4-6	0.50	0.22	0 - 3	16
Chinese Spring	10.68	1.03	3 - 18	16
Aroona	23.06	1.50	11 - 33	16
1R	16.74	2.30	3 - 44	23
3R	16.31	2.86	1 - 48	16
4R	19.44	2.26	1 - 44	27
5R	15.56	2.15	0 - 33	25
6R	1.45	0.18	0 - 4	53
7R	17.00	3.88	6 - 44	9

* Entries 1R, 3R, 4R, 5R and 7R refer to BC_1 plants with these respective rye chromosomes either singly or with other rye chromosomes provided 6R was absent. Entry 6R refers to BC_1 plants with that rye chromosome alone or in combinations with other rye chromosomes.

Fig. 12. Frequency distribution of the number of *H. avenae* females supported per plant by parents and BC_1 progenies in relation to the presence of rye chromosomes.

Entries 1R, 3R, 4R, 5R and 7R refer to BC_1 plants with these respective rye chromosomes either singly or with other rye chromosomes provided 6R was absent. Entry 6R refers to BC_1 plants with that rye chromosome alone or in combinations with other rye chromosomes.


CHINESE SPRING
AROONA
7R
5R
4R
3R
1R
Number
of
Plants
0-1
4-5
8-9
12-13
16-17
20-21
24-25
28-29
32-33
36-37
40-41
44-45
48-49
Number of Females
T701-4-6
Number of Females
6R
Number of Females

of females per plant supported by Aroona in the tests was only 10.56. Nevertheless, it may still be significant that all the 89 plants in which 6R was identified supported either one or no females whereas plants without 6R carried relatively high numbers. Selfed progeny of a particular BC_1 plant, B83-9A-84-1, which had only chromosome 6 from the rye genome showed clear segregation between resistance and susceptibility. Six of them which retained chromosome 6R supported an average of 0.5 females (range of 0 to 1) whereas five plants, which did not, supported an average of 21.6 (range of 12 to 29). From their meiotic metaphase I configurations three of the resistant progeny were found to be monosomic additions of 6R to the complete wheat chromosome complement and another two were monosomic substitutions for wheat chromosomes. A BC_2 plant, B83-9A-254-1, was also found to have a 6R addition to the wheat genome and supported no nematode females. Plant B83-9C-136-3-1, a BC_1F_2 derivative from crosses involving Chinese Spring, had a total of 42 chromosomes, was disomic for 6R and supported no females. Its meiotic metaphase I configuration was mainly 20 bivalents of wheat chromosomes and one of rye. Its Glutamate oxaloacetate transaminase -2 isozyme pattern confirmed the presence of chromosome 6R and indicated the absence of 6D of wheat. The plant showed similar morphology and fertility to its wheat parent. Five other BC_1F_2 plants which were disomic for 6R allowed no female development whereas a sixth such plant carried one female.

No *H. avenae* females were observed on three BC_2 plants in which the long arm of 6R (i.e. 6RL) was identified and another one in which an isochromosome of the long arm was found in the absence of the short arm. However, two females were found on one BC_2 plant which had 6RL.

From the selfed progenies of BC_2 plants the disomic addition of 6R, the monotelosomic addition of 6RL, the monotelodisomic and monoisodisomic additions were selected. The last two addition plants had the short arm (6RS) as the misdivision product.

6.4 Discussion.

The requirement of embryo culture for the wheat X triticale crosses was probably due to genetic imbalance in the endosperm (Lukaszewski *et al*, 1982). The poor seed setting on the F_1 plants compares with 0.27 to 1.95 seeds per spike from a number of Triticale X wheat crosses reported by Lukaszewski *et al* (*loc. cit.*) and could be due to the imbalance caused by the high number of univalents from the rye genome and D genome of wheat.

The reaction of Chinese Spring to *H. avenae* in the test involving parents and F_1 progenies (table 2 and fig. 10) was interesting. The Chinese Spring seedlings exhibited slow early growth and possibly root production which may have contributed to poor support for growth of the females or restricted the number of invasion sites initially.

Subsequent tests (for instance, Table 3 and Fig. 11) showed higher numbers of females on the line but Aroona remained more susceptible than Chinese Spring in all tests.

The reaction of F_1 plants suggests dominant action of the resistance gene in T701-4-6 (Table 2 and Fig. 10). The wider range of reaction of the F_1 (T701-4-6 X Aroona) progeny of 0 to 4 females per plant compared to 0 to 2 for the resistant parent (Fig. 10) may be attributed to the change in the genetic background of the resistance gene and/or hemizygosity of the gene (Ellis and Brown, 1976).

The fact that the segregation of the BC_1 population was consistent with the expected Mendelian ratio for a single dominant gene could suggest the A or B genome of wheat in the resistant triticale parent as the source of resistance, so that segregation was uninfluenced by any unusual female transmission rate of a univalent chromosome from either the rye genome or D genome of wheat. However, the high correlation of the presence of chromosome 6 of rye (6R) with resistance to the nematode (Table 3 and Fig. 12) suggests strongly that that chromosome carries the gene for resistance. To be consistent with the total BC_1 data the sum of the female transmission rates of 6R and its critical arm from their F_1 parents must have been approximately 50 per cent, which is not unlikely. The observed transmission rate of 6R was 41.73 per cent. Lukaszewski et al (1982) reported a 27.8 per cent female transmission rate of 6R and

Lukaszewski and Gustafson (1983) speculated on a probable low misdivision rate of 6R. The difference between those reports and the current observation may be due to the different genetic backgrounds involved.

The BC_1 plants representing the lower end of the range of reactions of plants lacking 6R but having one or more of chromosomes 1R, 3R, 4R or 5R deviated from the general trend (Table 3 and Fig. 12). Considering the range of reaction of Chinese Spring (Table 3 and Fig. 12) it is not surprising that five out of the seven plants involved were derived from crosses to that line. The five plants could just be expressing the Chinese Spring gene(s). It can only be speculated that the remaining two BC_1 plants either partially escaped infection or contained translocations of the critical segment of 6R which were not identified. It must be noted that the low numbers of females on those plants were not associated with any specific combinations of chromosomes. Different combinations of the rye chromosomes (excluding 6R) were found in very susceptible plants (i.e. plants carrying more than 20 females per plant). It could also be argued that while some plants might have partially escaped infection and therefore produced only a few females the production of numerous females on a plant with a particular rye chromosome is a good case against that chromosome being the critical one. That situation is true of all the rye chromosomes except 6R (Table 3 and Fig. 12).

The apparent higher susceptibility of some BC_1 plants than their susceptible parents (Table 3 and Fig. 12) could be due to aneuploidy, high susceptibility of the wheat background of the triticale parent or higher tolerance of the BC_1 plants (probably derived from the rye genome) enabling them to support more females.

