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CHOCOLATE SPOT OF FABA BEANS IN SOUTH AUSTRALIA

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SUMMARY

Mycelial infections on crop residues of faba beans are the most important mechanism for summer survival of *Botrytis* spp which cause chocolate spot in southern Australia, and are the main source of primary inoculum. Up to 41 active colonies/ 200g of residue, and survival for more than 12 months in the field were recorded.

Host range studies showed that *Vicia narbonensis* Acc. SA 22648 and *V. sativa* cvs. Languedoc and Blanchfleur were as susceptible to *B. fabae* as *V. faba* cv. Fiord. Other grain legume species tested ,including *V. villosa* ssp. *dasycarpa* cv. Namoi and *V. benghalensis* cv.Popany, were resistant.

A model describing the relationship between infection % (I), temperature (T) and wet period (W) for *B. fabae* infection of *V. faba* cv.Fiord leaves in a controlled environment was determined as $I = 34.85T + 89.91(LnW) - 0.85T^2 - 2.69(LnW).T - 404.96$ ($R^2 = 0.93$). This showed significant correlation with field observations and predicted that the best conditions for infection would occur in wet spring weather.

A detached leaf inoculation technique was developed which was as effective as whole plant inoculations in screening for disease resistance. The technique was used to identify partially resistant *V. faba* accessions held in the Waite Agricultural Research Institute collection and to screen these against *B. fabae* and *B. cinerea* isolates collected from field surveys.

These tests and exposure of plants to natural infection in the field provided no evidence for races within *B. fabae*

and *B. cinerea*. They identified accessions derived from the ICARDA lines BPL 710 and BPL 261 as the best sources of resistance to chocolate spot in Australia. *B. fabae* was identified as the main disease causing organism while *B. cinerea* can cause the same symptoms but is usually less aggressive.

It was concluded that optimum disease control will require an integrated approach using disease resistance supplemented by fungicides as appropriate.

STATEMENT OF ORIGINALITY AND CONSENT TO PHOTOCOPY OR LOAN

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

I give consent for this thesis being made available for photocopying and loan.

Jeremy I Dennis

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CHAPTER ONE INTRODUCTION

1.1 LITERATURE REVIEW

1.1.1 Host

Description

Vicia faba L. is an erect, simple stemmed annual normally 50 to 200 cm high with one or more basal branches. The leaves are alternate and pinnate with two to eight oval leaflets up to 7.5 cm in length. It is mainly cross pollinating with short axillary racemes formed at flowering nodes, and seeds are produced in pods which vary in size with the number and size of seeds.

V. faba is the most isolated of the *Vicia* species with fewer and larger chromosomes (2n=12) than most other species within the genus (2n=14) (Ladizinsky, 1975). The only morphological characters in which sp. *faba* differs from all other *Vicia* species are the lack of tendrils, and the aspect of the hilum which is at right angles to the length of the seed (Gunn, 1978). No successful crosses have been made between *V. faba* and other *Vicia* species, although other *Vicia* species have been intercrossed (Lawes *et al*, 1983). At least one author (Ladizinsky, 1975) has suggested a separate genus for this species.

Some distinct groups within the species are recognised and the classification of Muratova (1931), based mainly on seed size, has been widely used. He recognised two subspecies, viz. *paucijuga* (2-2.5 pairs leaflets) and *eu-faba* (3-4 pairs

leaflets). Ssp. *eu-faba* was subdivided into var.minor with small round seeds (1.0 cm long), var. equina with medium size seeds (1.5 cm long) and var. major with large broad flat seeds (2.5 cm long) (Lawes *et al*, 1983). Cubero (1974) altered the classification to the four botanical varieties faba, equina, minor and paucijuga in the subspecies *minor* and *faba* from the genetics of 17 quantative characteristics. Var. major, however, is synonymous with var. faba (Sirks, 1931) and is in more general use, while Hanelt (1972) considered var. paucijuga to be a geographical race of ssp. *minor*.

Not all authors have used the same limits of seed length and weight to define the three botanical varieties of *V. faba*, viz. major (large), equina (medium) and minor (small seeded) (Hebblethwaite, 1983). These inconsistencies and the development of cultivars from intervarietal crosses have made these groupings almost impossible to maintain. There has also been a need to distinguish individual cultivars for Plant Variety Rights. Higgins *et al* (1981) examined 11 discontinuous characters on 128 Western European cultivars and found seed testa colour, seed hilum colour and melanin spots in flowering petals useful for classification. On the basis of these they produced 16 possible groups, nine of which contained known cultivars. They also suggested the use of the determinate growth character (Sjodin, 1971) for further classification. This system is limited in that it does not reflect evolutionary pathways or genealogical relationships between groups. It is, however, useful for distinguishing cultivars, many of which are populations, by determining not only presence or absence of a character, but also its frequency. Isozyme patterns from seed extracts have also been used to

distinguish some cultivars (Bassiri and Rouhani, 1977; Gates and Boulter, 1979, 1980).

Currently only two varieties are used in commercial crop production in Australia and these can be easily distinguished by seed type. The most commonly grown is cv. Fiord which is small seeded and commonly called "faba beans" and the other is cv. Aquadulce which is large seeded and known as "broad beans".

Distribution

The cultivated faba bean has been grown in the Mediterranean Basin and Europe since the Bronze Age but its origins are uncertain in the absence of a wild progenitor. Most authors (Cubero, 1973, 1974; Zohary and Hopf, 1973; Zohary, 1977) consider evolution and domestication occurred from a central *Vicia* nucleus in the Middle East. Ladizinsky (1975), however, favours domestication in Central Asia and introduction into Europe with invading tribes. Faba beans have been recorded in China and India for over a thousand years and were introduced into North and South America and Australasia with the first European migration.

