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Variation in resistance to Ascochyta blight in faba beans

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Thesis submitted for the degree of Doctor of Philosophy

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STATEMENT

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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SUMMARY

1. Ascochyta blight, caused by *Ascochyta fabae* is a serious disease of faba beans in southern Australia. It may be controlled by fungicidal sprays but their use adds to the cost of production. The breeding of resistant cultivars may overcome or reduce the need for these sprays. This study is concerned with various aspects of genetic resistance to Ascochyta blight and the means by which resistance may be bred into cultivars.

2. Variation for resistance to Ascochyta was found within and between accessions and cultivars of faba beans. From a genetic study of an F2 segregating population, obtained following crossing of parents selected to be highly resistant (plants from Acc 970) or to be highly susceptible (plants from Acc 811) and tested against a single isolate of *A. fabae*, it was concluded that resistance was controlled by a single dominant gene. The phenotypic expression of this single dominant gene for resistance may be influenced by the time of evaluation after inoculation.

3. The genetic study was extended to include many plants of Acc 970 and 811 which had shown different levels of resistance when inoculated with one isolate. F1 and F2 populations were produced following crossing of these plants, which in Acc 970 had been classified: Highly resistant, Resistant, Moderately resistant, Moderately susceptible and in Acc 811: Resistant, Moderately resistant, Moderately susceptible, Susceptible and Highly susceptible. Several sets of distinct segregation ratios were obtained in the F2 generations of the crosses between the different parents of the two accessions.

It was concluded that there were different genes for resistance in Acc 970 and Acc 811. In Acc 970, there was evidence for a single dominant gene conferring resistance, with most plants being homozygous for this gene but some were heterozygous. Acc 811 had the recessive, susceptible allele to this gene but there was also evidence that some plants of Acc 811 carried two or more complementary recessive genes which also conferred resistance.

4. Thirty-one selections were available for study from a mass-selection program aimed at increasing resistance to Ascochyta in the cultivar Fiord. Three cycles of selection had been practised in the field where natural infection had been encouraged and amplified with artificial inoculation. The selections were compared with the original Fiord cultivar. The selections as a group were clearly more resistant than Fiord, but within the selections individual plants differed in their levels of resistance. Furthermore there was evidence that the plants of any one selection differed in their resistance and further selection would increase the homogeneity of the resistance. No significant correlation was found between the level of infection of the plants scored 21 days after inoculation and infection of the seed at harvest.

5. The morphology of 254 single-spore isolates from 52 colonies of *A. fabae* collected from 21 bean growing areas in South Australia was compared. There was as much variation in morphology amongst isolates derived from single spores, collected from the same colony, as between isolates from different colonies collected from the same or from different locations. Hence the variation was not related to the location of origin. The variation in morphological features of the cultures confirmed the great variability of *A. fabae* in South Australia where the sexual stage has been reported.

6. Components of resistance including incubation period, lesion size, lesion number, disease efficiency (the ratio of lesions that develop pycnidia to total number of lesions) and disease infection type (score) were studied for 8 isolates of *A. fabae* on 8 accessions of faba beans grown in the glasshouse. There was pathogenic variability amongst the 8 isolates. From the 8 isolates and 8 accessions, 7 groups of isolates were designated. Within each faba bean accession, heterogeneity of response was found.

Resistance was expressed firstly as the inhibition of infection, the plants did not become infected as shown by the variation in incubation period and lesion number. Secondly resistance was evident as reduced production of spores. Lesion size was not associated with the resistant or susceptible condition. If the infection occurred on otherwise resistant material large lesions could develop. There was evidence that in

some instances an accession would have few lesions on the stems and many on the leaves, while another accession would have the opposite reaction when exposed to a specific isolate. Lesion number and disease efficiency seemed to be the best indicators of resistance, as they were highly correlated to the disease score.

7. A detached-leaf and a detached-stem technique were developed that differentiated successfully between resistance and susceptibility to Ascochyta. The method of inoculation, the age of the plants, the leaf's position or the stem's position on the plant before being excised and the conditions under which the material was incubated were investigated. Applying inoculum as a spray allowed good discrimination of resistance or susceptibility whilst applying inoculum in a drop did not. The younger parts of a plant, both its leaves and stems, were more susceptible than the older parts, even for susceptible material. For the best discrimination the younger parts of plants should be used. Plants only 3-5 weeks old provided good material for the test. Spore concentrations of 5×10^2 sp/ml were sufficient to infect material but the higher concentration of 5×10^4 sp/ml gave more lesions. With further increases in concentration the lesion number did not increase. The detached-stem or -leaf test will greatly facilitate the studies of host genotypes and isolates and be an aid to breeding programs.

8. In the Discussion it was concluded that because resistance is controlled by more than one gene, some of which interact, and because several isolates are present, breeding for resistance could proceed either by producing synthetic cultivars or by a procedure such as mass selection. The mass selection approach would be simpler and would not depend on a detailed knowledge of the genetics of each host and isolate.

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CHAPTER 1. INTRODUCTION

Ascochyta blight, caused by *Ascochyta fabae* Speg., is a common disease of faba beans throughout the world (Gaunt, 1983). There is a potential for serious loss of yield when the inoculum level builds up and the disease reaches epidemic proportions. Major outbreaks causing total loss of yields have been reported in China and Russia (Yu, 1947; Konstantinova, 1965).

Faba bean production in Australia on a significant scale began in 1980 with the release of the cultivar Fiord (Knight, personal communication). Before that time beans were grown in home gardens as a fresh vegetable, as a green manure crop in vineyards or on a small commercial scale as a fresh green bean in Tasmania. Following the release of Fiord the faba bean industry has expanded each year, first in South Australia and more recently in Victoria, NSW and WA, such that the crop in 1992 occupied approximately 70,000 ha, with a production of 100,000 t and a value to growers of $20 M annually. The grain is primarily exported for human consumption but there is also a significant domestic market for the beans in compounded animal feeds.

Ascochyta has been present in Australia for many years (Geard, 1962). Before the release of Fiord its seed stock had been grown and rogued to be totally free of Ascochyta. No infected crops of Fiord were seen until 1985 when some minor occurrences were reported. More were seen in 1986 and by 1987, many crops in South Australia were observed to be infected. Infection of crops is now expected every year in South Australia and in many areas of Victoria.

The disease causes a loss of yield as a result of damage to leaves, stems and pods and also results in a down-grading of the grain. Blemishes on the grain are a significant problem when the grain is being exported for human consumption. Losses in value occur when the grain is less attractive to the overseas consumer, when blemished grains have to be removed in the canning process or when the blemishes are so extensive as to lead to a down-grading to animal feed. Thus the extent of the commercial loss is governed by the end use of the crop. This in turn determines the degree of disease protection which is economic and the type of control strategies which give optimal benefit (Knight, personal communication). For example late sowing, late

June instead of early May, leads to less infection but also to a much reduced yield because of the shorter growing season.

Methods for controlling the disease have included chemical and integrated control methods (Kharbanda and Bernier, 1976; Wallen and Galway, 1977; Hampton, 1980; Anon, 1985a). In Australia, aerial spraying with the fungicide Dithane is common with an average cost of $20 per application. Two to three applications may be required during the growth of a crop and the cost severely reduces the financial return to the grower. Growers sometimes make more than three applications but then the intent has been also to control chocolate spot disease (*Botrytis fabae*) which may occur in some years and areas. None of the chemical fungicides completely controls Ascochyta and there is invariably some loss of yield. In addition there must always be concern for the development of resistance to the fungicide and for environmental damage. One means of avoiding the cost incurred in the fungicide and its attendant problems would be to develop and use resistant varieties. To be feasible, this approach requires much more knowledge of the disease including the genetics of the host and pathogen.

The outbreaks of Ascochyta blight since 1985 have led to many questions. Has the increase in the area and the frequency of growing the crop been the cause of the increased occurrence of the disease? Have new races of *A. fabae* developed, capable of overcoming any resistance that may have been present in Fiord? How difficult would it be to breed cultivars adapted to Australian conditions and resistant to the disease? If resistance were achieved, would it be quickly broken down by new races?

The present study is concerned with the question of breeding new cultivars resistant to the disease and covers four subjects:

1. The genetics of resistance in faba bean populations.
2. The effects of mass-selection on increasing resistance in Fiord.
3. The components of disease resistance when various faba bean accessions are inoculated with isolates of *A. fabae*
4. The development of efficient methods for evaluating material for its resistance to the disease.

The genetics of resistance was studied with the objective of understanding the effects of selection for resistance in breeding programs. Differences in resistance to

Ascochyta had been observed between accessions of faba beans grown in field trials in Australia (Knight, personal communication) and overseas there have been many reports of differences in resistance, although only limited information is available on the genetics of resistance.

Although by 1987, many crops of Fiord were seriously infected by Ascochyta, observation suggested there was variation for resistance within this cultivar. A study of mass selection as a method of increasing resistance within Fiord was initiated in 1988 by Dr. R. Knight. The aim in one component of the present study was to determine whether the simple mass-selection procedure had resulted in populations more resistant to Ascochyta blight than the original cultivar.

Overseas, there have been studies on the virulence and aggressiveness of *A. fabae* isolates, but nothing is known of the spectrum of variability in Australia. The expression of resistance on stems, leaves, pods and seeds is relevant to the damage that will result. If resistance is also expressed as a limitation to spore production it will inhibit the spread of the disease.

Since faba beans are partially cross-pollinated and most accessions or cultivars are mixtures of genotypes, individual plants need to be subjected to replicated evaluations for such characteristics as disease resistance. This would be feasible if resistance could be determined on a detached leaf, as the many leaves of a plant would provide for the desired replication. Such a test would be particularly valuable if it gave results correlated with ratings in the field. In recent years selection for resistance has relied on field or glasshouse conditions. A detached-leaf test was developed by Dodd (1971) but the lesions were atypical and he was unable to separate the cultivars on their resistance. This failure was mainly due to the senescence of the leaf before the disease could develop. One aim of the present study was to establish the conditions necessary for an efficient detached-leaf technique to be used in genetic studies of the host and pathogen races and in the breeding program.

CHAPTER 2. LITERATURE REVIEW

2.1 Introduction

Faba beans today are the world's fifth most important food legume (FAO, 1990). They are grown on 3.2 million hectares in about 50 countries and total production exceeds 4.3 million tonnes. Whenever the crop has been grown, it has a reputation for being unstable in it yield, partly as a result of the three diseases Ascochyta blight (*Ascochyta fabae* Speg.), chocolate spot (*Botrytis fabae* Sard.) and rust (*Uromyces viciae-fabae* (Pers.) Schroet.). These diseases vary in their incidence in different regions and years but all are reported to cause serious losses of production in Asia, North Africa and Europe (Anon, 1981; Anon, 1984; Anon, 1990).

The faba bean industry in Australia is relatively new but Ascochyta blight and Chocolate spot already have had serious effects on production. The Australian industry began with the release of Fiord in 1980 (Knight, personal communication). Disease resistance was not a direct breeding objective in the development of Fiord. The cultivar was released because of its high yield (a component of which may have been some disease resistance) and because of its relatively small seed (0.48 g) which enables farmers to sow and harvest the crop with conventional cereal seeders and harvesters. The area sown to Fiord has been increasing steadily and in 1992 was over 70,000 ha. The greatest limitation to the wider adoption of the crop by farmers has been the cost of controlling the fungal diseases Ascochyta blight and Chocolate spot.

In South Australia between 1980 and 1992 there was only one major outbreak of Chocolate spot which occurred in 1983. Before Fiord was released in 1980, its seed stock had been grown and rogued to be totally free of Ascochyta and no infected crops were seen until 1985. Every year since then, there has been a potential for damage from this disease. It has been contained by the aerial application of fungicides. This has been done at considerable cost to the farmer who may make up to three applications of fungicide (Knight, Hawthorne, personal communication). Efforts are being made to breed cultivars resistant to both diseases but because Ascochyta appears every year and Chocolate spot is sporadic, Ascochyta has been judged the

more serious disease in South Australia. The present study is concerned with an understanding the genetics of resistance to Ascochyta, information which will be used in the development of cultivars resistance to the disease.

Chocolate spot and Ascochyta blight occur in other bean growing areas of the States of Vic, NSW and WA. The diseases vary in their relative importance in the different areas. For instance Chocolate spot, until now, has been more important than Ascochyta in northern NSW (Simmons and Marcellos, 1989).

2.2 Ascochyta blight

The disease was first described in 1899 by Carruthers in England and Spegazzini in Argentina (reviewed by Dodd, 1971).

2.2.1 Yield losses

Losses in yield as a result of Ascochyta infection understandably are variable depending on the host cultivar, the races of the pathogen and environmental conditions. Yield losses of between 5 and 50% have been reported (Yu, 1947; Gaunt *et al.*, 1978; Zakrzewska, 1986). Hanounik (1980b) showed that by artificial inoculation of the susceptible cultivar (Giza 4) and a local more resistant cultivar in Syria, yield losses of 91% and 50% respectively occurred when compared with plants of these cultivars sprayed each week with the fungicide, Dithane M-45. Little is known about the relative effect of the presence of leaf, stem or pod lesions on yield reduction. In one trial, when the seeds sown were artificially inoculated with *A. fabae*, it was found that green yield per plant was reduced by 35%, and grain yield by 34% (Hampton, 1980). It was suggested the yield reduction resulted from damage to the leaf tissue. A similar conclusion was reached by Madeira *et al.* (1988) who found the leaf area index and dry matter production were reduced and the loss of yield was 15%. Gaunt (1983) reported that yield losses were evident as a reduction in the number of pods, with lesser effects on the number of seeds per pod and seed weight. The early stages of infection were the most important in terms of yield loss. However, Wallen and Galway (1977) reported that infection levels of 4% to 10% in field plots caused no significant loss of yield. These authors did not state when the infection was scored

and this somewhat contrary result may indicate that the timing of the infection relative to pod development is important.

In addition to reducing yield, Ascochyta infections cause spotting on the grain, which reduces quality when the grain is being used for human consumption and especially when the grain is to be used in canning (Knight, personal communication).

2.2.2 Symptoms

Ascochyta fabae attacks the aerial parts of the plants; the leaves, stems and pods. Root infection has not been recorded and attempts to inoculate the root were unsuccessful (Yu, 1947).

The lesions on the leaves are usually circular, up to 35 mm in diameter, brown in colour and often paler in the centre. Later, the centre may become white. In conditions favourable to the fungus the lesions will enlarge and become irregular. Lesions can coalesce to produce a large area of necrosis. At a more advanced stage of infection, pycnidia are produced in the centre of the lesions and are visible as small black spots.

Stem lesions have symptoms similar to those on the leaves, but are less abundant and are sunken deeper into the host tissue. Most lesions are elongated and are parallel with the stem. In a badly infected stem, the lesion may be up to 25 cm long (Yu, 1947). The infection weakens the stem of the plant and it is easily broken in windy conditions (Yu, 1947; Beaumont, 1950; Dodd, 1971; Gourley and Delbridge, 1973; Punithalingam and Holliday, 1975; Liew, 1981; Gaunt, 1983; Omar, 1986).

Pod lesions are circular to oval, dark brown in colour and deeply sunken. If lesions develop on the line of dehiscence of the pods they are usually oval and the pod may split as the seeds are filled. The severity of the disease depends on the stage of development of the pod when attacked by the fungus. At an early stage of infection, the whole pod may become yellow. The seeds are not set and the pod aborts. At a more mature stage, mycelia often penetrate the pod and enter the seed. Infected seeds may shrivel and have dark brown blemishes on the seed coat. Pritchard *et al.* (1989) suggested the fungus infected **only** the testa. However, Gaunt (1983) observed that in severely infected seeds the cotyledons were also infected. Similarly, Kharbanda and Bernier (1979) showed that *A. fabae* is often present under the seed coat.

2.3 Classification and morphology of *Ascochyta fabae*

The genus Ascochyta was established as *Ascoxyta* by Libert in 1830. In 1833, Link altered the spelling to Ascochyta and this was later accepted by all, including Libert (reviewed by Punithalingam, 1979). The species *Ascochyta fabae* Speg. was named by Spegazzini in 1899 (reviewed by Beaumont, 1950).

The teleomorph of *A. fabae* was reported for the first time on over-wintering straw of *V. faba* in Cambridge by Jellis and Punithalingam (1991) and given the name *Didymella fabae* Jellis & Punith. It was found on infected stems which had been collected and put in open-mesh net bags laid on a grassy bank near a field trial. Three months later, immature ascomata were observed on the stems. Ascomata were not found on the pods, seeds or leaves, even when examined 5 months after collection. These authors stated "Since the initial find in the UK, pseudothecia have already been found in Australia (Dennis, personal communication) and they may well be widespread in over wintering bean straw". Although the sexual stage was found in South Australia by J. Dennis he has not conducted any further studies on the fungus. The occurrence of pseudothecia, their importance in the dispersal of the pathogen, and in creating variation in the pathogen over time, still needs to be determined.

2.3.1 Teleomorph description

The teleomorph was described by Jellis and Punithalingam (1991):

*"**Ascomata** arranged in rows on bean straw, immersed, becoming partially erumpent, dark brown to blackish brown, subglobose, single, separate, sometimes in groups, 180-240 x 130-150 μm, with short necks, ostiolate.*

***Ostiole** nearly circular, 35-50 μm wide, surrounded by dark brown cells.*

***Asci** arranged in a relatively flat layer, hyaline, cylindrical to subclavate, bitunicate, eight-spored, 55-70 x 10-14 μm, usually constricted near the base to form a distinct foot.*

***Ascospores** irregularly distichous, hyaline, smooth, slightly biconic, broadly ellipsoid, two-celled, constricted at the septum, with the upper cell broader than the lower cell, 15-18 x 5.5-6.5 μm. Naturally discharged ascospores on beans straw later turn yellowish brown to dark brown and sometimes three-septate".*

2.3.2 Anamorph description

A culture of *A. fabae* on malt agar develops circular colonies with a peripheral band of white aerial hyphae of various widths. The mycelium spreads from the centre and pycnidia are produced abundantly. They often are arranged concentrically (Dodd, 1971). The mycelium of *A. fabae*, when grown on potato dextrose agar (PDA), is white or light grey with abundant pycnidia scattered within the mycelium (Punithalingam and Holliday, 1975), but colonies on oat agar are yellowish, mycelia are hyaline to yellowish brown and smooth with branched and septate hyphae (Jellis and Punithalingam, 1991).

The optimum temperature range for growing mycelium was found to be 20°-25°C. At 5°C and 35°C no growth was observed (Dodd, 1971). Similar results were obtained by Ali (1985). Wallen and Galway (1977) also reported that 20°C was the optimum temperature for growing mycelium. Yu (1947) found that the fungus grew best between 20°-26°C with an optimum of 25°C, a maximum of 32°-33°C and minimum about 8°C. A pH of 6.8 was found to be the optimum for growth of the fungus, but it could grow in a wide range of pH from 3.8-9 (Yu, 1947).

The generally light-brown pycnidia are flask-shaped to globose or depressed globose and ostiolate (Sprague, 1929; Yu, 1947; Dodd, 1971; Liew, 1981; Omar, 1986). Many variations in the size of pycnidia have been recorded by different authors: 80-250 µm (Sprague, 1929); 172-178 µm (Yu, 1947); 100-160 µm with the ostiole 20-50 µm (Dodd, 1971); 150-220 µm (Omar, 1986); 250-360 x 300-420 µm, with ostiole 35-36 µm (Jellis and Punithalingam, 1991). On the host, pycnidia appear on stems, leaves and pods (Punithalingam and Holliday, 1975).

The pycnidiospores are oblong with rounded ends, hyaline and 1-3 septate (Yu, 1947; Beaumont, 1950; Punithalingam and Holliday, 1975; Omar, 1986). There is variation in the size of pycnidiospores; 14-25 x 3.5-6 µm (Sprague, 1929), 18.6 x 4.5 µm (Bondartzeva-Monteverde and Vassilievsky, 1940), 17.9 x 5.9 µm (Yu, 1947), 14-21 x 2.9-5.4 µm (Omar, 1986), 16-24 x 3.5-6 µm (Punithalingam and Holliday, 1975), 16-19 x 3.5-4.5 µm (Jellis and Punithalingam, 1991). Dodd (1971) reported that different isolates had different sizes. The optimum temperature for pycnidiospores to germinate was 25°C. Yu (1947) found that 22°C was the optimum, but the spores could germinate over a wide range of temperature from 14°-32°C. It was observed that

cultures which had originated from pycnidiospores produced pycnidia more readily than those originating from mycelia (Dodd, 1971).

2.4 Pathogen variability

Plant pathogens are known for their ability to vary. The failures that occur, following the breeding of resistant varieties, are due to the appearance of new races of the pathogen that overcome the resistance of the host plant. It is important therefore to understand pathogen variation.

Variation within populations of plant pathogens is attributable to the ability to create new populations and the ability of new populations to increase in frequency and become dominant members of the species (Nelson, 1971). Mutation, heterokaryosis and somatic recombination can cause variation in the fungi imperfecti, and sexual reproduction is important in fungi of other classes. Changes in virulence and host range, however, have been reported, and some have been attributed to mutation, heterokaryosis and parasexuality (Tinline and MacNeill, 1969).

2.4.1 Variability in *Ascochyta fabae*

It is important to have information on variability in *A. fabae* when attempting to breed resistant cultivars. From studies of single-spore cultures of *A. fabae,* found on seed obtained from several regions of the world, Kharbanda and Bernier (1980) concluded that *A. fabae* has many biotypes with differences in their cultural characteristics such as growth rate of mycelium, sporulation and size of conidia. Differences between the isolates did not seem to be related to the geographic origin of the seed. Pathogenicity tests showed that there was a wide range of pathogenic variability and no isolate gave a uniform reaction on any of the faba bean cultivars tested. In this study the attempt to identify races by means of host differentials was not successful, but physiological specialisation was observed to exist within *A. fabae.*

Kharbanda and Bernier (1978b) found that isolates which produced brown pigmentation in the culture medium (Potato Dextrose Agar), were relatively less virulent on the cultivar 'Ackerperle' than isolates that produced black pigmentation. Ali (1985) reported that aggressiveness of *A. fabae* isolates was not related to their

cultural characteristics such as production of mycelium or ability to grow rapidly in the media.

Hanounik and Maliha (1984) demonstrated a wide range of pathogenic variability in naturally occurring populations of *A. fabae* using a two-cycle screening technique. The same paper presented the reaction of faba bean genotypes in different countries. In Canada, Rashid and Bernier (1985) were able to identify ten races of *A. fabae* using 15 inbred lines of *V. faba*. Hanounik and Robertson (1989) also developed a set of host differentials and designated four races of *A. fabae* in Syria on a basis of their reaction on five faba bean accessions (Table 2.1).

Recently, ICARDA reported the reaction of these differential faba bean lines to *A. fabae* in the Mediterranean region. Races 3 and 4 were reported to be prevalent in Italy and France, and Races 2 and 4 in Tunisia, Syria and Italy. Line BPL 471, while resistant in many countries, showed a susceptible reaction in Italy, which suggested a new virulence (Anon, 1990).

There is ample evidence therefore that pathogenically, *A. fabae* is extremely variable. There is, however, no agreement as to how to interpret this variability and it is extremely difficult to compare and reconcile the results of workers in different countries.

Table 2.1 Reaction of differential faba bean lines to *A. fabae* races (Source: Hanounik and Robertson, 1989).

ICARDA accession number	Disease reaction to *A. fabae* race number			
	1	2	3	4
BPL 471	R	R	R	R
BPL 2485	R	R	R	R
BPL 818	R	R	S	S
ILB 1814	R	S	R	S
Giza 4	S	S	S	S

2.5 Epidemiology

The present study is not concerned with the epidemiology of the disease, but the various means of spread need to be considered in studies evaluating genetic differences in infection, if these studies are to be relevant in the field.

2.5.1 Seed borne transmission

Until recently, when the sexual stage was found (Jellis and Punithalingam, 1991), it was believed Ascochyta blight was mainly a seed-borne disease and that seeds were the primary source of inoculum (Beaumont, 1950; Hewett, 1973; Punithalingam and Holliday, 1975; Wallen and Galway, 1977; Gaunt *et al.*, 1978). Pritchard *et al.* (1989) suggested the disease transfer to the emerging seedlings was by physical contact with the infected seed testa. However the transmission rate from seed to seedlings is low (Hewett, 1973; Wallen and Galway, 1977). Wallen and Galway (1977) demonstrated that when 13% of the seeds were infected, only 1-3% of seedlings grown in the glasshouse from these seeds were infected. Kharbanda and Bernier (1979) also found that from a seedlot in which 30% of the seeds were infected only 3% of the seedlings developed primary infections in the glasshouse. This low rate of transmission may be associated with the hypogeous nature of the bean, with the testa remaining attached to the cotyledons under the soil surface rather than emerging from the ground with the highly susceptible plumule.

Nevertheless, even a very low level of infection in seedlings can develop in time into a severe infection of the crop. When only 9% of the seeds were infected, 5 to 6 months later 20-30% of the plants could be infected (Hewett, 1966). Gaunt and Liew (1981) also found that infected seeds as low as 1% in winter-sown beans have been shown to cause severe epidemics and significant yield losses. In 1973, Platford illustrated that when 5% of the seeds sown in the field were infected, under conditions of frequent rains the fungus was dispersed and the disease increased dramatically so that between 28% to 35% of the harvested seeds were infected (reviewed by Kharbanda and Bernier, 1979).

2.5.2 Transmission from debris and volunteer plants

Even before the discovery of the teleomorph it was evident that plant debris was source of infections in ensuing crops. Frequently, outbreaks have arisen from apparently disease-free seed and other outbreaks have been more severe than expected from the amount of seed infected. Other sources of inoculum have been suspected such as the debris, volunteer plants or soil. Geard (1962) concluded that infected crop debris was an important means of transmission. Gourley and Delbridge (1973) reported that the fungus persisted from season to season on debris as well as on the seed. Michail *et al.* (1983) and Tivoli *et al.* (1987) also suggested that the primary inoculum came either from infected debris or seed. As the teleomorph has now been found on debris, it confirms the importance of debris in the dispersal of the pathogen (Jellis and Punithalingam, 1991).

Bond and Pope (1980) suggested the spread of disease from volunteer plants was a more important source of infection than infected seed. Gaunt (1983) also considered volunteer plants may be an important source if they occurred in the vicinity of new crops.

A secondary spread of the disease may be by rain-splash with spores being dispersed further by wind-blown rain (Dodd, 1971). Over-head irrigation would have a similar effect (Gaunt *et al.*, 1978). There have been variable reports on the distance the disease is spread. Dodd (1971) found that under optimal conditions spores may be splash-dispersed at least 1 m from the inoculation source, while Hewett (1973) found that the spread occurred up to 10 m. However, Bond and Pope (1980) reported that the disease could spread from volunteer plants for a distance of at least 120 m into a field.

Beaumont (1950) said there was no evidence of infection taking place from the soil in the absence of debris, but gave no details to support the statement.

2.5.3 The survival of *Ascochyta fabae*

The mechanism of survival of *A. fabae* during the intercrop period, when faba bean plants are absent, is still a controversial subject. No alternative hosts have been reported. The relative role of spores or mycelia as sources of infection is often discussed. The disease was found to be transmitted from infected seeds to seedlings

from the mycelium on or in the seeds (Yu, 1947). He said the fungus may remain in the seed coat in the form of dormant mycelium until the seed germinates but he also found that spores could remain viable on stems and pods of plants through the winter. No pycnidia were reported on or in seed (Punithalingam and Holliday, 1975). Various studies have been made on the viability of stored beans and the viability of infections on these beans. They have confirmed the viability of *A. fabae* on stored bean seeds for 12 months (Hewett, 1966), for 30 months (Dodd, 1971) and for nine years (Sprague, 1929).

Dodd (1971) studied the survival of *A. fabae* on debris buried in soil and kept at a range of temperatures. No viable *A. fabae* colonies were observed at any time at -20°C and -10°C. Viable colonies were observed for one and two months only at 0°C and 20°C respectively and after 4 months at 10°C. Under field conditions, over-winter survival of *A. fabae* was no longer than 4 months. The survival on infected debris was linked with the time for decomposition of the debris. When debris had decomposed in the soil, the fungus could no longer survive. Dodd also reported that neither chlamydospores nor pycnidia were found on debris in the soil and only mycelia gave rise to colonies. Wallen and Galway (1977) also could not find *A. fabae* in soils where infected debris had been ploughed into the soil. Dodd (1971) suggested the limited survival of *A. fabae* was due to an inability to form chlamydospores. Chlamydospores have been found on aging cultures (Dodd, 1971) and in lesions on maturing faba bean stems naturally infected in the field (Kharbanda and Bernier, 1978a). It was observed that chlamydospores could survived a 96 h exposure to -19°C.

In Australia, modern farming techniques have led to more debris being left in the paddocks. Grazing by sheep could destroy the debris, but there has been a reduction in sheep numbers with many farmers eliminating their sheep altogether to adopt continuous cropping. The dry weather experienced in the summer does not lead to decomposition of the debris so it is possible that the pathogen builds up and survives on the debris throughout the year.

Many studies have been conducted on the survival of other *Ascochyta* spp. on other legume plants. The survival of *A. pisi* in the soil was shown to be limited by its inability to form chlamydospores when compared with *A. pinodes* and *A. pinodella* which survived a wide range of temperatures including heat treatment of 100°C for 12

to 15 h (Wallen and Jeun, 1968). It was suggested that chlamydospores of these fungi could withstand high temperatures and survive temperature extremes (Wallen *et al.*, 1967). Similarly, Dickinson and Sheridan (1968) confirmed that the limited ability of *A. pisi* to survive in soil was because it was a poor saprophyte and also could not form chlamydospores. In Iran, *Ascochyta rabiei*, the causal agent of Ascochyta blight in chickpeas, was able to survive for more than two years at various temperatures up to 35°C, relative humidity of 30% or less, and on the soil surface (Kaiser, 1973).

2.6 The infection

2.6.1 Host specialisation

The only true host of *A. fabae* is the faba bean. The artificial inoculation of pea (*Pisum sativum* L.) has occasionally been successful but the pathogen retained its specific character and was quite distinct from *A. pisi* (Sprague, 1929; Beaumont, 1950; Dodd, 1971; Punithalingam and Holliday, 1975). Lasman (1965) showed that in inoculation tests on faba beans, peas, vetches, wild peas, lentils, chickpeas and kidney beans, all monospore lines of *A. fabae* infected only faba beans, and *A. pisi* infected only peas. Similarly, Yu (1947), Sundheim (1973) and Wallen and Galway (1977) found that isolates of *A. pisi* did not infect faba beans and *A. fabae* did not infect peas. Gossen *et al.* (1986) compared 68 isolates of *A. lentis* obtained from around the world with 13 isolates of *A. fabae*. Multivariate analysis of the cultural and morphological characters could not separate the two species. However, differences in host specificity were clear; isolates of *A. fabae* infected only faba beans whereas *A. lentis* infected only lentils (*Lens culinaris* Medik.).

2.6.2 The infection process

After inoculation, germ tubes are produced. Pycnidiospores of *A. fabae* usually produce one to three germ tubes (Dodd, 1971). The appressoria grow from these germ tubes, penetrate the plant surface, and then grow into the lumen of the epidermal cells. Hyphae of *A. fabae* have been observed growing into stomatal openings and presumably penetration occurs in this way. The host tissue is disorganised by the fungus and lesions begin to form. The mycelium in plant tissue grows at the edge of

the lesion while at the centre, pycnidia develop. There was no evidence of systemic infection of the plant and, apart from spores, any disease transfer was probably by physical contact (Pritchard *et al.*, 1989). Dodd (1971) found that when plants were washed in running tap water for 4 h after inoculation, they still showed severe infection and the spores were not removed by the washing. Infection is influenced by host, pathogen and environmental factors. Their interaction ultimately determines the number of infections.

2.6.3 Factors affecting inoculation

Weather conditions affect the rate of development and spread of Ascochyta lesions (Hewett, 1973). Cool and wet conditions were considered favourable for the spread of the disease and in dry weather the spread ceased (Gourley and Delbridge, 1973; Nene *et al.*, 1988). Geard (1962) observed a severe outbreak of *A. fabae* in 1949 in Tasmania attributed to unusually prolonged and frequent periods of high humidity. Van Breukelen (1985) investigated, in control growth chambers, the effect of humidity and temperature on the screening of seedlings. High humidity for as little as one day was sufficient for the development of lesions, but longer periods of high humidity of up to three days gave increasing numbers of lesions. A period of seven or eleven days almost doubled the number of lesions compared with a three-day period. High humidity for three days gave good discrimination between susceptible and resistant genotypes within 14 days. Symptoms developed earlier at 20°C than at 15°C, but the final number of lesions was not higher (Van Breukelen, 1985). Wallen and Galway (1977) also found that the optimum temperature for infection was 20°C compared with 15°C and 25°C. At this temperature, well defined fruiting lesions were produced on leaves and stems 14 days after inoculation. Other workers have also found that low temperatures favoured the development of the disease (Van Breukelen, 1985; Tivoli *et al.*, 1988). Pritchard *et al.* (1989) reported that the successful establishment of the disease required leaf wetness, for periods of 2-4 h at 20°-25°C and 12 h at 10°C.