While the eleven selfed progeny of plant B83-9A-84-1 was only a small population their reactions provided strong supporting evidence of the role of 6R. Even in the weak tests, there was clear separation between the mean of 0.5 females per plant on plants with 6R (6 plants) and 21.6 on those without it (5 plants).

The influence of 6RL alone could not be conclusively established owing to the inefficiency of the tests in which they occurred and the fact that short arm telocentrics of 6R were not positively identified in any susceptible plants. Further tests are required to confirm the arm location of the resistance gene.

The 6R (-6D) disomic substitution plant, B83-9C-136-3-1, will be useful for induction of homoeologous recombination between 6R and wheat chromosomes, especially 6D, through crossing with Sears' ph mutant line.

Since the first three seminal roots of each seedling were considered essential for the nematode assay only a

single root per seedling could be sacrificed for cytological analysis. Consequently, in situations where a particular root tip showed only a few dividing cells fixed at the metaphase stage the number of cells available and suitable for chromosome identification was restricted. For identification of chromosomes 4R and 6R there were supporting data from isozyme analysis and 5R identification was supported by pubescence of the peduncle of the mature plant. In the few instances where there was doubt about the identity of a rye chromosome, some of which could have been centric-fusions between arms of different chromosomes (Sears, 1972), the reaction of the plant to the nematode was ignored in relation to that chromosome. Hence figure 12 does not include all the plants whose reactions are presented in figure 11. The frequencies of the various rye chromosomes in the BC_1 population, as shown in table 3 and figure 12, are influenced by their ease of identification.

CHAPTER SEVEN

DIFFICULTIES WITH SCREENING FOR RESISTANCE.

7.1 Introduction.

Fisher (1982) listed the causes of variation in the number of *H. avenae* females on a cultivar as: escape from infestation, genetic variation in seed, variation in methods of assessment, existence of different biotypes and/or different proportions of biotypes, different starting materials (cysts or larvae), different initial densities of the nematode, and environmental factors like temperature, nutrition and parasites.

Methods reported for the screening of plants for resistance to *H. avenae* include use of infested soil in clay pipes (Slootmaker *et al*, 1974; Andersen, 1963), infested soil in pots (Brown, 1974; Cotten, 1967; Cotten and Hayes, 1969, 1972; Chew *et al*, 1981; Cook and York, 1982), test-tube or petri-dish culture (Brown, 1974; Person-Dedryver and Doussinault, 1984), field tests (Brown and Meagher, 1970; O'Brien and Fisher, 1974, 1977, 1979), and inoculation of plants in plastic tubes with larvae (O'Brien *et al*, 1980; Fisher, 1982).

Use of infested soil makes control of the initial larval density difficult as it relies on uniform hatching of the eggs. This factor could confound differences in the number of females eventually produced per plant. Control of water relations is difficult in the test-tube or petri dish culture method. This also suffers from non-uniform hatching where cysts are used. Moreover, problems of maintaining aseptic conditions while keeping the seedlings adequately supplied with nutrients cannot be ignored. Field conditions are too variable to give a reliable indication of the reaction of individual plants. Infestation can be very patchy and interactions of the plants' reactions with uncontrolled parasites and climatic factors could provide misleading results.

In the light of the foregoing disadvantages of the other methods, the method reported by Fisher (loc. cit.) was used as detailed in section 3.3. This permits greater control of both the environmental conditions and the initial density of larvae.

The efficiency of the assay dropped dramatically part-way through the work reported in this thesis and also in three other research programmes on H. avenae at the same Institute; all these programmes relied on the same soil and similar growth conditions as described in section 3.3. Constraints in supply had necessitated substitutions for some of the original ingredients in the soil (e.g. blood meal as

source of nitrogen). The low numbers of females supported by standard susceptible cultivars could have been caused by a low percentage of larvae establishing in the roots or a low proportion of established larvae developing into mature females. However that may be, the soil used in the assay is the most likely source of the limiting factor(s).

7.2 Variation in Initial Density.

7.2.1 Materials and Methods.

The assay reported in section 3.3 was used for this experiment except that three different larval densities were tried: 100, 125 and 150 larvae/ml. of water. Six seedlings each of T701-4-6, T1006, T1116, Aroona, Warigal and Halberd were inoculated with each of the three larval densities. The experiment was arranged in a randomised complete block design.

7.2.2 Results.

Analysis of variance, followed by Duncan's new multiple range test, showed that differences among mean numbers of females on the different cereal cultivars/lines were highly significant (appendix 1 and table 4). The resistant line, T701-4-6, allowed development of a mean of 0.28 females per plant which was significantly fewer than the mean numbers supported by any of the other cultivars/lines. The significant differences between the cultivars/lines are shown in table 4. The different initial larval densities did not affect the numbers of females produced (appendix 1). No significant interaction between larval density and cultivar/line was detected.

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TABLE 4.

EFFECT OF LARVAL DENSITY ON THE NUMBER OF *H. AVENAE* FEMALES SUPPORTED BY SIX CEREAL CULTIVARS/LINES.

Cereal Cultivar or Line	Larval Density (Larvae/ml.)			Cultivar/Line Means	
	100	125	150		
T701-4-6	0.00	0.50	0.33	0.28	a *
T1006	7.83	4.00	7.00	6.28	b
Warigal	8.33	10.17	7.50	8.66	bc
T1116	9.33	10.50	12.66	10.83	bcd
Halberd	15.16	14.50	10.66	13.44	cd
Aroona	13.16	18.66	13.50	15.11	d
Larval Density Means	8.97	9.72	8.61		

LEAST SIGNIFICANT RANGES (LSR) FOR COMPARISONS BETWEEN ANY TWO RANKED LINE MEANS USING DUNCAN'S NEW MULTIPLE RANGE TEST.

Number of Ranked Means Separating Any Two Line Means Being Compared	0	1	2	3	4
LSR (five per cent level)	5.26	5.54	5.72	5.85	5.96
LSR (one per cent level)	6.98	7.26	7.48	7.64	7.74

* Cultivar/line means followed by the same letter do not differ significantly.

For analysis of variance table see appendix 1.

7.3 Numbers of Larvae Establishing in Roots.

7.3.1 Materials and Methods.

Twelve seedlings of Halberd wheat were inoculated at sowing with 100 larvae/ml. of water per seedling. Apart from harvesting at twenty days after sowing the set-up and management of the trial were as detailed in section 3.3.

At harvest the roots of each seedling were washed, boiled in lactophenol cotton blue for three minutes and then destained in clear lactophenol for not less than three hours (Goodey, 1963). Nematodes within the roots were counted.