Faba bean production is now widespread in temperate and subtropical regions with a wide diversity of types grown. Small seeded cultivars are predominant in northern Europe , Nile Valley, Ethiopia, Afghanistan, India, North America and Australia. Large seeded types are most important in the Mediterranean Basin, western Asia, China and Latin America. Most production is as rain-fed crops in areas with >350mm rainfall with some irrigation in dry inland areas.

In Australia faba beans are grown mostly in South Australia and Victoria on neutral to alkaline soil in high rainfall (>400mm) cropping regions as a break for cereals. Production in South Australia is divided between the central region (12,000 ha in 1989) and the south-eastern region (9,000 ha in 1989) with some faba beans grown on the eastern Eyre Peninsula (500 ha in 1989) (Ragless, 1989).

Production

FAO (1988) statistics for 1987 area, yield and production are summarised for major geographical regions in Table 1.1

TABLE 1.1 Faba bean area, yield and production in 1987

REGION	AREA (1000ha)	YIELD (kg/ha)	PRODUCTION (1000t)
AFRICA	854	1620	1384
NORTH /CENTRAL AMERICA	97	936	91
SOUTH AMERICA	207	517	107
ASIA	1766	1393	2461
EUROPE	297	2070	614
AUSTRALIA	50	1260	63
USSR AND EASTERN EUROPE	20	1868	37
WORLD TOTAL	327	1443	4720

Since 1979 there has been a reduction in area in Europe (Northern and Eastern) , USSR and China with increases in North America, Africa and Australia. This has produced an overall world reduction in area, principally through trends in China which still produces 52% of the world's faba beans. Improved yields, however, have resulted in an overall increase in production (FAO, 1988). The decline in most European countries has been attributed to the instability in yields

relative to other crops and competition from cheap protein imports (Hawtin and Hebblethwaite, 1983).

Faba beans are normally planted in spring in Northern Europe but there has been a move to autumn sown varieties in the UK and France because of their 20-25% yield advantage despite greater risk of disease and frost damage (Anon., 1987a). In Southern Europe and the Mediterranean Basin they are usually grown as a rain-fed winter crop while in central Asia and Egypt they are grown under irrigation. In China, which still produces 1700,000ha (FAO, 1988), faba beans are either autumn sown following rice, or spring sown following wheat in areas with severe winters (Tao, 1981).

The area of faba beans grown in southern Australia has expanded rapidly since 1980 when their potential in cereal rotations was recognised (Laurence, 1979) and a variety suitable for broad acre farming practices developed. Production is currently dominated by the early maturing semi-determinate cultivar Fiord which was selected from an introduction from the Greek island of Naxos and has an average seed size of 0.45g (Knight, 1988). Larger seed types which attract a price premium are being developed. Area of production in 1987 was 35,000ha, 3,000ha and 7,700ha in South Australia, Victoria and New South Wales respectively (Rees, 1988) with an average yield of 1816kg/ha (FAO, 1988). Area in South Australia, however, had declined to 21,000ha in 1989 (Ragless, 1989) because of erratic yields through poor seasons and disease problems and is likely to stabilise at this level. In Australia faba beans are usually grown as a rain-fed winter crop sown in autumn.

Uses

Legumes have been used in rotations to improve cereal yields for a long time. This has been attributed to their ability to fix nitrogen through association with *Rhizobium* bacteria, and to act as a break in cereal diseases (Prew and Dyke, 1979). In temperate Australia emphasis has been on pasture legumes, but recently there has been a move to grain legumes in response to a developing stock feed market and the need for alternative crops to counter falling wheat prices (Rees, 1988).

Studies on nitrogen fixation by faba beans showed a range of 30-600 kg/ha with an average of 57 kg/ha (Hamblin, 1987). Most of this is removed with the grain but soil fertility in terms of total soil nitrogen and organic matter levels will be maintained and under low yielding conditions sufficient nitrogen will be provided for the following cereal crop (Anon., 1987b). At Rothamsted the effect of a faba bean crop on the yield of a following barley crop was equivalent to 40 kg/ha of N-fertilizer (Prew and Dyke, 1979). It also reduced incidence of take-all disease (*Gaeumannomyces graminis* var. *tritici*) in a wheat crop to 1% of that recorded when the previous crop was barley (Prew, 1980).

Faba beans form a minor component of human diets in many countries but are an important substitute for meat in the Eastern Mediterranean and Middle East regions (Simpson, 1983). In 1978 79% of the 250,000 tons of faba beans produced in Egypt was used for human consumption (Gabrial, 1982). 100g of cooked faba beans will supply, with the exception of methionine and threonine, most of the daily requirements for

essential amino acids (Ali *et al*, 1982). Faba beans also contain antinutritional factors and excessive consumption of fresh beans can cause the disease known as favism (Marquardt, 1982).

The major use of faba beans is in animal food as a protein supplement. They are suitable for ruminants and pigs at up to 20% of the compound feed (Simpson, 1983) and may be used at higher rates for poultry (Lindgren, 1975). Various cooking processes are often used to reduce antinutritional factors and amino acid additives are sometimes required (Simpson, 1983). In Australia sheep may be grazed on cereal stubbles with faba beans supplied as a protein supplement, or on faba bean stubbles where spilt grain is the major nutritional component (Anon., 1987b).