The age and physiological properties of infected tissue, together with environmental conditions, affect the period of incubation, that is the time from inoculation to the appearance of symptoms (Konstantinova, 1965). Young leaves took the shortest time and the old leaves the longest time. Several studies have shown the leaves on the upper parts of the plant were more seriously infected than those on the

lower parts (Dodd, 1971; Ali, 1985; Van Breukelen, 1985; Tivoli *et al.*, 1987; Pritchard *et al.*, 1989). Dodd (1971) reported that the response varied with the age of the plant. Plants, inoculated when they were 8-11 days old, all died, whereas plants 12-32 days old developed symptoms but did not die. Plants inoculated at the two-leaf stage showed the most lesions on the third and fourth leaves, which had been visible but were folded at the time of inoculation (Van Breukelen, 1985; Pritchard *et al.*, 1989). Ali (1985) found that only young plant tissue was susceptible at any growth stage, even on susceptible cultivars. In contrast, Hanounik (1980a) found that disease severity increased with the age of plants, from two to seven weeks. Plants with a high nitrogen content have been shown to be more susceptible to the disease than those with a low, but not deficient, level of nitrogen (Gaunt, 1983). Wallen and Galway (1977) reported that wounds were not necessary for infection.

The spore concentration of an inoculum and its age affect the extent of the infection. A tenfold increase in the concentration of the spore suspension resulted in approximately a doubling of the number of lesions per leaf within the tested range of 1×10^4 - 15×10^5 spore/ml (Van Breukelen, 1985). An increase in the inoculum dose from 0.5 ml to 1.0 ml per plant doubled the number of lesions, but a further increase in the dose to 2.0 ml had a small or nil effect. Dodd (1971) reported that virulence of *A. fabae* fell slightly over the period of time of subculture involved. Spores 7 to 14 days old were more infective than spores 21, 28, 35 and 42 days old. The older the spores the less the infectivity. Spore concentrations of 100-1000 spore/ml were required to cause infection on whole plants using a spray application method, whereas only 10-100 spore/drop (1×10^3 - 1×10^4 spore/ml, with a drop volume of 0.01 ml) were required to infect detached leaves (Dodd, 1971).

2.7 Control of Ascochyta blight

Methods for controlling Ascochyta blight have included chemical and integrated control methods. Many kinds of fungicides and methods of application have been tried (Konstantinova, 1963; Kharbanda and Bernier, 1976; Wallen and Galway, 1977; Bernier, 1980; Hampton, 1980; Hanounik, 1980b; Liew and Gaunt, 1980; Liew, 1981; Michail *et al.*, 1983; Gärber and Motte, 1990). Systemic fungicides appeared to give better control than non-systemics, but their persistence was too brief to give adequate

protection (Kharbanda and Bernier, 1976; Kharbanda and Bernier, 1979). Nevertheless, no single chemical method has been completely effective and many are expensive to apply. Integrated control, when a chemical treatment and host resistance have been combined, has been effective (Anon, 1985a).

Cultural methods for disease control include the use of clean seed (Hewett, 1973; Gaunt and Liew, 1981) and a lower plant density (Konstantinova, 1963). However as indicated earlier there is often not a strong correlation between the extent of infection of seed and the subsequent infection of the crop.

The use of resistant cultivars appears to offer a relatively cheap and effective method of control. Resistant lines have been reported among ICARDA germplasm (Anon, 1981; Anon, 1984; Hanounik and Maliha, 1984). Several lines, identified as resistant in Syria by ICARDA, were also found to be resistant in Canada, England and Sweden (Hanounik and Maliha, 1984).

2.8 Disease resistance in plants

According to Nelson (1973), disease resistance may be divided into two types of host response. The first is host resistance to infection by restricting the infection site and the infection process and is known as hypersensitivity, specific resistance, non-uniform resistance, vertical resistance or major gene resistance. The second type, resistance to colonisation and growth of the parasite subsequent to a successful infection, is known as tolerance, field resistance, general resistance, non-specific resistance, partial resistance, uniform resistance, horizontal resistance, multigenic or polygenic resistance, and minor gene resistance.

This multiplicity of names arose as a result of different authors attempting to express forms of resistance in various ways according to the mechanisms of resistance, the mode of inheritance or epidemiology. The terminology has been discussed by Robinson (1969). In fact resistance is relative and the types of resistance may be placed on a continuous scale. A plant may possess several types of resistance that come into play after infection sites are established. These mechanisms tend, in general, to restrict the extent to which pathogens can colonise host tissue and the degree to which they are able to produce inoculum for subsequent infection in the disease cycles within a single growing season.

When selecting or breeding resistant cultivars the mode of inheritance will determine the methods used. Three modes of inheritance were classified by Day (1974).

Oligogenic resistance is determined by one or a few genes with major effects and is the simplest form of inheritance (Day, 1974; Johnson, 1987). A single dominant gene is common in the determination of resistance but there are some instances of resistance due to a single recessive or incompletely dominant gene (Hooker and Saxena, 1971). A reversal of dominance may also be found in an allelic relationship. Environmental factors have been reported as causing a change from dominance to recessiveness (Hooker and Saxena, 1967).

Polygenic resistance is determined by many genes and is considered to be a quantitative expression (Day, 1974). Polygenic resistance tends to be a potentially more valuable genetic resource in the development of long-lasting and effective resistance against all races of a particular pathogen (Hooker and Saxena, 1971). Host-parasite systems showing polygenically determined resistance have been listed by Christ *et al.* (1987).

Extranuclear or cytoplasmic resistance is determined by organelles or their interaction with nuclear genes. A genotype in one cytoplasm may function differently when in another cytoplasm. Such effects are clearly maternally inherited (Strickberger, 1985) and are very rare in plants (Lunsford *et al.*, 1975).

2.8.1 Expression of resistance

Resistance to a disease can be expressed by a plant at the stages of infection, colonisation, or during subsequent sporulation. In most cases the disease systems or reactions are assessed with the assumption that they reflect quantitative growth of the pathogen in the host. Resistance may be measured in terms of infection frequency, the length of the incubation period, the latent period, lesion size, spore production and the infectious period (Parlevliet, 1979).

The incubation period (ICP) and latent period (LP): ICP is the time from inoculation to the first appearance of symptoms. LP is the time from inoculation to sporulation. ICP and LP are assumed to reflect differences in growth rate of the pathogen in the host. In most cases ICP and LP vary in a parallel way, and because of the difficulty of measuring LP, ICP is more commonly measured (Parlevliet, 1979).

When measuring components the plants compared should be at the same stage since the development of the plant and age of the leaf can be important factors (Parlevliet, 1979; Zadoks and Schein, 1979). An increase in ICP and LP will reduce the number of disease cycles and sporulation, resulting in reduced disease levels.

Lesion number (LN), Disease efficiency (DE), or Infection frequency (IF): These terms and their definitions vary depending on the authors and the nature of the diseases. The terms are broadly similar in that they are a measure of the success of infection from colonisation by the pathogen up to the production of reproductive organs. They are measured as ratios or proportions per unit area. DE is a ratio of the lesions that produce spores to the total number of lesions (Ali, 1985). IF was measured as the number of uredosori per unit leaf area, representing the successful infection of leaf rust in barley (Parlevliet and Kuiper, 1977). The other terms synonymous with IF are infection efficiency and infection ratio (Jeger and Groth, 1985). Differences in DE, IF or LN reflect differences accumulated over the various development stages, from establishment to sporulation of the pathogen and are thus affected not only by the host genotype, but also by its developmental stage and the environmental conditions. In the present study, LN is taken as the number of lesions formed on the leaves or stems and thus indicates the ability of the pathogen to infect, while DE is the proportion of lesions that sporulate and thus indicates the ability of the pathogen to reproduce, which is particularly important from the point of view of causing serious secondary spread of infection.

Lesion size (LS): Lesion size refers to the area showing disease symptoms. It has been measured in various ways including area, diameter, length or an assessment using a scale devised for the purpose. In faba beans, as mentioned previously, yield loss was shown to be proportional to the amount of damage of leaf tissue (Hampton, 1980; Madeira *et al.*, 1988) and thus lesion size is important in the present study. In many plant species, resistance may operate after successful infection, in the form of cork layers, abscission layers, lignotubers and tyloses (Jones, 1987). These resistance mechanisms can result in restriction of the lesion but may not eliminate it completely.

Spore production (SP): Spore production has been expressed per unit leaf area, per lesion or pustule, per unit area of lesion and per unit area of sporulating surface. Furthermore it can be per unit of time or over the whole period of infection. These

various ways of expressing SP reflect its dependence on many factors including environmental conditions and the plant's age.

Infectious period (IP): This is the period during which infectious propagules are produced by the pathogen. The infectious period and spore production are often negatively correlated with the infection frequency. At high infection frequency, the leaves are apparently exhausted sooner. A long infectious period may result in high total spore production. Sporulation may be terminated by the exhaustion or death of the infected plants, senescence or by environmental conditions. The last two components of resistance, SP and IP, were not studied in this study as they were difficult to measure and other components such as IP and DE would give adequate information.

2.8.2 The association between components of resistance

A cultivar with a high level of resistance would have a low infection frequency, a long latent period, small lesion size, reduced spore production and short infectious period (Parlevliet, 1979). Different components may reinforce each other, thereby resulting in a higher level of resistance. Some components of resistance may be correlated and selection for one component such as LP may be adequate for evaluation of resistance levels amongst cultivars in a breeding program. Nevertheless this is not always so and several components may need to be measured. Apart from the technical problems involved in making many detailed assessments of resistance, there are problems in terms of interpreting the results. Firstly, the components of resistance are not always associated and some are independent (Jeger and Groth, 1985). Secondly, assessment of components made in a glasshouse and on juvenile plants may not be correlated with the field results. Thirdly, the expression of the components is often environmentally influenced and interactions occur. It is important to consider the many factors which could affect the expression of the disease: temperature, rain, humidity, resistance or susceptibility of the host, its nutritional status and the virulence and quantity of the pathogen (Parlevliet, 1979; Van der Plank, 1984).

Ali (1985) found that the incubation period (ICP) seemed to be the most reliable component for evaluating resistance levels in various combinations of faba bean genotype and *A. fabae* isolate, with very little variation in the results from two successive inoculations of plants in the field and growth room. The poor correlation

between ICP and components of resistance in the field, however, suggested that components such as LD (lesion diameter) and LN (lesion number) also needed evaluation. Furthermore, stems and leaves showed different components of resistance, indicating some specificity between lines and isolates. Lesion number of Ascochyta on faba bean has been reported to depend on environmental conditions and pathogen races (Dodd, 1971; Wallen and Galway, 1977; Ali, 1985; Van Breukelen, 1985; Pritchard *et al.*, 1989).

2.9 Inheritance of resistance in faba beans to *Ascochyta fabae*

Although resistant lines have been reported, there has been only a limited number of studies of the inheritance of resistance.

Variation in resistance has been found both within and between cultivars (Lockwood *et al.*, 1985; Pritchard *et al.*, 1989), but no completely resistant cultivars have been reported. Some lines with the *ti-1* (terminal inflorescence) gene, which induces determinate growth and reduces the straw length, have been shown to be more susceptible to infection than indeterminate lines. Jellis *et al.* (1985) confirmed the association between long straw and low incidence of infected pods, which Lockwood *et al.* (1985) attributed to disease escape, but biochemical differences between plants are also partly responsible for the susceptible or resistant reaction. Zakrzewska (1987) found that brown-flowered mutants were appreciably more resistant than the white-flowered lines.

Both general and specific resistance have been found among lines of *V. faba* (Hanounik and Robertson, 1989). They reported that the use of mixed inocula favoured the detection of the different types of resistance. A faba bean cultivar is a heterogenous population and its component genotypes may differ in their reaction to *A. fabae*. This makes the inheritance of resistance difficult to study. In a paper published after the present studies were commenced in 1989, Rashid *et al.* (1991b) found that for some genotypes the resistant reaction was evident for only the stems and they suggested that the resistance in leaves and stems may be under different genetic control. Three cycles of mass selection improved the level of homogeneity, but no cultivar was homogenous for resistance to Ascochyta.

2.10 Screening of parents and segregating populations for resistance

The detection of suitable parents in a breeding program is an important step which can be done only when the host and pathogen are brought together in a disease-inducing environment. Screening for disease resistance is carried out on segregating populations grown under environmental conditions favourable for the disease to induce the interaction phenotype, so that resistant individuals can be identified. As in all studies of plant disease, the screening involves four components: host, pathogen, environment, and time.

2.10.1 Methods of screening for resistance to Ascochyta blight

Relying on natural infection in the field is not a totally satisfactory way of screening faba beans for resistance to *A. fabae*. Ideally, screening should be carried out in the field with natural epidemics, but adverse environmental conditions and the absence of the pathogen can seriously decrease the effectiveness of such tests. Artificial inoculation of a field trial is feasible but its success is dependent on ensuing environmental conditions. These problems can be avoided by screening in controlled conditions reflecting the conditions encountered in the field. The conditions chosen will always be a compromise as it is difficult to imitate the fluctuations that occur in natural environments. It is not feasible to have totally controlled fluctuating conditions for the large number of plants required in breeding programs. Many techniques have been investigated in the field and in the laboratory. Jellis *et al.* (1982), when investigating field resistance, found that spraying every fifth plant in a row with a spore suspension and covering the plants with polythene bags for five days, produced a significant increase in the proportion of infected plants. Under English conditions, the method of spraying and bagging, or of applying a sand-maize meal mixture inoculated with *A. fabae* around the base of each plant, was effective in increasing pod infection. Kharbanda and Bernier (1980) and Tivoli *et al.* (1987) demonstrated that an effective simulation of infection following field inoculation could be achieved by growing plants in pots in the glasshouse and spraying them with a spore suspension. The method can be used to rank the genotypes for field performance.

Hanounik and Maliha (1984) developed a "two-cycle screening" technique. In the first cycle a mixed inoculum of *A. fabae* was used. Resistant and highly resistant selections on which a few lesions developed, were believed to have been infected by more virulent pathotypes present in the original mixed inoculum. Isolates selected from such lesions, were then used in a second screening cycle to re-inoculate progenies from resistant selections made in the first cycle. The technique was used to screen for resistance to a wide range of pathogenic variability.

Detached leaves and other organs have been used in many studies of host-pathogen interactions especially for obligate pathogens. With this technique, host-pathogen interactions can be studied in controlled conditions and leaves of the same age from different cultivars can be inoculated. The more controlled comparison leads to greater efficiencies in breeding programs. The method does not destroy whole plants, so those with resistance can be transferred into potting soil for generation advancement. The technique may be rapid and requires little space, but it has its limitations when the disease is one in which symptoms develop slowly and are not apparent before senescence of the detached leaf.

As senescence has a role in inducing disease symptoms, many attempts have been made to culture leaves and inhibit senescence. Benzimidazole, kinetin and cytokinin were used for culturing infected leaves of wheat, barley, beans, tobacco, tomato and sugar beet with considerable success (Person *et al.*, 1957).

The infection that develops on detached leaves depends on many factors. For *A. fabae*, Dodd (1971) used a technique developed by Heath and Wood (1969) for *A. pisi* and *Mycosphaerella pinodes* on detached pea leaves. Dodd found that the lesions of *A. fabae* began to show 48 h after inoculation but it was not until 10-12 days after inoculation that symptoms were clearly expressed. The lesions on detached leaves were atypical, they never developed pycnidia and he suggested this was probably due to the waterlogged condition of the leaves. Leaf age was reported to have no effect on the reaction to the pathogen. A concentration of 10-100 spore/drop was used to infect the detached leaves and spores that were 7 days old gave a higher percentage of infection than older ones. His attempts to investigate physiological specialisation and genetic resistance were unsuccessful.

2.11 Variability within populations of *Vicia faba*

Studies on *V. faba* need to take account of the variation that occurs within accessions or cultivars of the species due to the partially cross-pollinated nature of the breeding system. Pollination is mediated by bees and in Australia the honeybee is the only known pollen vector (Stoddard, 1991). Values of the extent of cross-pollination in faba beans vary from 3 to 84% with an average of 35%, but many factors such as location of the plants within field, plant density and position of inflorescence can influence this (Bond and Poulsen, 1983).

Because within a cultivar some of the plants have been selfed and others crossed in the previous generation, most faba bean cultivars have levels of heterogeneity, heterozygosity and heterosis which are intermediate between those present in completely outbreeding and completely inbreeding crop species (Lawes *et al.*, 1983). It is thought that this provides a buffer against the varying and unpredictable numbers of insects available for pollination.

The disadvantages arising from the cross-pollinated habit include unrepeatability and non-uniformity of evaluation in genetic studies, genetic drift and the segregation of recessive genes from heterozygotes. ICARDA has attempted to minimise these problems by producing pure lines (Anon, 1985b) although the inbreds may be expected to be lower in vigour than the mixed populations (Lawes *et al.*, 1983).

2.12 Conclusions from the literature review

Ascochyta blight is an important disease of faba beans and can cause significant losses in production. Chemical and integrated control methods can provide partial crop protection, but a form of genetic resistance that was not rapidly overcome by the disease would provide a cheaper and more environmentally acceptable solution. Comprehensive genetic studies of both the pathogen and the host are required to determine the feasibility of stable resistance.

The mixed pollination system of the faba bean means that any cultivar is a mixture of genotypes and this introduces a complication to studies on disease resistance. Nevertheless there is evidence that certain lines of faba beans carry different kinds of resistance to Ascochyta, including both general and specific resistance. This resistance

has not been strongly characterised genetically nor heavily used by breeders either overseas or in Australia. Therefore part of the experimentation in the present thesis was designed to investigate the genetics of Ascochyta resistance in different faba bean populations, with the particular objective of incorporating this resistance within the breeding program.

There is also published evidence that there is variability of pathogenicity within the fungus and sets of differential cultivars have been developed in both Syria and Canada to distinguish the races or biotypes. The variability of *A. fabae* within Australia has not been established, so several isolates were developed in this thesis and used for pathogenicity tests on several faba bean populations. This part of the project also was designed to test the components of resistance, which have not been well described in the faba bean - Ascochyta interaction.

Environmental conditions, plant growth stage and pathogen race play an important role in disease interaction. Many different methods of inoculation and assessment have been reported and screening for disease resistance in the glasshouse has given results similar to screening in the field. An attempt in the past to develop a detached-leaf test for Ascochyta was not fully successful but showed the potential for rapid and repeatable tests from detached organs. The development of lines resistant to *A. fabae* requires a quick and simple test so that plant selection can be based on reliable and reproducible criteria. Another part of this project therefore focuses on the optimisation of detached-leaf and related tests for evaluating breeding stocks for their resistance to the disease.

The various experiments in this thesis therefore were aimed to give valuable information on the genetics of Ascochyta resistance in faba beans which would be of use to breeders in Australia and overseas.

CHAPTER 3. GENETICS OF RESISTANCE TO ASCOCHYTA BLIGHT IN TWO POPULATIONS OF FABA BEAN

3.1 Introduction

A few years after the release of FIORD in 1980, Ascochyta blight became a serious problem, especially in southern Australia. For the initiation of programs to breed resistant cultivars, it was necessary to understand the inheritance of resistance, for example whether it was determined by a single gene or many genes. If it were the former, selection would be simpler and the classes of resistance would be distinct, but probably any resistance bred into a cultivar would be overcome in a relatively short time. If its inheritance were polygenic, resistance would be expressed quantitatively and selection may need to be based on relatively small differences between plants, but a cultivar with a high level of resistance may be expected to remain resistant for a longer period of time. Other issues that would arise included the number and virulence of the races of the pathogen and the fact that a cultivar of faba beans is a mixture of genotypes. Mixtures tend to remain resistant for longer periods when resistance is determined by oligogenes or by polygenes. If resistance were determined by oligogenes, with further knowledge, it may be feasible to constitute mixtures of genotypes carrying different resistances. This chapter is devoted to the first question of the number of genes determining resistance.

The faba bean breeding program at the Waite Agricultural Research Institute has introduced over one thousand accessions from around the world, many because of their reputation for disease resistance. Preliminary tests had shown that variation for resistance to Ascochyta occurs within many of these reputedly resistant populations and that there were no accessions in which all plants were completely resistant, probably because of the partially cross-pollinated nature of faba beans. Several ICARDA accessions listed as inbred lines showed variation for resistance. One of the most resistant was accession 970 (ILB 752 of ICARDA). One inbred line, BPL 471, classified as resistant, has been shown to have a low proportion of susceptible plants by Hanounik and Robertson (1989). Further selfing and selection to produce uniform

homozygous genotypes for this study would have taken considerable time and could have led to inbreeding depression (Lim and Knight, 1980).

One possible method of obtaining more homogenous plants is to screen the parents and to select ones that show the same level of infection. The mode of inheritance of resistance to *A. fabae* in faba beans was studied using plants selected in this way. Initially, highly resistant plants from Accession 970 and highly susceptible ones from Acc 811 were crossed. Subsequently, plants of several levels of resistance from each of these two populations were crossed. F_1 and F_2 progenies were screened for their level of resistance. A single isolate of *A. fabae*, designated A26, was used for these experiments.

The literature review showed that volunteer or neighbouring plants were an important source of inoculum and that rain or overhead irrigation was a factor in the spread of disease. It was therefore of interest to determine whether a border of the resistant Acc 970, or the susceptible Acc 811, had a detectable influence on the disease scores in the plots. If such an effect occurred it should be progressively more pronounced with time, and the difference would be greater at 45 DAI than at 21 DAI.

3.2 Materials and methods

3.2.1 Crosses of highly resistant and highly susceptible parents

Accession 811, a small-seeded accession from Afghanistan, otherwise designated by the ICARDA number NEB 463, was used as the susceptible source material. Accession 970 (ICARDA line ILB 752) was chosen as a known source of resistance. A pilot experiment had shown individual plants of each of these accessions had different levels of resistance to *A. fabae*. Selection was practised in a glasshouse to find appropriate parents to use in crosses. In April 1990, 30 plants of Acc 811 and 21 plants of Acc 970 were sown three per 25 cm pot containing UC soil with fertiliser (Appendix 3.1). Plants were inoculated when they had 3-4 leaves (4 weeks after sowing).

Ascochyta fabae, isolate A26, was subcultured and multiplied on 2% PDA and incubated under continuous fluorescent light at 25°C for 2 weeks. Pycnidiospores were harvested by flooding the surface of the agar with sterile RO (Reverse osmosis)

water and scraping with a glass rod. Mycelial debris was removed from the water by filtration through two layers of muslin. The concentration of the pycnidiospores was adjusted to 3-5 x 10^5 sp/ml, using a haemocytometer. Plants were inoculated by spraying at dusk to take advantage of the cooler temperatures and higher humidity overnight. After inoculation the plants were covered with plastic bags for three days to maintain high humidity.

At 21 days after inoculation (DAI), the plants were evaluated for their disease score following the criteria in Table 3.1, where scores of 0-2 indicated the absence of pycnidia and hence resistance, while 3-5 showed pycnidia to be present and hence susceptibility. Resistant plants of Acc 970 and susceptible plants of Acc 811 were chosen to be used as parents. Fourteen plants of Acc 970 which had no lesions were chosen although some had flecking or bronzing on the stem (score 0 or 1). Ten plants of Acc 811 which were highly infected were chosen. They had broken stems and more than 30% of the leaf area was infected (score 4 or 5). After the level of disease was recorded, the infected shoots were cut off to allow the development of new shoots which were used for crossing.

Flowers were emasculated before the anthers dehisced and were pollinated at anthesis with pollen either from another plant of the same line or from the other line (Appendix 3.2). Numerous pairs of parents were used for each cross and in addition selfed seed was collected from each parent.

All the crosses were kept separate. Three to five F_1 seeds from a cross were sown in pots, 3 seeds per pot, containing UC soil with fertiliser. The plants were grown in a bee-proof glasshouse in December 1990. F_2 seeds were obtained by selfing the F_1 plants. The flowers were self-pollinated by manual 'tripping' of the petals.

The parents, F_1 hybrids and F_2 populations of the crosses were sown in an open-mesh bird-proof cage at the Institute on 19 June 1991. There were three replicates, each divided into two main plots. In the first, alternate rows consisted of the susceptible parent and in the second, of the resistant parent. The experimental rows consisted of the F_1 hybrids and F_2 progenies, allocated in a randomised design. The space between plants was 20 cm in a row and 25 cm between rows. The rows were 4 m long so each row contained 20 seeds.

Table 3.1 Classification of disease reactions on faba bean stems and leaves (after Kharbanda and Bernier, 1980).

Description of stem reaction	Description of leaf reaction	Reaction class	Score
Healthy, no infection.	Healthy, no infection.	Highly resistant (HR)	0
Flecking or light bronzing.	Flecking or bronzing.	Resistant (R)	1
Localised and non-penetrating lesions, or high bronzing ; no pycnidia.	Localised lesions, < 3 mm in diameter; < 5% leaf area infected; no pycnidia.	Moderately resistant (MR)	2
Localised, penetrating lesions, 2-3 mm in diameter; pycnidia present.	Lesions > 3 mm in diameter, 6-30% leaf area infected; pycnidia present.	Moderately susceptible (MS)	3
Spreading, and deep penetrating lesions, 3-5 mm in diameter; pycnidia present.	Lesions > 3 mm in diameter, 31-50% leaf area infected; pycnidia present.	Susceptible (S)	4
Large coalescing lesions, > 5 mm in diameter, stem constriction and girdling; pycnidia present.	Lesions > 3 mm in diameter, coalescing; > 51% leaf area infected; pycnidia present.	Highly susceptible (HS)	5

In this experiment some waterlogging occurred which delayed germination and the plants were inoculated 6 weeks after sowing to allow the later germinated plants to reach the 2-3 leaf stage. The *Ascochyta fabae* isolate A26 was subcultured and multiplied using the method described above. Inoculation was carried out on 31 July 1991 using a hand sprayer. For the following three days an overhead sprinkler was turned on for 15 min every 2-3 h. Subsequently, the plants were watered for 1-2 h by

the overhead sprinkler once a week. No fertilisers or pesticides were used throughout the experiment.

Disease scores were assessed at 15 DAI, 21 DAI and 45 DAI following the criteria in Table 3.1. By 45 DAI secondary infection should have had time to develop and the score should reflect the susceptible or resistant effect of neighbouring plants resulting from the splash of water from the overhead irrigation. The assessment of disease per plant was obtained by calculating a leaf and stem average for the recorded values. Plants which scored 0, 1, 2 were classified as resistant and those which scored 3, 4 and 5 as susceptible.

Chi-square tests were used to determine goodness of fit of observed to hypothetical segregation ratios in the F_2 populations, with continuity correction (Strickberger, 1985).

3.2.2 Crosses of parents with different levels of resistance

The faba bean Accessions 811 and 970 were again used in this study. In August 1990, 57 plants of Acc 811 and 51 plants of Acc 970 were sown in 25 cm pots containing UC soil with fertiliser, 3 plants per pot in a glasshouse. Plants were inoculated at the 3-4 leaf stage (4 weeks after sowing) by the procedure described above.

At 21 DAI, disease scores were assessed as in the previous experiment. Scores of 0-3 were found for plants of Acc 970 and 1-5 for Acc 811 (Table 3.2). The diseased shoots were cut off and the regenerated shoots used for crossing as described before. Two to nine F_1 seeds of each cross were grown in a bee-proof glasshouse and the flowers were tripped to proved F_2 seed, as described above.

Seeds of the parents, F_1 hybrids and F_2 progenies were sown on 9 August 1991 in an open-mesh, bird-proof cage using a randomised block design. Insufficient seed was available of the F_1 hybrids of Acc 970 x Acc 970, but all other F_1 hybrids and all four classes of F_2 progenies were included. Altogether, 88 F_1 hybrids and 91 F_2 progenies were tested. The numbers of plants differed, but when there were 50 plants, they were arranged as 10 plants to a plot in 5 replications. In each plot there were 2 rows 25 cm apart of 5 plants 20 cm apart. Plants were inoculated 4 weeks after sowing when they were at the 3-4 leaf stage and disease scores were assessed on each plant at 15 and 21 DAI as described above.

Table 3.2 Disease scores of Acc 970 and Acc 811 material used as parents.

Accession	Score	Plant designation number
Acc 970	HR (0)	6, 16, 51
	R (1)	5, 9, 12, 38, 39,41, 43, 50
	MR (2)	29, 48
	MS (3)	25, 36
Acc 811	R (1)	3, 8, 21
	MR (2)	19, 35, 51
	MS (3)	1, 2, 9, 10, 20, 22, 23, 25, 27, 29, 37, 42, 46, 47, 52, 57
	S (4)	17, 26, 28, 30, 38, 39, 45
	HS (5)	4, 5, 6, 11, 12, 13, 14, 18, 32, 33, 34, 43, 44, 48, 50, 54, 55, 56

3.3 Results

The technique used for infecting the plants in the field was effective and symptoms typical of natural infection first appeared 10 days after inoculation. On infected plants the damage increased with time and lesions on leaves and stems expanded in area. Deep lesions on the stems caused them to break, with wind contributing to the breakage. Resistant plants showed flecks or red patches without developing lesions. It was noted that occasionally on plants which were expected to show a resistant reaction, the lesions did not remain restricted, but expanded at a slower rate than on susceptible plants.

The plants were scored for infection at 15 DAI which had been found adequate for symptoms to develop in the glasshouse, but in the field it was insufficient time and the results are not presented.

3.3.1 Crosses of highly resistant and highly susceptible parents

At 21 DAI, all the plants of Acc 970 were scored resistant, most with a score of 0 (Table 3.3, Figure 3.1 and Appendix 3.2). On Acc 811 large lesions with abundant sporulation were produced and nearly all the plants were scored susceptible, 3 or higher. Two plants of the 335 of Acc 811 were given scores of 2, moderately resistant.

Table 3.3 Disease reactions of the parents, F1 hybrids and F2 populations 21 DAI. Plants scored 0-2 were classed as resistant (R) and those scored 3-5 as susceptible (S).

Population	Number of plants		Mean score		Expected ratio	χ^2	p-values
	R	S	R	S			
Acc 970	290	0	0.1	—	All R		
Acc 811	2	333	2.0	3.5	All S		
F1 970 x 970	87	0	0.02	—	All R		
F1 811 x 811	0	39	—	3.5	All S		
F2 970 x 970	131	0	0.2	—	All R		
F2 811 x 811	4	190	2.0	3.5	All S		
F1 970 x 811	74	0	0.1	—	All R		
F1 811 x 970	158	0	0.2	—	All R		
F2 970 x 811	169	58	0.6	3.2	3R : 1S	0.01	0.80-0.90
F2 811 x 970	175	57	0.3	3.2	3R : 1S	0.005	0.90-0.95

F1 hybrids and F2 populations from crosses within accessions gave similar results to the parental material. All of the plants from Acc 970 x Acc 970 scored 0 or 1 and all from Acc 811 x Acc 811 scored 3 or more, except four individuals with scores of 2 (Figure 3.1, Table 3.3).

The F1 hybrids of both Acc 970 x Acc 811 and Acc 811 x Acc 970 were all resistant, with scores of 2 or less, indicating that resistance was dominant to susceptibility. The reciprocal crosses gave similar results, indicating the absence of maternal effects in resistance. The F2 progenies of both crosses segregated with a good approximation to 3 resistant : 1 susceptible. This result would be expected when resistance was due to a single dominant gene (Table 3.3 and Figure 3.2). The present results suggested that resistance to *A. fabae* isolate A26 was due to a single dominant gene in Acc 970.

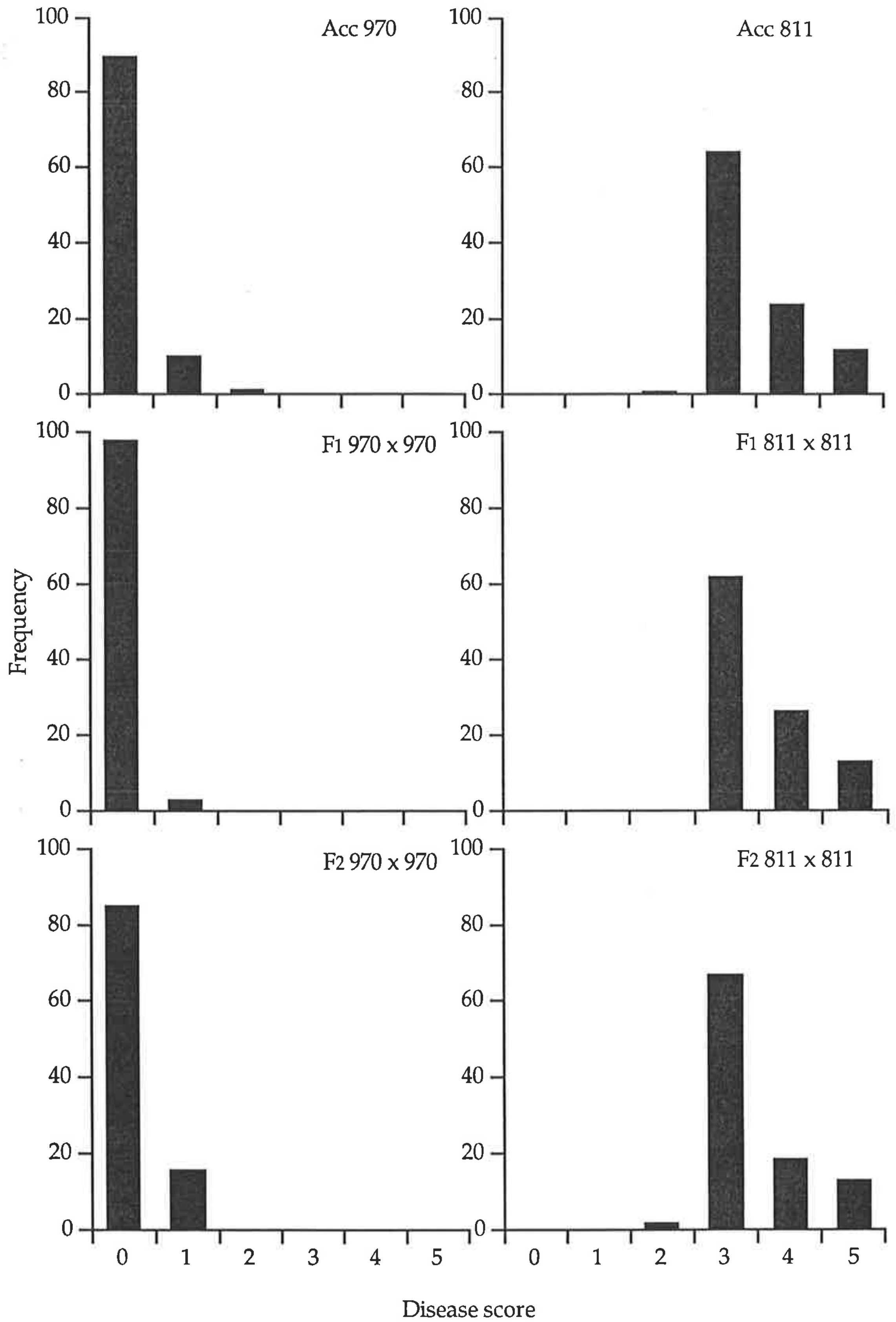


Figure 3.1 The frequency distributions of disease scores from parents, F1 hybrids and F2 populations of the crosses R x R or S x S 21 DAI.