7.3.2 Results.

The mean number (± standard error) of developing larvae per Halberd seedling twenty days after inoculation was 50.08 ± 10.18 with variation from 35 to 66. This represented approximately 50 per cent of the number inoculated.

7.4 Effects of Soil Type, Nutrition and Larval Density on Number of Developed Females.

7.4.1 Materials and Methods.

The testing procedure was basically the same as in section 3.3. Aroona was the test cultivar and a split-split-plot design incorporating a randomised complete block design was used with six replications. Four different soil types used in the base trays, constituting the main units (a) with their fertiliser treatments, were as follows:

a_1: Unsterile international grade concrete sand with less than 5 per cent clay and fertilised by full-strength Hoagland's solution (Hoagland and Snyder, 1933) with iron supplied (Jacobson, 1951) before planting and then at half-strength every ten days.

a_2: Standard soil was a sterilised mixture of equal parts (by volume) of sand and loam to which the following nutrients were added per cubic metre: 0.51kg. of potassium nitrate, 0.30kg. of potassium sulphate, 0.55kg. of superphosphate and 0.20kg. of lime. As well, a soluble feed of nitrogen prepared at 2g/l. of solution of a 60:11:9 mixture (by weight) of ammonium sulphate, potassium nitrate and mono-ammonium phosphate was added before planting and every ten days.

a_3: This was the same as a_1 except that the sand was sterilised before use.

a_4: A modified U.C. mix (Baker, 1957) prepared as a sterilised mixture of equal parts (by volume) of sand and peat with the following fertilisers added per cubic metre: 0.12kg. each of potassium nitrate and potassium sulphate, 0.24kg. of magnesite, 0.92kg. of plaster of Paris, and 1.40kg. each of reverted superphosphate, blood meal and hydrated lime. Soluble fertilisers were added as for a_2. All sterilisation was by means of aerated steam at about 75°C. The fertiliser solutions were added to the surface of the soil.

Sub-units (b) were made up of three different soils used in the tubes; standard soil was b_1, standard soil occupying the bottom three-quarters of the tube and topped up with sand was b_2 and b_3 was sterile sand.

Three different larval densities were used for inoculation as sub-sub-units (c). Treatment c_1 was 100 larvae/ml. of water applied five times at three-day intervals starting from the date of planting. Treatment c_2 was 150 larvae/ml. of water applied as for c_1. Finally, c_3 was 200 larvae/ml. of water applied at planting, repeated seven days later, and then 100 larvae/ml. of water applied ten days after planting.

7.4.2 Results.

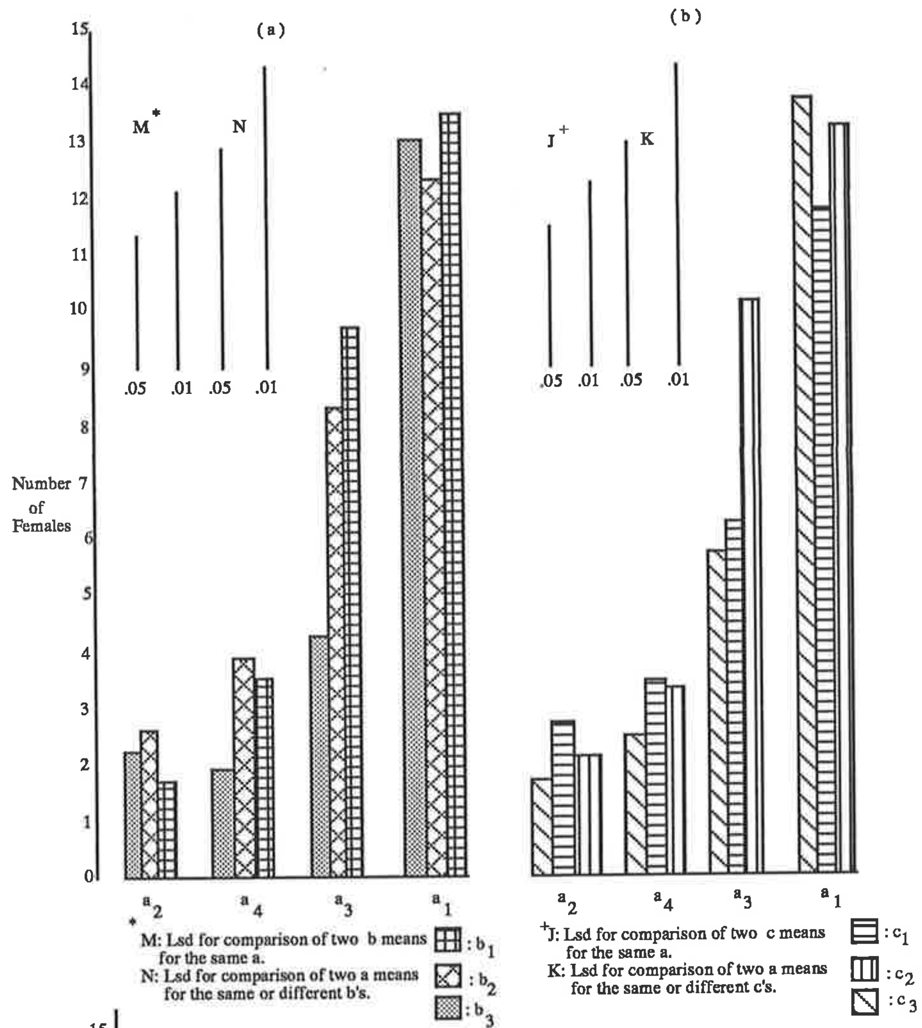
No three-factor interaction was detected but three two-factor interactions were (appendix 2). These were the reduced number of females in sterile sand in the base tray (a_3) when sand was used in the tube (b_3); the increased number of females when sterile sand in the base tray (a_3) was coupled with an inoculum density of 150 larvae/ml. (c_2); and the reduced number of females for the combination of sand in the tube (b_3) with an inoculum density of 150 larvae/ml. (c_2). The treatments a_3, b_3 and c_2 were the only ones which contributed to the significant interactions (figs. 13 a-c and tables 5 a-c).