1.1.2 Disease

Symptoms

Chocolate spot disease of faba beans is mostly associated with leaves, but under favourable conditions stems, pods and flowers can be affected. Symptoms range from minor necrosis to complete destruction of large areas of host tissue. The origin of the common name is unknown, but it now has universal usage and refers to the red/brown discrete lesions exhibited at the onset of the disease. Two phases of the disease are recognised and were described by Wilson (1937) as aggressive and non-aggressive. The non-aggressive phase appears as rust coloured to dark brown discrete spots which expand to a diameter of 1-10 mm. They occur on both the upper and lower leaf surfaces,

but are frequently more numerous on the upper side. Stem lesions are usually superficial and may extend in streaks for several centimetres, while petiole infections can cause complete tissue collapse. Pods and seed coats may show brown blemishes. Lesions often show concentric dark and light zones (Sardina, 1932; Wilson, 1937; Yu, 1945; Anon., 1987b). The non-aggressive disease phase occurs in most faba bean crops every year and lesions increase in number as the season progresses, but usually cause little damage (Wilson, 1937; Harrison, 1981).

The aggressive phase occurs under conditions of continuous high humidity when discrete lesions coalesce and expand rapidly, often causing defoliation and eventually killing the whole shoot system (Wilson, 1937; Yu, 1945; Harrison, 1980a). This is responsible for most of the loss caused in epidemic outbreaks of chocolate spot. The pathogens often sporulate on infected tissues and sclerotia can be found in the dead stems (Yu, 1945; Harrison, 1979).

Effect on Yield

The effect of chocolate spot on yield is variable and depends on the onset and severity of disease relative to crop growth. Epidemics in England and Wales occur when severe spring frosts are followed by warm, wet weather and yield losses have been estimated at 40-75% (Wilson, 1937; Moore, 1949). Moore (1949) also points out that badly affected crops are often ploughed in giving a 100% loss. Severe epidemics usually only happen in 1 out of 6 years although individual crops may be affected more often, especially when nutrient

deficient (Grainger, 1950; Soper, 1952). Yield losses of greater than 50% have been reported from China (Yi, 1986) and of 60-100% from Turkey (Harrison, 1988) but are considered to be insignificant in Canada (Gaunt, 1983). Losses measured in field experiments with severe chocolate spot in the UK have varied from 25% (Bainbridge *et al*, 1985) to 70% (Fitt *et al*, 1986). In Australia losses of 27% have been reported by Williams (1975) and up to 50% by Mayfield (1985).

Studies on components of yield in faba beans have shown the most important factor to be pod number (Ishag, 1973a; Kambal, 1969). Maximum pod set is dependent on a large leaf area before and during flowering, especially at podding nodes (Ishag, 1973b), while defoliation above the podding zone has little effect on yield (McEwen, 1972). Similarly, the greatest yield loss from chocolate spot is caused by reduction in pod number when infection occurs during flowering and pod development. Infection at later stages of development has little effect on yield (Grainger, 1945; Williams, 1975; Griffith and Amin, 1977). Seed weight is usually only a significant factor in yield loss when disease severity is low (Mayfield, 1985; Fitt *et al*, 1986). Losses can also occur through direct infection of flowers which causes pod and seed rotting, and flower and pod abortion (Leach, 1955; Jellis and Bond, 1980).

1.1.3 Causal Organism

Description

In early studies the cause of chocolate spot was

attributed to either *Botrytis cinerea* Pers. (Wilson, 1937; Moore, 1944; Slope, 1957) or *B. fabae* Sardina (Sardina, 1930; Yu, 1945). It is now recognised that the non-aggressive phase of the disease can be caused by both organisms while *B. fabae* is usually responsible for the aggressive phase (Sardina, 1932; Ogilvie and Munro, 1947; Leach, 1955; Sundheim, 1973; Harrison, 1981). *B. cinerea* can, however, produce spreading lesions under ideal conditions (Harrison, 1984a) and is more often responsible for flower infection and pod rotting (Leach, 1955; Jellis and Bond, 1980). Chocolate spot should, therefore, be considered as caused by both *B. cinerea* and *B. fabae*, either singly or in combination.

B. cinerea occurs both as a parasite and a saprophyte on a wide range of host plants and was first described by Persoon in 1822 (Ellis and Waller, 1974a). Groves and Drayton (1939) showed that *B. cinerea* was an asexual form of the ascomycete *Sclerotinia fuckeliana* (de Bary) Fuckel. The taxonomy of *Botrytis* has recently been reviewed and *S. fuckeliana* has now been superseded by *Botryotinia fuckeliana* (de Bary) Whetzel (Jarvis, 1980) although *B. cinerea*, the name of the more common conidial form is still most often used. In 1929 Sardina described a new species which was a more specialised pathogen of faba beans and which he named *Botrytis fabae* Sardina. So far no teleomorph (sexual form) of this species has been reported.

In the asexual form both species grow on host material as mycelia which produce abundant conidiophores with grey/greyish brown spherical and ellipsoid conidia. Mycelia also produce dense black sclerotia which can vary considerably in size and shape. These are important for survival and produce

conidiophores and/or mycelium under favourable conditions. The two *Botrytis* species are best distinguished by conidium size (Harrison, 1983a), with those of *B. cinerea* ranging from 6-18x4-11 (mostly 8-14x6-9)μm (Ellis and Waller, 1974a) compared to 14-29x11-20 (mostly 16-25x13-16)μm (Ellis and Waller, 1974b) for *B. fabae*.

Host Range

B. cinerea is an unspecialised pathogen with a host range of over 200 species including *V. faba* (Ellis and Waller, 1974a; Jarvis, 1980). Wilson (1937) reported a host range restricted to a few legume species for *B. cinerea* isolates from faba beans but it now seems likely that he was using *B. fabae* (Harrison, 1988).

B. fabae is a specialised pathogen of *V. faba* although other *Vicia* species, particularly *V. sativa* (Yu, 1945; Bremer, 1954; Harrison, 1979) have been reported as hosts. Other reported hosts are *V. cracca*, *V. grandiflora*, *V. narbonensis*, *Phaseolis vulgaris*, *Lens culinaris* and *Pisum sativum* (Sardina, 1932; Yu, 1945; Bremer, 1954; Tivoli *et al*, 1986). Raspberry flowers have also been successfully infected with *B. fabae* (Harrison and Williamson, 1986). Harrison (1979) pointed out that most species were tested under artificial conditions and the only alternative host likely to be important is *V. sativa*.