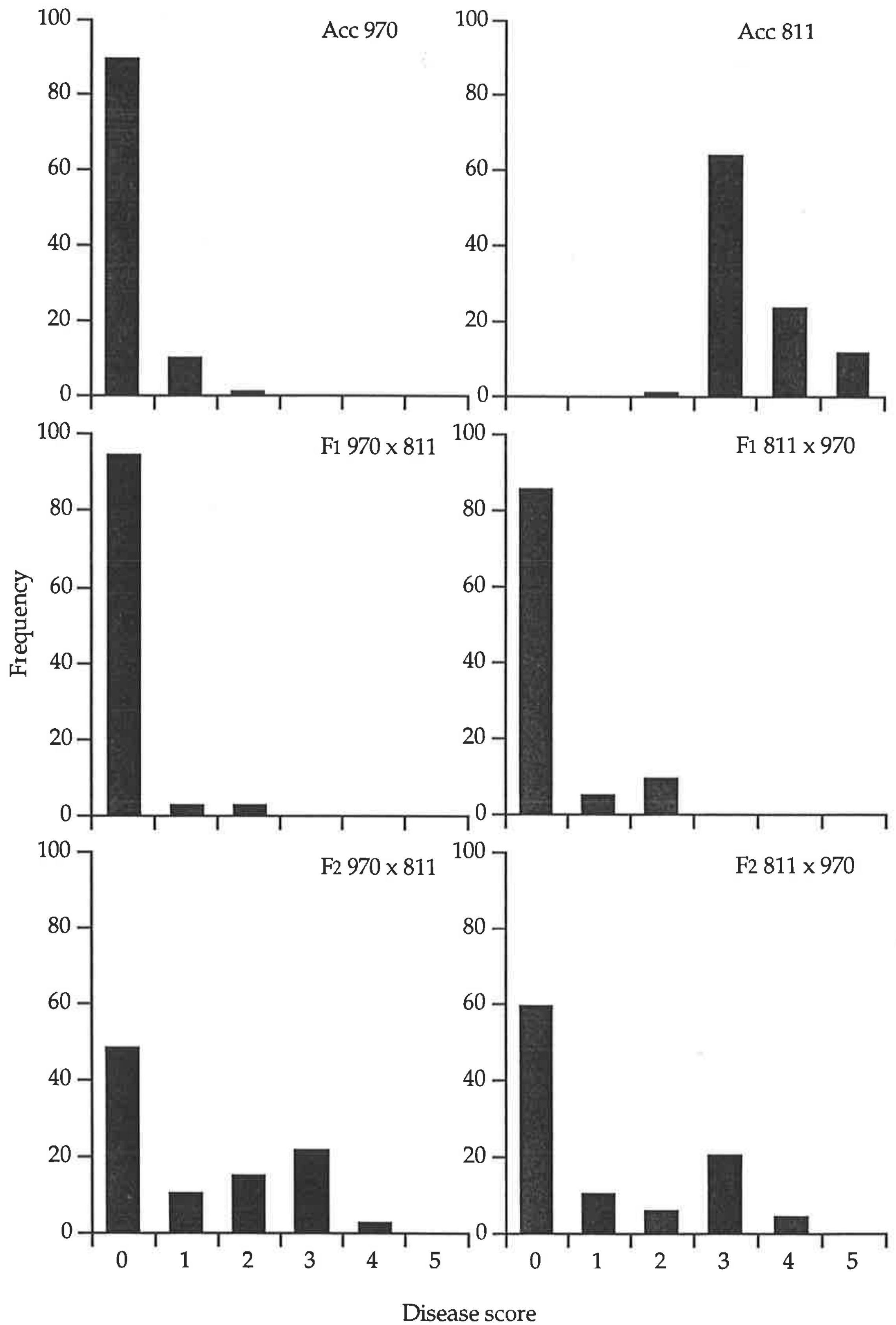


Figure 3.2 The frequency distributions of disease scores from parents, F1 hybrids and F2 populations of the crosses R x S or S x R 21 DAI.

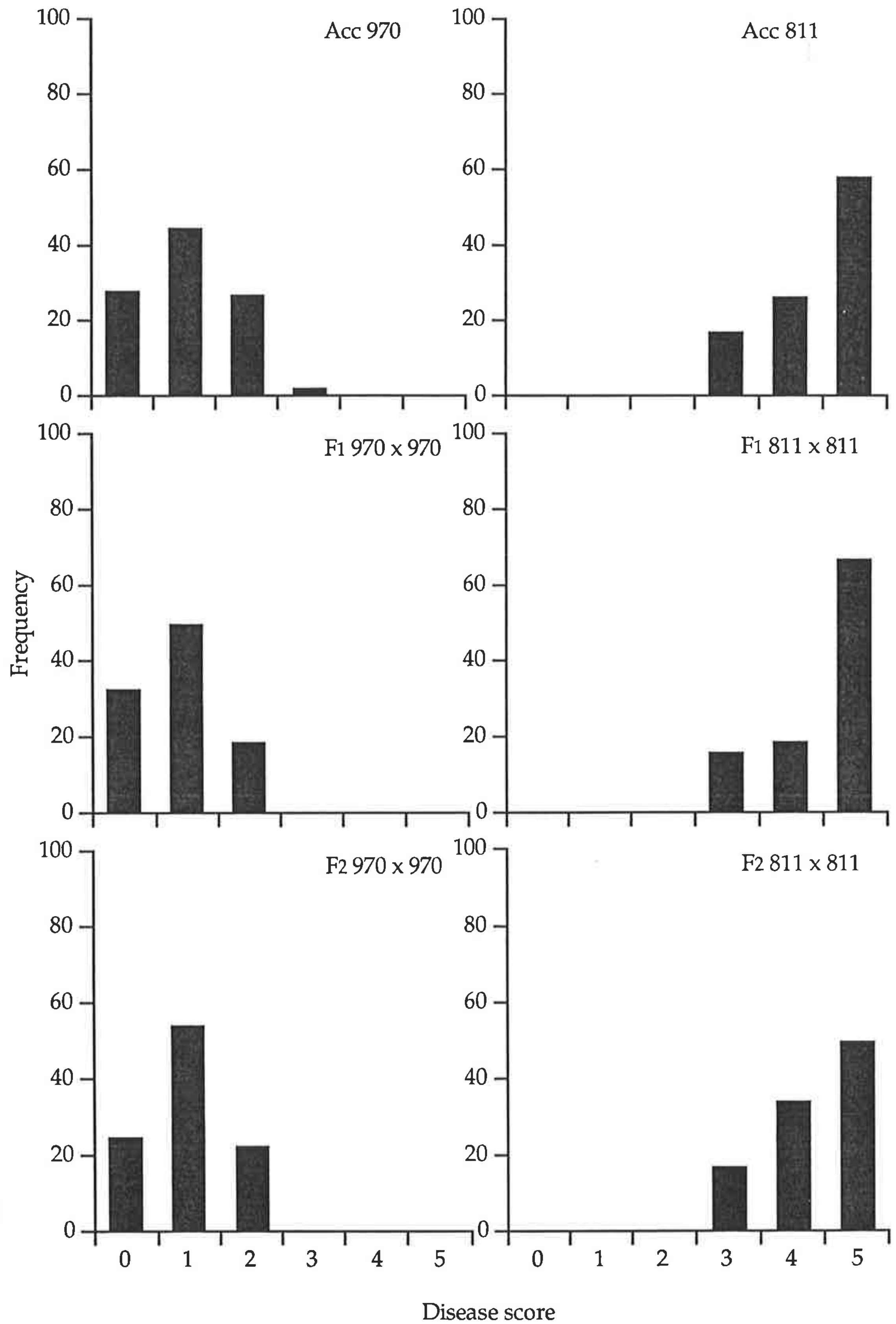


Figure 3.3 The frequency distributions of disease scores from parents, F1 hybrids and F2 populations of the crosses R x R or S x S 45 DAI.

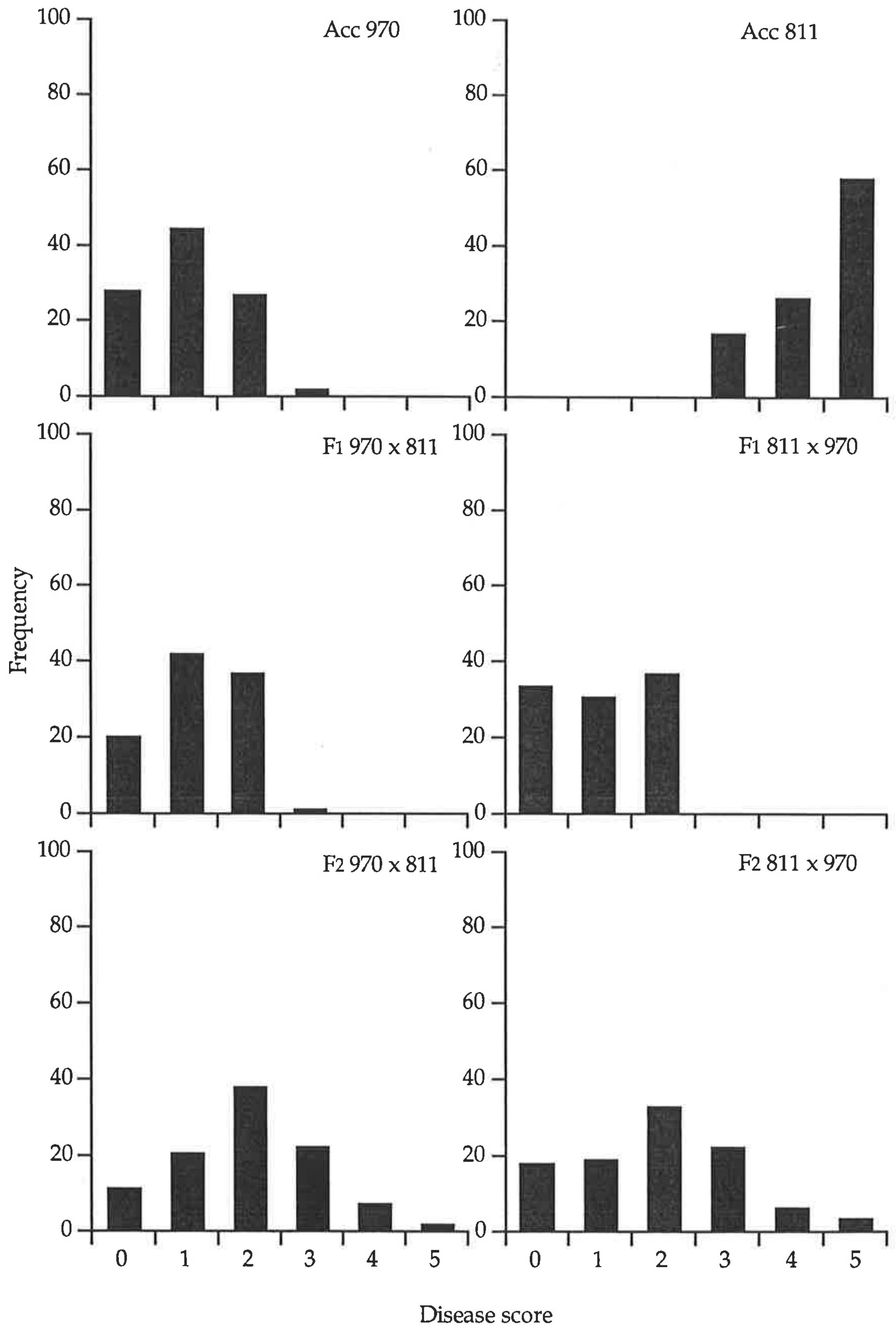


Figure 3.4 The frequency distributions of disease scores from parents, F1 hybrids and F2 populations of the crosses R x S or S x R 45 DAI.

Table 3.4 Disease reactions of the parents, F1 hybrids and F2 populations 45 DAI. Plants scored 0-2 were classed as resistant (R) and those scored 3-5 as susceptible (S).

Population	Number of plants		Mean score		Expected ratio	χ^2	p-values
	R	S	R	S			
Acc 970	285	5	1.0	3.0	All R		
Acc 811	0	335	—	4.4	All S		
F1 970 x 970	87	0	0.9	—	All R		
F1 811 x 811	0	39	—	4.5	All S		
F2 970 x 970	131	0	1.0	—	All R		
F2 811 x 811	0	194	—	4.3	All S		
F1 970 x 811	73	1	1.2	3.0	All R		
F1 811 x 970	158	0	1.0	—	All R		
F2 970 x 811	157	70	1.4	3.3	3R : 1S	3.81	0.05-0.10
F2 811 x 970	160	72	1.2	3.4	3R : 1S	4.19	0.025-0.05

At 45 DAI, disease severity had increased and the plants were given higher scores (compare Figure 3.3 with Figure 3.1). For Acc 970 the most common score was 1, but few plants scored greater than 2, while for Acc 811 most plants scored 5. Again, progenies of 970 x 970 and 811 x 811 behaved like their parents and the F1 hybrids of both 970 x 811 and 811 x 970 were still as resistant as 970 itself (Appendix 3.2), except for one individual from the cross of Acc 970 (13) x Acc 811 (11).

For the F2 plants of these two crosses, however, no simple segregation was evident as it had been at 21 DAI (*cf.* Figures 3.2 and 3.4). The χ^2 and p-values would lead to a rejection of the hypothesis that a single dominant gene was determining resistance (Table 3.4). Closer inspection of the data (Appendix 3.2) provides evidence of differences between the crosses. All the F2 plants studied of Acc 970 x Acc 811 involved Acc 970 (13) but among the reciprocal, Acc 811 x Acc 970, one family was derived from Acc 970 (13) and two families had other parents of Acc 970. When

classified into R or S the 970 (13) progeny were in the ratio 51:34, which deviates significant from 3:1, whereas the other two families were 55:17 and 54:21, which are not significantly different from 3:1.

3.3.2 The effect of resistant or susceptible border plants

At 45 DAI, the effect of the neighbouring plants and the overhead irrigation should have been evident as a secondary infection and indeed secondary infection had occurred, as lesions were observed on new leaves. Nevertheless, the different border plants were associated with no detectable difference in scores of F2 progenies. Plants with susceptible or resistant border rows had similar severities of infection (Figures 3.5 and 3.6). Both at 21 DAI and at 45 DAI the progenies of the F2 generation of Acc 970 x Acc 811 with borders of Acc 811 or Acc 970 had a similar pattern of segregation. The segregation was similar in other crosses.

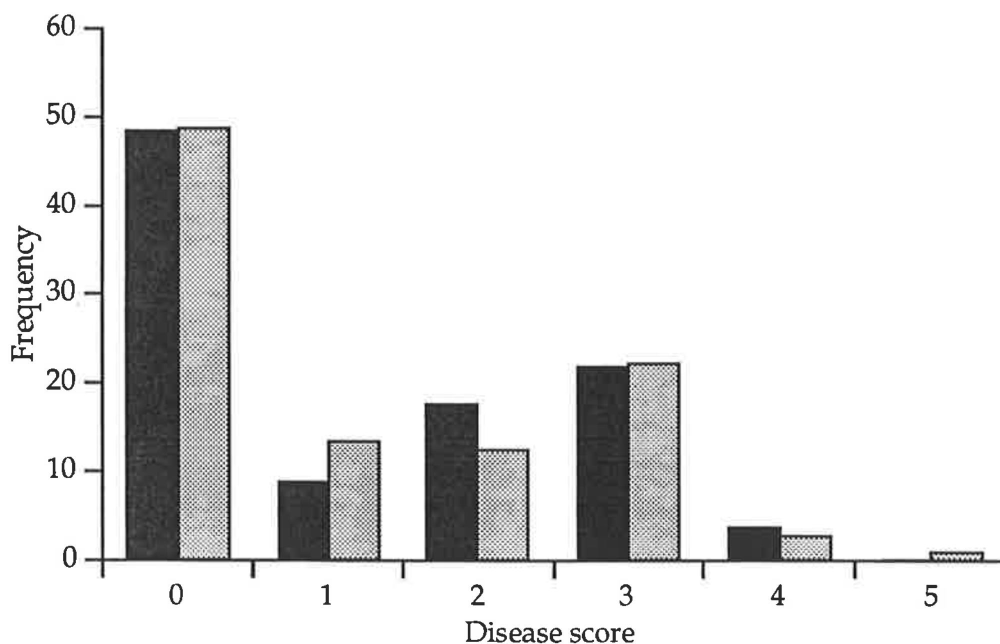


Figure 3.5 Effect of border plants of susceptible (Acc 811, ■) or resistant (Acc 970, ▩) accessions on the frequency distribution of disease scores 21 DAI of the F2 progenies of Acc 970 x Acc 811.

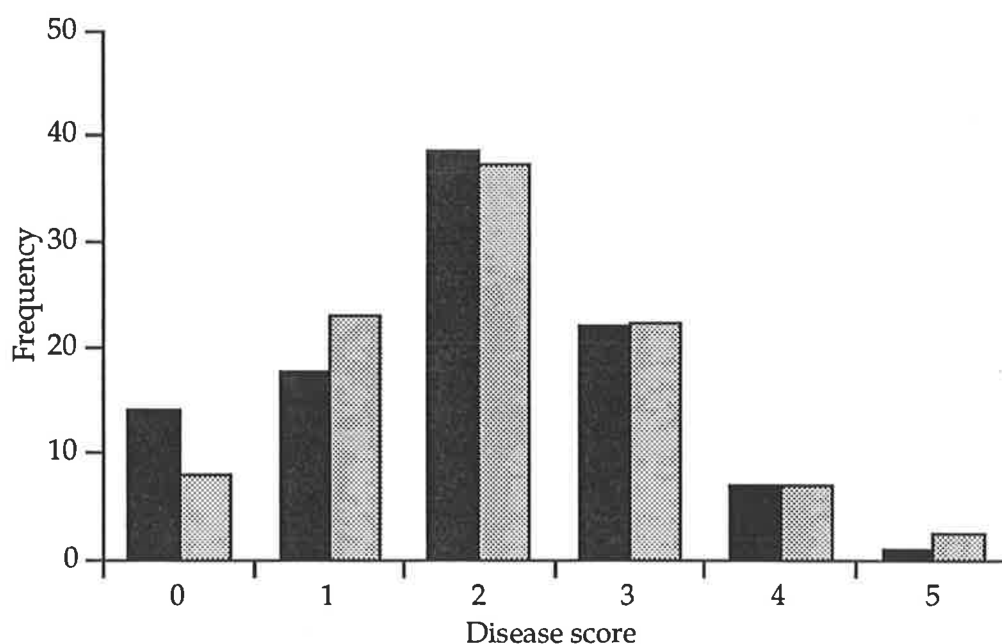


Figure 3.6 Effect of border plants of susceptible (Acc 811, ■) or resistant (Acc 970, ▩) accessions on the frequency distribution of disease scores 45 DAI of the F2 progenies of Acc 970 x Acc 811.

Table 3.5 The disease score distribution of three F2 populations of 970 x 970 at 21 DAI.

Cross		Number of plants with disease score								
		0	1	2	3	4	5	R (0-2)	S (3-5)	Total
R x R	970(39) x 970(41)	22	18	9	0	0	0	49	0	49
MR x R	970(29) x 970(12)	41	7	2	0	0	0	50	0	50
MR x MR	970(48) x 970(29)	39	9	2	0	0	0	50	0	50

3.3.3 Within-accession crosses of parents with different levels of resistance

The results presented here are from the disease reaction evaluated at 21 DAI (Plate 3.1). The F1 hybrids of 970 x 970 could not be evaluated. The F2 progenies of 970 x 970 were all resistant (Table 3.5).

Plate 3.1 F_2 progenies of 3 crosses of faba beans following inoculation with *Ascochyta fabae*: (A), Acc 970 (Resistant) x Acc 970 (Resistant), (B), Acc 811 (Susceptible) x Acc 811 (Susceptible) and (C) Acc 970 (Resistant) x Acc 811 (Susceptible).

The F_1 hybrids of 811 x 811 were all susceptible, even when one or both of the parents had been scored as resistant (Table 3.6), but few plants were available for scoring. The F_2 progenies of 811 x 811 showed a number of segregation ratios but there were few resistant plants. Different parents resulted in F_2 populations with different levels of resistance (Figure 3.7). Crosses of resistant plants gave very low numbers of resistant progenies in two crosses and higher in a third. Where R x S and S x S segregated resistant progeny, they were of the low numbers (ca. 3 of 50) expected from a two-gene system where both alleles had to be homozygous recessive (1:15). These results contrast with the 3 resistant : 1 susceptible obtained where Acc 970 was a parent.

Because of the susceptibility of the F_1 hybrids of R x R, a simple 2-gene system may not be adequate to account for resistance in this accession. Two pairs of two genes are necessary to account for the low numbers in the crosses of (3) x (21) and its reciprocal. The higher numbers in (51) x (21) would imply that these two plants were homozygous recessive for one of the four genes so there was a 3-gene segregation. In a

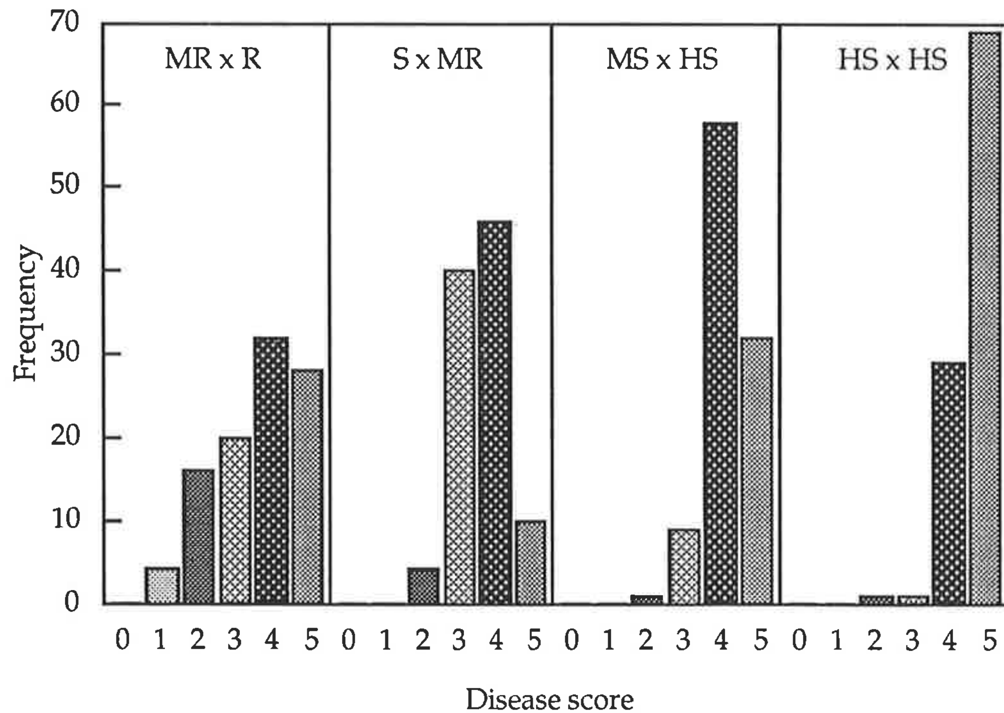


Figure 3.7 The frequency distributions for F_2 progenies of crosses 811 x 811 where the parents had different disease scores.

A

B

C

Table 3.6 The disease score distribution of 25 F_1 hybrids and 32 F_2 populations of 811 x 811 at 21 DAI and the total resistant (R) or susceptible (S) plants; • indicates that the cross was not evaluated.

Cross		Number of F_1 plants with disease score									Number of F_2 plants with disease score								
		0	1	2	3	4	5	R (0-2)	S (3-5)	Total	0	1	2	3	4	5	R (0-2)	S (3-5)	Total
R x R	811(3) x 811(21)	•	•	•	•	•	•	•	•	•	0	0	1	18	14	15	1	47	48
R x R	811(21) x 811(3)	0	0	0	0	2	0	0	2	2	0	1	3	18	19	9	4	46	50
R x MR	811(21) x 811(51)	0	0	0	2	1	0	0	3	3	•	•	•	•	•	•	•	•	•
MR x R	811(51) x 811(21)	0	0	0	4	1	0	0	5	5	0	2	8	10	16	14	10	40	50
R x MS	811(3) x 811(27)	0	0	0	0	0	1	0	1	1	0	1	5	13	18	13	6	44	50
R x MS	811(21) x 811(42)	0	0	0	4	1	0	0	5	5	0	0	4	12	30	4	4	46	50
MS x R	811(22) x 811(21)	•	•	•	•	•	•	•	•	•	0	0	0	0	19	31	0	50	50
R x HS	811(3) x 811(14)	•	•	•	•	•	•	•	•	•	0	0	6	19	17	7	6	43	49
R x HS	811(21) x 811(11)	•	•	•	•	•	•	•	•	•	0	0	0	0	16	33	0	49	49
HS x R	811(5) x 811(8)	0	0	0	1	0	0	0	1	1	0	0	3	26	18	3	3	47	50
HS x R	811(14) x 811(8)	0	0	0	1	1	0	0	2	2	•	•	•	•	•	•	•	•	•
HS x R	811(50) x 811(8)	0	0	0	1	1	1	0	3	3	•	•	•	•	•	•	•	•	•
MR x MS	811(19) x 811(23)	0	0	0	1	0	0	0	1	1	•	•	•	•	•	•	•	•	•
MR x MS	811(19) x 811(25)	0	0	0	0	1	0	0	1	1	0	0	3	20	17	10	3	47	50
MR x MS	811(51) x 811(22)	0	0	0	1	0	0	0	1	1	•	•	•	•	•	•	•	•	•
MS x MR	811(10) x 811(19)	•	•	•	•	•	•	•	•	•	0	0	0	1	16	33	0	50	50
MR x S	811(19) x 811(17)	•	•	•	•	•	•	•	•	•	0	1	1	19	19	10	2	48	50
S x MR	811(26) x 811(51)	•	•	•	•	•	•	•	•	•	0	0	2	20	23	5	2	48	50
MR x HS	811(51) x 811(14)	•	•	•	•	•	•	•	•	•	0	1	0	21	21	7	1	49	50
HS x MR	811(6) x 811(19)	0	0	0	0	2	0	0	2	2	0	1	3	16	19	11	4	46	50
HS x MR	811(55) x 811(19)	0	0	0	0	1	1	0	2	2	2	1	1	8	28	10	4	46	50
MS x MS	811(37) x 811(23)	•	•	•	•	•	•	•	•	•	0	0	0	7	32	11	0	50	50
MS x MS	811(52) x 811(37)	•	•	•	•	•	•	•	•	•	0	0	2	24	23	1	2	48	50
S x MS	811(17) x 811(10)	•	•	•	•	•	•	•	•	•	0	0	0	2	34	14	0	50	50
S x MS	811(45) x 811(52)	•	•	•	•	•	•	•	•	•	0	0	4	15	29	2	4	46	50
MS x HS	811(10) x 811(43)	0	0	0	0	4	0	0	4	4	0	0	0	1	30	18	0	49	49
MS x HS	811(22) x 811(48)	•	•	•	•	•	•	•	•	•	0	0	0	3	29	18	0	50	50
MS x HS	811(47) x 811(43)	•	•	•	•	•	•	•	•	•	0	0	1	9	28	12	1	49	50

Table 3.6 continued.

	Cross	Number of F1 plants with disease score									Number of F2 plants with disease score								
		0	1	2	3	4	5	R (0-2)	S (3-5)	Total	0	1	2	3	4	5	R (0-2)	S (3-5)	Total
MS x HS	811(57) x 811(18)	0	0	0	1	2	3	0	6	6	•	•	•	•	•	•	•	•	•
HS x MS	811(6) x 811(10)	•	•	•	•	•	•	•	•	•	0	0	2	1	38	9	2	48	50
HS x MS	811(34) x 811(10)	•	•	•	•	•	•	•	•	•	0	0	0	1	21	28	0	50	50
S x S	811(39) x 811(26)	•	•	•	•	•	•	•	•	•	0	0	0	4	20	26	0	50	50
S x HS	811(17) x 811(32)	0	0	0	0	2	0	0	2	2	0	0	1	0	30	19	1	49	50
S x HS	811(26) x 811(14)	0	0	0	0	1	1	0	2	2	•	•	•	•	•	•	•	•	•
S x HS	811(38) x 811(13)	0	0	0	0	1	0	0	1	1	0	0	0	1	22	27	0	50	50
S x HS	811(39) x 811(32)	0	0	0	0	2	0	0	2	2	0	0	0	0	22	28	0	50	50
S x HS	811(39) x 811(43)	0	0	0	0	1	0	0	1	1	•	•	•	•	•	•	•	•	•
S x HS	811(45) x 811(48)	0	0	0	0	2	0	0	2	2	•	•	•	•	•	•	•	•	•
HS x HS	811(11) x 811(44)	0	0	0	1	1	0	0	2	2	•	•	•	•	•	•	•	•	•
HS x HS	811(13) x 811(5)	0	0	0	0	1	1	0	2	2	•	•	•	•	•	•	•	•	•
HS x HS	811(18) x 811(50)	0	0	0	0	3	1	0	4	4	•	•	•	•	•	•	•	•	•
HS x HS	811(34) x 811(12)	0	0	0	0	1	1	0	2	2	0	0	0	0	9	41	0	50	50
HS x HS	811(55) x 811(56)	•	•	•	•	•	•	•	•	•	0	0	1	1	17	31	1	49	50
HS x HS	811(6) x 811(5)	•	•	•	•	•	•	•	•	•	0	0	0	1	18	31	0	50	50

cross of AABBccdd x aabbCCDD where either aabb or ccdd is required to confer resistance, the F1 hybrid would be AaBbCcDd and thus susceptible. The F2 generation would have the complex segregation of 81 A-B-C-D- : 27 of each of the four single homozygous recessives : 9 of each of the six double homozygous recessives, of which only aabb and ccdd would be resistant : 3 of each of the four triple homozygous recessives, all of which would be resistant : 1 aabbccdd, resistant. Thus 31 of the 256 F2 types would be resistant, or about 6 of 50. In a three-gene system of AABBcc x aabbCC (perhaps with dd in both parents) where either aabb or cc is required to confer resistant, the F2 generation would consist of 27 A-B-C- : 9 of each of the three single homozygous recessives, of which cc would be resistant : 3 of each of the three double homozygous recessives, of which aabb would be resistant : 1 aabbcc, resistant, for a total of 13 resistant of 64 types, or about 10 of 50. Nevertheless this is a

cumbersome model and the numbers of F_1 hybrids evaluted were too small to confirm it.

All of the other crosses segregating resistant plants were compatible with two gene differences between the parents.

3.3.4 Between-accession crosses of parents with different levels of resistance

Crosses of Acc 970 plants 6, 9, 16, 41, 43 and 50 with any Acc 811 plants gave no susceptibles in the F_1. The ratio of resistants to susceptibles was not significantly different from 3:1 (31 to 44 of the 50 plants were resistant) in the F_2 families of 21 of these 24 crosses. The other three families had more resistant individuals than were expected from a 3:1 ratio. One of these was from a cross with 811 (3), a resistant plant, and the other two were with 811 (37) and 811 (42), which were not otherwise used in crossing. These additional resistant individuals may easily be accounted for if the Acc 970 parent plant were heterozygous or homozygous recessive for one or both of the complementary recessive resistance genes (e.g. was RR Aa bb) and if the Acc 811 parent plant were similarly heterozygous for both or homozygous for one of the complementary recessive resistance genes (e.g. was rr AA bb). Since most Acc 811 plants were used to produce only one or two F_2 populations, it is difficult to confirm the suggested genotype of a given individual.