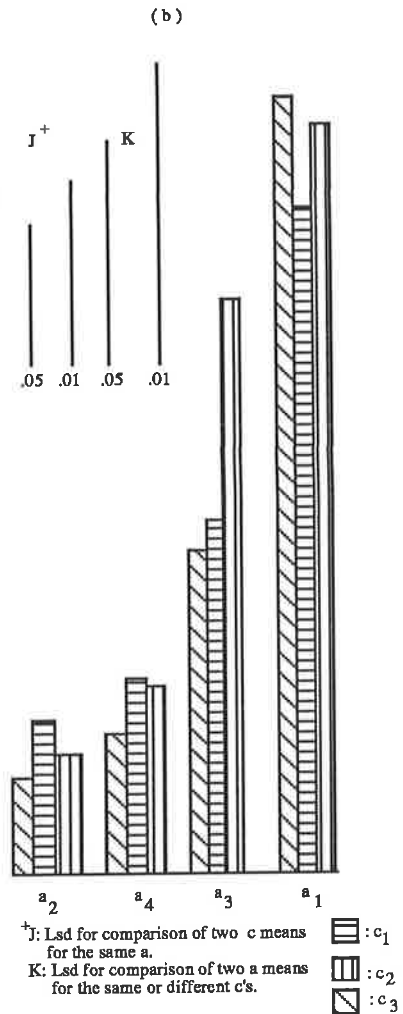
The major effects were complex (figs. 13 a-c; tables 5 a-c). None of the different combinations of treatments allowed the development of an adequate or expected number of females. Of the soils in the base tray, the standard soil (a_2) and U.C. mix (a_4) consistently produced poor results with all the tube soils and all the inoculum densities. Unsterile sand (a_1) in the base tray produced the best results and more females than sterile sand, except when standard soil (b_1) was used in the tubes or an inoculum density of 150x5 larvae (c_2) was used. Sterile sand proved better than U.C. mix (a_4) or standard soil (a_2) in the base tray.

Fig. 13a. Interaction between the effects of soil on the tray (a) and soil in the tube (b) on numbers of females per plant.

Fig. 13b. Interaction between the effects of soil on the tray (a) and inoculation (c).

Fig. 13c. Interaction between the effects of soil in the tube (b) and inoculation (c).


(a)
Number of Females
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
M*
N
.05
.01
.05
.01
a2
a4
a3
a1
* M: Lsd for comparison of two b means for the same a.
N: Lsd for comparison of two a means for the same or different b's.
: b1
: b2
: b3


(b)
J+
K
.05
.01
.05
.01
a2
a4
a3
a1
+J: Lsd for comparison of two c means for the same a.
K: Lsd for comparison of two a means for the same or different c's.
: c1
: c2
: c3

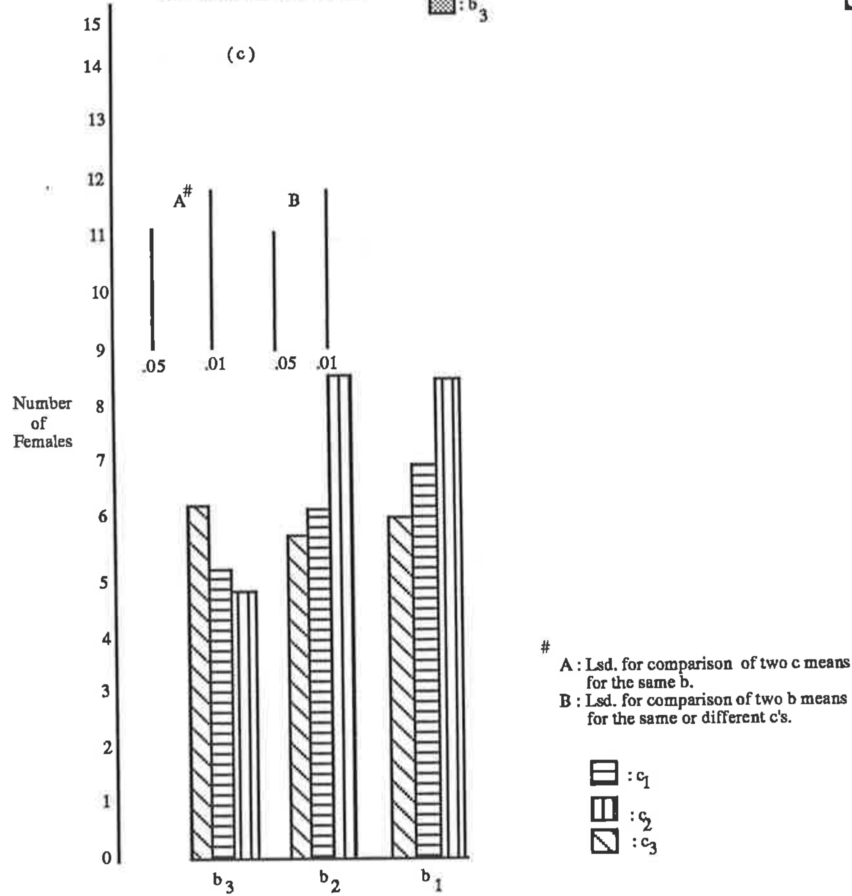

(c)
Number of Females
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
A#
B
.05
.01
.05
.01
b3
b2
b1
#
A : Lsd. for comparison of two c means for the same b.
B : Lsd. for comparison of two b means for the same or different c's.
: c1
: c2
: c3

TABLE 5.

MEAN NUMBER OF *H. AVENAE* FEMALES SUPPORTED BY PLANTS GROWN IN DIFFERENT SOILS AND INOCULATED WITH DIFFERENT LARVAL DENSITIES.

(5a)
Interaction Between Base Tray Soil and Tube Soil

Soil on base tray	Soil in Tube			Means for base tray soil
	b_3 (sand)	b_2 (Standard soil + sand)	b_1 (Standard soil)	
a_2 (Standard Soil)	2.28	2.66	1.72	2.22 a *
a_4 (U.C. Mix)	1.94	3.94	3.50	3.12 a
a_3 (Sterile Sand)	4.22	8.22	9.72	7.38 b
a_1 (Non-Sterile Sand)	13.00	12.28	13.44	12.90 c
Means for Soil in Tube	5.36d	6.78e	7.10e	

* Two treatment means followed by the same letter do not differ significantly.

LEAST SIGNIFICANT DIFFERENCES (LSD)

Difference Between:	Level of Significance	
	0.05	0.01
Two base tray soil means	3.3539	4.6380
Two tube soil means	1.1842	1.5845
Two tube soil means for the same base tray soil	2.3686	3.1690
Two base tray soil means for the same or different tube soils	3.8705	5.3074

(5b)
Interaction Between Base Tray Soil and Inoculation

Soil on base tray	Inoculation Procedure			Means for base tray soil
	C_3(200, 200,100)	C_1 (100x5)	C_2 (150x5)	
a_2	1.72	2.78	2.16	2.22 a
a_4	2.56	3.44	3.38	3.12 a
a_3	5.78	6.28	10.11	7.38 b
a_1	13.78	11.72	13.22	12.90 c
Means for Inoculation	5.96	6.05	7.22	

LEAST SIGNIFICANT DIFFERENCES (LSD)

Difference Between:	Level of Significance	
	0.05	0.01
Two inoculation means for the same base tray	2.4289	3.2104
Two base tray means for the same or different inoculations	3.8942	5.3202

(5c)

Interaction Between Tube Soil and Inoculation.