Survival

Epidemics of chocolate spot depend on the survival of the

dominant causal organism, *B. fabae*, between crops. This can be over winter in cold conditions as in northern Europe or over summer in hot, dry conditions as in Australia.

B. fabae produces both macroconidia and microconidia (Harrison and Hargreaves, 1977) but neither of these are considered important for overwintering in Europe. Several studies (Last and Hamley, 1956; Last, 1960; Harrison, 1983b) have shown that macroconidia lose viability and infectivity when exposed to adverse conditions for more than a few weeks whereas microconidia are unlikely to infect plants (Harrison, 1988). The most important means of overwintering is by sclerotia in crop debris (Yu, 1945; Harrison, 1979). Their survival has been recorded as over 1 year at low temperature and they will produce infectious conidia for up to 9 months (Harrison, 1979). Mycelial infections in plant debris have also been reported to sporulate after surviving winter in Europe (Ogilvie and Munro, 1946; Harrison, 1979), although this did not occur in Norway (Sundheim, 1973). Some inoculum may survive on volunteer faba beans and alternate hosts but this form of survival is unlikely to be significant in the production of epidemics (Harrison, 1979).

It is not known how important these forms of survival are in regions with a hot, dry summer between crops. Sclerotia, however, have been found in dry stems in Argentina (Harrison, 1988) and sclerotia and infected crop debris are reported as a means of survival of *B. fabae* in Tasmania (Geard, 1960).

B. fabae and *B. cinerea* have been isolated from commercially produced faba bean seed (Neergaard, 1977) and this has been suggested as a means for disease carryover (Moore, 1944; Geard, 1960). A detailed study by Harrison

(1978) showed that both the level of infection per seed and the percent of infected seed were low and unlikely to produce many infected plants. This confirmed earlier work by Ogilvie and Munro (1944), who also showed that plant infections transmitted from seed often failed to develop. Surveys in Australia (Cook, 1987) have also reported low incidence of *B. fabae* and *B. cinerea* in commercial seed. Infected seed is considered to be of minor significance in pathogen survival between crops.

Dispersal

Conidia are the most important means of dispersal of *B. fabae* and *B. cinerea* because of their ability to survive and travel long distances in the air (Wilson, 1937; Pady and Kelly, 1954; Harrison, 1983b) and their high rate of retention on impact with faba bean leaves (Rapilly and Foucault, 1976). Optimum temperature for their production has been determined as 15°C for *B. cinerea* (Jarvis, 1977) and 17°C for *B. fabae* (Harrison, 1981). Sporulation is stimulated by high relative humidity (RH) (Purkayastha and Deverall, 1964) and Harrison (1984b) showed that *B. fabae* sporulated only between 83-100% RH. He also noted that the optimum humidity for sporulation of both species was below saturation and that *B. fabae* did not sporulate on lesions when free water was present on the surface. Exposure to light is necessary to induce sporulation and is most effective in the near-UV wavelengths (Tan, 1976; Harrison and Heilbronn, 1988).

Conidia are released by vigorous hygroscopic movement of conidiophores during rapid changes in RH between 85 and 65%

(Jarvis, 1960). These conidia are then dispersed by air currents, rain splash or mechanical shock. Spore trapping studies (Jarvis, 1962; Fitt *et al*, 1985) have shown a large peak in numbers around midday, attributed to changing humidity and increasing wind speeds. Increasing numbers of conidia are blown away as wind speed increases whereas release of conidia into air at a constant wind speed is sustained over a long period (Harrison and Lowe, 1987). Many, however, remain trapped within the crop because they are released in a sheltered part of the canopy, or they stick together to form clumps which are not as easily carried by the wind (Jarvis, 1962; Harrison and Lowe, 1987).

Rain is important in the dispersal of conidia by dislodging them and causing them to become airborne through impact (Fitt *et al*, 1985). They can also attach to splash droplets which have been shown to travel as far as 5m (Jarvis, 1962; Stedman, 1980).

Airborne ascospores are produced by *Botryotinia fuckeliana*, the sexual form of *B. cinerea*, but their ability to infect faba beans has not been determined. The ability of *B. cinerea* and *B. fabae* to survive on seed and crop debris (Harrison, 1978, 1979) may also be important for dispersal, especially the introduction of the disease into new areas.

1.1.4 Host-pathogen Interaction

Infection Conditions

The temperature range for germination of *B. cinerea* conidia has been determined *in vitro* and on various plant

tissues in several studies (van den Berg and Lentz, 1968; Hyre, 1972; Yoder and Whalen, 1975; Dennis and Cohen, 1976; Bulger *et al*, 1987; Salinas *et al*, 1989). It is considered to be 0-30°C with an optimum near 20°C, although differences in germination and growth rates at any given temperature may occur between strains (Dennis and Cohen, 1976). *B. fabae* has not been studied as extensively as *B. cinerea* but investigations by Yu (1945) and Wilson (1937), whose *B. cinerea* isolate is now considered to have been *B. fabae* (Harrison, 1988), indicate that its temperature requirements are similar to those of *B. cinerea*.