Crosses involving Acc 970 plants 12, 38, 39 and 51 all produced some susceptible F_1 individuals, suggesting that these parent plants may have been heterozygous for the dominant resistance gene. From these crosses, 26 F_2 populations were evaluated. Twelve of these segregated in a 3R : 1S ratio, suggesting that the F_1 hybrids chosen for producing the F_2 populations were heterozygous for the dominant resistance gene. The remaining F_2 populations segregated in other ratios compatible with the F_1 hybrids having been mixed homozygotes and heterozygotes for the dominant resistance gene.

Acc 970 plants 25 and 36 were scored as moderately susceptible. The only evaluated progeny of Acc 970 (36) was in the cross with the resistant Acc 811 (21). The F_1 of this cross was fully resistant and the F_2 segregated in a 3R : 1S ratio. These results imply that Acc 970 (36) carried the complementary recessive resistant genes in a form such as Aabb. In that way, the F_1 of a cross to a resistant aabb would segregate dominants and recessives and those that were grown on to produce F_2 seed could have been

Table 3.7 The disease score distributions of 16 F1 hybrids and 14 F2 populations of 970 x 811 at 21 DAI and the total resistant (R) or susceptible (S) plants; • indicates that the cross was not evaluated.

Cross		Number of F1 plants with disease score									Number of F2 plants with disease score								
		0	1	2	3	4	5	R (0-2)	S (3-5)	Total	0	1	2	3	4	5	R (0-2)	S (3-5)	Total
HR x MS	970(16) x 811(47)	0	0	1	0	0	0	1	0	1	14	10	9	9	7	1	33	17	50
HR x MS	970(51) x 811(20)	1	0	2	1	0	0	3	1	4	•	•	•	•	•	•	•	•	•
R x R	970(43) x 811(21)	0	0	1	0	0	0	1	0	1	•	•	•	•	•	•	•	•	•
R x MR	970(12) x 811(19)	6	2	0	0	0	0	8	0	8	22	16	9	1	1	1	47	3	50
R x MS	970(9) x 811(1)	1	0	0	0	0	0	1	0	1	16	10	15	7	1	0	41	8	49
R x MS	970(39) x 811(23)	0	0	2	0	0	0	2	0	2	7	21	16	6	0	0	44	6	50
R x MS	970(39) x 811(25)	0	0	1	1	0	0	1	1	2	•	•	•	•	•	•	•	•	•
R x MS	970(43) x 811(46)	•	•	•	•	•	•	•	•	•	13	20	10	5	2	0	43	7	50
R x S	970(38) x 811(26)	•	•	•	•	•	•	•	•	•	16	18	9	4	3	0	43	7	50
R x S	970(43) x 811(17)	•	•	•	•	•	•	•	•	•	17	12	11	9	1	0	40	10	50
R x HS	970(9) x 811(34)	0	2	0	0	0	0	2	0	2	20	12	11	3	4	0	43	7	50
R x HS	970(9) x 811(44)	0	2	2	0	0	0	4	0	4	•	•	•	•	•	•	•	•	•
R x HS	970(9) x 811(55)	0	1	1	0	0	0	2	0	2	•	•	•	•	•	•	•	•	•
R x HS	970(12) x 811(14)	1	1	0	0	0	0	2	0	2	18	20	5	6	1	0	43	7	50
R x HS	970(12) x 811(33)	2	0	0	0	0	0	2	0	2	16	17	12	5	0	0	45	5	50
R x HS	970(12) x 811(43)	1	1	0	0	0	0	2	0	2	•	•	•	•	•	•	•	•	•
R x HS	970(12) x 811(48)	•	•	•	•	•	•	•	•	•	20	14	10	6	0	0	44	6	50
R x HS	970(12) x 811(55)	•	•	•	•	•	•	•	•	•	19	13	10	8	0	0	42	8	50
R x HS	970(38) x 811(4)	•	•	•	•	•	•	•	•	•	30	7	7	3	3	0	44	6	50
R x HS	970(39) x 811(56)	0	0	1	1	0	0	1	1	2	•	•	•	•	•	•	•	•	•
R x HS	970(43) x 811(11)	0	0	2	0	0	0	2	0	2	•	•	•	•	•	•	•	•	•
R x HS	970(43) x 811(34)	•	•	•	•	•	•	•	•	•	17	7	12	12	1	1	36	14	50
R x HS	970(43) x 811(55)	0	1	1	0	0	0	2	0	2	•	•	•	•	•	•	•	•	•

Table 3.8 The disease score distributions of 47 F1 hybrids and 42 F2 populations of 811 x 970 at 21 DAI and the total resistant (R) or susceptible (S) plants; • indicates that the cross was not evaluated.

	Cross	Number of F1 plants with disease score									Number of F2 plants with disease score								
		0	1	2	3	4	5	R (0-2)	S (3-5)	Total	0	1	2	3	4	5	R (0-2)	S (3-5)	Total
R x HR	811(3) x 970(6)	5	0	0	0	0	0	5	0	5	32	12	4	2	0	0	48	2	50
R x HR	811(8) x 970(6)	•	•	•	•	•	•	•	•	•	16	14	10	8	2	0	40	10	50
R x HR	811(21) x 970(6)	•	•	•	•	•	•	•	•	•	13	9	12	6	9	1	34	16	50
R x R	811(3) x 970(12)	5	1	0	0	0	0	6	0	6	22	22	5	1	0	0	49	1	50
R x R	811(3) x 970(39)	0	2	3	0	0	0	5	0	5	0	18	24	8	0	0	42	8	50
R x R	811(8) x 970(9)	•	•	•	•	•	•	•	•	•	13	26	4	7	0	0	43	7	50
R x R	811(21) x 970(39)	•	•	•	•	•	•	•	•	•	2	3	11	16	11	7	16	34	50
R x MS	811(8) x 970(25)	0	2	3	0	0	0	5	0	5	•	•	•	•	•	•	•	•	•
R x MS	811(21) x 970(36)	0	2	1	0	0	0	3	0	3	14	14	11	6	4	1	39	11	50
MR x MS	811(51) x 970(25)	2	0	0	0	0	0	2	0	2	17	10	18	5	0	0	45	5	50
MR x R	811(35) x 970(38)	0	1	3	0	0	0	4	0	4	•	•	•	•	•	•	•	•	•
MR x R	811(51) x 970(12)	•	•	•	•	•	•	•	•	•	32	15	2	1	0	0	49	1	50
MS x HR	811(23) x 970(51)	0	0	2	3	0	0	2	3	5	0	4	13	22	11	0	17	33	50
MS x HR	811(37) x 970(51)	0	1	4	0	0	0	5	0	5	•	•	•	•	•	•	•	•	•
MS x HR	811(52) x 970(51)	•	•	•	•	•	•	•	•	•	3	6	22	13	6	0	31	19	50
MS x R	811(2) x 970(12)	3	2	0	0	0	0	5	0	5	•	•	•	•	•	•	•	•	•
MS x R	811(9) x 970(12)	2	1	0	0	0	0	3	0	3	26	13	11	0	0	0	50	0	50
MS x R	811(9) x 970(43)	0	1	0	0	0	0	1	0	1	•	•	•	•	•	•	•	•	•
MS x R	811(10) x 970(43)	0	1	1	0	0	0	2	0	2	14	18	11	7	0	0	43	7	50
MS x R	811(20) x 970(12)	1	1	1	0	0	0	3	0	3	•	•	•	•	•	•	•	•	•
MS x R	811(20) x 970(39)	0	0	2	0	0	0	2	0	2	•	•	•	•	•	•	•	•	•
MS x R	811(22) x 970(9)	0	1	1	0	0	0	2	0	2	10	12	19	7	2	0	41	9	50
MS x R	811(22) x 970(39)	0	2	0	0	0	0	2	0	2	0	5	12	18	12	3	17	33	50
MS x R	811(23) x 970(41)	0	2	0	0	0	0	2	0	2	•	•	•	•	•	•	•	•	•
MS x R	811(29) x 970(43)	3	3	0	0	0	0	6	0	6	17	16	8	8	1	0	41	9	50
MS x R	811(37) x 970(12)	2	1	0	0	0	0	3	0	3	•	•	•	•	•	•	•	•	•
MS x R	811(37) x 970(43)	0	4	1	0	0	0	5	0	5	21	16	9	3	1	0	46	4	50
MS x R	811(42) x 970(43)	0	2	0	0	0	0	2	0	2	23	12	13	2	0	0	48	2	50
MS x R	811(52) x 970(12)	2	0	0	0	0	0	2	0	2	•	•	•	•	•	•	•	•	•

Table 3.8 continued

	Cross	Number of F1 plants with disease score									Number of F2 plants with disease score								
		0	1	2	3	4	5	R (0-2)	S (3-5)	Total	0	1	2	3	4	5	R (0-2)	S (3-5)	Total
MS x R	811(57) x 970(12)	3	1	0	0	0	0	4	0	4	20	17	8	4	1	0	45	5	50
MS x R	811(57) x 970(38)	1	1	1	1	0	0	3	1	4	1	4	15	24	6	0	20	30	50
MS x R	811(57) x 970(39)	0	0	2	0	0	0	2	0	2	0	8	12	21	8	1	20	30	50
MS x MS	811(29) x 970(25)	•	•	•	•	•	•	•	•	•	0	0	13	32	5	0	13	37	50
S x HR	811(28) x 970(51)	0	0	3	1	0	0	3	1	4	1	8	20	19	2	0	29	21	50
S x HR	811(30) x 970(51)	0	2	2	0	0	0	4	0	4	•	•	•	•	•	•	•	•	•
S x HR	811(45) x970(51)	0	0	0	2	0	0	0	2	2	0	7	8	25	9	1	15	35	50
S x R	811(17) x 970(43)	0	2	2	0	0	0	4	0	4	16	14	10	8	2	0	40	10	50
S x R	811(28) x 970(43)	0	1	1	0	0	0	2	0	2	10	18	16	4	2	0	44	6	50
S x R	811(38) x 970(43)	0	2	2	0	0	0	4	0	4	12	13	17	8	0	0	42	8	50
HS x HR	811(5) x 970(6)	0	1	3	0	0	0	4	0	4	13	10	13	7	7	0	36	14	50
HS x HR	811(14) x 970(51)	0	0	0	1	0	0	0	1	1	0	3	15	23	9	0	18	32	50
HS x HR	811(44) x 970(51)	0	1	0	1	0	0	1	1	2	•	•	•	•	•	•	•	•	•
HS x HR	811(48) x 970(51)	0	0	1	0	0	0	1	0	1	0	9	21	13	7	0	30	20	50
HS x R	811(5) x 970(12)	•	•	•	•	•	•	•	•	•	22	8	12	5	3	0	42	8	50
HS x R	811(5) x 970(50)	•	•	•	•	•	•	•	•	•	15	14	11	5	4	1	40	10	50
HS x R	811(6) x 970(43)	2	1	0	0	0	0	3	0	3	15	15	10	7	3	0	40	10	50
HS x R	811(11) x 970(43)	1	1	0	0	0	0	2	0	2	•	•	•	•	•	•	•	•	•
HS x R	811(12) x 970(12)	•	•	•	•	•	•	•	•	•	23	13	5	7	2	0	41	9	50
HS x R	811(13) x 970(9)	0	1	0	0	0	0	1	0	1	•	•	•	•	•	•	•	•	•
HS x R	811(13) x 970(43)	2	1	1	0	0	0	4	0	4	14	9	13	11	3	0	36	14	50
HS x R	811(18) x 970(39)	0	0	0	1	0	0	0	1	1	1	3	17	24	5	0	21	29	50
HS x R	811(48) x 970(12)	1	0	0	0	0	0	1	0	1	•	•	•	•	•	•	•	•	•
HS x R	811(48) x 970(50)	2	3	0	0	0	0	5	0	5	10	13	15	10	2	0	38	12	50
HS x R	811(50) x 970(12)	4	0	0	0	1	0	4	1	5	•	•	•	•	•	•	•	•	•
HS x MS	811(5) x 970(25)	0	1	0	1	0	0	1	1	2	•	•	•	•	•	•	•	•	•
HS x MS	811(14) x 970(25)	•	•	•	•	•	•	•	•	•	0	1	8	22	18	1	9	41	50
HS x MS	811(32) x 970(25)	0	0	0	4	0	0	0	4	4	9	15	16	8	2	0	40	10	50
HS x MS	811(48) x 970(25)	0	1	1	2	0	0	2	2	4	13	20	13	4	0	0	46	4	50
HS x MS	811(54) x 970(25)	•	•	•	•	•	•	•	•	•	12	7	5	14	11	1	24	26	50

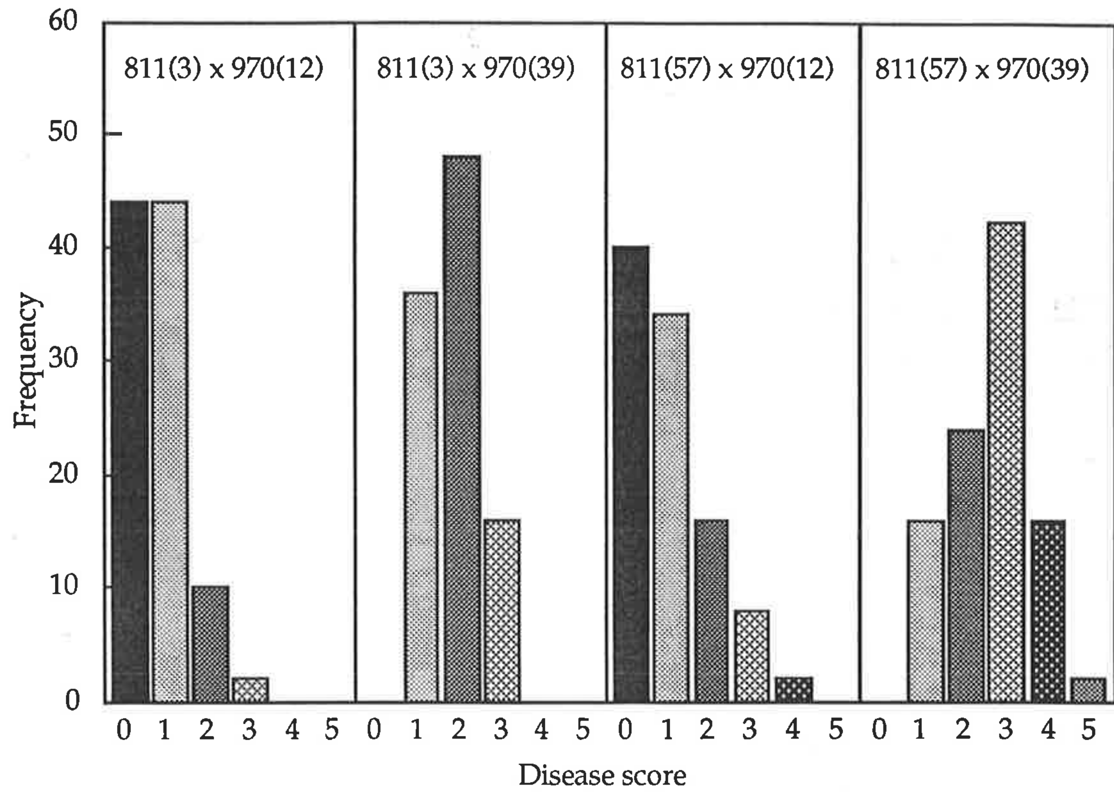


Figure 3.8 The segregation of the F2 progenies of crosses of 811 (3) (R) and 811 (57) (MS) x 970 (12) (R) and 970 (39) (R).

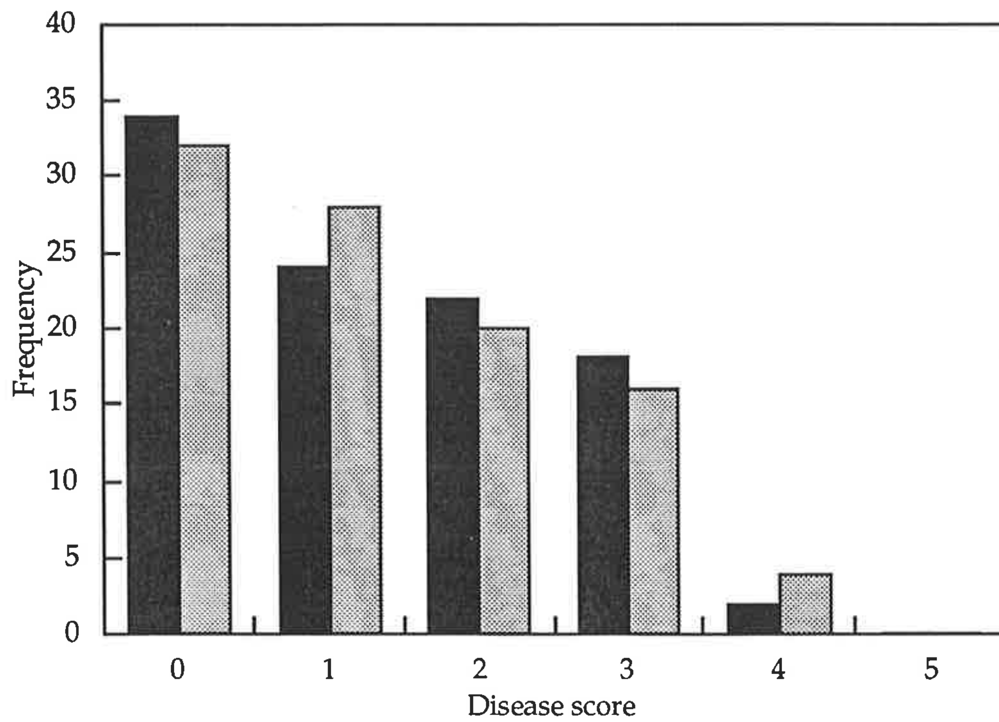


Figure 3.9 The distribution of the F2 progenies of the crosses of 970 (43) x 811 (17) (■) and 811 (17) x 970 (43) (▩) at 21 DAI.

largely the recessives. Similarly, Acc 970 (25) when crossed with the resistant Acc 811 plants 8 and 51 produced only resistant F1 progeny. Acc 970 (25) also produced some resistant F1 plants when crossed with the susceptible Acc 811 plants 5 and 48, which might therefore also have had a genotype such as Aabb. In the F2 generation, progenies of Acc 970 (25) segregated in a variety of ratios, including 1R : 3S (with 811 (32) and (48)), 1R : 1S (with 811 (54)), 3R : 1S (with 811 (32) and (51)) and >3R : 1S (with 811 (48)). Since the F1 generation was already segregating, a wide range of F2 segregation ratios was likely.

Where reciprocal crosses were prepared, the segregation patterns of the progenies were nearly identical (Figure 3.9), indicating a lack of maternal effects in resistance.

3.4 Discussion

The first part of this study has showed that when (1) only a restricted number of crosses are assessed, (2) a single race of the pathogen is used as inoculum, (3) assessment is made 21 days after inoculation and (4) the crosses are between parents that have been selected for clear expression of resistance or susceptibility, the results can be explained by segregation of a single dominant gene for resistance to Ascochyta blight in faba beans. When assessment was delayed to 45 DAI, however, the results of some crosses did not conform to this hypothesis. Furthermore, the examination of crosses between other parent plants suggested that at least one pair of complementary recessive genes could also confer resistance.

Every aspect of the results obtained with the first set of crosses 21 days after inoculation supported a single gene hypothesis. The resistant parent Acc 970 and the F1 hybrids and F2 progenies arising from crossing between plants of this accession were resistant. The susceptible parent Acc 811 and the F1 and F2 generations of plants of this accession were susceptible. In addition the F1 hybrids resulting from crossing the resistant Acc 970 parents with the susceptible 811 were resistant. The F2 population of the reciprocal crosses of Acc 970 with Acc 811 showed a segregation ratio that fitted a 3R : 1S.

The results obtained 45 DAI for the most part may be explained also by a single gene but not all crosses were consistent and in some the F2 populations did not fit a 3:1 segregation. In the interval between 21 and 45 DAI the lesions on both resistant

and susceptible plants had expanded and in a few instances plants which had been scored resistant were now scored susceptible, all originating from plant 13 of Acc 970. Among the 27 selfed progeny of this parent, 5 plants were scored susceptible and the F2 progenies of this parent showed more susceptibles than compatible with a 3R : 1S ratio. Evidently there were differences between the Acc 970 parents which were only evident when assessment was delayed to 45 DAI. Thus plant 13 was probably heterozygous. Similarly, in the second part of this study, several plants of Acc 970 were shown to be heterozygous for resistance.

Increases in scores with time, such that resistance appears to be determined by a single dominant gene at one time and some other genetic mechanism at another, have been found in barley. With scald (*Rhynchosporium secalis*) of barley, Habgood and Hayes (1971) found that scores made 14 DAI indicated a single dominant gene was segregating 3:1 in the F2, but at 28 DAI there were increases in scores such that a segregation of 1:2:1 became apparent, implying that an incompletely dominant gene was operating. At 14 DAI the incomplete dominance was not evident in the scores in the heterozygotes but at 28 DAI an intermediate reaction which developed slowly with time was evident. In crosses involving parents with other genes for resistance and where again there were differences in scores with time, two complementary genes were postulated, one incompletely dominant and the other completely dominant, but operating only in conjunction with the first. Its presence was not apparent 14 DAI but prevented the later development of symptoms when in either the homozygous or heterozygous state. Its presence had not been detected until their study as its effect was only apparent if assessment was delayed beyond that normally used, when seedlings generally would have been discarded.

Results obtained by Lockwood *et al.* (1985) and Jellis *et al.* (1985) also showed that the longer the time the higher the score. However, they were not trying to evaluate segregating populations and their results have no bearing on the genetics of resistance to Ascochyta. Hunter *et al.* (1981) studied a method to screen *Phaseolus vulgaris* and *P. coccineus* for resistance to white mold (*Sclerotinia sclerotium*). They found the rating 10 days after inoculation gave higher scores than at 6 days after inoculation and the method of inoculation had a great effect on the frequency and severity of the induced disease.

As scores can increase with time it is to be expected environmental conditions will have an effect. The spore age and concentration of an inoculum have been reported to affect the disease reaction (Dodd, 1971; Van Breukelen, 1985). Both the incubation period and the degree of resistance varied according to the age of the plant tested, the organ examined and the plant's overall growth habit (Konstantinova, 1965; Dodd, 1971; Wallen and Galway, 1977; Hanounik, 1980a; Hanounik and Maliha, 1984; Van Breukelen, 1985; Ali, 1985; Jellis *et al.*, 1985; Tivoli *et al.*, 1987; Pritchard *et al.*, 1989). The environmental conditions such as humidity and temperature are important factors for infection and colonisation of the plant (Dodd, 1971; Hewett, 1973; Van Breukelen, 1985; Tivoli *et al.*, 1987).

It has been reported for other species that plants which are resistant under one set of conditions are not necessarily equally resistant under other environmental conditions altering the expression of inherited resistance (Arnold and Brown, 1968; Ellingboe, 1981; Hall, 1985). As a result of environmental action, the different genotypic classes become "blended" into a distribution of phenotypes which may or may not correspond with the underlying distribution of genotypes. The degree of correspondence will obviously depend upon the relative roles of genotypic and environmental effects on the expression of resistance. No complete resistance has been found to Ascochyta. When extremely favourable environments have been applied in glasshouse tests even highly resistant plants of Acc 970 may show visible symptoms. Similarly Hanounik and Robertson (1989) found within the pure line, BPL 471, a line classified as resistant that resistant plants could be rendered more susceptible by different treatments.

It is concluded that delaying evaluation for a long time or providing very favourable conditions for the pathogen may enable symptoms to develop sufficiently for an otherwise resistant plant to be scored as susceptible and the segregation rates to be affected.

There was no evidence in this study of the effect of neighbouring plants on the spread of infection. It was expected that from 21 DAI onwards, new symptoms might develop from secondary infections from pycnidiospores of the first infection, and the disease could be further distributed by physical contact with neighbours or as a result of overhead irrigation. The reasons for the lack of an effect might be: (1) close

proximity to an infected plants is not important if the conditions for disease infection were already extremely favourable and the plant genotypes studied had marked genetic differences in resistance; and (2) the area of the experiment was small and the spores could spread and infect all the plants in the trial. The spread of the disease seemed to be even throughout the trial. It has been demonstrated by Dodd (1971) that spores may disperse at least 1 m from the inoculum source by rain splash and Bond and Pope (1980) suggested other forms of dispersal such as the wind are involved which could spread the disease up to 100 m from volunteer plants. The increase in the severity of the infection that occurred between 21 DAI and 45 DAI could be interpreted as the effect of the water from overhead sprinkler which carried or splashed spores from one part of a plant to another. It was evident that new leaves became infected during this time.

In terms of the development of a field epidemic, the resistance of plants of Acc 970 45 DAI was important. It was established in the preliminary studies in controlled environments that infection could take place in Acc 970 if spores were available and the environment was very favourable. In the field the resistant plants of Acc 970 still showed their resistant reaction 45 DAI even when adjacent to susceptible plants which were severely infected. Symptoms may be apparent but were of no significance to yield or quality of the grain.

To standardise assessments of disease resistance the plants age, method of inoculation, inoculum concentration, temperature, humidity, days following inoculation should be specified.

The aim of the second part of this study was to confirm whether there were genetically determined differences in resistance to Ascochyta within accessions. Such differences clearly occurred in Acc 811. In the F_1 generation of crosses between plants of this accession, no resistant plants were found and the resistance reaction appeared to be recessive, although this conclusion was based on relatively few plants. In the F_2 generation, resistant plants were detected and the distribution illustrated thats there were genetic differences among the parents crossed and that resistance was recessive. The data were compatible with a pair of complementary resistance conferring resistance, giving a 1:15 segregation in an F_2 from a double-heterozygous F_1. Some of the data implied that there were two pairs of complementary recessive genes for

resistance, as this was the only model that accounted for resistant parents, a susceptible F_1 and a small number of resistant F_2 individuals. Nevertheless the complexity of this model is unappealing and is particularly dependent on the susceptibility of an F_1 generation of only 10 plants. The working model to account for resistance in Accession 811 is thus of a simple pair of complementary recessive genes.

The results showed that most plants of Acc 970 were homozygous for the dominant resistance gene. All of the F_2 plants resulting from crosses within this accession were resistant to Ascochyta blight. Some crosses between Acc 970 and Acc 811 produced unexpected ratios of resistants to susceptibles. It has already been shown that Acc 811 carried complementary recessive genes for resistance and if they were present at a low level in Acc 970 as well, additional resistant individuals would segregate.

There are two simple models which may be advanced to account for the presence of excess susceptible segregants in the F_2 generation. The first is that some plants of Acc 970 were heterozygous for the resistance gene. Since a small number of F_1 plants was harvested to produce the F_2 generation and since these F_1 plants were not tested for their resistance, a variety of segregation ratios could arise if one of the parents were heterozygous.

The second model is that there is a dominant inhibitor of resistance at a low frequency in one or both of the accessions. Acc 811 has two alleles of the resistance gene, one the usual recessive susceptible, the other a dominant susceptible. While this study was in progress, Rashid *et al.* (1991) published that among inbred lines of faba beans, three susceptible (lines 20, 21 and 22) to Ascochyta blight and the fourth (line 9) resistant, resistance was dominant in two crosses (9 x 20 and 9 x 21) and recessive in the third (9 x 22). Acc 970 in the present study is the same ICARDA population as line 9 of Rashid *et al.* and both Acc 811 and line 22 came from Afghanistan. Acc 970 plant 36 would fit this model, being itself susceptible but producing resistant progeny, and so would Acc 811 (32) and (48) which produced ratios of 1R : 3S in the F_2 generation. The findings of Rashid *et al.* concerning lines 9 and 22 confirm the results of this study that there are complicated interactions among the genes conditioning resistance to Ascochyta in faba beans.

To resolve the matter it may be necessary to concentrate on fewer crosses and to use a detached-organ test, described later, to increase the precision of classification of the parents, which had been based on results for them as single plants. It would also be necessary to include the selfed progeny of the parents as a means of determining whether they were homozygous or heterozygous. Rashid *et al.* (1991a) used inbred lines, which reduced the problem of heterozygosity and allowed the authors to repeat assessments of the parents. It also enabled them to undertake backcrosses. Inbred lines are constant across generations and backcrosses can be produced. It is not normally feasible to backcross an F1 to its parent in an annual outbreeding species such as the faba bean.

The disease scores used in this experiment were averages from leaves and stems which occasionally differed in their reactions. Many susceptible plants had higher scores on the stems than on the leaves and the spore suspension was sometimes held in the axil of the petiole and stem, increasing the likelihood of stem lesions. Lesions often developed on the younger leaves which had been folded at the time of inoculation. Apart from any greater susceptibility, folded leaves could hold the suspension for a longer time than unfolded leaves. This indicates that the expression of the disease was dependent on the environmental conditions. Further research is necessary to establish whether differences sometimes observed in scores on stems and leaves were purely environmental or had a genetic basis and the detached-organ tests (Chapter 6) were designed to investigate this.

The investigations in this chapter were based on a particularly virulent isolate of *A. fabae*, A26. Further studies were necessary to establish the variability present among Australian collections of this pathogen and are described in Chapter 5.

This study has shown the presence of one major dominant gene conferring resistance to Ascochyta blight in faba beans and a further two complementary recessive genes with the same effect. The existence of multiple genes in an outcrossing species makes it likely that mass selection will be an appropriate technique to increase the level of resistance in a partially resistant population and the next chapter investigates this possibility.

CHAPTER 4. MASS SELECTION FOR RESISTANCE TO ASCOCHYTA BLIGHT IN THE FABA BEAN CV FIORD

4.1 Introduction

Before the faba bean cultivar Fiord was released in 1980, its multiplication plots had been thoroughly rogued for several seasons so they were completely free of Ascochyta blight. The plants were examined at fortnightly intervals and any which showed symptoms of Ascochyta blight were immediately uprooted and destroyed. Nevertheless, by 1985, symptoms of Ascochyta blight had been found in farmers' crops and by 1987, widely distributed crops were infected. Further trials showed that Fiord was more Ascochyta-resistant than most other accessions and it was clear that plants of this cultivar varied widely in their resistance.

Mass selection has often been used in heterogeneous populations, such as those of faba beans, for the enhancement of certain characteristics, including disease resistance (Rashid and Bernier, 1984; Rashid *et al.*, 1991b). As the faba bean is partially cross-pollinated it is difficult to produce homogeneous populations and variation is expected to be present in all populations. Hanounik and Robertson (1989) demonstrated the presence of considerable heterogeneity in *V. faba* for resistance to *A. fabae*. The mixed-breeding system of *V. faba* allows for considerable recombination of minor genes, including those affecting disease reaction, at every generation. Thus it would be necessary to remove susceptible plants before flowering, so they did not pollinate the more desirable resistant plants. Regardless of whether minor genes or major genes were involved, strong selection pressure in a mass-selection system would be expected to result in increased resistance to *A. fabae* in a faba bean cultivar. This was tested using cv. Fiord. Three cycles of mass selection had been conducted in the field and progress was due for evaluation in comparison with the parent cultivar.

4.2 Materials and methods

4.2.1 Selection history

In 1987, a heavily infected crop of Fiord was harvested and 35% of the seeds bore Ascochyta lesions. From this lot, 12,000 seeds free of lesions were selected and sown at the Mortlock Experiment Station, near Clare, South Australia, where natural infection occurs regularly. The seeds were sown in rows 25 cm apart with 20 cm between seeds in a row. Every third row in the plot was a spreader row of Acc 811, which is highly susceptible. The entire plot was artificially inoculated with a spore suspension as described below. Before flowering commenced, all rows of Acc 811 and all plants of Fiord which showed any symptoms of Ascochyta infection were removed, leaving 393 plants which were pollinated normally by bees. The plants were harvested individually and evaluated for both yield and seed lesions, which were found although no lesions had been detected on the plants.

The best 145 progenies were selected and sown at the Mortlock site in 1989. Each single-plant progeny was sown in a plot with two rows 5 m long, giving 50 plants at a spacing of 25 x 20 cm. The experiment was a randomised complete block design with four replicates which were incomplete owing to inadequate seed of some progenies. Spreader rows and inoculation were as in the previous year. The selection criterion was the complete absence of Ascochyta blight in all plots of a progeny and only 8 progenies fulfilled this so all others were removed before flowering. From these 8, a total of 157 single plants were harvested.

The progeny of these 157 selections were sown in two replicates at the Mortlock site in 1990. Spreader rows, plot size and inoculation were as in the previous years. Where more than 10 plants were infected in a single plot of a progeny, both plots were removed before flowering and where 10 or fewer were infected, the individual plants were removed. This left a total of 65 selections, representing all eight families.

Inoculum of *A. fabae* was collected by isolating from infected plants in the Clare region of South Australia, multiplied on 2% Potato Dextrose Agar (PDA) and incubated in a growth chamber at 20°C for 2 weeks. Pycnidiospores were harvested by flooding the surface of agar with sterile RO water and scraping with a glass rod. Mycelial debris was removed by filtration through two layers of muslin. The

concentration varied from year to year depending on the production of pycnidia, but was never less than 1 x 10^5 sp/ml. Enough suspension was prepared to allow the use of 2-3 ml per plant. Even coverage of the field experiment was assured by spraying up and down the trial. The inoculation was undertaken by J. Dennis. The trial was inoculated each year although it was observed that the susceptible spreader Acc 811 already bore symptoms from natural infection at the time of artificial inoculation.