Soil in tube	Inoculation Procedure			Means for soil in tube
	C_3(200, 200,100)	C_1 (100x5)	C_2 (150x5)	
b_3	6.20	5.12	4.75	5.36 d
b_2	5.66	6.16	8.50	6.78 e
b_1	6.00	6.88	8.42	7.10 e
Means for Inoculation	5.96	6.05	7.22	

LEAST SIGNIFICANT DIFFERENCES (LSD)

Difference Between:	Level of Significance	
	0.05	0.01
Two inoculation means for the same tube soil	2.1035	2.7802
Two tube soil means for the same or different inoculations	2.0862	2.7680

The type of soil in the tube had little effect on numbers of females produced although sand (b_3) was worse than the other two types when combined with sterile sand on the base tray (a_3) or five inoculations of 150 larvae/ml. (c_2) (tables 5a and 5b; figs. 13a and 13b).

The different inoculation procedures did not produce significant differences in numbers of females apart from the interactions of five inoculations of 150 larvae/ml. with sterile sand on the base tray (a_3) and sand in the tube (b_3) (tables 5b and 5c; figs. 13b and 13c).

7.5 Discussion.

The results presented in table 4 illustrate the difficulties in screening during the latter part of the work reported in this thesis. The susceptible cultivars or lines allowed the development of more females per plant than the resistant line (T701-4-6) but generally the numbers of females obtained were fewer than expected (table 4). Moreover, there was no clear difference between the reactions of the moderately susceptible cultivars/lines (Warigal, T1006 and T1116) on the one hand and the standard susceptible cultivars (Aroona and Halberd) on the other. The results (table 4) also indicated that the density of larvae used for inoculation may not be a significant factor in the reduction in the numbers of females supported by susceptible cultivars. Inoculating with more than 150 larvae/ml. of water could affect plant growth adversely and thus reduce female development, especially for an intolerant cultivar like Aroona. The lack of response to increased larval density by the susceptible cultivars suggested that probably restrictions on larval establishment within the roots and/or environmental factors which adversely affect female development to maturity were responsible for the reduced numbers of females obtained.

The establishment trial (section 7.3.) showed that approximately 50 per cent of the larvae were established twenty days after a single inoculation. This compares

favourably with the 60 per cent reported by Fisher (1982) using, basically, the same assay and the 58 per cent reported from another laboratory assay (Chew, 1979; quoted by Cook and York, 1982). However, the variation (from 35 to 66 per cent; standard error of mean = 10.18) was quite high. Nevertheless, about 50 per cent larval establishment from the first inoculation plus numbers established from the subsequent four inoculations should have been sufficient to ensure much better separation between the mean numbers of females supported by resistant and susceptible lines in section 7.2. Therefore, there must have been factors restricting larval development to maturity. This conclusion assumes no sex reversal of female larvae on the susceptible hosts. Since temperature, light or photoperiod conditions had not been changed, the soil used in the assay was the probable source of the limiting factor(s). However, it would be interesting to repeat the experiment reported in section 7.3 and stain roots of samples of plants twenty days after planting and about every ten days after that until mature females were obtained. Correlation of the numbers of developing larvae at the various times with final numbers of mature females could indicate a critical survival stage after initial establishment of the larvae. It would also be useful to establish the effectiveness of the four later inoculations relative to the first one by varying the number of inoculations, using the same larval density, on a single susceptible cultivar and relating these to the number of females obtained.

In section 7.4 unsterile sand in the base tray (a_1) fertilised with Hoaglands's solution was shown to be more suitable for the assay than standard soil (a_2) or U.C.mix (a_4) fertilised with the soluble feed when considered in relation to the different soils in the tube or different larval densities. This may be due to the physical properties of sand, the efficiency of the nutrient supply from the Hoagland's solution, microbial activity in the unsterile sand and/or toxicity in the standard soil and/or U.C. mix. While some limited chlorosis was observed on plants growing in sand no symptoms of nutrient deficiency or toxicity were seen on plants grown in U.C. mix or standard soil. Since aerated steam was used for sterilisation (at about 75°C) it seems unlikely that sterilisation per se resulted in toxic factors in the U.C. mix and standard soil. The influence of sterile sand in the base tray (a_3) was complicated. It showed a negative interaction with sand in the tube (b_3) (figure 13a) but a positive one with five inoculations of 150 larvae/ml. (c_2) (figure 13b). The former interaction may be a result of inadequate nutrition for the plants since the sand in either the tubes (b_3) or tray (a_3) had almost no innate nutrients. The nutrient supply from Hoagland's solution could only reach the developing roots by capillary action from the base tray soil through the tube soil in the initial stages of growth. The inconsistency of this explanation with the effect of combining unsterile sand in the base tray (a_1) with sand in the tube (b_3) (figure 13a) may be because the factor(s) which made unsterile sand

in the base tray (a_1) so efficient was capable of making up for the defects of having sand in both the base tray and tube. The positive interaction between sterile sand in the base tray (a_3) and five inoculations of 150 larvae/ml. (c_2) is rather puzzling but suggests that increased larval density may help to overcome the limiting factor(s), particularly in sterile sand. If root growth is restricted in sterile sand then increased larval density may play a compensatory role, to some extent. However, the poor results of the combinations of sterile sand in the base tray (a_3) and the two other larval densities were surprising.

The overall reaction of plants grown on U.C. mix in the base tray (a_4) was also surprising. The combination of this soil and standard soil in the tube (b_1) approximated the set-up in section 7.2. While Aroona supported a mean of 15.11 females per plant in the experiment reported in section 7.2 it supported only 3.50 females per plant in the above-mentioned combination in section 7.4 (table 5a). The major known difference between the soils in the two experiments was that the nutrient supply was boosted with soluble feed in section 7.4 but not in section 7.2. However, since they were different batches of soil, prepared months apart, there could have been other differences. Nevertheless, the comparison raises serious questions about the influence of the soluble feed. Only the numbers of females obtained from the combinations of unsterile sand on the base tray (a_1) with the three soils in the tubes were comparable to the reaction of Aroona in section 7.2 (tables 4 and 5a).

The negative interactions of sand in the tube (b_3) with sterile sand in the base tray (a_3) (fig. 13a) and also with five inoculations of 150 larvae/ml. (c_2) (figure 13c) may explain the suggestion in table 5a that sand is the worst medium in the tube for the assay. Putting sand on top of standard soil in the tube (b_2) was expected to improve aeration and prevent crusting at the soil surface but no significant advantage of this has been detected.