B. fabae and *B. cinerea* conidia require free water or high humidity to produce infections (Wilson, 1937; Snow, 1949; Mansfield and Deverall, 1974a; Yoder and Whalen, 1975) with germination rarely occurring below 92% RH (Harrison, 1984c). The time for which these conditions must be maintained for infection to occur varies with the temperature. In optimum conditions conidial germination in both species begins by 3 hours while 24 hours is usually sufficient for maximum infection (Deverall and Wood, 1961; Harrison, 1984c; Bulger *et al*, 1987; Salinas *et al*, 1989). The pattern of germination on faba bean leaves is similar for both species but *B. fabae* germtubes develop faster and are more likely to produce infections (Purkayastha and Deverall, 1965a; Mansfield and Hutson, 1980). The rate of conidia germination and infection development decreases as the temperature deviates from the optimum.

The longer *B. cinerea* conidia have been detached from conidiophores the longer they take to germinate (van der Spek, 1960) whereas *B. fabae* conidia from 25 and 35 day old cultures

are only 10% and 1%, respectively, as infective as those from 10 day old cultures (Last, 1960). Harrison (1983) showed that the time over which *B. fabae* conidia are infective decreased faster at 22°C than 10°C.

The number of lesions which develop is proportional to the conidium concentration in the inoculum (Last and Hamley, 1956; Deverall and Wood, 1961) although self-inhibitory effects have been observed at high concentrations (Deverall and Wood, 1961; Yoder and Whalen, 1975). Wastie (1962) showed that lesions could be produced in faba bean leaves by single spores of *B. fabae* and *B. cinerea* but their respective success rates were 13% and 0.9%. He estimated that at least four *B. fabae* and 500 *B. cinerea* conidia/inoculum drop were necessary to develop lesions at half the inoculation sites.

Germination of conidia and infection development can be enhanced by the addition of plant exudates and other compounds to the inoculum. Blakeman (1975) showed this effect was related directly to an increase in available nutrients. Several studies have shown that the most effective additives are sugars, inorganic N and mineral salts (Blackman and Welsford, 1916; Deverall, 1960; Deverall and Wood, 1961; Sol, 1965; Rossall and Mansfield, 1981) while Chou and Preece (1968) achieved the same effect by exposing conidia to pollen grains. The presence of exogenous nutrients is more important for germination and infection by conidia of *B. cinerea* than *B. fabae* (Last, 1960) and may be related to their smaller size and low internal energy reserves. Barash *et al* (1963) suggested that this may explain why *B. cinerea* was more often associated with infection of flowers which provided a greater nutrient source than leaves.

Physiology of Infection

McKeen (1974) suspected that the cuticle on faba bean leaves was dissolved by enzymes produced at the tip of *B. cinerea* germ tubes and not penetrated mechanically as previously thought (Brown, 1916). Microscopic studies by Mansfield and Richardson (1981) supported McKeen's findings and showed that the means of infection by *B. fabae* was similar to *B. cinerea*. They noted that, after penetration of the cuticle, hyphae expanded within the walls of epidermal cells which became extensively vesiculated before collapsing and dying. Frequently *B. cinerea* infections fail to develop further and the infection hyphae die, particularly when the number of conidia in the infection droplet is low (Abu-Zinada *et al*, 1973; Mansfield and Deverall, 1974b; Mansfield and Hutson, 1980; Mansfield, 1981).

Host penetration rarely occurs via stomata for *Botrytis* spp. but wounded and damaged leaves are an important means of entry (Verhoeff, 1980). This allows faster establishment and spread of *B. fabae* infections on faba beans but those of *B. cinerea* usually remain limited (Deverall and Wood, 1961a; Purkayastha and Deverall, 1965a). Hyphal infection through contact with *Botrytis* infected tissues such as fallen flowers lodged on leaves may also occur but this has not been investigated in faba beans (Verhoeff, 1980).

Blackman and Welsford (1916) observed death of faba bean leaf cells in front of advancing *Botrytis* hyphae and speculated that this was caused by the diffusion of an enzyme from the fungus. Later research confirmed this and showed that several cell degrading enzymes were produced, the most

important being polygalacturonase (Deverall and Wood, 1961b; Balasubramani *et al*, 1971; Harrison, 1983c). Harrison (1980b and 1983c) also identified heat stable toxins and concluded that cell death in advance of fungal hyphae is caused by the combined effects of cell wall degrading enzymes, phytotoxins and reduced osmotic potential.

The optimum temperature for lesion growth is between 15 and 20°C with a minimum and maximum of <4°C and 30°C respectively (Harrison, 1980). Rate of lesion growth is proportional to humidity between 70% and 100% with little growth at <66% RH. *B. cinerea* infections can develop into the aggressive phase provided the humidity after inoculation is maintained near saturation (Brown and Swinburne, 1982; Harrison, 1983a) and dormant infections of both *Botrytis* spp. can be induced to produce spreading lesions when transferred to high humidity (Harrison, 1984a). Harrison (1980) suggested that these effects may be caused by an influence of humidity on the movement of cell degrading factors within the leaf.

Faba bean cells respond to *Botrytis* infection by producing phytoalexins, the most important of which has been identified as wyerone acid (Purkayastha and Deverall, 1965b; Deverall, 1967; Letcher *et al*, 1970 and Hargreaves *et al*, 1977). *B. fabae* is more tolerant to wyerone acid than are other *Botrytis* species. The concentrations required to kill germinated spores exposed for 24 hours are 55μg and 35μg/ml for *B. fabae* and *B. cinerea* respectively (Rossall and Mansfield, 1978). The ability of *B. fabae* to suppress phytoalexin production during infection development, however, is considered more important than phytoalexin metabolism in explaining its greater pathogenicity (Mansfield *et al*, 1974;

Hargreaves *et al*, 1977; Rossall and Mansfield, 1978). Rapidly expanding lesions probably occur when faba bean cells are killed by cell wall degrading enzymes and phytotoxins before they can synthesise significant quantities of phytoalexins (Harrison, 1988). Oldest faba bean leaves are most susceptible to *Botrytis* infection (Purkayastha and Deverall, 1965a; Creighton *et al*, 1986; Omar *et al*, 1986) which may be due to lower levels of phytoalexin production relative to younger leaves (Heilbronn and Harrison, 1989). Disease development is also greater in plants deficient in potassium and phosphorus (Glassock *et al*, 1944; Leach, 1955), calcium (Deverall, 1960) or growing in low pH soils (Elliott and Whittington, 1978). This could be caused by nutrient deficient cell walls being more susceptible to degradation by pectic enzymes produced by the pathogen (McGuire and Kelman, 1984).