4.2.2 Ascochyta blight test in 1991

The effectiveness of mass selection was evaluated using 31 selections from the 1990 harvest in comparison with remnant material from the 1987 population of Fiord. Seeds of some of the selections had spots which could be confused with an Ascochyta blight infection (Plate 4.1) and the causal agent was investigated. The seeds were surface sterilised in 10% NaOCl for 5 min and rinsed with sterilised RO water 3 times, then plated on 2% PDA and incubated at 25°C. When colonies of fungi appeared there were subcultured to new PDA and incubated at 25°C under continuous fluorescent light for 10 days. The mycelia and spores were examined. *A. fabae* was obtained from more than 90% of the seeds which had typical dark brown lesions. The agent causing small spots on the seed could not be identified as no fungal mycelium or bacterial ooze could be detected. The selections were classified according to the different seed lesions before sowing (Table 4.1). The objective was to examine the relation, if any, between these spotted lesions and Ascochyta.

The trial was sown in an open bird-proof cage at the Waite Agricultural Research Institute on 2 May 1991. There were two replications in a completely randomised design. Each replication contained 52 plots comprising the 31 selections and 21 plots of the original (1987) seed stock of Fiord. A plot consisted of 2 rows 25 cm apart of 5 plants 20 cm apart. Between the test plots was a row of Acc 811 to act as a spreader of the disease. No fungicides or insecticides were used in the experiment.

Ascochyta fabae isolate A26 was subcultured onto 2% PDA and incubated in continuous fluorescent light at 25°C for 2 weeks. Pycnidiospores were harvested by flooding the surface of the agar with sterile RO water and scraping the surface with a glass rod. Mycelial debris was removed by filtration through two layers of muslin. The concentration of pycnidiospores was adjusted to 5 x 10^5 sp/ml and confirmed with a haemocytometer. When the plants were at the 3-4 leaf stage, four weeks after

sowing (30 May 1991), they were inoculated at dusk by spraying 3 ml of pycnidiospore suspension on each plant, or until run-off, using a hand-pressure sprayer.

Table 4.1 The 31 selections of faba bean cv. Fiord, arranged in their 8 families and according to the presence or absence of seed spots attributed to the unknown causal agent.

Family	Very clean selections	Clean selections	Slightly spotted selections	Heavily spotted selections
24	86, 134	132	-	-
120	-	87	53, 93	51
181	9, 10, 54	-	5	-
193	15	95	144	58
194	-	60	61, 110	109
260	-	25, 67, 70	114	-
262	157	71, 122	-	155
314	80	131	82	84

The following day and for three days, the plants were sprayed during the day with a fine mist using an overhead sprinkler, for 15 min every hour, to maintain a high humidity and to encourage the development of the disease.

Each plant was given a disease score at 15 and 21 days after inoculation (DAI) following the standard criteria (Table 3.1), except that scores of 3, 4 and 5 were pooled to give a category of 3+. An assessment of overall disease per plant was obtained by calculating a leaf and stem average. Plants were harvested individually and counts made of the seeds infected with Ascochyta and those affected by the unknown agent.

Statistical analyses were carried out using GENSTAT. A Poisson model was fitted and comparisons made of the observed χ^2 values using accumulated analysis of

Plate 4.1. Ascochyta infected seeds (A) and the unknown causal agent on seeds (B).

A

B

deviance. The number of plants of the selections (616) and the original Fiord population (420) were not equal and to enable comparisons, the distributions are presented as the frequencies of plants with each score. Correlation coefficients between seedling infection at 15 DAI, 21 DAI and seed infection were calculated using the mean values of each selection.

4.3 Results

At 15 DAI, most plants of the selections had a score of 0 or 1 while plants of the original population had scores of 1 or 2 (Figure 4.1, Table 4.2, Appendix 4.1). The susceptible accession, Acc 811, included in the trial to spread the disease, had scores of 2 or 3, indicating that the inoculation had been effective.

By 21 DAI, the disease severity had increased in the selections and the original population (*cf*. Figures 4.1 and 4.2) and there were major differences between the selections and the original population. On Acc 811, many large lesions with abundant sporulation were evident on both leaves and stems with most plants scoring 4 or 5. Many of the infected stems died when they broke at the lesion (Plate 4.2).

In the analysis of deviance of these results (Table 4.3), neither the main effects of groups nor of scores had any fundamental biological significance and it was only the group x score interaction that was of interest, as it indicated whether the selection-group or original-group distribution differed for the various scores. The analysis showed that the selections had a significantly different score distribution from the original cultivar and the data showed that this change was toward lower scores.

The results were further analysed to determine whether there were differences between the 31 selections and also between the 21 entries of the original population grown in the trial (Tables 4.4 and 4.5). No differences were found between entries of the original population, as expected since each entry was a random sample from that population. For the original Fiord population the interaction between entries and score was found to be non-significant at both 15 and 21 DAI (Table 4.5). The interaction between selection and score was highly significant ($p << 0.001$) indicating that the selections varied in their score distribution.

Plate 4.2. The Ascochyta blight test trial in 1991. The susceptible control, Acc 811 (arrows) was severely infected compared with the selected or original plants.

Table 4.2 The distribution of plants of the selected and original populations of Fiord at different scores at 15 DAI and 21 DAI.

	Population	Number of plants with score of				Total
		0	1	2	3^+	
15 DAI	Selection	288	287	39	2	616
	Original	35	292	74	19	420
	Total	323	579	113	21	1036
21 DAI	Selection	135	266	209	6	616
	Original	8	104	200	108	420
	Total	143	370	409	114	1036

Table 4.3 Accumulated analysis of deviance of disease scores for the selected and original populations at 15 DAI and 21 DAI.

	Change	d.f.	deviance	mean deviance
15 DAI	population	1	37.31***	37.31
	score	3	781.26***	260.42
	population*score	3	215.81***	71.94
	Total	7	1034.38	147.77
21 DAI	population	1	37.31***	37.31
	score	3	280.69***	93.56
	population*score	3	283.87***	94.62
	Total	7	601.87	85.98

Table 4.4 Accumulated analysis of deviance of disease scores for the 31 selections at 15 DAI and 21 DAI.

	Change	d.f.	deviance	mean deviance
15 DAI	selection	30	0.18ns	0.01
	score	3	593.41***	197.80
	selection*score	90	220.54***	2.45
	Total	123	814.13	6.62
21 DAI	selection	30	0.18ns	0.01
	score	3	343.92***	114.64
	selection*score	90	158.78***	1.76
	Total	123	502.88	4.09

Table 4.5 Accumulated analysis of deviance of disease scores for the 21 entries of the original Fiord population at 15 DAI and 21 DAI.

	Change	d.f.	deviance	mean deviance
15 DAI	entry	20	0.00ns	0.00
	score	3	403.66***	134.55
	entry*score	60	76.48ns	1.28
	Total	83	480.14	5.79
21 DAI	entry	20	0.00ns	0.00
	score	3	220.65***	73.55
	entry*score	60	76.49ns	1.28
	Total	83	297.13	3.58

ns non significant; *** $p << 0.001$

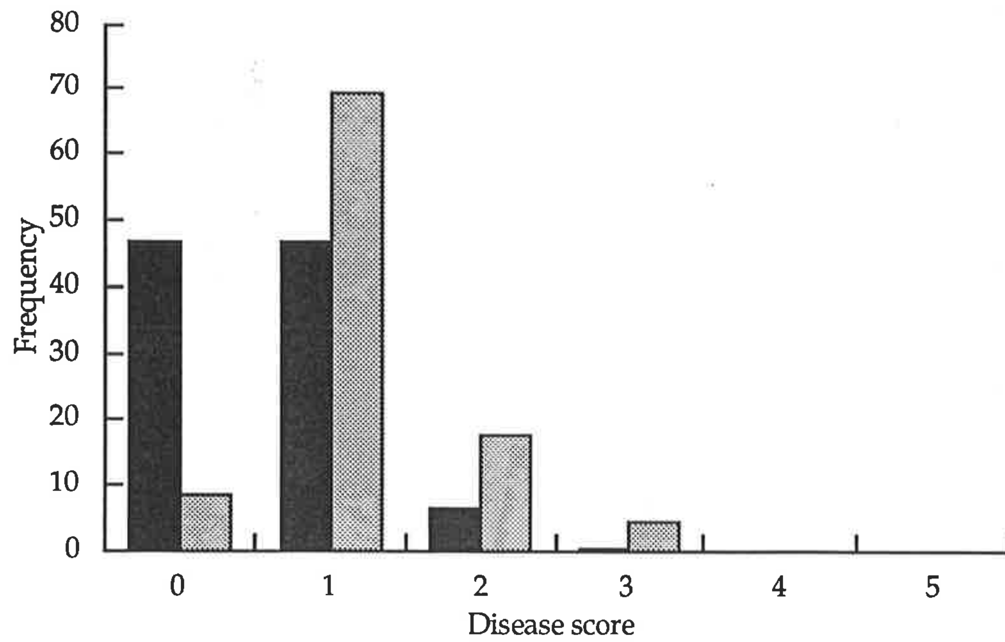


Figure 4.1 The frequency distributions for disease scores of the selections (■) and original (▩) Fiord population 15 DAI.

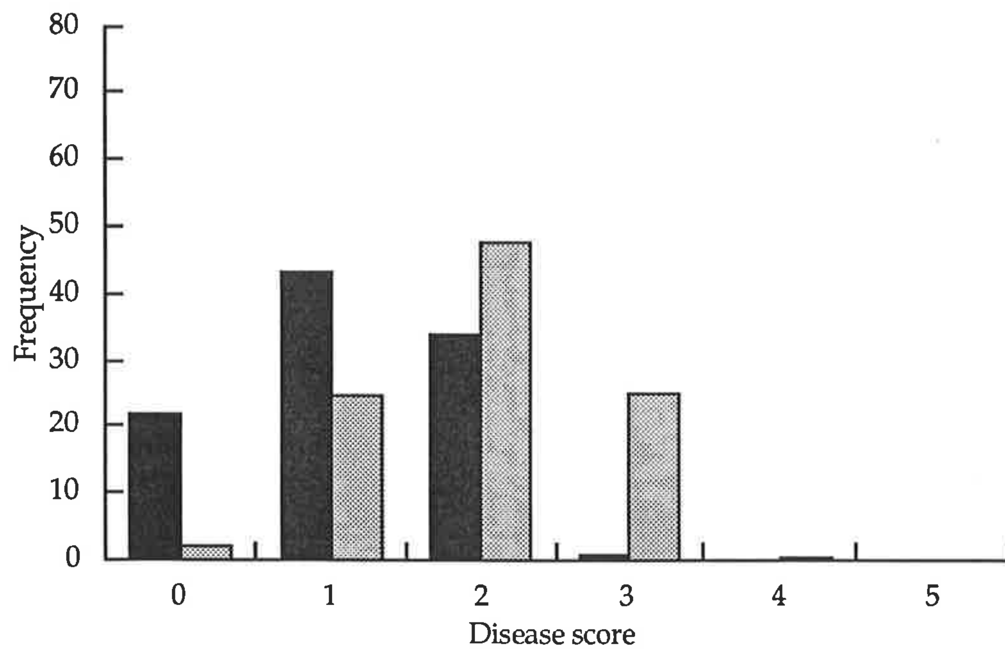


Figure 4.2 The frequency distributions for disease scores of the selections (■) and original (▩) Fiord population 21 DAI.

Three generations of selection reduced the mean score (21 DAI) from 1.97 to 1.14, increased the proportion of resistant plants from 74.3% to 99.0% and percentage of uninfected plant from 1.9% to 21.9% (Table 4.2). From the original population of Fiord 20% of the seeds were infected with Ascochyta, while the infection of the 31 selections ranged from 0.9 to 34% (Table 4.6). Most of the plants of the susceptible spreader, Acc 811, died before podding, but a few survived or developed new basal shoots to have seeds at harvest and 25% of these seeds were infected with Ascochyta. This percentage was lower than expected because many of the late shoots escaped infection.

Seedling scores of the selections were not significantly correlated with the percentage of seed infected by Ascochyta (r = 0.17, Table 4.7). Some selections with scores at 21 DAI between 1.1 and 1.5 had 20-34% of their seeds infected while other selections with scores of 1.0-1.3 had only 0.9-1.7% infected seed. Selections 67 and 132 which had the two highest seedling scores of 1.8 and 1.7 had only 2.9 and 3.5% respectively of their seeds infected.

The disease scores at 15 DAI and 21 DAI were highly correlated (Table 4.7). There was no indication that the incidence of either the unknown causal agent or of seed infection with *A. fabae* was significantly correlated with the seedling score at either 15 DAI or 21 DAI. The incidence of the unknown spot was not significantly correlated with any other measured variable, nor was it related to the original incidence. For example selection number 54, categorised as having no spots before sowing (Table 4.1), showed the highest percentage of seed with spots at harvest (Table 4.6).

In this study the 31 selections were derived from 8 families. It was found that there was a variation in disease level of selections within a family. Family 260 showed great differences among its component selections 25, 67, 70 and 114. Selection 25 had the lowest disease score and 67 the highest score among all the selections (Table 4.6). Similarly, for seed infected with *A. fabae,* family 24 showed great differences among its progeny, with selection 134 showing the lowest percentage of seed infection, 132 intermediate, and 86 the highest.

Table 4.6 Ascochyta blight disease scores of the 31 selections from eight different families at 15 and 21 DAI, together with the percentage of seed infected with *A. fabae* and affected by the unknown causal agent. The figures are means of 19-20 plants.

Family	Selection	Average disease score		% of infected seed	
		15 DAI	21 DAI	Ascochyta	Unknown
24	86	0.7	1.5	20.0	3.7
	132	1.1	1.7	3.5	3.2
	134	0.3	1.0	1.1	0.5
120	51	1.1	1.4	7.0	1.4
	53	0.5	0.8	7.5	4.2
	87	0.5	1.0	2.7	1.1
	93	0.8	1.3	8.9	2.9
181	5	0.3	1.3	2.0	1.5
	9	0.8	1.3	4.5	0
	10	0.6	1.0	0.9	0.2
	54	0.1	0.8	1.6	10.2
193	15	0.6	1.0	7.4	0.7
	58	0.7	1.6	5.6	3.2
	95	0.6	1.0	2.9	4.9
	144	0.8	1.2	5.1	1.9
194	60	0.3	0.7	3.1	0.3
	61	0.5	1.2	5.4	7.0
	109	0.6	0.8	13.2	0.5
	110	0.5	1.0	5.3	4.6
260	25	0	0.4	9.8	2.5
	67	1.3	1.8	2.9	3.1
	70	1.0	1.4	34.0	7.6
	114	0.3	1.1	7.6	2.4
262	71	0.3	0.7	3.1	3.7
	122	0.5	1.1	26.4	0
	155	0.3	0.9	5.5	10.2
	157	0.9	1.3	1.7	0.7
313	80	0.8	1.4	6.8	5.1
	82	1.0	1.4	5.8	4.5
	84	0.7	1.1	4.8	2.0
	131	0.8	1.5	17.9	0

Table 4.7 The correlation coefficients of the average disease scores at 15 DAI, 21 DAI, the percentage of seed infected with *A. fabae* and the percentage of seed affected by the unknown causal agent, for the 31 selections from cv. Fiord.

Variable	Disease score 15 DAI	Disease score 21 DAI	Ascochyta seed lesions	Unknown seed spot
Disease score 15 DAI	1			
Disease score 21 DAI	0.85**	1		
Ascochyta seed lesions	0.17	0.17	1	
Unknown seed spot	-0.14	-0.06	0.05	1

** $p < 0.01$

4.4 Discussion

The results show that mass selection over three generations had been effective at increasing the level of resistance to Ascochyta blight, with the selected populations being more resistant than the original population. The rapid success of the mass-selection program would indicate that relatively few genes were involved. Variation among the selections remained, both within each selection and between selections from the same family derived from a single original parent plant. Further selection beyond the three cycles would thus be warranted. The continued variation suggests that resistance to Ascochyta blight was dominant in Fiord and that the variation was due to segregation from resistant heterozygotes. The variation could also be a consequence of escapes, with undetected susceptible plants producing susceptible offspring. If these plants were cross-pollinated with resistant plants there would be segregation for resistance in the next generation.

In those species which are highly cross-pollinated and resistance to a disease is dominant, self-pollination may be practised to reveal the recessive susceptible individuals. In Fiord and other faba beans, where self-pollination occurs naturally and

is commonly of the order of 60-70%, this may not be necessary as sufficient selfing occurred during the mass-selection procedure.

Other workers (Kharbanda and Bernier, 1980; Zakrzewska, 1986; Hanounik and Robertson, 1989) have noted variation in disease resistance within cultivars. The variation may arise from genetic differences in resistance to infection or from morphological features which might facilitate disease escape, for example dwarf entries showed greater susceptibility (Lockwood *et al.*, 1985).

This study did not reveal any association between leaf infection and seed infection as the correlation between seedling score and seed infection score among the 31 selections was not significant. Jellis *et al.* (1985) also found no association between leaf and pod infection in spring beans. Similarly, Van Breukelen (1985) reported the absence of any correlation between early leaf score and pod score but found a moderate correlation with a later leaf score. However, Lockwood *et al.* (1985) found that seed infection was strongly correlated with leaf and pod infection in winter beans. The reasons for the discrepancies in these results might be the differences in environmental conditions during leaf development and pod growth. The winter bean paper reported much more infection than the spring bean papers, which could be associated with the cooler and wetter conditions during pod setting for winter beans than spring beans. In South Australia where weather conditions are often quite dry, the spread of the pathogen may not be promoted, but in wetter conditions, e.g. northern NSW, there may be a stronger correlation between leaf infection and pod or seed infection.

It is also possible that resistance in the leaves and pods is determined by different plant characters and genetic systems. If the plants being studied were very different in their levels of resistance and severe infection in the seedling stage caused the main shoot to be replaced by secondary shoots, the pods would be formed under different environmental conditions. These plants would confound the correlation between leaf, pod and seed infection.

It is commonly observed that broad bean stain virus (BBSV) as well as other viruses, chocolate spot and rust can give some staining of pods and seeds. So can physiological damage from e.g. oedemas, hail or *Heliothis* caterpillars. Nevertheless, these different causes can be distinguished by standard methods of culturing the seeds

and in this case the seed spotting caused by the unknown agent was not associated with Ascochyta blight. Nor was there a good association between spots on the seed that were sown with spots on the seed harvested. Since producers aim to have seed that is completely free from spots and blemishes, the cause of this unidentified spot needs to be resolved.

Mass selection is also useful for traits other than disease e.g. maturity, plant type and chemical composition. The only published results on mass selection for resistance to Ascochyta have been the present one on Fiord and the one undertaken by Rashid *et al.* (1991b). Mass selection could have been successful only because the accessions were heterogeneous and were carrying a small number of major genes for resistance and thus it will not always be equally successful in other populations. The matter is important as there are many cultivars, well adapted to local environments with suitable seed sizes, colour and chemical composition but with uniform susceptibility to Ascochyta. When the simple procedure of mass selection results in cultivars with the same desirable attributes, and with Ascochyta resistance, it provides a simpler approach to breeding than crossing of the cultivars to parents with known resistance and selecting in the segregating generations.

The results from this study suggest that the following strategy could be used with success in selecting for resistance to *A. fabae*. To minimise restrictions on the number of plants evaluated, the progenies of selected plants could be tested in the seedling stage in outdoor nurseries. The resistant selections could be transferred before flowering to pots in a bee-proof enclosure, to ensure self-pollination. This procedure would enable the detection of any families that were misclassified originally as a result of escaping the infection. Following nursery evaluations, selection would be grown in an open field trial to allow inter-pollination to reduce the inbreeding depression and to evaluate other agronomic characteristics. The possible advantage of this procedure over mass selection in the field is that self pollination is strictly imposed and with a pedigree type of selection program heterozygous material could be efficiently identified. In conclusion, mass selection is ideal for use in certain populations of faba beans since it will make rapid progress towards disease resistance.

CHAPTER 5. CULTURAL AND PATHOGENIC VARIABILITY OF *ASCOCHYTA FABAE* ISOLATES

5.1 Introduction

One of the causes of the failure of plants to maintain disease resistance is the variability of the pathogen. Mutation, heterokaryosis and hybridisation can all contribute to the development of new races of a fungal pathogen such as *Ascochyta fabae*. Both the sexual and asexual stages of this fungus have been found in Australia, indicating that sexual recombination can contribute to the development of variability, but there have been no studies as yet published on the races of *A. fabae* present in this country. This information needs to be established in order to ensure that testing for resistance conducted overseas, particularly at the large genetic resource collection of ICARDA, will be valid for Australian conditions. This chapter deals with attempts to distinguish races on morphological characters when grown in culture and when inoculated onto plants of different accessions. It also deals with the expression of some of the components of resistance.

5.2 Materials and methods

5.2.1 Growth in culture of isolates of *Ascochyta fabae*

Parts of bean plants, infected with Ascochyta, were collected from 21 different areas of SA and also one each from NSW and WA. In each area attempts were made to obtain samples from stems, leaves, pods and seeds. The plant parts were surface-sterilised in 10% sodium hypochlorite (NaOCl) solution for 5 min, rinsed in sterile RO water three times and plated on 2% PDA. It was not always possible to isolate and obtain viable cultures from each of the plant parts and 52 colonies were produced. After incubation for 5-7 days at 20°C, pure colonies were transferred to a new PDA plate. Subcultures of these pure colonies were incubated for 14 days, then they were streaked onto a new PDA plate. Three days later, three to five single spores were isolated from each of these colonies and incubated onto new plates. After incubation

for 21 days at 20°C, an agar disc from the margin of the single-spore isolate was placed in the centre of a 90 mm plate containing 20 ml of medium and incubated at 20°C, under continuous light. This was replicated five times. Altogether therefore there were 52 colonies, each one represented by 3-5 single-spore cultures (total of 254 spores) with 5 replicates of each.

Growth of the culture was evaluated by measuring two perpendicular diameters of each colony at 5, 8, 11 and 14 days after incubation. For sporulation assessment, each Petri dish was flooded with 50 ml water and then filtered through 2 layers of muslin. The concentration of harvested spores was estimated using a haemocytometer, twice for each Petri dish. Spore size was measured using micrometre eyepiece on 50 spores for each culture (Petri dish) so there were 250 spores per sample and the range and mean were calculated. Scores were made of the amount of aerial mycelia and the numbers of pycnidia produced at 14 days, in a similar method to that described by Kharbanda and Bernier (1980).

Data were subjected to analysis of variance and the variance components estimated (Appendix 5.1).

5.2.2 Evaluating the components of resistance of faba beans and the differences in pathogenicity of isolates of *Ascochyta fabae*

Eight faba bean accessions, representing the known range of resistance to Ascochyta were evaluated (Table 5.1). Four accessions were selected because they were the host differential set studied by Hanounik and Robertson (1989). They found Acc 299 to be resistant to all isolates of *A. fabae* and Acc 508 together with Acc 333 were resistant to some isolates, but not all. Acc 342 was susceptible to all isolates. Acc 290 was introduced recently as a supposedly resistant selection from ICARDA. The other three accessions were from the Waite Institute collection and have been tested for their resistance. They were the highly resistant Acc 970, the highly susceptible Acc 811 and the moderately resistant Fiord, which is the only widely grown cultivar in Australia.

Seeds of the accessions were scarified to ensure even germination, surface sterilised with 10% NaOCl for 5 min, rinsed in sterile RO water three times and soaked in water overnight. They were sown 3 cm deep in plastic trays filled with UC soil, four accessions to a tray and five plants of each accession. The experiment was arranged in

a split-plot design in two replicates in a glasshouse with eight isolates as the main plots and eight accessions as subplots. Thus there were ten plants per treatment, except Acc 333, where there were fewer, because of poor germination.

Table 5.1 The origins of the eight faba bean accessions.

Accession	Other Designations	Country of origin
Acc 290	ICARDA, BPL 230 (A 884)	Unknown
Acc 299	ICARDA, BPL 471 (A 8810)	Lebanon
Acc 333	ICARDA, ILB 1814	Syria
Acc 342	Giza 4, ICARDA ILB 1820	Egypt
Acc 508	ICARDA, BPL 818 (A 8815)	Ethiopia
Acc 811	NEB 463	Afghanistan
Acc 970	ICARDA, ILB 752	Syria
Fiord	-	Naxos, Greece

Table 5.2 The origins of the eight *A. fabae* isolates.

Isolate	Location	Collected part
A11	Coonalpyn, SA	single spore isolated from stem
A14	Keith, SA	single spore isolated from pod
A26	Mintaro, SA	single colony isolated from seed, provided by J. Dennis
A28	Naracoorte, SA	single spore isolated from pod, provided by J. Heap
A41	Spalding, SA	single spore isolated from seed
A48	Auburn, SA	single spore isolated from pod
NSW	Cowra, NSW	single spore isolated from the culture isolated from leaf, provided by Dr. A. Nikandrow
WA	Katanning, WA	single spore isolated from seed, provided by G. Walton

Eight isolates of *A. fabae* were selected to cover a wide range of locations of origin and cultural characteristics (Table 5.2). They were cultured on 2% PDA in a 25°C room for 2 weeks. The spore suspension was prepared as described in Chapter 3. The concentration of spore suspension was adjusted to 4-5 x 10^5 sp/ml for each isolate. Plants were inoculated with the spore suspensions, using a hand sprayer, at the 2-leaf stage, three weeks after sowing. For the next three days, during daylight hours the plants were sprayed from an overhead sprinkler for 5 min, every hour, to maintain a high humidity. Incubation period, lesion number, lesion size, disease efficiency and disease score were assessed in the following ways.

The incubation period (ICP) was the time in days from inoculation to the appearance of the first symptom. Flecking and bronzing were not considered to be symptoms and the results for infected plants only were analysed. If no symptoms were visible on a plant, it was given the value 0. These results have not been analysed as a two-way analysis of variance because of the many missing values that occurred when the plots were resistant. The standard error of the means given in the table may be used to judge whether any two results are significantly different.

The lesion number (LN) was recorded on leaves (LLN), stems (SLN) and the whole plant (WLN) fifteen days after inoculation (15 DAI). Where lesions had coalesced, the numbers of foci were counted.

The lesion size (LS) was recorded on stems and leaves separately, 15 DAI. Stem lesion size (SLS) was measured as the length of the lesion. Leaf lesion size (LLS) was determined as the mean of two perpendicular diameters. Again, where lesions had coalesced, their sizes were estimated using the number of visible foci.

The disease efficiency (DE) was estimated as the proportion of lesions bearing pycnidia at 15 DAI on stems (SDE), leaves (LDE) and the whole plant (WDE). The ANOVA and method of calculation of Tukey's Honestly Significant Difference Test are given in Appendices 5.2 and 5.3 respectively. The character did not warrant rigorous statistical analysis given the large differences involved and that the ratios were based on a varying, and in some instances, a very small number of lesions. Furthermore a value of zero could be obtained because an accession/isolate combination had not developed any lesions or because lesions had developed but no

pycnidia had been formed. The marginal means were calculated as the average of DE from all plants tested as given in Table 5.4.

The disease score was assessed for each plant at 15 DAI for stems (SS), leaves (LS) and as an average for the whole plant (WS), following the description in Table 3.1. The ANOVA and Tukey's Honestly Significant Difference Test are given in Appendix 5.4.

In the analyses and in averaging the results for some characters, account was taken of the number of plants infected. For DE and Score, the averages are for all the plants tested, but for ICP, LS and LN only the infected plants were averaged and analysed (*i.e.* on the basis of numbers given in Table 5.4). Because of this variation in numbers, weighted mean values were obtained for the accessions and isolates.

Means were calculated for the components and overall disease scores for each isolate on each host. The correlations between the components were calculated, using the results from infected plants only. Mean scores were also calculated across all of the tested plants in each combination and scores of 0-2.0 were classified as resistant and scores of 2.1-5 as susceptible.

5.3 Results

5.3.1 Growth in culture of isolates of *Ascochyta fabae*

At 14 days, there were highly significant differences in colony size among the 52 colonies (Table 5.3). Furthermore, there was significant variation among cultures from single spores taken from the same colonies. The colonies contributed the greater proportion of the variance.

Pycnidiospore production varied from none to 1 x 10^6 sp/ml, even among replicate cultures from a single-spore isolate, so statistically significant variation was not found. The mean size of pycnidiospore varied much less, with a range of 3-6 x 10-26 µm, but again no statistically significant differences were obtained. The amount of aerial mycelia and the number of pycnidia in the cultures varied from abundant to sparse. It was noted that some of the pycnidia were brown and others black.

Table 5.3 Summary analysis of variance of colony size (mm) in cultures of *A. fabae* measured 14 days after incubation.

Source of variation	d.f.	SS	MS	F	% total variance
Colonies	51	306498.47	6009.77	18.3***	75.05
Single spores	202	66399.74	328.71	22.7***	20.28
Error	1016	14715.50	14.48		4.67
Total	1269	387613.72			

*** $p < 0.001$

Differences between the colony or single spore cultures were not related to the locality of origin of the infected plants, as single spores from the same colony behaved differently. This study illustrated that there were differences in cultural characters but no attempts have been made to group or designate the isolates because of the great variation present.

5.3.2 Rate of infection of plants

Accession 970 was the most resistant with only 4 plants infected and Acc 811 the most susceptible with only 3 plants not infected, closely followed by Acc 342 (Table 5.4). Isolate A26 was the most virulent, closely followed by A14, while isolate WA infected the fewest plants. There was evidence of a host x pathogen interaction, with Acc 290 not being infected by isolate A28 while Fiord was not infected by WA. Although most of the accessions were supposed to be pure lines, most responses to the isolates were quantitative with only a proportion of plants becoming infected.

5.3.3 Incubation period

First symptoms were visible about 7 days after inoculation. The longest incubation period was 15 days. On average, Acc 811 had the shortest ICP and Acc 970 the longest. Among isolates, A14 had the shortest ICP and WA the longest. The ICP for Acc 811 and Acc 342, the two susceptible accessions, were always in the first four rankings for all the isolates tested, with, in each instance, a shorter ICP for Acc 811

Table 5.4 Infection of eight faba bean accessions with eight isolates of *A. fabae*. Ten plants were used for each accession / isolate combination except for Acc 333 in which there were fewer plants available.

Accession	Proportion of plants infected by isolate								Infected/
	A11	A14	A26	A28	A41	A48	NSW	WA	total
Acc 290	0.20	0.30	0.70	0	0.50	0.30	0.30	0.40	27/80
Acc 299	0.60	1.00	0.80	0.60	0.50	0.60	0.10	0.20	44/80
Acc 333	0.38	0.77	0.77	0.75	0.83	0.50	0.55	0.14	37/62
Acc 342	0.90	1.00	0.90	1.00	1.00	0.60	1.00	0.70	71/80
Acc 508	0.40	0.20	0.50	0.20	0.30	0.20	0.10	0.10	20/80
Acc 811	1.00	1.00	1.00	1.00	0.90	1.00	0.80	1.00	77/80
Acc 970	0.20	0.20	0	0	0	0	0	0	4/80
Fiord	0.40	1.00	0.90	0.40	0.80	0.50	0.50	0	45/80
Infected/ total	40/78	54/79	55/79	38/78	45/76	35/76	33/79	25/77	325/622

than for Acc 342. These two accessions were similar therefore in the proportion of plants that became infected, their response to the different isolates were in the same rank order, but each isolate showed symptoms more rapidly on Acc 811 than on Acc 342. The average value of ICP, based only on infected plants ranged from 7.6 to 14 days for the accession/isolate combinations (Table 5.5).

5.3.4 Lesion number

On average, leaves bore twice as many lesions as stems. Accession 342 had the most lesions on leaves and stems, while Acc 290 had fewer lesions per plant, on average, than the few infected plants of Acc 970. Among the isolates A26 produced most lesions and WA the fewest. Isolate A14 infected Acc 970 only on the leaf and A11 only on the stem, while isolate WA infected the stem of Acc 299 and Acc 508 but not the leaves. Furthermore, isolate NSW produced very many lesions on the stems of

Table 5.5 Incubation period (days) of eight faba bean accessions inoculated with eight isolates of *A. fabae*. The values presented are the means ± standard errors of the infected plants only; • indicates that no lesions were observed; W. mean = Weighted mean.

Accession	Incubation period (days) following inoculation with isolate								W. mean
	A11	A14	A 26	A28	A41	A48	NSW	WA	
Acc 290	12.0±2.0	9.0±0.0	9.9±0.5	•	9.8±0.5	13.0±0.6	13.0±0.6	12.0±0.7	10.9±0.4
Acc 299	13.3±0.3	8.7±0.3	9.5±0.3	9.0±0.3	8.8±0.4	10.3±0.6	9.0	12.5±1.5	9.9±0.3
Acc 333	13.0±1.0	10.4±0.3	10.1±0.8	10.3±0.8	10.2±0.4	8.7±0.3	10.8±0.7	14.0	10.5±0.3
Acc 342	9.1±0.5	8.3±0.3	9.9±0.4	8.7±0.3	9.0±0.4	9.5±0.6	9.5±1.0	10.4±0.5	9.2±0.2
Acc 508	11.5±1.5	11.5±2.5	10.4±1.1	10.5±1.5	10.3±0.9	11.0±3.0	10.0	9.0	10.7±0.5
Acc 811	8.0±0.4	7.6±0.5	8.8±0.5	8.7±0.6	8.6±0.5	8.6±0.3	9.3±0.5	10.2±1.0	8.7±0.2
Acc 970	12.5±0.5	10.0±0.0	•	•	•	•	•	•	11.3±0.8
Fiord	10.5±0.3	9.8±0.4	10.2±0.5	9.5±0.9	9.9±0.5	9.4±0.5	10.6±0.9	•	10.0±0.2
W. mean	10.5±0.4	9.0±0.2	9.8±0.2	9.2±0.3	9.4±0.2	9.7±0.3	10.1±0.4	10.8±0.5	9.7±0.1

Acc 333 with very few on the leaves, while it produced numerous lesions on Acc 299 leaves but few on the stems (Table 5.6).