The interactions of inoculum density with the soils used indicate that it would be incorrect to consider larval density as an insignificant factor in the low numbers of females obtained from susceptible plants as the results of section 7.2 suggested.

In conclusion, it must be reiterated that the numbers of females supported per plant by susceptible cultivars were about half the expected numbers. Factors restricting female development to maturity, most probably in the soil, are the most likely causes. While the best combination of the appropriate levels of the soils, nutrients and larval densities may not have been found it is possible that there are other important factors which have not been identified. The probable involvement of microbial activity, as suggested by the overall reaction of plants grown on non-sterile sand in the base tray, requires further investigation.

CHAPTER EIGHT

INDUCTION OF HOMOEOLOGOUS RECOMBINATION

8.1 Introduction.

Alien chromosome additions or substitutions, irradiation-induced translocations, union of telocentrics and induction of homoeologous pairing and recombination have been either tried or successfully used for alien gene transfer into wheat (Riley and Kimber, 1966; Knott and Dvořák, 1976; Lacadena, 1978; Sears, 1972, 1981). According to Sears (1972) the most efficient of these methods is the induction of allosyndesis which may be followed by crossing over if the alien chromosome has enough affinity with one of its wheat homoeologues to permit frequent pairing between them. Methods of inducing allosyndesis include suppressing the activity of the major homoeologous pairing inhibitor gene (Ph) on wheat chromosome arm 5BL using the genome of Aegilops speltoides, removing chromosome 5B or using Sears' ph1b ph1b mutant line (Sears, 1977) which is deficient for the Ph1 locus (Sears, 1981).

With respect to the material in which allosyndesis is induced, Sears (1981) described the following increasing order of precision for obtaining wheat-alien recombination: wheat-alien hybrids, disomic alien additions, monosomic alien

additions, monosomic alien substitutions, monotelosomic alien substitutions possessing the critical alien chromosome or misdivision product. However, Anderson (1985) found that in three recombinant lines selected by Koebner (1985) from progeny of crosses between Sears ph1b ph1b mutant line and a plant which was heterozygous for chromosomes 1RL and 1DL, all the wheat homoeologous group 1 chromosomes appeared to have been involved in separate recombination events with the rye chromosome arm.

8.2 Materials and Methods.

Crosses were made between the disomic 6R(-6D) substitution line, B83-9C-136-3-1, reported in chapter 6 and Sears' *ph1b ph1b* mutant line with the aim of inducing pairing and recombination of 6R with 6D or other wheat chromosomes. Embryos from immature F_1 seeds were excised for culture on artificial growth media in order to save time (section 3.2.4). Selfed seeds were collected from five F_1 plants. One hundred F_2 seedlings from these seeds were screened for the presence of chromosomes 6R and 6D mainly by electrophoresis for glutamate oxaloacetate transaminase isozyme analysis (section 3.2.5). A few of them were also screened by cytological examination of C-banded somatic chromosomes (section 3.2.2). The meiotic metaphase 1 configurations of 24 selected F_2 plants were analysed. On the basis of these configurations, eight plants, four of which were considered probable *ph1b* homozygotes, were crossed as females to *Aegilops variabilis* (= *Triticum kotschyi*) for further evidence of their *ph1b* status. In order to save time, embryos from the developing intergeneric F_1 seeds were cultured on artificial growth media (section 3.2.4). Meiotic pairing of the resulting F_1 hybrids were analysed. Many of the eight F_2 plants crossed to *Aegilops variabilis* were partially self-sterile so, for maximum production of F_3 seeds, tillering had to be sustained by applying fertiliser to the plants and harvesting the seeds as soon as they were considered mature enough to be viable after oven-drying.

As a second approach, with the ultimate aim of recombining 6R with 6A or 6B, disomic 6R addition lines were crossed as males to the following Chinese Spring Wheat monosomics: monosomic 6A, monosomic 6B, double monosomic 5B, 6A and double monosomic 5B, 6B.

8.3 Results

Five F_1 progeny from the crosses between the disomic 6R(-6D) substitution line and Sears' *ph1b ph1b* mutant line produced 1525 selfed seeds.

Twenty-four F_2 plants were selected from the isozyme and/or cytological analysis of 100 seedlings as having both 6R and 6D. From meiotic analysis five of them were found to have 43 chromosomes and 19 had the required 42. Two of the five with 43 chromosomes were disomic for 6R. Most of the 24 F_2 plants had some pollen mother cells (PMCs) with multivalents at first meiotic metaphase so it was difficult to use that criterion (Sears, 1977) for the selection of *ph1b* homozygotes. The mean meiotic metaphase 1 configuration, on the basis of 50 PMCs each, typical of most of the F_2 plants was:

1.85^{I*} $(0\text{-}5)^{\#}$ + $2.20^{\lfloor II \rfloor}$ (0-6) + $17.00^{\textcircled{II}}$ (12-20) + 0.51^{III} (0-2) + 0.04^{IV} (0-1).

However, there were four plants which, on the basis of 50 PMCs each, had the following mean configuration:

2.92^{I} (1-5) + $5.21^{\lfloor II \rfloor}$ (2-11) + $13.82^{\textcircled{II}}$ (8-17) + 0.14^{III} (0-1) + 0.07^{IV} (0-1).

---

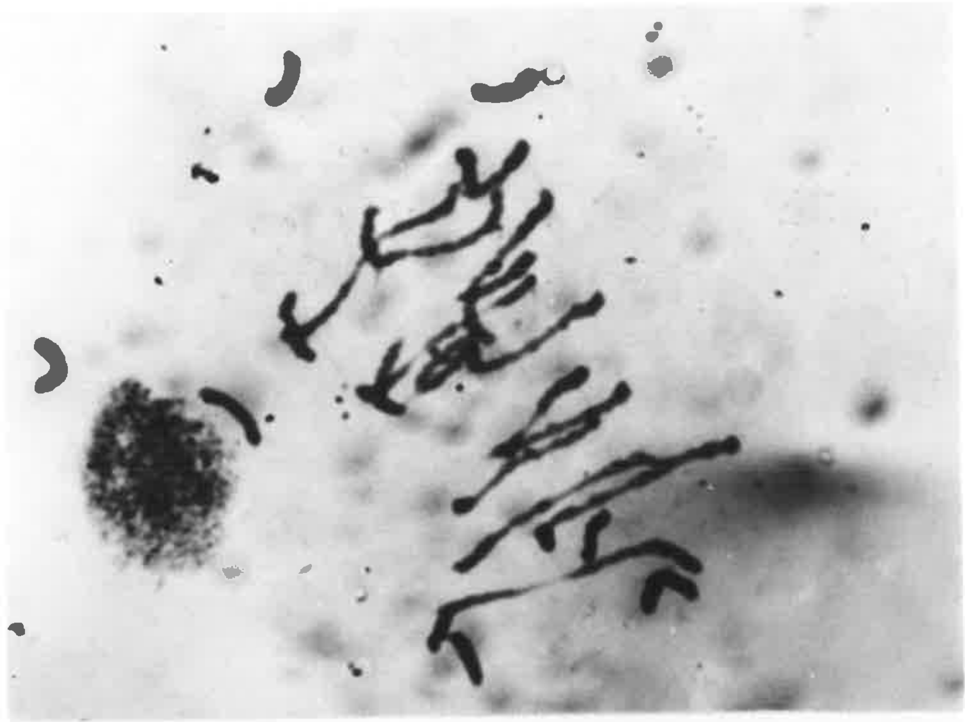
* I=univalents, $\lfloor II \rfloor$=open bivalents, $\textcircled{II}$=closed bivalents, III=trivalents, IV=quadrivalents; # =range.