Variation in the Host

Variation in faba beans to chocolate spot was reported in England by Smith (1968), who observed greater susceptibility in the cultivars Herz Freya, Stubes and Francks Ackerperle. Since then *V. faba* genotypes have been screened extensively, especially at the International Centre for Agricultural Research in Dryland Areas (ICARDA), to find sources of resistance to *B. fabae*. No completely resistant genotypes have been found but reported sources of partial resistance are given in Table 1.2

TABLE 1.2 *V. faba* genotypes partially resistant to *B. fabae*

GENOTYPE	ORIGIN	SOURCE	REFERENCE
BPL 1179	NEB 938	Egypt	El-Sherbeeny and Mohamed,1980
Minica	-	?	Khalil and Harrison, 1981
BPL 710	ILB 438	ICARDA	Hanounik and Hawtin ,1982
BPL 261	-	ICARDA	Hanounik *et al*, 1984
L83107	-	ICARDA	Bond *et al*, 1986
L83108	-	ICARDA	Bond *et al*, 1986
Bourbon	-	France	Tivoli *et al*, 1986
S45	-	England	Tivoli *et al*, 1986
BPL 1196	-	ICARDA	Hanounik and Maliha, 1986
ILB 2822	-	ICARDA	Hanounik and Robertson, 1988
ILB 3025	-	ICARDA	Hanounik and Robertson, 1988
ILB 3026	-	ICARDA	Hanounik and Robertson, 1988

N.B. NEB 938 is synonymous with ILB 938 (Jellis *et al*, 1982)

BPL 710 and BPL 1179 are considered most resistant and are reported by Bond *et al* (1986) as having common resistance genes derived from ILB 438. Other genotypes such as BPL 261 are resistant in Africa but susceptible in Europe (Hanounik and Maliha, 1986).

Bond *et al* (1972), using a detatched leaf method to test the F2 generation derived from the Plant Breeding Institute, Cambridge (PBI) inbred line 67, suggested that chocolate spot resistance was determined by two genes. The inheritence of resistance in this and other PBI lines was investigated more fully by Elliott and Whittington in 1979. In field conditions resistance was controlled mainly by dominant alleles within a system of partial dominance. Detached leaf tests indicated that within virtually complete dominance systems, dominant alleles in the host facilitated fungal penetration and induced a hypersensitive response within the leaf. Control of further lesion development was effected by a virtually complete

additive system. Later studies using the resistant ICARDA line IBL 938 (Mohamed, 1982; Khalil *et al*, 1986) suggested that chocolate spot resistance in this genotype was dominant.

The physiological mechanisms which produce chocolate spot resistance in faba beans are not clear. Recent studies (Hanounik and Hasanain, 1986; Anon., 1988) showed that the leaves of the resistant genotypes BPL 710, 1179 and 261 produced water soluble diffusates which suppressed *B. fabae* spore germination and germtube elongation. This effect was enhanced under field conditions through an interaction between diffusates and endophytic micro-organisms. Tivoli *et al* (1986) found genotypes resistant to infection but susceptible to lesion development (and *vice versa*) and concluded that infection and lesion development were influenced by different mechanisms. This observation supports the earlier genetic studies of Elliott and Whittington (1979).

Variation in the Pathogen

Paul (1929) studied the growth characters of five isolates of *B. cinerea* and recognised three morphological strains which he classified as sclerotial (Sc), sporulating (Sp) and mycelial (M). In pure culture on different agar growth media the degree of development of these characters changed but the basic expression of the three types remained. Degree of pathogenicity (aggressiveness) also varied with morphology, with the M type being most aggressive. Variation in morphology and aggressiveness between isolates of *B. fabae* has subsequently been reported (Hutson and Mansfield, 1980; Mohamed *et al*, 1981; Hanounik *et al*, 1984).

An explanation of this instability was given by Hansen and Smith (1932) who showed that cells of *B. cinerea* were multinucleate and that each nucleus could be genetically different (heterokaryosis). They suggested that a multinucleate spore was not an individual but a colony which could not produce a genetically pure culture unless all its nuclei were genetically identical. Nuclei can migrate between strains of the same species (Hansen and Smith, 1932) and occasionally between species (Mezinger, 1966) via hyphal bridges (anastomosis).

Hutson and Mansfield (1980) used a genetic approach to analyse pure strains of *B. cinerea* and *B. fabae* and concluded that both species were heterokaryotic for aggressiveness on faba bean leaves. Aggressiveness has, however, also been correlated with number of nuclei and spore size, both of which can be influenced by nutrients in the growth media (Phillips *et al*, 1988).

The occurrence of physiological races of *B. fabae* on faba beans has been reported by Hanounik and Maliha (1986) and Hanounik and Robertson (1988). They claimed that four races could be differentiated on the basis of reaction to three ICARDA accessions (BPL 1763, 1821 and ILB 1814) and that these races were more common in England and the Netherlands than in Egypt and Syria. These observations, however, could have been confounded by variation for aggressiveness in *B. fabae*, and so far there have been no other reports of a race structure for either *B. fabae* or *B. cinerea* on *V. faba*.