5.3.5 Lesion size

The average stem lesion length was twice the average leaf lesion diameter (Table 5.7). The few lesions to form on Acc 970 were very big, Acc 811 had the next biggest stem lesions and isolate A28 made the biggest lesions. There was a tendency for the susceptible accessions, Acc 342 and Acc 811 to have larger lesions on the stems than the more resistant accessions but this was not found with leaf lesions. The most virulent isolates A26 and A14 did not result in larger lesions than the less virulent WA and NSW.

Table 5.6 Numbers of lesions on stems, leaves and whole plants of eight faba bean accessions inoculated with eight isolates of *A. fabae* evaluated 15 DAI. The values presented are the means ± standard errors of the infected plants only; • indicates that no lesions were observed; W. mean = Weighted mean.

Accession	Mean lesion number following inoculation with isolate								
	A11	A14	A26	A28	A41	A48	NSW	WA	W. mean
Stem									
Acc 290	4.0±3.0	2.7±1.2	4.4±1.3	•	3.3±1.5	1.7±0.3	•	2.0±0.4	3.1±0.5
Acc 299	1.8±0.3	4.1±0.9	5.1±1.1	3.2±0.9	2.3±0.3	4.0±1.5	1.0	2.0±1.0	3.6±0.4
Acc 333	5.0±2.0	5.0±1.7	6.2±2.5	3.5±0.7	8.0±2.0	4.0±1.0	12.0±0.0	•	5.6±0.8
Acc 342	9.0±2.4	6.9±1.9	7.8±3.9	5.1±1.5	10.4±4.3	4.2±1.5	3.3±1.2	4.0±1.1	6.9±1.0
Acc 508	2.5±1.5	2.0±0.0	12.5±2.5	7.0±0.0	12.0±0.0	2.0±0.0	•	2.0	6.1±1.7
Acc 811	6.4±1.7	9.7±1.4	5.4±2.1	2.7±0.9	3.4±1.9	5.0±1.7	2.5±0.5	3.3±0.8	5.3±0.6
Acc 970	6.0±5.0	•	•	•	•	•	•	•	6.0±5.0
Fiord	2.0±0.0	3.7±1.3	6.5±3.9	6.0±3.0	2.3±0.8	3.0±1.0	2.5±1.5	•	3.8±0.8
W. mean	5.8±1.0	5.6±0.7	6.2±0.9	4.3±0.6	5.9±1.5	3.9±0.7	3.7±1.2	3.1±0.5	5.1±0.3
Leaf									
Acc 290	•	•	2.7± 1.2	•	2.0±0.5	12.0±0.0	1.0±0.0	•	2.8±0.8
Acc 299	8.0±5.0	8.5±4.3	10.4± 6.7	15.0±12.0	11.7±7.2	3.8±1.1	12.0	•	9.2±2.2
Acc 333	4.0±1.2	3.2±0.9	32.2±24.8	14.4± 6.2	3.5±1.3	7.0±2.0	2.0±0.6	1.0	10.0±4.4
Acc 342	11.9±3.3	31.0±5.9	15.7± 8.0	15.4± 4.3	16.2±3.6	11.0±7.3	11.9±5.4	2.0±0.6	15.8±2.1
Acc 508	5.3±3.0	3.0±0.0	9.0± 5.8	1.0± 0.0	4.7±2.0	2.0±1.0	4.0	•	5.2±1.8
Acc 811	11.5±2.3	18.2±3.3	23.8± 3.4	3.3± 1.3	29.1±7.7	9.2±2.0	8.4±3.5	3.8±1.1	14.3±1.7
Acc 970	•	6.5±4.5	•	•	•	•	•	•	6.5±4.5
Fiord	5.0±2.0	4.9±1.9	11.2± 3.6	3.7± 1.7	4.8±1.4	1.5±0.5	2.8±0.9	•	5.7±1.1
W. mean	9.2±1.4	14.6±2.4	15.0±3.2	10.1±2.2	13.2±2.5	7.0±1.6	6.9±1.9	2.9±0.7	11.1±0.9

Table 5.6 continued

Accession	Mean lesion number following inoculation with isolate								
	A11	A14	A26	A28	A41	A48	NSW	WA	W. mean
Whole plant									
Acc 290	4.0±3.0	2.7±1.2	5.9±2.2	•	3.3±0.8	5.7±4.2	1.0±0.0	2.0±0.4	3.8±0.8
Acc 299	3.8±1.9	8.8±2.7	11.6±4.4	7.7±5.1	8.4±4.5	5.8±1.9	13.0	2.0±1.0	7.8±1.4
Acc 333	7.3±3.2	5.1±1.0	29.7±23.1	14.3±5.3	6.0±1.3	11.0±3.0	4.4±2.7	1.0	11.3±4.1
Acc 342	19.9±4.1	37.9±6.7	20.9±10.7	19.5±4.3	25.6±6.7	12.7±7.6	13.0±5.6	4.6±0.8	20.3±2.5
Acc 508	5.3±3.3	2.5±0.5	14.0±7.8	4.5±3.5	8.7±5.8	3.0±2.0	4.0	2.0	7.2±2.3
Acc 811	16.6±3.7	25.0±4.6	25.2±5.1	3.1±0.9	31.0±8.1	12.3±2.3	9.0±3.7	4.9±1.3	15.9±1.8
Acc 970	6.0±5.0	6.5±4.5	•	•	•	•	•	•	6.3±2.8
Fiord	5.5±2.3	6.5±1.6	14.1±5.4	7.0±4.5	5.4±1.4	2.4±0.6	3.2±0.6	•	7.0±1.3
W. mean	11.3±1.7	15.6±2.4	17.6±3.5	10.5±2.0	15.1±2.7	8.6±1.6	7.7±2.0	3.8±0.6	12.3±0.9

5.3.6 Disease efficiency

Accessions 342 and 811 both had high DE on stems and leaves, while Acc 970 had low DE (Table 5.8). Isolate A14 gave high DE on stems and A26 on leaves. DE was low from isolate NSW on stems but moderately high on leaves while the lowest DE on leaves was from isolate WA. Acc 290 with isolate A26 had no lesions with pycnidia on the stem but had a high DE on the leaves.

5.3.7 Disease score

Accessions 970 and 290 had low scores on both stems and leaves (Table 5.9). Some interactions were evident, for example, Acc 811 and Acc 342 with isolate NSW had a low score on stems and a high score on leaves. Acc 299 had higher scores on stems than on leaves, the reverse of Fiord. On average, Acc 342 and Acc 811 had the two highest scores and isolates A26 and A14 were associated with the two highest scores.

Table 5.7 Lesion size (mm) on stems and leaves of eight faba bean accessions inoculated with eight isolates of *A. fabae* evaluated 15 DAI. The values presented are the means ± standard errors of the infected plants only; • indicates that no lesions were observed; W. mean = Weighted mean.

Accession	Lesion size (mm) following inoculation with isolate								
	A11	A14	A26	A28	A41	A48	NSW	WA	W. mean
Stem									
Acc 290	3.2±0.9	4.3±0.3	4.9±1.0	•	5.1±2.5	3.5±0.9	•	7.5±1.3	5.0±0.6
Acc 299	5.0±1.9	6.6±0.9	4.8±0.7	4.7±0.9	4.0±1.2	4.5±1.0	4.0	4.2±1.9	5.1±0.4
Acc 333	2.7±0.1	4.8±1.1	5.9±1.3	6.4±0.5	3.4±0.2	5.9±0.7	6.0±0.0	•	5.2±0.5
Acc 342	7.0±0.6	6.9±0.9	5.5±0.8	10.0±1.1	6.9±0.9	4.8±0.8	5.8±1.9	5.9±1.1	6.9±0.4
Acc 508	6.3±0.3	10.0±0.0	7.1±0.1	4.6±0.0	5.6±0.0	8.0±0.0	•	2.0	6.3±0.7
Acc 811	9.2±1.3	7.7±0.8	10.3±2.5	6.8±2.3	9.5±1.3	7.8±1.3	6.9±3.9	7.2±1.2	8.4±0.6
Acc 970	10.8±7.3	•	•	•	•	•	•	•	10.8±7.3
Fiord	6.0±0.0	2.5±0.2	5.8±2.5	6.0±2.1	5.2±1.4	5.5±1.5	3.5±0.5	•	4.6±0.6
W. mean	6.9±0.7	5.9±0.4	6.3±0.7	7.1±0.7	6.2±0.6	5.8±0.6	5.3±1.0	6.3±0.7	6.3±0.2
Leaf									
Acc 290	•	•	3.3±0.7	•	2.0±0.1	2.4±0.0	3.0±0.0	•	2.8±0.3
Acc 299	1.8±0.1	3.1±1.0	2.5±0.4	2.1±0.4	2.6±0.5	2.2±0.3	1.2	•	2.5±0.3
Acc 333	2.8±0.9	3.4±0.7	4.5±1.4	2.7±0.3	3.1±0.4	1.3±0.1	4.5±0.7	2.0	3.4±0.3
Acc 342	3.5±1.1	2.9±0.3	3.6±0.3	3.7±0.5	3.3±0.2	2.0±0.3	4.7±1.0	4.8±1.3	3.5±0.2
Acc 508	2.0±0.5	1.3±0.0	3.1±0.4	3.0±1.0	2.9±0.5	3.0±0.0	1.5	•	2.7±0.2
Acc 811	2.8±0.2	3.3±0.1	3.3±0.3	3.3±0.5	3.2±0.2	2.6±0.2	3.6±0.5	3.0±0.6	3.1±0.1
Acc 970	•	4.2±0.8	•	•	•	•	•	•	4.2±0.8
Fiord	4.0±1.0	3.5±0.4	3.1±0.3	4.5±1.0	3.3±0.3	3.4±0.6	1.9±0.4	•	3.3±0.2
W. mean	3.0±0.4	3.2±0.2	3.3±0.2	3.4±0.3	3.0±0.1	2.5±0.2	3.6±0.4	3.5±0.6	3.2±0.1

Table 5.8 Disease efficiency values (DE) for stems, leaves and whole plants of eight faba bean accessions inoculated with eight isolates of *A. fabae* evaluated 15 DAI. The values presented are the means of the infected plants only; • indicates that no lesions were observed.

	Accession	Disease efficiency following inoculation with isolate								
		A11	A14	A26	A28	A41	A48	NSW	WA	Mean
Stem	Acc 290	0.00	0.04	0.00	•	0.00	0.00	•	0.03	0.01i
	Acc 299	0.00	0.11	0.22	0.07	0.05	0.14	0.00	0.00	0.07i
	Acc 333	0.05	0.15	0.20	0.21	0.10	0.12	0.10	•	0.12i
	Acc 342	0.66	0.61	0.31	0.64	0.57	0.19	0.15	0.31	0.43j
	Acc 508	0.03	0.00	0.13	0.04	0.07	0.00	•	0.00	0.03i
	Acc 811	0.63	0.63	0.45	0.07	0.39	0.59	0.07	0.29	0.39j
	Acc 970	0.13	•	•	•	•	•	•	•	0.02i
	Fiord	0.00	0.18	0.19	0.11	0.13	0.05	0.00	•	0.08i
	Mean	0.19pq	0.21q	0.19pq	0.14pq	0.16pq	0.14pq	0.04p	0.08pq	
Leaf	Acc 290	•	•	0.43	•	0.08	0.04	0.30	•	0.11ij
	Acc 299	0.02	0.21	0.24	0.07	0.15	0.09	0.00	•	0.10ij
	Acc 333	0.13	0.22	0.40	0.30	0.33	0.00	0.39	0.00	0.22jk
	Acc 342	0.46	0.58	0.59	0.59	0.50	0.19	0.43	0.27	0.45m
	Acc 508	0.02	0.03	0.28	0.10	0.12	0.13	0.00	•	0.09ij
	Acc 811	0.47	0.56	0.50	0.27	0.40	0.37	0.46	0.29	0.42km
	Acc 970	•	0.10	•	•	•	•	•	•	0.01i
	Fiord	0.32	0.59	0.54	0.20	0.29	0.30	0.10	•	0.29kl
	Mean	0.18pq	0.29pr	0.37r	0.19pr	0.23pr	0.14pq	0.21pr	0.07p	
Whole plant	Acc 290	0.00	0.02	0.22	•	0.04	0.02	0.15	0.02	0.12ik
	Acc 299	0.01	0.16	0.23	0.07	0.10	0.12	0.00	0.00	0.09ik
	Acc 333	0.09	0.18	0.30	0.25	0.22	0.06	0.24	0.00	0.17jk
	Acc 342	0.56	0.60	0.45	0.62	0.54	0.19	0.29	0.29	0.44l
	Acc 508	0.03	0.02	0.21	0.07	0.10	0.07	0.00	0.00	0.06ij
	Acc 811	0.55	0.60	0.48	0.17	0.40	0.48	0.27	0.29	0.40l
	Acc 970	0.07	0.05	•	•	•	•	•	•	0.01i
	Fiord	0.16	0.39	0.37	0.16	0.21	0.18	0.05	•	0.19k
	Mean	0.18pr	0.25qr	0.28r	0.17pq	0.20pq	0.14pr	0.12pq	0.07p	

For the last column (mean) and the last row (mean), the numbers followed by the same letters are not significantly different by Tukey's Honestly Significant Difference Test.

Table 5.9 Disease scores for stems, leaves and whole plants of eight faba bean accessions inoculated with eight isolates of *A. fabae* evaluated 15 DAI. The values presented are the means of the infected plants only.

	Accession	Disease score following inoculation with isolate								
		A11	A14	A26	A28	A41	A48	NSW	WA	Mean
Stem	Acc 290	1.1	1.2	1.4	1.0	1.2	1.0	0.9	1.4	1.2ij
	Acc 299	1.4	2.6	2.2	1.8	1.3	1.4	0.4	1.2	1.5j
	Acc 333	1.6	1.6	2.1	1.5	1.5	1.2	1.1	0.6	1.4j
	Acc 342	3.1	3.4	2.1	3.1	3.1	1.7	1.0	2.1	2.5k
	Acc 508	1.3	0.9	1.5	0.8	0.7	0.9	0.5	1.1	1.0ij
	Acc 811	3.3	3.1	2.5	0.7	1.9	2.9	0.7	2.5	2.2k
	Acc 970	1.2	0.8	0.8	0.5	0.7	0.5	0.3	0.7	0.7i
	Fiord	0.8	1.8	1.6	0.9	1.6	0.9	0.6	0.8	1.1ij
	Mean	1.7q	1.9q	1.8q	1.3pq	1.5pq	1.3pq	0.7p	1.3pq	
	Acc 290	0.6	0.5	1.9	0.2	1.4	0.3	1.1	0.7	0.8ij
Leaf	Acc 299	1.1	1.9	1.8	1.1	1.3	1.2	0.4	0.4	1.2jk
	Acc 333	1.1	1.8	2.0	2.0	1.8	0.8	1.8	0.6	1.5k
	Acc 342	2.8	3.2	2.8	2.9	3.0	1.4	2.5	1.5	2.5l
	Acc 508	0.9	0.4	1.4	0.7	0.9	0.6	0.4	0.5	0.7ij
	Acc 811	2.9	3.1	2.8	1.9	3.1	2.6	2.2	2.0	2.6l
	Acc 970	0.0	0.6	0.0	0.0	0.0	0.2	0.0	0.2	0.1i
	Fiord	1.4	2.3	2.5	0.8	1.8	1.2	0.9	0.1	1.4k
	Mean	1.3pr	1.7qr	1.9r	1.2pr	1.7qr	1.0pq	1.2pr	0.8p	
	Acc 290	0.9	0.9	1.7	0.6	1.3	0.4	1.0	1.1	1.0ik
Average	Acc 299	1.3	2.3	2.0	1.5	1.3	1.3	0.4	0.8	1.3ik
	Acc 333	1.3	1.7	2.0	1.8	1.7	1.0	1.4	0.6	1.4ik
	Acc 342	3.0	3.3	2.5	3.0	3.1	1.6	1.8	1.8	2.5k
	Acc 508	1.1	0.7	1.5	0.8	0.8	0.8	0.5	0.8	0.8ij
	Acc 811	3.1	3.1	2.7	1.3	2.5	2.8	1.5	2.3	2.4jk
	Acc 970	0.6	0.7	0.4	0.3	0.4	0.4	0.2	0.5	0.4i
	Fiord	1.1	2.1	2.1	0.9	1.7	1.1	0.8	0.5	1.3ik
	Mean	1.5pq	1.8pq	1.8q	1.2pq	1.6pq	1.1pq	0.9p	1.0pq	

For the last column (mean) and the last row (mean), the numbers followed by the same letters are not significantly different by Tukey's Honestly Significant Difference Test.

Table 5.10 The correlation coefficients of components of resistance evaluated on faba bean accessions inoculated with *A. fabae*. ICP (incubation period), SLS (stem lesion size), LLS (leaf lesion size), SLN (stem lesion number), LLN (leaf lesion number), WLN (whole plant lesion number), SDE (stem disease efficiency), LDE (leaf disease efficiency), WDE (whole plant disease efficiency), SS (stem score), LS (leaf score), MS (mean score). Treatment means were used, giving 62 degrees of freedom for most pairs of variates, 54 for those involving ICP and WDE, 50 for SLS and SDE, 48 for LLS and LDE and 44 for those involving either SLS or SDE with either LLS and LDE.

	ICP	SLS	LLS	SLN	LLN	WLN	SDE	LDE	WDE	SS	LS	MS
ICP	1											
SLS	-0.22	1										
LLS	-0.19	0.14	1									
SLN	-0.28	0.19	0.38	1								
LLN	-0.46	0.33	0.13	0.38	1							
WLN	-0.53	0.41	0.21	0.56	0.94	1						
SDE	-0.56	0.52	0.25	0.46	0.56	0.76	1					
LDE	-0.48	0.36	0.61	0.47	0.48	0.62	0.69	1				
WDE	-0.57	0.46	0.46	0.53	0.63	0.77	0.93	0.91	1			
SS	-0.47	0.40	0.20	0.80	0.63	0.73	0.90	0.64	0.83	1		
LS	-0.59	0.36	0.44	0.94	0.71	0.79	0.81	0.90	0.94	0.80	1	
MS	-0.58	0.41	0.34	0.96	0.70	0.80	0.90	0.82	0.94	0.94	0.96	1

5.3.8 Correlations among the components of resistance

The incubation period (ICP) was negatively correlated with all other components, with particularly strong correlations with SDE, WDE, LS and MS and weak ones with lesion size (SLS, LLS) (Table 5.10). The lesion size of stems (SLS) and leaves (LLS) were not significantly correlated with each other whereas lesion numbers for the two

organs were 0.38. The very high correlation between SLN and WLN shows that most of the lesions were on leaves rather than on stems.

There was a stronger correlation of lesion size on stems and leaves with their respective DE (0.52, 0.61) compared to lesion number on stems and leaves and the correlation with their respective DE (0.46, 0.48).

Disease score was highly correlated with the number of lesions and the disease efficiency, had a lower correlation with lesion size and was negatively correlated with incubation period. A high disease score was associated with a shorter ICP.

5.3.9 Differential hosts test

Isolates A11 and A48 gave the same pattern of resistances and susceptibilities, but each of the others gave a different pattern (Table 5.11). Acc 970 was resistant to all isolates, Acc 290 and Acc 508 susceptible to isolate A26 only. Acc 811 was resistant to isolates A28 and NSW. Acc 342 was susceptible to isolate NSW while all other accessions were resistant. Similarly, Acc 811 was susceptible to isolate WA but all others were resistant.

5.4 Discussion

The results have shown Ascochyta to be a very variable organisms, both in culture and on the host. This variation led to a wide range of responses by the various bean populations, which were also variable as has been shown in previous chapters. This variability has important implications for resistance breeding and also disease control.

The variability of the isolates shown in culture indicated that *A. fabae* is highly variable within South Australia and probably consists of many biotypes. Variation was evident even among the single-spore cultures derived from the same initial colony. These results confirm earlier reports of variability of the fungus in culture (Kharbanda and Bernier, 1980; Hanounik and Maliha, 1984), where there was no indication of the source of the variation.

It was feasible to differentiate between isolates in the study of eight accessions and eight isolates in the pathogenicity test. This illustrated the variation that exists between isolates in their aggressiveness and physiological specialisation, and in the components of resistance and susceptibility present in the hosts.

Table 5.11 Disease reactions for eight faba bean accessions inoculated with eight isolates of *A. fabae* evaluated 21 DAI. The values in parentheses are the mean disease scores of the plants tested as shown in Table 5.4.

Accession	Disease reaction to isolate							
	A11	A14	A26	A28	A41	A48	NSW	WA
Acc 290	R	R	S	R	R	R	R	R
	(1.2)	(1.3)	(2.7)	(1.0)	(1.9)	(1.2)	(1.3)	(1.5)
Acc 299	R	S	S	R	R	R	R	R
	(1.6)	(3.0)	(2.8)	(2.0)	(1.8)	(1.8)	(0.6)	(1.2)
Acc 333	R	S	S	S	S	R	R	R
	(1.8)	(2.6)	(2.5)	(2.1)	(2.5)	(2.0)	(1.9)	(1.0)
Acc 342	S	S	S	S	S	S	S	R
	(3.8)	(4.3)	(3.3)	(4.1)	(4.2)	(2.3)	(2.2)	(2.0)
Acc 508	R	R	S	R	R	R	R	R
	(1.6)	(1.0)	(2.2)	(1.1)	(1.1)	(1.3)	(0.7)	(1.1)
Acc 811	S	S	S	R	S	S	R	S
	(4.2)	(4.0)	(3.6)	(2.0)	(3.3)	(3.8)	(2.0)	(3.0)
Acc 970	R	R	R	R	R	R	R	R
	(1.0)	(1.1)	(0.8)	(0.5)	(0.7)	(0.7)	(0.3)	(0.7)
Fiord	R	S	S	R	S	R	R	R
	(1.6)	(3.0)	(3.1)	(1.3)	(2.5)	(1.8)	(1.1)	(0.8)
Group	A	B	C	D	E	A	F	G

One factor needing consideration when evaluating differences in the components of resistance was the variation present within accessions. Although accessions 290, 299 and 508 were inbred lines, they gave non-uniform responses, with a few plants becoming infected by most isolates when one would expect either all or none to develop the disease. Since the experimental conditions were uniform, the treatments were randomised and certain other responses were uniform, it is unlikely that either the non-infected plants in these three lines had escaped the disease or that the infected

ones were in particularly susceptible locations. Variation within an accession in its response to an isolate is therefore a component of its resistance. It is not known whether the non-infected or resistant plants of an accession, when exposed to the different isolates, were of the same genotype. This matter could be resolved for an accession such as Acc 508 by propagating all the non-infected plants and testing them by the detached-organ test.

Some of the measured components of resistance, such as incubation period and lesion number, were closely correlated with the overall classification of the accessions as resistant or susceptible while others were not. In particular, lesion size was independent of the other resistance components and not significantly correlated with overall resistance. It appeared that when the spores germinated and infected the plant, the size of the lesion was not influenced by any resistance mechanism and large lesions developed on otherwise resistant genotype. Further evidence of the lack of association with the known resistance was that the isolates with a higher rate of infection did not produce larger lesions than those with a lower rate of infection.

Disease efficiency, measured as the proportion of lesions that developed pycnidia, was another component that reflected the resistance of the accessions. The significance of the DE was its indication of the potential damage which could be done to the crop. When host resistance has inhibited the production of spores, the damage has been limited to that arising from the initial infection from the source of spores, onto the susceptible individuals within the crop.

Some components seemed to reinforce each other, resulting in a higher level of resistance or susceptibility. Accession 970 had a useful combination of few lesions, long ICP and low DE so it will not spread the disease, whereas Acc 290 had many lesions, quite long ICP but also a low DE which suggested it would also restrict the spread of the disease. The resistance of Acc 290 and Acc 970 took different forms. In Acc 970, lesions formed on very few plants and when they did they were large, numerous and produced average numbers of pycnidia. In Acc 290, in contrast, lesions were formed by most isolates but they were few in number, small and generally produced few pycnidia. Both results are valid to the epidemiology of the disease as no further spread of infection occurs. DE is important for secondary infections of the

crop, but nothing is known of the quality of the spores produced on the different accessions and whether they will have the same pathogenicity.

Stem lesions on plants may result in severe damage through breaking of the stems which leads to a heavy loss of yield. It is evident that some accessions such as 290, 299 and Fiord had a low number and small size of lesions on their stems. These accessions might be useful in developing new varieties that do not show heavy losses of yield. The weak correlation of the size and number of lesions on stems and leaves may again indicate these components are controlled by different genes.

The differential host study confirmed the presence of pathogenic variability in *A. fabae* as reported by other workers (Kharbanda and Bernier, 1980; Hanounik and Robertson, 1989; Rashid *et al.*, 1991b). Isolates used in this study included six obtained from the areas of South Australia where beans have been grown for a decade and one each from Western Australia and New South Wales where beans have only recently been grown. These two isolates showed less virulence than the others and gave fewer lesions, a low disease efficiency and low average disease score. It is possible that the pathogen has increased its virulence over time in South Australia as a result of mutation or sexual reproduction and selection. The isolates A11, A48 and NSW were recognised as being possible Race 1 and isolates A28 and A41 were recognised as possibly being Race 2. The other two isolates could not be related to any Race in the set by Hanounik and Robertson (1989). Acc 299 has been classified as resistant in most countries tested and therefore, its susceptible reaction to isolate A26 suggested a new virulence as was found in Italy (Anon, 1990).

Differential hosts have been used in many studies of crop species to determine the number of races that might be present and the genetic resistance or susceptibility of a set of host genotypes. In the present study, the results for the differential hosts are not as readily interpreted. The results suggest at least seven races are present but it is evident that occasionally only about a half of the tested plants became infected. It is believed this represents genetic variation within the accessions. When an average is calculated, to determine whether the response is resistant or susceptible, small variations in the average can influence this classification. In view of the heterogeneity within accessions, further tests using homozygous lines are required to clarify the

relationship among different races of *A. fabae*. Alternatively it could be accomplished by exposing individual plants to several races using a detached-organ test.

This study has shown that *A. fabae* exhibits great variability, but because of the non-uniform reaction within the accessions tested, there remains some uncertainty about the number of races that were distinguished.

CHAPTER 6. DETACHED-ORGAN TECHNIQUES FOR EVALUATING FABA BEANS FOR RESISTANCE TO ASCOCHYTA BLIGHT

6.1 Introduction

Populations of faba beans vary for their resistance to Ascochyta and populations of Ascochyta also vary in their virulence (Chapters 3, 4 and 5). Thus, for mass selection and certain aspects of cultivar development, testing for resistance requires large-scale field trials and early screening of small populations can be done in glasshouse conditions. More detailed studies, however, will require smaller-scale experiments. For example, repeated challenges of the same bean genotype with a range of disease genotypes require either defined inbred lines or some way of multiple use of the same plant. The previous chapter has shown that even inbred lines retain some variability in disease reactions, so the most practical way to handle this variability will be the development of detached-organ tests. Testing of individual leaves of a single plant against several pathogen isolates would allow a detailed description of its genotype, which could be confirmed in its progeny. Similarly, since the reactions of stems and leaves often differed (Chapter 5), a stem-segment test may also be necessary to allow detailed analysis of the genetics and physiology of the overall host-pathogen reaction.

There are technical drawbacks to this approach. First, the previous chapters have shown that it takes about 10 days for disease symptoms to develop fully, so the leaves would have to be kept in good condition for at least that long. In contrast, resistance to chocolate spot disease caused by *Botrytis fabae* can be evaluated on detached leaves in as little as 7-8 hours (Tivoli *et al.*, 1986). Second, use of stem segments destroys the stem and this method requires conditions that promote strong tillering for the rest of the plant to remain otherwise useful. Third, the conditions may be so strongly altered in favour of either the pathogen or the host that the results do not adequately correlated with those obtained in normal field conditions. Thus Dodd

(1971) was unable to develop a detached-leaf technique for investigating physiological specialisation and genetic resistance to the disease.

Another difficulty experienced when trying to develop a detached-leaf technique was, that leaves of faba beans are very sensitive to surface sterilants such as ethanol and NaOCl and even low levels can damage the leaves. In the literature on detached-leaf testing for *Botrytis fabae*, no mention is made of surface sterilants (El-Sherbeeny and Mohamed, 1980; Heilbronn and Harrison, 1989) and in the present experiments surface sterilisation was not undertaken. Plants were grown in the glasshouse to ensure a minimum of infection from other diseases. It was also found necessary to handle the leaves with care to avoid damage. For these reasons, the potential was investigated for detached-leaf and detached-stem methods of evaluating faba beans for resistance to Ascochyta blight. Plant age at the time of organ removal, inoculum concentration, inoculation method and incubation conditions were all varied.

6.2 Materials and methods

6.2.1 Plant material

Seeds of Acc 970 were harvested from plants determined to be highly resistant in Chapter 3. Similarly, seeds of Acc 811 were harvested from plants scored as highly susceptible. Fiord was used as the moderately resistant material. Seeds were surface sterilised with 10% NaOCl for 5 min, washed in RO water 2-3 times and 20-30 of a single accession were sown in each plastic tray (35 x 25 x 12 cm), which contained UC soil with fertiliser. Leaves were detached when the plants reached the 2-leaf stage, 3 weeks after sowing. Surface sterilisation of the detached organs was always lethal so was discontinued.

6.2.2 Preparation of inoculum

A. fabae isolate A26 was subcultured and multiplied on 2% PDA for 14 days at 25°C. Pycnidiospores were harvested by flooding the surface of the agar with sterile RO water and scraping with a glass rod. Mycelial debris was removed by filtration through two layers of muslin. The spore suspension concentration was adjusted to 5 x 10^5 sp/ml and confirmed using a haemocytometer.

6.2.3 Preliminary experiments

In pilot experiments, several media for keeping leaves alive were tried, namely Benzimidazole in 2% agar, 2% agar, kinetin in water, sand with water, germination paper, moist sponges, and water. None of them gave acceptable results as the leaves were damaged by the direct contact with the media. This problem was later solved by using a solid growing medium into which only the stems or petioles were pushed, leaving the leaves standing in the air while the medium provided water. The leaves lasted longer, occasionally roots were produced from the stems or petioles, and there was sufficient time for Ascochyta blight to develop.

Several methods of inoculation were also tested including applying the spores with a brush or in a drop or spray, or placing an agar plug containing mycelium on the leaves. The drop and spray methods of inoculation were compared using leaves from plants at the 2-leaf stage. There were 9 plants of Acc 970, 12 of Fiord and 14 of Acc 811. The spore suspension used was 5×10^5 sp/ml. Droplets of 20 μl were placed on the abaxial side of the leaves, using a pipette. One droplet was placed on each half leaf, so there were 4 droplets per bifoliate leaf. The spray was applied using a hand-sprayer until it started to run off.

Conditions for incubation were varied to find the optimal artificial environment for the growth of *A. fabae*. It was found that temperatures above 20°C allowed the leaves to wilt or yellow before lesions could develop and 18°C was the best temperature for lesions to develop and for the leaves to remain green. A saturated atmosphere was required for the development of the lesions and was achieved by covering the tray with a plastic bag and misting inside the bag. The bags were then tied in a way that protected the inoculated leaves from contact with the bag.

Optimum culture age was tested using *A. fabae* cultures that were 7, 14, 28 and 42 days old. They were sprayed onto detached leaves at the same spore concentration.

6.2.4 Inoculation method

Bifoliate leaves were excised intact with a short length of the internode below them. The leaves and stems were washed with sterile RO water and the stems then pushed into Oasis™ LC-1 growing medium which had a 60% draining capacity (Smithers-Oasis Australia Pty Ltd, Elizabeth West, South Australia). A block of

medium, 30 x 25 x 3 cm, was placed in a plastic container, measuring 35 x 25 x 12 cm, with 2 l of water. One tray could contain up to 32 bifoliate leaves. The original position of the leaf on the plant was recorded, with the unfolded leaf at the top of the plant being designated number one and older leaves were successively 2, 3, 4 etc. This numbering system has been used throughout these studies.

After inoculation, the tray was covered with a wet plastic bag and a high humidity established by misting inside the bag. The bags were then tied in a way that protected the inoculated leaves from contact with the bag. The trays were placed in a growth chamber at 18°C fitted with fluorescent tubes giving a 16 h day and 8 h night.