Owing to the higher incidence of univalents and open bivalents in the PMCs from these four plants they were considered probable homozygous _ph1b_ plants. The numbers of wheat x _Ae. variabilis_ hybrids derived from these four plants and analysed for the level of meoitic pairing were eight from plant B84-9CP-2-1-15b, five from plant B84-9CP-2-1-14a and four each from plants B84-9CP-2-1-7b and B84-9CP-2-1-11a. All these 21 wheat x _Ae. variabilis_ hybrids had high levels of pairing at metaphase I (figure 14a). For comparison wheat X _Ae. variabilis_ hybrids involving four sib F_2 plants showed extremely low frequencies of pairing (figure 14b). Assuming a 0.5 transmission rate of _ph1b_ through the eggs of the F_2 plants crossed to _Aegilops variabilis_ the probabilities of the four selected plants having been heterozygous (_Ph1b ph1b_) instead of homozygous (_ph1b ph1b_) are 0.0039 for B84-9CP-2-1-15b, 0.0312 for B84-9CP-2-1-14a and 0.0625 for each of B84-9CP-2-1-7b and B84-9CP-2-1-11a. From the meoitic analyses of the four F_2 plants B84-9CP-2-1-11a was found to be disomic for 6R and most probably monosomic for 6D, unless there had been a univalent shift, since the total number of chromosomes was 43. The other three plants were monosomic for both 6R and 6D. Thirty-seven selfed seeds were collected from B84-9CP-2-1-11a whereas the other three plants produced a total of 1220 selfed seeds all of which are available to be screened for recombination products.

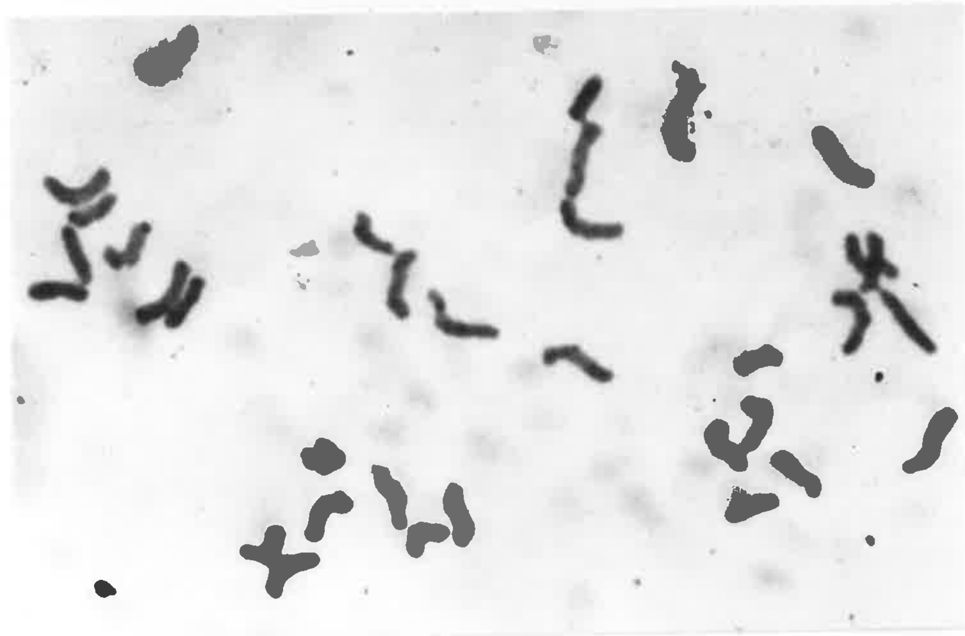
Fig. 14. Meiotic configuration in Wheat X *Aegilops variabilis* hybrids.

(a) High pairing

(b) Low pairing



a.



b.

In the second approach seeds from crosses between the Chinese Spring Wheat monosomics or double monosomics and disomic 6R addition have been harvested and are available for screening for double and triple monosomic F_1's.

8.4 Discussion

The occurrence of multivalents in PMCs of many of the F_2 plants at first meiotic metaphase could have resulted from translocations in the *ph1b ph1b* mutant stock that was used. The choice of the four probable *ph1b* homozygotes on the basis of higher incidence of univalents and open bivalents at meiosis (Driscoll *et al*, 1979; Giorgi, 1983; Yacobi and Feldman, 1983; Koebner and Shepherd, 1985) was vindicated by the meiotic configurations of F_1 hybrids from their test-crosses to *Ae. variabilis*. The probabilities of error in the selection of *ph1b* homozygotes are low (0.0625 - 0.0039) and the number of F_3 seeds available from the three double monosomic plants (1220) provides a good chance of obtaining some homoeologous recombinants.

Since it is reasonable to assume that 6R as a univalent would pair more frequently with its wheat homoeologues than when in a disomic condition, the 37 F_3 seeds from B84-9CP-2-1-11a could be sown for further crosses as males to Sears' *ph1b ph1b* mutant line. A lot of F_1 seeds could be produced which would be expected to be monosomic for both 6D and 6R and homozygous *ph1b ph1b*. Hence, it is possible to increase the number of seeds for screening for homoeologous recombinants. However, this should not be necessary.

Screening for recombinants could be done by a combination of C-banding and isozyme analysis. Only

glutamate oxaloacetate transaminase has been used in this work for the identification of 6R but preliminary trials have indicated that aconitate hydratase would be equally effective. In addition esterases, aromatic alcohol dehydrogenase and dipeptidase may be successfully used. The loci for all these isozymes are on the long arm of 6R which, from the limited evidence presented in chapter 6, may carry the gene for resistance to *H. avenae*. If, however, the short arm is found to be the critical arm, from tests scheduled to confirm the arm location, then the locus of aminopeptidase and the heterochromatic telomere may be relied upon for selection of recombinants. Also one of the isozymes on the long arm may be sufficiently close to the centromere to be useful for monitoring recombination in the short arm if this turns out to be the critical arm.