1.1.5. Disease Control

Cultural Methods

While cultural methods rarely give complete disease control they can reduce the risk and severity of chocolate spot. Strategies aim in the first instance to prevent exposure to primary *Botrytis* inoculum. Use of disease free seed is recommended (Anon., 1981, 1987) although the importance of infected seed in chocolate spot epidemiology is minor (see 1.3.3). The most important source of inoculum is infected stubble and this should be eliminated soon after harvest by either burial (Harrison, 1979) or use as stock feed (Anon, 1987). Faba beans should be rotated with non-host crops and in Australia a wheat-barley-faba bean system is common. Continuous cereal-grain legume rotations are sometimes used and faba beans are alternated with a grain legume such as peas which does not host the disease (Anon, 1987). Successive faba bean crops should be sited as far away as possible from each other (Harrison, 1979).

Cultural control strategies also rely on modification of the crop environment to reduce moisture retention and increase air flow. Ingram and Hebblethwaite (1976) showed that faba beans sown in rows at wide spacing (53cm) and low seeding rates (168kg/ha) reduced levels of chocolate spot in the crop. Delayed sowing will also reduce disease development but must be balanced against decreasing potential yields (Anon, 1987; Mohamed, 1982). Sufficient fertilizer, particularly potassium and phosphorus, should be applied to overcome the effects of nutrient deficiency on disease development (Moore, 1944;

Soper, 1951; Leach, 1955; Mohamed, 1982).

Fungicides

Wilson (1937) showed that both copper and sulphur could be used to control chocolate spot on inoculated plants in the glasshouse. Bordeaux mixture was the most effective but he considered it would be both uneconomic and impractical for field crops. Several sprays of 0.25% Bordeaux mixture have, however, been used successfully to control chocolate spot in Egypt (El-Helaly, 1950).

The advent of organic fungicides expanded possibilities for control of chocolate spot and several compounds are now recommended. Foliar application of the systemic fungicides benomyl and carbendazim will significantly reduce incidence of aggressive lesions on leaves and pods and increase yields when disease levels are high (Doto and Whittington, 1980; Elliott and Whittington, 1980). Protectant fungicides which have proved effective include vinclozolin (Hanounik, 1981), mancozeb (Mohamed, 1982), chlorothalinol and procymidone (Anon., 1987b). Greater efficacy can sometimes be achieved when a combination of fungicides, such as benomyl plus chlorothalinol, is used (Anon., 1987a). Mayfield (1985) showed that the most effective fungicide for chocolate spot control in South Australia was the specific protectant procymidone applied at flowering. The broad spectrum protectant mancozeb is usually preferred by farmers, however, because of its lower price and ability to control other diseases. Fungicide is usually applied by air and spray guidelines based on disease incidence, risk of development and crop value are used to

determine the most appropriate chemical control strategy (Hogg, 1988; Mayfield, 1988; Anon., 1987b). Fungicide application by seed treatment has had mixed results in Britain (Bainbridge *et al*, 1985) and is considered ineffective in South Australia (A. Mayfield, pers.comm.).

Excessive use of benomyl has resulted in fungicide resistant strains of *B. cinerea* and *B. fabae* occurring on faba beans (Harrison, 1984, 1988). These strains also show cross-resistance to other dicarboximide fungicides such as vinclozolin and procymodine (O'Brien and Glass, 1986). More consideration needs to be given to rotation of chemical groups and reduction in use to avoid selection for fungicide resistant strains.

Disease Resistance

Sources of partial resistance to *B. fabae* have been identified (Table 1.2) and used in breeding programs in an attempt to reduce chocolate spot severity (Bond *et al*, 1986; Khalil *et al*, 1986). Resistance in improved lines, however, has not been sufficient to prevent damaging attacks and so far no chocolate spot resistant cultivars have been produced. An alternative method to improve disease resistance and maintain high yields may be to use resistant genotypes as components of synthetic varieties (Bond, 1982). These would protect more susceptible components by interfering with disease spread in the crop.

Better sources of resistance are being sought and while these can be found in other *Vicia* species (e.g *V. narbonensis*) (Tivoli *et al*, 1986) they cannot be transferred to faba beans

until interspecific crosses are obtained. The possible occurrence of *B. fabae* races (Hanounik and Robertson, 1988) may also require the development of cultivars with several resistance genes to give effective disease control.

Other disease control strategies could be achieved through plant breeding. Earlier flowering would allow a high proportion of pods to be filled before the aggressive phase of disease development occurred, whereas a more open canopy and resistance to lodging would reduce humidity (Lawes *et al*, 1983). Better tolerance to other predisposing factors such as waterlogging, mechanical and herbicide damage, and nutrient deficiencies should also reduce chocolate spot severity.

Optimum control of chocolate spot will require an integrated approach using disease resistance, fungicides and cultural control methods unless complete host resistance is obtained.

1.2 PROJECT OBJECTIVES

1.2.1 Background

Fiord, a small seeded, semi-determinate faba bean cultivar adapted to the cereal regions of South Australia was released in 1980. This cultivar gained popularity as a grain legume for rotation with cereals and the area of production grew rapidly. In 1983, however, a serious epidemic of chocolate spot occurred which caused significant yield losses. Epidemics of varying severity have continued to occur and this disease is now recognised as a major constraint to the development of the faba bean industry in Australia. Subsequent research has shown that significant disease control can be achieved with fungicides (Mayfield, 1985) and delayed sowing (Anon., 1987b) but these reduce profitability and other control measures need to be developed for faba bean production to remain viable.

Chocolate spot on faba beans had been studied extensively in Europe and the Mediterranean Basin (Harrison, 1988). Studies in Australia had been limited to row crops in Tasmania (Geard, 1960; Williams, 1975) and little was known about its aetiology in broad-acre cropping under South Australian conditions. A greater understanding of the biology of chocolate spot in South Australia was needed so that alternative control strategies could be developed and the efficiency of current methods improved. The best method of control would be the use of resistant cultivars and sources of resistance had been reported from ICARDA (Hanounik and Maliha, 1986). These needed to be evaluated in Australia and their

potential for improved disease control determined. This research project was, therefore, undertaken with the following objectives so that the best strategies for controlling chocolate spot in faba beans in South Australia could be established.