6.2.5 The effect of age of leaves on *Ascochyta fabae* infection

Three ages of bean plants were compared, namely 3, 5 and 7 weeks after sowing, when the plants had 2, 3-4 and 6-7 leaves respectively. At 7 weeks after sowing the plants had reached the flowering stage. Leaves were excised and inoculated as described above. The test was conducted with an unequal number of leaves but attempts were made to make them comparable by having more replicates of the younger plants. For the 3-week-old plants 12 plants of Acc 970, 9 of Fiord and 18 of Acc 811 were used, while for the 5-week-old plants, the plant numbers were 4, 6 and 7 and for 7-week-old plants, 3, 3, and 3, respectively.

6.2.6 The effect of the concentration of the inoculum on infection of detached leaves

Five concentrations of inoculum, 2×10^6, 5×10^5, 5×10^4, 5×10^3, 5×10^2 sp/ml, were prepared by dilution of a 2×10^6 sp/ml suspension in RO water and the concentrations were confirmed using a haemocytometer. The controls in the experiment were detached leaves sprayed with RO water. Leaves from plants at the three-leaf stage (4-week-old) were used. The medium, leaf numbering, method of inoculation and incubation conditions were as described previously. Seven plants of each accession were used for each concentration.

6.2.7 The detached-stem test

Stem segments were taken from all parts of plants aged 3, 5, or 7 weeks old, giving 2, 4, and 6 stems respectively. The stems, following cutting, were about 3-6 cm long

and consisted of one node and an internode. The lower part of the cutting, which included the node, was pushed into the medium. The upper part of the internode was free in the air. The segments were sprayed with a spore suspension of 5 x 10^5 sp/ml. The medium, the inoculation and the incubation procedure were similar to the ones used in the detached-leaf method. At 3 weeks, 24 plants of Acc 970, 27 of Acc 811 and 32 of Fiord were used while for the 5- and 7-week treatments there were 12 plants of each accession. The segments were designated in a manner similar to the one used for the leaves, thus segment 1 was the youngest and segments (internodes) 2-6 were progressively older. Where lesions formed at the cut surface, they were first evaluated separately from the other lesions, then together with them. The results presented are the mean values.

6.2.8 Disease assessment

The detached-organ techniques were developed to distinguish resistant and susceptible plants in the laboratory, by the success of infection, colonisation and the development of spores. In all experiments, the percentage of organs infected, lesion number, lesion size and the proportion of lesions producing pycnidia were all evaluated ten days after inoculation. Treatment means were calculated but the unbalanced design of the experiments precluded analysis of variance.

6.3 Results

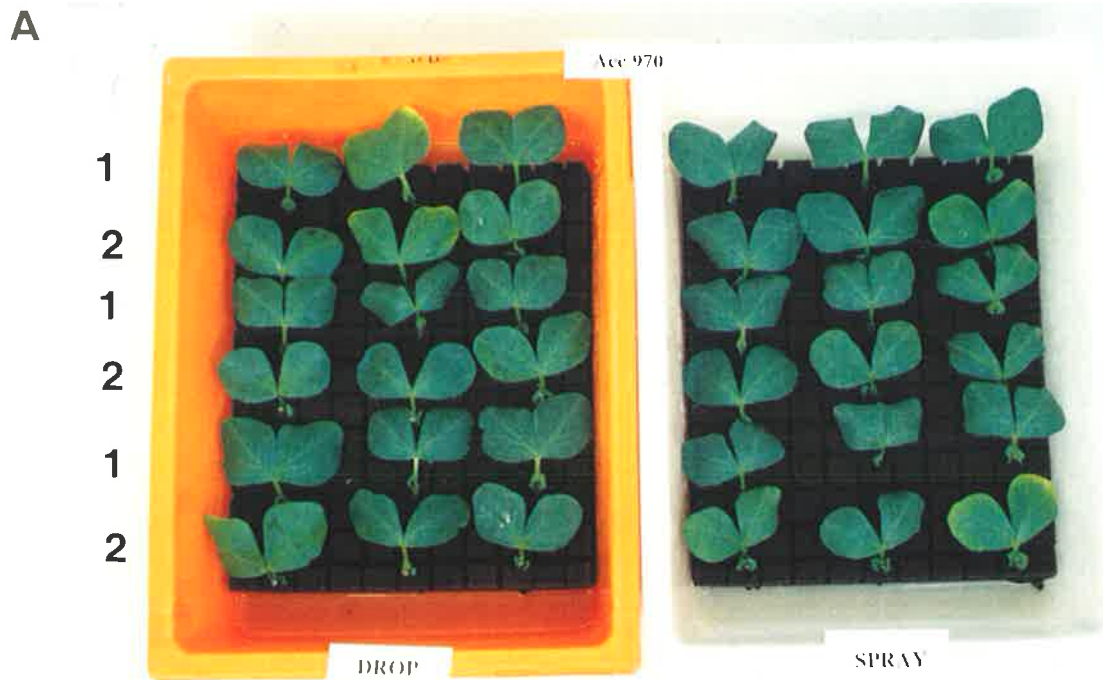
6.3.1 Preliminary experiments

The spray and brush methods of spore application gave similar results. Typical lesions were developed which produced pycnidia, but for the brush method it was not possible to quantify the inoculum. With the plug and drop methods, typical lesions did not develop, except on plants of Acc 811 where lesions were found with the drop method.

Brown areas developed underneath the drops on all the accessions tested about 3-4 days after inoculation. The symptoms were similar to the flecking sometimes observed, where brown pin-point spots condensed to form brown areas (Plate 6.1). All accessions showed a similar reaction to the pathogen with Acc 970 having as high

Plate 6.1. The comparison of drop and spray inoculation methods on Acc 970 detached leaves (A) and Acc 811 (B).

A



B

a percentage of browning as Acc 811 (Table 6.1). There were no differences between leaf positions 1 and 2, both showing brown areas. No typical lesions were formed from these brown areas on Acc 970 or Fiord even as late as 4 weeks after inoculation, but typical lesions were formed underneath 21.4% of the drops on the first leaves of Acc 811. No typical lesions were formed on the second leaves. When no brown area developed, the orientation of leaf to the stem had been such that the drop of inoculum would not have remained on the leaf indefinitely.

No lesions developed on sprayed leaves of Acc 970 (Table 6.1). Fiord was moderately infected while all leaves of Acc 811 were infected. The lesions were typical of natural infections and pycnidia were found in some of the lesions. The disease reactions were similar to those obtained with whole plants in the glasshouse and field.

6.3.2 The effect of leaf age on *Ascochyta fabae* infection

All leaves from 3-week-old plants were green at the time of evaluation (10 DAI). No lesions were found on Acc 970 while on Fiord and Acc 811, leaf 1 developed more symptoms than leaf 2 (Table 6.2). Lesion number and lesion size were also higher on leaf 1 than leaf 2.

The 5-week-old plants of Acc 970 and Fiord had four bifoliate leaves while Acc 811 had only three. The bottom leaves of Acc 970 and Fiord were wilting and

Table 6.1 Infection of detached leaves of faba beans by inoculum of *A. fabae* applied by drops and spray: the number of brown areas as a percentage of the number of drops applied in the drop test and the percentage of leaves producing lesions in the spray test.

Accession	Percentage of drop-treated leaves producing brown areas		Percentage of spray-treated leaves producing lesions	
	Leaf no. 1	Leaf no. 2	Leaf no. 1	Leaf no. 2
Acc 970	94.4	100.0	0	0
Fiord	87.5	93.8	25.0	6.3
Acc 811	92.5	98.1	100.0	100.0

Table 6.2 The infection of detached leaves of plants of three faba bean accessions 3, 5 or 7 weeks after sowing, measured as the percentage of leaves successfully infected, mean lesion number per leaf and mean lesion size (mm). Y indicates that the leaves were yellow and wilted before lesions could be observed; • indicates no lesion.

Infection criterion	Age	Accession	Infection of leaf number						
			1	2	3	4	5	6	7
Leaves infected (%)	3 weeks	Acc 970	0	0					
		Fiord	77.8	22.2					
		Acc 811	94.4	61.1					
	5 weeks	Acc 970	0	0	0	Y			
		Fiord	66.7	0	0	Y			
		Acc 811	100.0	71.4	42.9				
	7 weeks	Acc 970	0	0	0	0	Y	Y	Y
		Fiord	0	0	0	0	Y	Y	Y
		Acc 811	100.0	100.0	33.3	Y	Y	Y	
Lesions per leaf	3 weeks	Acc 970	0	0					
		Fiord	3.0	0.7					
		Acc 811	9.2	2.0					
	5 weeks	Acc 970	0	0	0				
		Fiord	28.0	0	0				
		Acc 811	10.0	5.7	3.4				
	7 weeks	Acc 970	0	0	0	0			
		Fiord	0	0	0	0			
		Acc 811	18.3	4.7	1.0				
Lesion size (mm)	3 weeks	Acc 970	•	•					
		Fiord	1.2	1.1					
		Acc 811	1.3	1.1					
	5 weeks	Acc 970	•	•	•				
		Fiord	1.0	•	•				
		Acc 811	1.4	1.2	1.1				
	7 weeks	Acc 970	•	•	•	•			
		Fiord	•	•	•	•			
		Acc 811	1.3	1.1	1.1				

yellowing a few days after being detached. No lesions appeared on any leaves of Acc 970. In Fiord, only the top leaves showed lesions. All leaves of Acc 811 from positions 1, 2 or 3 remained green. Lesions were most numerous and largest on the upper younger leaves and were fewer and smaller on successively older leaves.

At 7 weeks, the plants of Acc 970 and Fiord had 7 leaves while Acc 811 still had only 6 leaves. The lower leaves of all accessions tended to yellow and wilt after being detached. About 3-5 days after being detached they were senescent while the top three leaves from the plants were still green. At evaluation 10 DAI, no lesions had developed on leaves of Acc 970 or Fiord. Upper leaves of Acc 811 showed lesions about 5-7 DAI, with leaves from position 1 and 2 showing 100% infection. Mean lesion number and size were greater on the younger leaves from the top of the plant than on the lower older leaves. The results indicate that plants need only be kept 3-5 weeks after sowing to provide good material for the discrimination of accessions.

6.3.3 The effect of the concentration of the inoculum on infection of detached leaves

Leaves from plants of Acc 970 were resistant to every concentration, and no lesions developed. At high concentrations of inoculum, a flecking reaction was apparent but the flecks never developed into lesions (Table 6.3 and Plate 6.2).

The younger leaves from Fiord plants tended to have more symptoms than the older leaves. Flecking was found on the older leaves for concentrations between 5 x 10^3 and 2 x 10^6 sp/ml. The results were not consistent and there was variation in lesion size, lesion number and the percentage of lesions producing pycnidia among plants and leaf positions.

Leaves from plants of Acc 811 showed a high percentage of infection for each concentration. At the highest concentration of 2 x 10^6 sp/ml, flecking appeared as well as lesions on leaves 2 and 3. Leaf 1 developed more symptoms than leaves 2 and 3 for every concentration. The number of lesions increased up to the concentration of 5 x 10^4 sp/ml but did not change with further concentration. The lesions tended to be bigger on the younger leaves. The results for lesions with pycnidia were variable and there was no consistent indication that more pycnidia were produced as a result of either higher concentrations of spores or younger leaves.

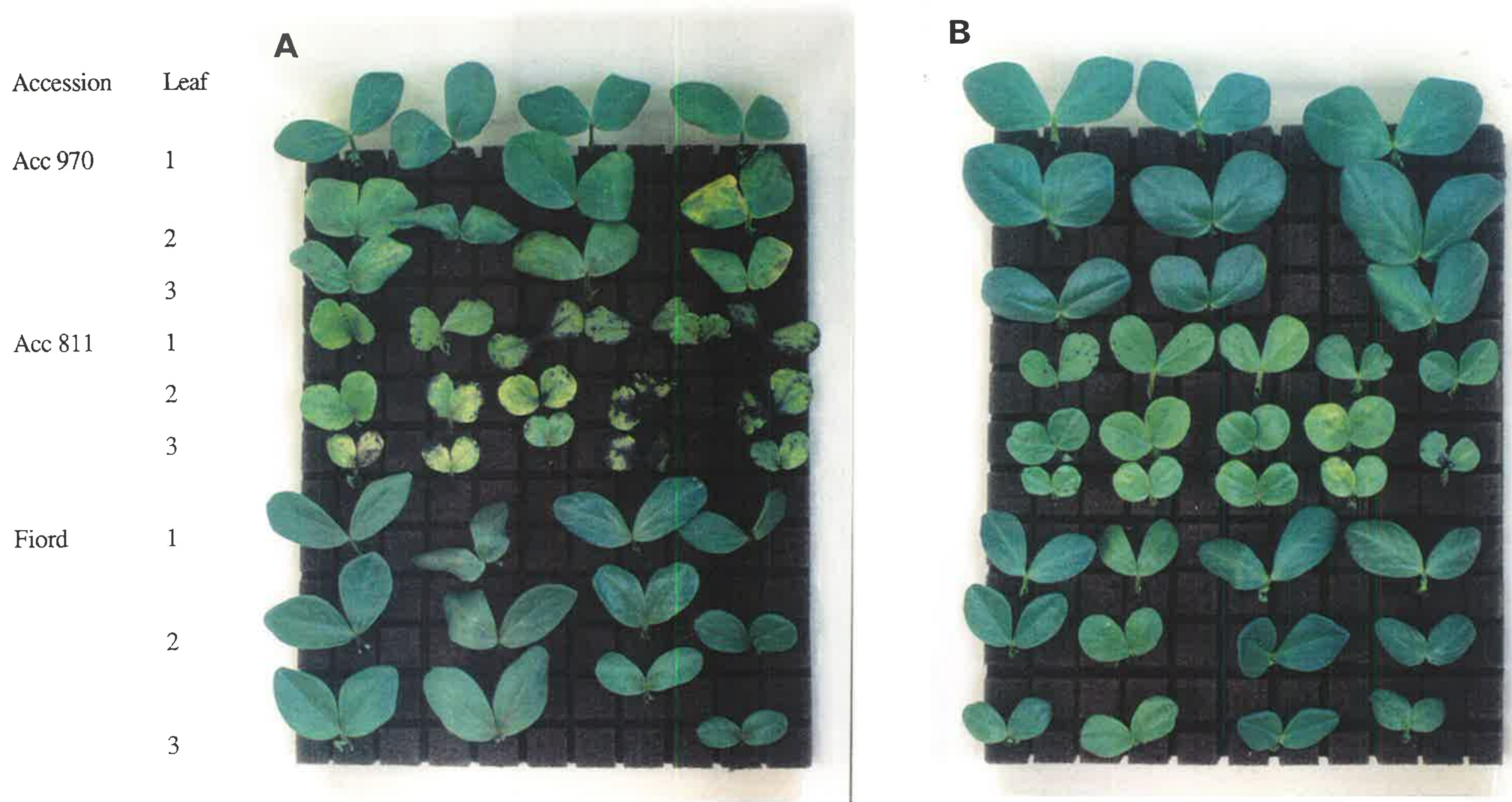


Plate 6.2. The effect of spore concentration on detached leaves of Acc 970, Acc 811 and Fiord with concentrations of 2×10^6 sp/ml (A) and 5×10^2 sp/ml (B).

Table 6.3 Percentage of infected leaves, mean lesion number, mean lesion size (mm) and percentage of lesions with pycnidia following inoculation with different concentrations of spores. F indicates flecking.

Infection criterion	Accession	Leaf #	Infection with spore concentration of					
			2×10^6	5×10^5	5×10^4	5×10^3	5×10^2	control
Leaf infection (%)	Acc 970	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
	Fiord	1	57.1	42.9	28.6	0	57.1	0
		2	0	0	14.3	14.3	57.1	0
		3	0	0	14.3	0	57.1	0
	Acc 811	1	100.0	100.0	100.0	100.0	100.0	0
		2	71.4	100.0	100.0	100.0	85.7	0
		3	71.4	71.4	100.0	85.7	57.1	0
Lesions per leaf	Acc 970	1	F	F	F	0	0	0
		2	F	F	0	0	0	0
		3	F	F	0	0	0	0
	Fiord	1	2.0	3.0	0.9	F	1.1	0
		2	F	F	1.3	0.3	1.7	0
		3	F	F	0.3	F	1.7	0
	Acc 811	1	23.9	24.3	27.4	4.1	6.6	0
		2	20.1	8.6	14.3	5.4	5.4	0
		3	11.7	7.1	12.4	3.3	2.7	0

Table 6.3 (continued) Mean lesion size (mm) and percentage of lesions with pycnidia following inoculation with different concentrations of spores; • indicates no lesion.

Infection criterion	Accession	Leaf #	Infection with spore concentration of					
			2×10^6	5×10^5	5×10^4	5×10^3	5×10^2	control
Lesion size (mm)	Acc 970	1	•	•	•	•	•	•
		2	•	•	•	•	•	•
		3	•	•	•	•	•	•
	Fiord	1	1.9	3.9	1.0	•	3.5	•
		2	•	•	1.0	1.0	1.0	•
		3	•	•	1.0	•	1.0	•
	Acc 811	1	2.5	1.3	1.3	1.2	1.2	•
		2	1.4	1.2	1.2	1.3	1.1	•
		3	1.5	1.1	1.0	1.2	1.4	•
Lesions with pycnidia (%)	Acc 970	1	•	•	•	•	•	•
		2	•	•	•	•	•	•
		3	•	•	•	•	•	•
	Fiord	1	0	26.7	0	•	0	•
		2	•	•	0	0	16.7	•
		3	•	•	0	•	0	•
	Acc 811	1	26.3	14.1	15.1	24.1	19.6	•
		2	42.5	18.3	7.0	0	28.9	•
		3	37.5	16.0	0	0	0	•

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6.3.4 The detached-stem test

The lesions on the stem segments developed 5 to 7 days after inoculation. Two types of lesions were observed. The first type was a wound lesion which appeared on the cut surface at the top of the stem (Plate 6.3). These lesions were atypical in shape and developed extensively. They started at the wound and progressed down until sometimes all of the stem was infected. This type of lesion produced many pycnidia on all accessions, including the otherwise resistant Acc 970. The second type was the typical lesion, similar to those in field crops. Most of them appeared on the younger stems and occurred sporadically. Pycnidia were produced also from this type of lesion.

Emphasis is placed on the results obtained for the typical lesions, as wound lesions are less likely to occur in the field. The results for the typical lesions are presented and considered (Tables 6.4 to 6.7) but for completeness the combined values are also given.

The youngest stem segment of the 3-week-old plants showed a higher percentage of infection than the older segments for all three accessions (Table 6.4). Acc 970 was the least infected, Fiord and Acc 811 were progressively more infected. These results were confirmed with the 5-week-old and 7-week-old plants. Including the wound lesions changed the numbers more for the older stem segments than for the younger ones.

These results for the percent infection were reflected in the results for the mean number of lesions per stem segment (Table 6.5). The same ranking for resistance was observed between accessions as was the effect of the age or position on the plant from which the segment was taken. In contrast the mean lesion size (Table 6.6) and the percentage of lesions with pycnidia (Table 6.7) were more variable, but these attributes were measurable only when infection had occurred which was very rare in Acc 970. Thus 67% of the lesions on segments from 7-week-old plants of Acc 970 bore pycnidia as compared with 30% on those of Acc 811, but these figures are based on an average of 1.2 lesions on 7 stems of 12 evaluated in Acc 970 as against an average of 10 lesions on all 12 stems of Acc 811.

Wound lesions were often larger than typical lesions and nearly all produced pycnidia. Older stem segments produced more wound lesions than younger ones.

Plate 6.3. The lesions on detached stems. A shows abundant pycnidia on detached stems of Acc 970. Two type of lesions on detached stems of Acc 811 (B); wound-lesion (a), Wound-lesion and typical lesion (b), the severe stage; lesions with pycnidia (c).

A

B

a

b

c

Table 6.4 Percentage of inoculated stem segments showing infection by Ascochyta blight, excluding or including the wound lesions. Each value for the 3-week-old plants is based on 24-32 stem segments and for 5- and 7-week-old plants on 12 stem segments.

	Age	Accession	Percentage of successful infections at internode number					
			1	2	3	4	5	6
Excluding wound lesions	3 weeks	Acc 970	20.8	0				
		Fiord	37.5	6.3				
		Acc 811	92.6	100.0				
	5 weeks	Acc 970	58.3	8.3	0	0	0	
		Fiord	58.3	25.0	0	0		
		Acc 811	66.7	58.3	50.0	0		
	7 weeks	Acc 970	58.3	25.0	0	0	0	0
		Fiord	50.0	33.3	16.7	0	0	0
		Acc 811	100.0	100.0	50.0	8.3	0	0
Including wound lesions	3 weeks	Acc 970	20.8	4.2				
		Fiord	43.8	18.8				
		Acc 811	96.3	100.0				
	5 weeks	Acc 970	58.3	8.3	0	0	0	
		Fiord	58.3	33.3	25.0	25.0		
		Acc 811	66.7	66.7	50.0	8.3		
	7 weeks	Acc 970	58.3	33.3	0	0	0	0
		Fiord	50.0	33.3	16.7	0	8.3	8.3
		Acc 811	100.0	100.0	66.7	25.0	16.7	8.3

Table 6.5 Mean lesion number, excluding or including the wound lesions, on inoculated stem segments. Each value for the 3-week-old plants is based on 24-32 stem segments and for 5- and 7-week-old plants on 12 stem segments.

	Age	Accession	Mean lesion number at internode number					
			1	2	3	4	5	6
Excluding wound lesions	3 weeks	Acc 970	0.3	0				
		Fiord	0.9	0.2				
		Acc 811	3.9	3.3				
	5 weeks	Acc 970	0.8	0.1	0	0	0	
		Fiord	0.8	0.3	0	0		
		Acc 811	1.8	2.6	1.4	0		
	7 weeks	Acc 970	1.2	0.4	0	0	0	0
		Fiord	1.5	0.6	0.4	0	0	0
		Acc 811	10.0	9.4	1.9	0.1	0	0
Including wound lesions	3 weeks	Acc 970	0.3	0.04				
		Fiord	0.9	0.3				
		Acc 811	4.1	3.3				
	5 weeks	Acc 970	0.8	0.1	0	0	0	
		Fiord	0.9	0.5	0.3	0.3		
		Acc 811	1.8	2.8	1.4	0.1		
	7 weeks	Acc 970	1.2	0.6	0	0	0	0
		Fiord	1.5	0.6	0.4	0	0.1	0.1
		Acc 811	10.0	9.4	2.2	0.3	0.2	0.1

Table 6.6 Mean lesion size (mm), excluding or including the wound lesions, on inoculated stem segments. Each value for the 3-week-old plants is based on 24-32 stem segments and for 5- and 7-week-old plants on 12 stem segments. • indicates no lesion.

	Age	Accession	Mean lesion size (mm) at internode number					
			1	2	3	4	5	6
Excluding wound lesions	3 weeks	Acc 970	4.2	•				
		Fiord	3.0	2.7				
		Acc 811	3.1	2.7				
	5 weeks	Acc 970	4.5	6.0	•	•	•	
		Fiord	3.6	5.5	•	•		
		Acc 811	4.4	2.8	2.8	•		
	7 weeks	Acc 970	4.9	4.8	•	•	•	•
		Fiord	3.3	3.3	3.8	•	•	•
		Acc 811	7.3	3.5	8.8	6.0	•	•
Including wound lesions	3 weeks	Acc 970	4.2	5.0				
		Fiord	3.5	9.5				
		Acc 811	3.1	2.7				
	5 weeks	Acc 970	4.5	6.0	•	•	•	
		Fiord	4.3	7.8	6.7	18.3		
		Acc 811	4.4	3.1	2.8	5.0		
	7 weeks	Acc 970	4.9	4.9	•	•	•	•
		Fiord	3.3	3.3	3.8	0	10.0	15.0
		Acc 811	7.3	3.8	9.7	7.0	7.5	3.0

Table 6.7 Percentage of lesions with pycnidia, excluding or including the wound lesions, on inoculated stem segments. Each value for the 3-week-old plants is based on 24-32 stem segments and for 5- and 7-week-old plants on 12 stem segments. • indicates no lesions.

	Age	Accession	Percentage of lesions with pycnidia at internode number					
			1	2	3	4	5	6
Excluding wound lesions	3 weeks	Acc 970	0	•				
		Fiord	25.0	0				
		Acc 811	17.0	50.0				
	5 weeks	Acc 970	0	0	•	•	•	
		Fiord	20.0	50.0	•	•		
		Acc 811	31.8	19.4	0	•		
	7 weeks	Acc 970	66.7	40.0	•	•	•	•
		Fiord	40.0	33.3	0	•	•	•
		Acc 811	30.0	20.0	26.1	0	•	•
Including wound lesions	3 weeks	Acc 970	0	100.0				
		Fiord	30.0	36.4				
		Acc 811	20.0	50.0				
	5 weeks	Acc 970	0	0	•	•	•	
		Fiord	27.3	66.7	100.0	100.0		
		Acc 811	31.8	21.2	0	100.0		
	7 weeks	Acc 970	66.7	42.9	•	•	•	•
		Fiord	40.0	33.3	0	•	100.0	100.0
		Acc 811	30.0	22.1	34.6	66.7	100.0	100.0

6.4 Discussion

Detached-organ techniques have been developed that are effective in the testing of faba bean genotypes for their resistance to Ascochyta blight. The medium used to support the detached leaf or stem, the inoculum, the method of its application and the age of the plant part all influenced the success of the test.

The method used to inoculate detached leaves had an effect on infection and if spores were applied in a drop, only a very few typical lesions developed and then only on the most susceptible material. It is not clear why a large drop does not provide a suitable environment for the development of symptoms. It is probable that penetration from the appressoria occurs beneath a drop, resulting in the brown area, but further development ceases. The drop method, as used here, did not differentiate between resistant and susceptible material. With the spray inoculation, however, it was possible to distinguish between resistant and susceptible accessions.

Consistent results were obtained with the spray inoculation and there were very clear effects due to leaf age and position, and the inoculum applied. Plants at the 2- to 3-leaf stage were the most efficient to use. They required less time to grow than older plants and all the leaves gave results. On more mature plants the older leaves were less suitable for use in the detached-leaf test as they showed fewer symptoms. The very oldest leaves from the bottom of plants soon yellowed, wilted and senesced after being detached. Leaves in positions 1-3 from the top of the plant were those which were most susceptible for all plant ages, and leaves from lower positions were more resistant even in the susceptible Acc 811. This variation in the reaction to the disease could be due to physiological, morphological or chemical differences in the leaves. Other studies, where whole plants have been tested have also found that leaves in different positions on the plants reacted differently to the disease (Ali, 1985; Van Breukelen, 1985; Pritchard *et al.*, 1989).

The detached-leaf test should give more reproducible results than tests of whole plants as the leaves from the different positions and accessions are tested together in a similar environment in a tray. On a whole plant, as it is only the younger and folded leaves that have the capacity for full development of symptoms, any factor such as

leaf angle that might reduce the level of inoculation could lead to a misclassification and false assessment of resistance.

There was no evidence in a preliminary experiment that the age of the cultures from which spores were obtained had any effect on infection. Dodd (1971), however, found that 7- and 14-day-old spores gave a similar total lesion number on whole plants and the number fell from 21 to 28 and 42 days. There was no obvious reason for the difference between Dodd's results and the present result, although spore production was lower in the younger cultures leading to difficulty in adjusting the concentration of the inoculum to be the same for all ages.

The flecking symptom was a consequence of inoculation, although it was not typically observed in the field. Flecks appeared on detached leaves of Acc 970 and Fiord when inoculated with a high spore concentration. Flecks were also observed on stems and leaves of whole plants inoculated by spraying. *A. fabae* apparently penetrates leaves causing the fleck symptom, but no further development occurs. Studies of chemical and physical differences by histological and cytological methods are need to investigate the present mechanism of resistance in faba beans. In a histological study of a related disease *Ascochyta rabiei* which develops on chickpeas, Höhl *et al.* (1990) found that a resistant cultivar produced necrotic areas resulting from cell death and a subsequent accumulation of phenolic compounds. The susceptible cultivar did not possess this defence mechanism.

The higher the concentration up to 5 x 10^4 sp/ml, the more lesions formed, but with further increase in concentration the lesion number remained the same. Van Breukelen (1985) reported that when whole plants were tested, a 150-fold increase in the concentration of the spore suspension from 1 x 10^4 to 1.5 x 10^6 sp/ml resulted in approximately a doubling of the number of lesions per leaf. The increase occurs up to a very high value and it is possible the detached-leaf test requires a lower concentration to obtain high lesion numbers than the whole-plant test, but a direct comparison under controlled conditions needs to be made. Van Breukelen also reported that the longer the period of high humidity, the higher the number of lesions on whole plants. In the detached-leaf test the material was maintained in a high humidity at all times and this might explain how a low spore concentration still

resulted in many lesions. There was no evidence that spore concentration had an effect on the proportion of the lesions that developed pycnidia.

The detached-stem test showed great promise as the disease was expressed on stems of Acc 970 and Fiord more clearly than on their leaves. The stem segments were easier to handle than detached leaves, which needed to be placed immediately into a suitable environment after they were cut, otherwise they would wilt and sometimes not recover in the tray. This problem did not arise with stem segments and there was no evidence of any condition comparable to wilting of leaves. The stem segments remained green and lesions developed as rapidly on them as on leaves. In Acc 970 and Fiord the stems developed more lesions than leaves. In the whole-plant test described in earlier chapters, the stems showed differences in disease resistance more clearly than leaves. In particular, pycnidia did not develop on lesions on stems of Acc 970, some did on those of Fiord and many did on those of Acc 811.

Of the four components of resistance evaluated in the detached-stem test, percentage infection and lesion number most closely emulated the corresponding results from the whole-plant test in Chapter 5, while lesion size and disease efficiency were less comparable between experiments.

As with the leaves, the age of the stem material had an effect on its susceptibility to infection, with younger stem segments more likely to develop typical lesions than older ones. When the wound lesions were included, the effect of age became somewhat less. Similarly, an age effect has been found in stem material of fruit trees exposed to Phytophthora infection (Utkhede and Quamme 1988, Scott *et al.* 1992). Scott *et al.* suggested that infection of young material was 'possibly due to the juvenile nature of the tissues, which presumably lack the structural and biochemical barriers to infection...'.

An essential factor in the success of the detached-stem and -leaf tests was the medium which supported the sample in an aerial environment. Oasis™ medium was first used in this project only in 1992 and, after its effectiveness was established, there was insufficient time to use it extensively. As the present study has established the significance of the age of leaves and stems, one important study yet to be undertaken would be the relationship between pod age and pod infection. A second study would be to establish the correlation between tests on leaves, stems and pods for a sample of

plants from a cultivar, such as Fiord, which shows a range of resistance to Ascochyta. From the results one could determine the sample sizes needed for discrimination between genotypes.

The advantage of using detached parts of a plant is that the evaluations of leaves, stems and pods could be made in the same environment, which should lead to more reproducible results. It overcomes the differences in the environment around leaves, stems and pods on the whole plants which hinder attempts to determine the genetics of resistance in the different organs. Uniformity of environment should also allow isolates of the pathogen to be tested efficiently and resolve interactions between host and pathogen genotypes. Furthermore tests could be made at any time of the year. The tests are destructive only of parts of the plant, not the whole, which is essential for the efficient evaluation of germplasm. The technique could be applied to other leaf diseases such as rust and chocolate spot. For Ascochyta, only the younger leaves or stems would be evaluated while other leaves or stems could be taken for rust or chocolate spot testing. A multiple evaluation of individual plants could be made in this way.

One problem experienced with faba beans is that no sterilisation could be made as the leaves were very sensitive to sterilising chemicals. It was found that chocolate spot occasionally disrupted experiments as this disease takes a shorter time to develop its lesions. The diseases were easy to distinguish so results were not confounded. This cross-infection may cause problems in future experiments if plants parts are collected from field plots or an open-air nursery.

It is evident from the results obtained for the detached stems that the components that most closely reflect the known differences in resistance among Acc 970, Fiord and 811 were the percentages of the stems infected and mean lesion number and not lesion size or percent of lesions with pycnidia. At first sight, this appears to be in conflict with the criteria for scoring stems in Table 3.1 where the separation of resistant from susceptible responses is based partly on size of the lesion and the presence of pycnidia. Table 3.1 does not use the criterion, percent of stems infected (it does not have the same meaning for a whole plant and detached stem segments) nor lesion number although numbers are considered when scores are made. On the detached stems, and because there were many fewer lesions on Acc 970 and Fiord than on Acc

811, the percentage of these lesions with pycnidia was not a meaningful attribute and did not reflect the known differences in resistance. For Fiord and Acc 970 it was a percentage of a very low number of lesions and for Acc 811 a percentage of a relatively high number. Absolute numbers of lesions with pycnidia were far less on Acc 970 and Fiord than on Acc 811. Mean lesion number together with the percentage of stems infected was more indicative of the resistances.

The technique requires a controlled environment such as a growth chamber and this may limit the amount of material that can be tested. Nevertheless symptoms develop more rapidly in this environment than in the field or glasshouse and any batch of leaves or stems occupies the growth chamber for only 10 days. Given that the technique requires a controlled environment facility, the detached-stem test, in particular, should be a valuable additional test to the screening of large numbers of genotypes in the field which can only be done effectively during the normal growing season and if climatic conditions are suitable for infection. The detached-stem or -leaf test should also provide more accurate evaluations of interactions between different isolates of the pathogen and genotypes of the host.