CHAPTER NINE

GENERAL DISCUSSION

It is difficult to quantify accurately the losses to Australian agriculture caused by the cereal cyst nematode owing to its occurrence with other soil pests and pathogens and also interactions of its effects with environmental factors. Nevertheless, the devastating effects of the nematode on cereal crops in Australia and other parts of the world are well documented (Fushtey and Johnson, 1966; Cotten, 1970; King *et al*, 1982). The best method of control would be the use of good host resistance, especially when associated with tolerance, in conjunction with cultural methods like crop rotation. However, the availability of only a single gene for resistance in wheat to the unique Australian pathotype is unsatisfactory. Moreover, that gene is not effective in keeping the nematode population below the economic injury level (Fisher, 1982). The resistance to *H. avenae* present in the triticale line, T701-4-6, has been shown in this thesis to be stronger than that in the most resistant wheat line, AUS 10894. Without going into the controversy over the genetic basis of durable resistance (*sensu* Johnson and Law, 1975; Lamberti *et al*, 1983), it is reasonable to suggest that, despite the monogenic inheritance and dominant action of this strong gene in T701-4-6, its deployment in conjunction with cultural control measures could ensure long-lasting stability of the resistance (de Ponti, 1983).

Moreover, the relatively slow multiplication rate and spread of the nematode and also the availability of some alternative hosts should lessen the dangers of 'break-down' of resistance. The usefulness of this resistance gene outside Australia, however, is yet to be established since the Australian pathotype of the nematode is a unique one.

The use of the homozygous resistant triticale lines produced in this study in any triticale breeding programme should provide a good chance for the release of triticale cultivars with higher resistance than is now available. Having originated from parents with considerable tolerance to the nematode it is very likely that these lines will also have good tolerance. The combination of tolerance and resistance in a single cultivar will give it the advantage of normal yield during its first year of use in an infested area, while still serving as a 'cleaning' crop and thus benefitting subsequent intolerant cultivars. The mutation of wheat stem rust strain 34-2,12 to the virulent 34-2,12,13 during the course of this project is just one more example of the frustration of combatting a quickly changing pathogen. The presence of resistance to strain 34-2,12 and *H. avenae* in a line like the one submitted to the National Rust Control Programme would facilitate progress towards multiple resistance in a single cultivar.

Rye cultivar South Australian has been known for years as resistant to *H. avenae* but this thesis presents the first known evidence of the location of a *H. avenae* resistance gene on a

particular rye chromosome (6R). The probable location of the resistance gene on the long arm requires confirmation. The presence of interstitial C-bands and loci of a number of isozymes on the long arm (6RL) could facilitate mapping of the resistance gene and characterisation of any future transfer chromosomes involving the critical rye segment and a wheat chromosome.

An excellent opportunity has been provided by this work for the use of rye resistance to the nematode in wheat cultivars in the near future. This would be most welcome since years of screening have resulted in only a single wheat line with, essentially partial, resistance to the pest. Chinese Spring wheat will obviously not be a good genetic background for the deployment of the resistance gene when a useful wheat-rye recombination is eventually obtained. Its value in cytogenetic analysis was given priority over its poor agronomic performance in the selection for this project. In fact, even Aroona wheat may not be the best possible genetic background available for use since it is known to be intolerant to cereal cyst nematode attack. Any useful recombinant line would have to be used in a backcross programme in which a tolerant wheat cultivar is the recurrent parent. Recombination of 6R with 6D is considered most probable since they were both univalents in three ph1b homozygotes whose selfed seeds are available for screening. However, recombination with either 6A or 6B is also possible and may be of significance in relation to the use of the gene in durum wheats.

Expression of the resistance in the Aroona genetic background was very good but there was a suggestion of incomplete epistasis in the Chinese Spring background. Only time will prove what deleterious or beneficial effects may be linked with the resistance to H. avenae when transferred to wheat. Obviously the lengths of the exchanged chromosome segments will be important. A linkage map of wheat presented by McIntosh and Cusick (1984) showed a number of loci for wheat stem rust and leaf rust resistance genes on homoeologous group 6 chromosomes. The short arm of 6B and the α-arms of 6A and 6D are also known to have loci for gliadin proteins which may have some relevance to bread-making quality (Shepherd, 1968; Wrigley and Shepherd, 1973).

The unfortunate deterioration of the assay during the course of the project slowed progress and led to some inconclusive results. The time required for the completion of a single test (75 days) made repetitions very wasteful of time. It also meant that experiments had to be set up often without waiting for the completion of an earlier one. While the soil seems to be the most likely source of the problem further investigation is required to identify the specific factor(s) and rectify the situation. An experiment specifically designed to assess the reaction of a single susceptible cultivar using the different assays reported by other workers and the one used in this work might have thrown some light on the problem. However, all the assays depend on maturation of the females and are either of

similar duration as the one used in this work or take even longer. Testing outside the laboratory was restricted by the seasons and with the short time left for completion of this study it was not possible to investigate the alternative assays properly or develop a new one.

APPENDIX 1

ANALYSIS OF VARIANCE FOR DATA SUMMARISED IN TABLE [illegible]

Source of Variation	df	SS	Mean Square	F
Blocks	5	83.71	16.74	0.80
Cereal cv./line	5	2591.94	518.38	24.66**
Inoculum Concentration	2	23.13	11.56	0.55
Cereal cv. X In. Conc.	10	267.98	26.80	1.27
Error	85	1787.12	21.02	
Total	107	4753.88		

** - significant at 1 per cent level.

APPENDIX 2

ANALYSIS OF VARIANCE FOR DATA SUMMARISED IN TABLE 2.5

Source of Variation	df	SS	Mean Square	F
Blocks	5	274.46	54.89	0.82
Soil on Base Tray (a)	3	3859.53	1286.51	19.24**
Error (a)	15	1003.23	66.88	
Soil in Tubes (b)	2	122.96	61.48	4.97*
Interaction, ab	6	228.34	38.06	3.08*
Error (b)	40	494.48	12.36	
Inoculation (c)	2	71.23	35.62	2.63
Interaction, ac	6	190.84	31.80	2.34*
Interaction, bc	4	137.93	34.48	2.54*
Interaction, abc	12	254.00	21.16	1.56
Error (c)	120	1625.33	13.54	
Total	215	8262.33		

* significant at 5% level

** significant at 1% level.

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