CHAPTER 7. GENERAL DISCUSSION

7.1 Introduction

This thesis reports on several important findings on the variability within both the host species *Vicia faba* and the pathogen *Ascochyta fabae* which affect the expression of the disease. These results show that it is possible to have good resistance in cultivars but that the pathogen is sufficiently variable that a continuous breeding effort will be necessary to maintain resistance.

7.2 Genetics of resistance in faba beans to *Ascochyta fabae*

It is concluded from this study that several genes are involved in the resistance of faba beans to *A. fabae*. This conclusion is in agreement with one made by Rashid *et al.* (1991a) published while the present study was in progress. They found that resistance in faba beans was determined by several genes, some dominant and some recessive. It may be of significance that Acc 811 where resistance was found to be recessive, and the material in Rashid *et al.*'s study which showed comparable recessive resistance, both came from Afghanistan. The sources of recessive resistance in both the present study and Rashid *et al.* may thus have a common origin.

In addition, disease levels on different parts of the plant are not always strongly correlated (Jellis *et al.*, 1985; Lockwood *et al.*,1985; Pritchard *et al.*, 1989). It has been suggested that the resistance on stems and leaves may be controlled by different genes (Rashid *et al.*, 1991b). The present study has corroborated this, with many plants showing symptoms only on leaves and others only on stems. Furthermore, detached stems of Acc 970 developed lesions although intact ones rarely did. Finally, it was easier to induce the expression of the symptoms on stems than on leaves under suitable conditions. Since environmental conditions strongly influenced the disease reaction, a uniform environment such as that provided in the detached-organ technique would allow the comparison of both leaves and stem from the same plant in the same conditions and thus elucidate the genetics of the different responses.

In investigations of the genetics of resistance to Ascochyta it is likely that segregation ratios will be variable, as both dominant and recessive gene action has been found. Other modifying genes have also been implicated, as heterozygotes were not distinguishable from homozygote dominants at 21 days after inoculation but were later. In addition, heterogeneity for resistance was found within most populations assessed. These three factors, taken together, indicate that simple segregation ratios are unlikely to be experienced in crosses in breeding programs, except in very special circumstances.

7.3 Variability within *Ascochyta fabae* populations

The variability of *A. fabae*, previously suggested (Kharbanda and Bernier, 1980; Hanounik and Maliha, 1984; Ali, 1985; Rashid and Bernier, 1985; Hanounik and Robertson, 1989), has been confirmed in Chapter 5 where eight isolates produced no fewer than seven distinct disease patterns when tested on eight faba bean populations. This wide variability, mostly from the limited geographical area of South Australia, would be unexpected in an asexual organism, so the recent discovery of the sexual stage of the fungus (Jellis & Punithalingam, 1991; Dennis, personal communication) helps to explain it. The sexual stage allows the continuous development of new races or pathotypes through recombination and selection, which gives a moving target for plant breeders who are attempting to develop widely resistant cultivars. Furthermore, *A. lentis* has recently been placed within *A. fabae* as *A. fabae f. sp. lentis*, since their morphological characteristics are similar (Gossen *et al.*, 1986) but the gene flow, if any, between these sections of the species has not been determined. However, the pathogenicity test showed that *A. fabae* only infected faba beans and *A. lentis* only infected lentils. The literature also supported that *A. fabae* was highly specialised to faba beans (Yu, 1947; Lasman, 1965; Sundheim, 1973; Wallen and Galway, 1977).

For breeding for Ascochyta resistance to be efficient, detailed studies on the genetics of *A. fabae* are essential. Several critical questions need to be answered:

1) Do strains capable of parasitising different varieties of faba bean or different legume species exist within a single pathogen population?

2) If so, do these strains represent taxonomic varieties of *A. fabae* or are they different pathotypes (races) of the one variety?

3) Is the genetic diversity of populations of *A. fabae* parasitising highly susceptible varieties of faba bean comparable with that of populations on less susceptible ones?

4) What are the genetic relationships between the different varieties and races of *A. fabae*, in particular, are any reproductively isolated?

To answer these questions we need to screen pathogen populations with molecular markers which can resolve different levels of genetic diversity, such as isozymes, restriction fragment length polymorphisms (RFLPs) and polymerase chain reaction (PCR) markers. The value of these methods is emphasised by the lack of success at answering similar questions by using morphological characteristics (Gossen *et al.*, 1986; Rashid *et al.*, 1991b). Differentiation between varieties and pathotypes of the pathogen with such markers will provide valuable information on blight epidemiology and will indicate the factors responsible for changes in the composition of pathogen populations.

Additionally, the reproductive rate and mode of dispersal of the pathogen need to be better understood. It was noted that sporulation occurred as soon as 4 days after inoculation in extremely favourable conditions but it remains to be established how virulent these fresh spores are and how long both they and the lesions that produce them are viable. The literature reviewed indicated that the infection process is strongly affected by environmental conditions, especially humidity (Wallen and Galway, 1977; Pritchard *et al.*, 1989).

7.4 The expression of disease

The results in this study showed that field trials are of limited value in studies of the mode of inheritance of resistance, because the expression of the disease reaction is strongly affected by environmental variation. Furthermore, cultivars of faba beans are mixed populations with considerable genetic variation among the individual plants and populations of the disease organism are also variable. Finally, there is opportunity for foreign inoculum to cross-contaminate a field trial. Intensive studies on the genetics of Ascochyta resistance are therefore probably better done with single-

spore isolates on inbred lines in partially controlled environments such as glasshouses. Thus it is perhaps not surprising that field results were not consistent with detached-leaf results and glasshouse results for the same combination of host and pathogen genotypes (compare Chapters 3 and 6). Artificial inoculation of a field trial is, however, also important if plant breeders are going to apply the conclusions of this thesis, as they will need to be able to test their improved breeding materials. Nevertheless, this study showed that a field trial was effective in ranking genotypes of faba bean for their disease reaction and can thus be used by breeders for this purpose.

Inconsistent results on the expression of disease resistance to Ascochyta were obtained in field when compared with detached-leaf tests, because both the host plant (Chapter 3) and the disease organism (Chapter 5) responded differently. Although parent plants of Acc 970 had been chosen for their resistance and those of Acc 811 for their susceptibility in glasshouse tests, their progeny did not produce comparable results in a field trial. The detached-leaf technique gave a consistent discrimination between Acc 970 (uniformly resistant/highly resistant) and Acc 811 (uniformly susceptible/highly susceptible), but it was less consistent for Fiord which was also shown by mass selection to have a proportion of resistant plants. The reason could be that the environmental conditions at 21 DAI in field tests were not favourable for the pathogen to infect, colonise or sporulate on the plant.

It is unfortunate that the detached-organ tests were developed quite late in the program, so only a few experiments could be done. Several further studies need to be undertaken with the detached-leaf or -stem test before they are of use in investigations on the genetics of resistance and the disease reaction. The number of samples required to discriminate between genotypes with a particular level of statistical confidence needs to be established for the efficient use of this technique in breeding programs.

As the youngest leaves and parts of the stem provided the best material for testing by this technique, experiments should be undertaken to determine the means by which many stems could be encouraged to develop on a plant. The great advantage of a detached-organ test over a whole-plant test is that it enables multiple assessments to be made of an individual under strictly controlled conditions. The more stems that can be induced to develop, the greater the number of assessments. The technique

should greatly facilitate the studies of the interaction of the host genotypes with isolates of the pathogen.

7.5 Mechanisms of resistance

Plants possess a number of different resistance mechanisms of which the primary are structural and physiological resistance (Jones, 1987). The actual mechanisms through which resistance operates should be studied and characterised in detail for successful breeding for resistance to this pathogen. *Ascochyta fabae* has been reported infecting bean by penetrating through the plant surface and/or through stomata (Dodd, 1971). While wounding is uncommon in natural conditions and is not necessary for infection (Wallen and Galway, 1977), the present study showed that it helped induce infection and sporulation, perhaps by providing both sap in which the spores germinate and unrestricted access for the hyphae into the plant tissue. For the detached-stem test, the results suggested that wounding by cutting the stem resulted in larger lesions and the production of pycnidia. To simulate the natural situation the cut surfaces might need to be sealed by using paraffin or plastic film. Since Acc 970 became infected only at cut surfaces, its resistance may have to do with the structure or chemistry of its cuticle or epidermis.

A very preliminary experiment with exciting promise showed that filter-sterilised extracts of bean leaves differentially enhanced growth of *A. fabae* on PDA, with most growth from Acc 811, less from either Acc 970 or Fiord, and least from unsupplemented medium. This result, which needs confirmation and amplification, indicates that chemical components as well as morphology of the host plant could influence the infection by the pathogen. Once an infection had occurred, it was not obvious that any resistant reaction was present to limit the size of the lesion. This observation, together with the finding that the younger parts of a plant are more readily infected should provide an indication of where to study the mechanism of resistance. From this evidence, both chemical and morphological aspects of the host plant need to be investigated. Further histological studies would help to explain the host defence mechanisms.

7.6 Quantifying the response

The assessment key adapted from the literature was developed in order to describe infection types and to quantify disease severity. While this is basically descriptive and relies upon subjective observation, it has been transformed into coded scores (infection type) for ease and speed of use. The several grades of increasing infection fall into two categories of great agricultural significance, those where no spores are formed (resistance) and those where spores form (susceptible) and further infection can occur. Disease assessment keys have been constructed for many pathogens and their use for particular diseases has been standardised to ensure that assessment may be compared.

The expression of disease in glasshouse and field may be different according to environmental conditions. In addition, it was found that in detached-organ tests, where environmental conditions were extremely favourable, colonisation and sporulation were easier to induce than in field tests and lesion size was not correlated with sporulation. In whole plants, the intact leaves often droop allowing the inoculum to run away, while normal atmospheric conditions dry out the inoculum before the spores can germinate. In the detached-organ test, however, leaves were supported holding the inoculum and humidity was maintained at a high level, which allowed infection to occur even in the resistant accession. Since the lesions in this case were different from those on the whole plant as they were fewer, larger and sporulating, perhaps the best criteria for distinguishing resistant from susceptible materials would be the percentage of successfully infected leaves/stems and lesion number.

In summary, environmental conditions have a strong influence on the disease reaction and hence on the evaluation of host resistance. Furthermore, both the pathogen and host showed great variability, making it difficult to standardise the criteria for evaluation. Time after inoculation could not be specific, because it depended on environmental conditions. Single recordings may suffice if made at the time of maximum disease expression but, as this is difficult to judge, inclusion of commercial cultivars and known susceptible and resistant accessions in the tests would provide controls and assist in defining the most appropriate time of evaluation.

7.7 Breeding faba beans for resistance to Ascochyta blight

7.7.1 Mass-selection method

It is evident that three cycles of mass selection have significantly raised the level of resistance in Fiord. Mass selection is used in cross-pollinated species when it is believed that the chosen character is controlled by many genes (Allard, 1960). The genetic studies presented here together with those of Rashid *et al.* (1991a) indicate that several genes are involved in resistance of faba beans to Ascochyta. This is particularly relevant when resistance is being sought simultaneously to several different pathotypes as appears to be the case in South Australia. The success of the selection after three cycles together with the evidence that there continues to be some variation among the selections suggests there are probably several genes for resistance within Fiord, so mass selection will increase the resistance to Ascochyta in the most suitable background.

Alternatively, mass selection can be applied within populations that are already mixed. Encouragement of disease infection, both natural and by artificial inoculation, could be used to reveal resistant plants. The Waite Institute's bean selection program has adapted the selection method in glasshouse or open field by sowing bean plants in plastic trays, and artificial inoculation is done at an early stage (2 weeks after germination), then healthy individual plants are transplanted and subjected to controlled pollination. The method is providing speed, ease of handling and the flexibility to be done at any time of the year. Since the pathogen has both sexual and asexual stages, there is great possibility for development of races. The mass-selection procedure as reported in this study was simple to undertake and it would be useful if it could be continued indefinitely to provide material resistant to new isolates as they develop. Nevertheless, mass selection is only effective if it involves variation that is genetically controlled. Environmental variation and escape from infection can easily be mistaken for resistance.

7.7.2 Development of synthetic varieties from inbred lines

The evidence that several genes are involved, with some dominant and others recessive, that gene interaction occurs, that there are several isolates, and that there is

a sexual stage in the fungal life cycle, all have bearing on the efficiency of breeding programs. This diversity in pathogenicity of *A. fabae* has important ramifications for the breeding of resistant faba bean cultivars because those with race-specific resistances will not control the disease for long after their release into commercial cultivation. The selected inbred lines can be used as sources of foundation stocks for synthetic or composite varieties.

Following the development of known inbred lines with known resistances to known pathotypes, they can be mixed to form a composite variety (Allard, 1960) and to recombine the resistance genes. This procedure has been used in Europe to make major advances in bean yields, for which mass selection is impractical because the outcrossed individuals in any population are the vigorous ones, rather than those which have genes for high vigour (Bond, 1982).

7.8 Implications for chemical control of Ascochyta blight

This investigation supports the view that Ascochyta is a disease which primarily infects young tissue. An increased resistance was found in older leaves even in the susceptible accessions, as reported elsewhere (Ali, 1985; Van Breukelen, 1985; Pritchard *et al.*, 1989). The nature of the development of the disease make it difficult to apply non-systemic fungicides as an efficient control measure. Non-systemic fungicides have been reported to be unable to control the disease and the only effective fungicides reported have been systemic (Kharbanda and Bernier, 1976; Kharbanda and Bernier, 1979). A systemic fungicide has the distinct advantage over an eradicant and traditional protectant that it is internally distributed and hence not subject to weathering. A systemic substance should be absorbed sufficiently, be translocated from the point of application to the site of the pathogen and have a considerable degree of stability within the host plant. These aspects of performance of systemic fungicides within faba beans need to be understood if they are to give efficient control of the disease. Even plants which had no apparent symptoms upon their foliage before harvesting had symptoms upon the seeds, which makes them unsuitable for the major human-consumption market. Since there are no available cultivars with effective resistance, short-term disease control will require reduction or prevention of infection. For this procedure to be effective it is essential that the behaviour of systemic

fungicides be understood, which would allow farmers to apply them with maximum economic efficiency to control the disease.

7.9 Conclusions

Resistance to Ascochyta blight has been found in faba beans, the resistance is controlled by several genes; some are dominant and some recessive. The analysis of the genetics of resistance is complicated by the existence of numerous pathogenic races of *Ascochyta fabae* and by the dependence of the disease reaction on environmental conditions. Thus there would appear to be considerable potential for minimising disease losses through plant breeding. In recognition of this potential, the majority of Ascochyta blight-resistance breeding programs may be accomplished by breeding strategies involving either composite (synthetic) varieties or mass selection. Efficient long-term disease control may come about only by developing a greater understanding of the genetic determinants of pathogenicity and the genetic control of host defence mechanisms in response to infection. The use of systemic fungicide would provide a short-term of disease control. It is hoped that the findings in this study would benefit the bean breeder for effective control and research programs.

APPENDICES

Appendix 3.1. The University of California Mix (UC Soil)

2.3 cubic metres of washed coarse sand is sterilised at 100° C for 1/2 hour in a sterilising mixer. One bale of Peatmoss (1/6 cubic metre which expands to 1/3 cubic metres) is added and mixed for 10 seconds. The combined temperature drops to about 75 ° C.

After about 10 minutes (more cooling) the following fertilisers are added and mixed with the sand and peat for 20 seconds. The pH should be about 6.5.

Fertilisers.

Calcium Hydroxide	700 g
Calcium Carbonate	480 g
Nitrophoska (15-4-12)	600 g

Appendix 3.2 Numbers of plants at each disease score, 21 days after inoculation (DAI) and 45 DAI, of the parent populations Acc 970 and Acc 811, their F1 hybrids and F2 populations.

Population	Number of plants with disease score at 21 DAI of						Number of plants with disease score at 45 DAI of						Total
	0	1	2	3	4	5	0	1	2	3	4	5	
Acc 970													
970(2)	23	1	0	0	0	0	16	7	1	0	0	0	24
970(3)	8	0	0	0	0	0	0	3	5	0	0	0	8
970(4)	20	0	0	0	0	0	8	7	5	0	0	0	20
970(5)	15	25	0	0	0	0	2	34	4	0	0	0	40
970(6)	5	0	0	0	0	0	2	1	2	0	0	0	5
970(7)	6	0	0	0	0	0	1	3	2	0	0	0	6
970(9)	29	0	0	0	0	0	2	20	7	0	0	0	29
970(10)	26	0	0	0	0	0	6	7	13	0	0	0	26
970(11)	4	0	0	0	0	0	2	2	0	0	0	0	4
970(13)	25	1	1	0	0	0	0	6	16	5	0	0	27
970(14)	23	0	0	0	0	0	7	12	4	0	0	0	23
970(15)	11	0	0	0	0	0	3	4	4	0	0	0	11
970(16)	44	1	1	0	0	0	28	8	10	0	0	0	46
970(17)	20	1	0	0	0	0	3	14	4	0	0	0	21
total	259	29	2	0	0	0	80	128	77	5	0	0	290
Acc 811													
811(11)	0	0	0	43	9	6	0	0	0	3	8	47	58
811(14)	0	0	0	0	0	3	0	0	0	0	0	3	3
811(16)	0	0	0	32	11	7	0	0	0	12	9	29	50
811(17)	0	0	1	17	10	5	0	0	0	0	6	27	33
811(18)	0	0	0	14	8	1	0	0	0	1	3	19	23
811(19)	0	0	0	35	18	8	0	0	0	9	23	29	61
811(20)	0	0	1	54	11	3	0	0	0	29	32	8	69
811(21)	0	0	0	13	5	2	0	0	0	0	2	18	20

Appendix 3.2 (continued)

Population	Number of plants with disease score at 21 DAI of						Number of plants with disease score at 45 DAI of						Total
	0	1	2	3	4	5	0	1	2	3	4	5	
811(27)	0	0	0	6	8	4	0	0	0	1	4	13	18
total	0	0	2	214	80	39	0	0	0	55	87	193	335
F1 970 x 970													
970(2) x 970(11)	4	0	0	0	0	0	0	0	4	0	0	0	4
970(2) x 970(13)	4	0	0	0	0	0	0	4	0	0	0	0	4
970(3) x 970(5)	1	0	0	0	0	0	1	0	0	0	0	0	1
970(3) x 970(6)	3	0	0	0	0	0	1	2	0	0	0	0	3
970(3) x 970(7)	4	0	0	0	0	0	2	1	1	0	0	0	4
970(4) x 970(11)	2	0	0	0	0	0	1	1	0	0	0	0	2
970(5) x 970(9)	4	0	0	0	0	0	0	2	2	0	0	0	5
970(7) x 970(3)	3	0	0	0	0	0	2	1	0	0	0	0	3
970(9) x 970(5)	3	0	0	0	0	0	2	1	0	0	0	0	3
970(10) x 970(15)	5	0	0	0	0	0	1	3	1	0	0	0	5
970(11) x 970(4)	4	0	0	0	0	0	3	1	0	0	0	0	4
970(11) x 970(9)	4	0	0	0	0	0	3	1	0	0	0	0	4
970(11) x 970(14)	1	0	0	0	0	0	1	0	0	0	0	0	1
970(13) x 970(14)	10	0	0	0	0	0	2	6	2	0	0	0	10
970(13) x 970(16)	4	0	0	0	0	0	2	2	0	0	0	0	4
970(14) x 970(7)	5	0	0	0	0	0	0	4	1	0	0	0	5
970(14) x 970(16)	4	0	0	0	0	0	0	3	1	0	0	0	4
970(15) x 970(10)	6	0	0	0	0	0	0	3	3	0	0	0	6
970(16) x 970(13)	2	0	0	0	0	0	1	1	0	0	0	0	2
970(16) x 970(14)	8	0	0	0	0	0	4	4	0	0	0	0	8
970(17) x 970(5)	4	2	0	0	0	0	2	3	1	0	0	0	6
total	85	2	0	0	0	0	28	43	16	0	0	0	87

Appendix 3.2 (continued)

Population	Number of plants with disease score at 21 DAI of						Number of plants with disease score at 45 DAI of						Total
	0	1	2	3	4	5	0	1	2	3	4	5	
F1 811 x 811													
811(3) x 811(18)	0	0	0	1	0	0	0	0	0	1	0	0	1
811(11) x 811(16)	0	0	0	3	4	1	0	0	0	0	1	7	8
811(11) x 811(19)	0	0	0	1	0	2	0	0	0	0	1	2	3
811(16) x 811(11)	0	0	0	0	2	0	0	0	0	0	0	2	2
811(16) x 811(27)	0	0	0	1	1	0	0	0	0	1	1	0	2
811(18) x 811(20)	0	0	0	4	0	0	0	0	0	0	0	4	4
811(19) x 811(11)	0	0	0	3	0	0	0	0	0	0	0	3	3
811(19) x 811(18)	0	0	0	2	0	0	0	0	0	0	0	2	2
811(20) x 811(21)	0	0	0	5	0	1	0	0	0	0	1	5	6
811(27) x 811(20)	0	0	0	4	3	1	0	0	0	4	3	1	8
total	0	0	0	24	10	5	0	0	0	6	7	26	39
F2 970 x 970													
970(6) x 970(10)	41	4	0	0	0	0	23	13	9	0	0	0	45
970(13) x 970(14)	42	0	0	0	0	0	4	22	16	0	0	0	42
979(17) x 970(5)	28	16	0	0	0	0	5	35	4	0	0	0	44
total	111	20	0	0	0	0	32	70	29	0	0	0	131
F2 811 x 811													
811(18) x 811(20)	0	0	0	16	5	8	0	0	0	3	5	21	29
811(20) x 811(18)	0	0	2	28	1	0	0	0	0	6	8	17	31
811(20) x 811(21)	0	0	1	53	6	7	0	0	0	15	19	33	67
811(27) x 811(20)	0	0	1	32	24	10	0	0	0	8	34	25	67
total	0	0	4	129	36	2	0	0	0	32	66	96	194

Appendix 3.2 (continued)

Population	Number of plants with disease score at 21 DAI of						Number of plants with disease score at 45 DAI of						Total
	0	1	2	3	4	5	0	1	2	3	4	5	
F1 970 x 811													
970(3) x 811(16)	2	0	0	0	0	0	1	0	1	0	0	0	2
970(3) x 811(18)	11	0	0	0	0	0	2	5	4	0	0	0	11
970(4) x 811(11)	5	0	0	0	0	0	2	2	1	0	0	0	5
970(5) x 811(20)	3	1	0	0	0	0	0	2	2	0	0	0	4
970(7) x 811(16)	3	0	1	0	0	0	0	3	1	0	0	0	4
970(7) x 811(19)	2	0	0	0	0	0	0	0	2	0	0	0	2
970(7) x 811(20)	6	0	0	0	0	0	0	3	3	0	0	0	6
970(9) x 811(18)	3	1	0	0	0	0	1	3	0	0	0	0	4
970(10) x 811(18)	2	0	0	0	0	0	0	1	1	0	0	0	2
970(11) x 811(16)	2	0	0	0	0	0	0	1	1	0	0	0	2
970(13) x 811(11)	9	0	1	0	0	0	5	1	3	1	0	0	10
970(13) x 811(17)	9	0	0	0	0	0	1	6	2	0	0	0	9
970(14) x 811(20)	2	0	0	0	0	0	2	0	0	0	0	0	2
970(17) x 811(16)	2	0	0	0	0	0	0	1	1	0	0	0	2
970(17) x 811(20)	9	0	0	0	0	0	1	3	5	0	0	0	9
total	70	2	2	0	0	0	15	31	27	1	0	0	74
F1 811 x 970													
811(11) x 970(3)	8	0	1	0	0	0	2	4	3	0	0	0	9
811(11) x 970(4)	2	0	1	0	0	0	2	0	1	0	0	0	3
811(11) x 970(7)	18	0	2	0	0	0	9	6	5	0	0	0	20
811(11) x 970(13)	9	0	3	0	0	0	2	2	8	0	0	0	12
811(16) x970(3)	4	0	0	0	0	0	1	2	1	0	0	0	4
811(16) x 970(7)	6	1	1	0	0	0	3	3	2	0	0	0	8
811(16) x 970(13)	5	0	0	0	0	0	0	2	3	0	0	0	5
811(17) x 970(3)	4	0	0	0	0	0	2	2	0	0	0	0	4
811(17) x 970(7)	2	0	0	0	0	0	1	0	1	0	0	0	2

Appendix 3.2 (continued)

Population	Number of plants with disease score at 21 DAI of						Number of plants with disease score at 45 DAI of						Total
	0	1	2	3	4	5	0	1	2	3	4	5	
811(17) x 970(13)	3	0	0	0	0	0	0	1	2	0	0	0	3
811(18) x 970(13)	6	0	0	0	0	0	0	1	5	0	0	0	6
811(19) x 970(3)	4	0	0	0	0	0	0	2	2	0	0	0	4
811(19) x 970(7)	25	1	0	0	0	0	11	12	3	0	0	0	26
811(19) x 970(17)	4	0	2	0	0	0	0	0	6	0	0	0	6
811(20) x 970(3)	8	0	0	0	0	0	4	2	2	0	0	0	8
811(20) x 970(7)	8	1	1	0	0	0	3	2	5	0	0	0	10
811(20) x 970(16)	2	0	1	0	0	0	2	0	1	0	0	0	3
811(21) x 970(7)	5	0	0	0	0	0	4	1	0	0	0	0	5
811(21) x 970(13)	0	2	0	0	0	0	0	1	1	0	0	0	2
811(21) x 970(16)	4	1	1	0	0	0	2	1	3	0	0	0	6
811(27) x 970(7)	4	0	0	0	0	0	1	2	1	0	0	0	4
811(27) x 970(13)	4	2	2	0	0	0	3	2	3	0	0	0	8
total	135	8	15	0	0	0	52	48	58	0	0	0	158
F2 970 x 811													
970(13) x 811(11)	37	7	17	7	4	0	10	17	25	13	5	2	72
970(13) x 811(17)	27	9	5	12	3	0	6	13	19	13	4	0	56
970(13) x 811(19)	11	2	5	10	0	0	2	3	16	6	1	0	28
970(13) x 811(20)	35	7	7	21	0	1	7	13	26	18	5	2	71
F2 811 x 970													
811(11) x 970(7)	50	0	6	14	2	0	18	16	21	9	3	5	72
811(20) x 970(3)	45	6	1	21	2	0	14	9	31	15	6	0	75
811(27) x 970(13)	42	18	7	12	6	0	9	18	24	27	5	2	85
total	137	24	14	47	10	0	41	43	76	51	14	7	232

Appendix 4.1 Numbers of plants at each disease score, 15 days after inoculation (DAI) and 21 DAI, of the 31 selections from Fiord grouped in their eight families.

Family	Selection	Number of plants with disease score at 15 DAI of				Number of plants with disease score at 21 DAI of			
		0	1	2	3+	0	1	2	3+
24	86	7	12	1	0	1	9	10	0
	132	2	14	4	0	0	7	13	0
	134	15	5	0	0	7	7	6	0
120	51	3	13	4	0	2	8	10	0
	53	10	8	1	0	8	7	4	0
	87	10	10	0	0	6	8	6	0
	93	6	12	2	0	4	6	10	0
181	5	14	6	0	0	4	7	9	0
	9	6	13	0	1	2	11	6	1
	10	10	9	1	0	6	8	6	0
	54	19	1	0	0	8	9	3	0
193	15	8	12	0	0	3	14	3	0
	58	7	12	1	0	0	9	11	0
	95	8	12	0	0	4	12	4	0
	144	7	11	2	0	4	8	8	0
194	60	15	5	0	0	9	9	2	0
	61	11	9	0	0	5	6	9	0
	109	8	12	0	0	6	12	2	0
	110	10	10	0	0	6	8	6	0
260	25	19	0	0	0	11	8	0	0
	67	2	12	5	1	1	4	13	2
	70	4	13	3	0	2	9	8	1
	114	14	6	0	0	5	9	6	0
262	71	14	5	0	0	8	9	2	0
	122	11	9	0	0	3	12	5	0
	155	14	6	0	0	6	11	3	0
	157	4	12	3	0	2	9	8	0
314	80	8	9	3	0	3	7	9	1
	82	9	3	8	0	4	5	10	1
	84	7	13	0	0	4	10	6	0
	131	6	13	1	0	1	8	11	0

Appendix 5.1 The calculation of expected mean square.

Source of variation	d.f.	MS	F	E[MS]
Colonies	51	6009.77	18.3***	$n_o \sigma_C^2 + n \sigma_{SS}^2 + \sigma^2$
Single spore	202	328.71	22.7***	$n \sigma_{SS}^2 + \sigma^2$
Error	1016	14.48		σ^2
Total	1269			

$$n_o = \frac{1}{a-1}\left(\sum^a n_i - \frac{\sum^a n_i^2}{\sum^a n_i}\right)$$

Where: 2 colony x 3 single spore

2 colony x 4 single spore

48 colony x 5 single spore

$\Sigma n_i = 1270$

$\Sigma n_i^2 = 31250$

$$n_o = \frac{1}{52-1}\left(1270 - \frac{31250}{1270}\right) = 24.42$$

So:

$328.71 = 5\,\sigma_{SS}^2 + 14.48$

$\sigma_{SS}^2 = 62.846$

$6009.77 = 24.42\,\sigma_C^2 + 5(62.84) + 14.48$

$\sigma_C^2 = 232.640$

Total variance $= \sigma_C^2 + \sigma_{SS}^2 + \sigma_P^2$

$= 232.640 + 62.846 + 14.48$

So: The percentage of variation due to colony is 75.05%, single spore 20.28% and the error 4.67%.

Appendix 5.2 ANOVA of disease efficiency.

Source of variation	d.f.	SS	MS	v.r.	F-pr
Stem					
rep	1	0.00109	0.00109		
isolate	7	1.93682	0.27669	4.68	0.030
residual	7	0.41369	0.05910		
accession	7	15.89243	2.27035	33.10	<0.001
isolate*accession	49	6.42786	0.13118	1.93	0.009
residual	56	3.84064	0.06856		
rep*isolate*accession*plant	494(18)	32.96667	0.06673		
Total	621(18)	61.43530			
Leaf					
rep	1	0.09831	0.09831		
isolate	7	4.68260	0.66894	7.07	0.01
residual	7	0.66186	0.09455		
accession	7	14.81658	2.11665	21.37	<0.001
isolate*accession	49	5.52293	0.11271	1.14	0.318
residual	56	5.54582	0.09903		
rep*isolate*accession*plant	494(18)	37.83567	0.07659		
Total	621(18)	68.43346			
Whole plant					
rep	1	0.03002	0.03002		
isolate	7	2.50191	0.35742	5.68	0.018
residual	7	0.44072	0.06296		
accession	7	14.67632	2.09662	36.32	<0.001
isolate*accession	49	4.26879	0.08712	1.51	0.068
residual	56	3.23280	0.05773		
rep*isolate*accession*plant	494(18)	20.27809	0.04105		
Total	621(18)	45.14410			

Appendix 5.3 Method of calculating Tukey's Honestly Significant Difference Test.

$$\text{Tukey's} = \frac{\sqrt{\text{Res MS}}}{\sqrt{n}} \times q_{k,v}$$

Where:

$q_{k,v}$	=	value from table Studentized range
k	=	number of treatments
v	=	Residual d.f.
n	=	number of values that made up each mean
Res MS	=	Residual MS from ANOVA table

Thus to calculate Tukey's Honestly Significant Difference Test for Stem DE in Appendix 5.2:

isolate*accession
$$= \frac{\sqrt{0.06856}}{\sqrt{10}} \times q_{50,56}$$
$$= \frac{0.2618}{3.1623} \times 5.3$$
$$= 0.44$$

accession
$$= \frac{\sqrt{0.06856}}{\sqrt{80}} \times q_{8,56}$$
$$= \frac{0.2618}{8.994} \times 4.48$$
$$= 0.13$$

isolate
$$= \frac{\sqrt{0.05910}}{\sqrt{80}} \times q_{8,7}$$
$$= \frac{0.24310}{8.994} \times 5.80$$
$$= 0.16$$

Appendix 5.4 ANOVA of disease score.

Source of variation	d.f.	SS	MS	v.r.	F-pr
Stem					
rep	1	0.001	0.001		
isolate	7	84.670	12.096	6.40	0.013
residual	7	13.233	1.890		
accession	7	207.110	29.587	21.33	<0.001
isolate*accession	49	113.569	2.318	1.67	0.032
residual	56	77.674	1.387		
rep*isolate*accession*plant	494(18)	580.533	1.175		
Total	621(18)	1071.178			
Leaf					
rep	1	0.0975	0.0975		
isolate	7	83.5282	11.9326	4.47	0.033
residual	7	18.6858	2.6694		
accession	7	405.2130	57.8876	43.80	<0.001
isolate*accession	49	88.8805	1.8139	1.37	0.126
residual	56	74.0195	1.3218		
rep*isolate*accession*plant	494(18)	460.7167	0.9326		
Total	621(18)	1120.6832			
Average					
rep	1	0.0720	0.0720		
isolate	7	69.8540	9.9791	4.81	0.028
residual	7	14.5116	2.0731		
accession	7	292.6495	41.8071	40.86	<0.001
isolate*accession	49	81.0059	1.6532	1.62	0.042
residual	56	57.2991	1.0232		
rep*isolate*accession*plant	494(18)	323.2625	0.6544		
Total	621(18)	832.9843			

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