1.2.2 Objectives

1. Establish means of summer survival and sources of inoculum.

In order to perpetuate in South Australia, *B. fabae* and *B. cinerea* must survive hot, dry summer conditions in the absence of actively growing hosts. Both fungal species were known to survive on harvested seed at low frequencies (Cook, 1987) but survival in the field as sclerotia or mycelial infections on crop debris had not been investigated.

The role of alternate hosts in the survival of the pathogen and development of chocolate spot in Australia was also unknown. Other grain legumes had been reported to be potential hosts (see 1.1.3) and since these were often grown in rotation with faba beans their susceptibility needed investigation.

2. Define temperature and moisture conditions for infection and disease development.

Germination and infection by *B. fabae* and *B. cinerea* spores, and subsequent lesion development can only occur when suitable temperature and moisture conditions are met (see 1.1.4). An understanding of these conditions and their

interaction would help in defining disease risk situations in crops. This would identify possible changes in cropping practices to avoid high risk situations and allow more efficient use of fungicides.

3. Examine the resistance/virulence interaction between *V. faba* accessions and *B. fabae* and *B. cinerea* isolates.

Several *V. faba* genotypes with partial resistance to chocolate spot have been identified by ICARDA and tested in northern Europe and the Mediterranean Basin (see 1.1.4). Accessions from these had recently been introduced into Australia but had not been tested against local *B. fabae* and *B. cinerea* isolates. The Waite Agricultural Research Institute (WARI) also held a collection of *V. faba* accessions which had not been screened for reaction to chocolate spot. Accessions with resistance to South Australian isolates of *B. fabae* and *B. cinerea* could be used in a breeding program to develop locally adapted disease resistant cultivars. A suitable screening method would need to be developed to identify resistance and screen breeders' lines.

CHAPTER TWO EXPERIMENTAL

2.1 ORIGIN AND MAINTENANCE OF FUNGAL CULTURES

2.1.1 Collection of Isolates.

A total of 70 *B. fabae* and 46 *B. cinerea* isolates obtained from fresh and dry faba bean material were collected in South Australia and western Victoria. These covered the major faba bean production areas and many were isolated from samples collected for survival studies described in 2.2. Other isolates were obtained from infected seed supplied to the South Australian Department of Agriculture (SADA) for seed pathogen testing. These included several *B. cinerea* isolates from other grain legume species. The origin of isolates used in experiments described in this chapter is shown in Appendix 3.

Stubble samples wetted with distilled water were incubated on wet paper towels in 21cm² metal boxes covered with clear plastic until sporulating *Botrytis* colonies were observed. This usually required 3 days on laboratory benches at approximately 20°C with natural lighting. Sporulation was induced on infected leaves, flowers and seeds collected from the field by incubating them on wet filter paper in petri dishes at 20°C under a 12 hours dark/12 hours fluorescent light cycle. This was the standard incubator setting for fungal culture used in this study unless otherwise specified. Spores from selected colonies were examined using the microscope and classified as *B. fabae* or *B. cinerea* on the basis of size (Harrison, 1983).

Selected colonies were subcultured on to 2% Malt Extract Agar (MEA) in petri dishes by transferring conidiophores and spores using sterile forceps. These cultures were incubated under standard conditions until sporulation occurred. Actively growing pieces of mycelium were transferred to 2% Potato Dextrose Agar (PDA) slopes after cultures had been checked microscopically for species and contamination. PDA slope cultures were incubated under standard conditions for 7 days after which they were stored at 4°C until required.

2.1.2 Reference Isolate

A *B. fabae* reference isolate (F0.5.1) stable for pathogenicity and growth habit during culture was developed from a field isolate by a series of single spore isolations. Isolate F0.0.1, collected from faba bean stubble at Millicent, S.A., was chosen as the parent culture because of its association with a major bean growing area, high degree of pathogenicity on cv. Fiord leaves and its ability to grow and sporulate readily on MEA.

Spores from a culture of F0.0.1 grown for 14 days on MEA were shaken on to 1% distilled water agar in a petri dish and left to germinate overnight at room temperature. With the aid of a binocular microscope 10 single germinated spores were then removed and each placed into a separate petri dish containing 2% MEA. These cultures were then grown for 14 days on a laboratory bench by which time spores and sclerotia had formed. The cultures were compared for mycelial growth pattern, sporulation and sclerotial production and size.

A spore suspension from each single spore isolate was

made by shaking sporulating culture pieces in 2 ml of sterile distilled water. Each suspension was filtered through cheese cloth then adjusted to 10,000 spores/ml with the aid of a haemocytometer. A 100μl drop from each suspension was placed at a marked site on the upper surface of a cv. Fiord leaf on wet filter paper in a 9cm petri dish. The petri dish was sealed and kept at room temperature with natural lighting for 24 hours. The pathogenicity of each culture was determined and compared by assessing lesion development under each inoculum drop (Appendix 1).

One culture which displayed both abundant sporulation and a high degree of pathogenicity was chosen as isolate F0.1.1 and single spore cultures developed from this as above. This process was repeated for five generations, each time selecting a culture typical of F0.1.1. By this time uniform cultures were produced and one of these was selected as the reference isolate F0.5.1.

2.2 SUMMER SURVIVAL AND SOURCE OF INOCULUM

2.2.1 Alternate Hosts.

Aim

To determine the susceptibility of selected grain legumes to *B. fabae* and *B. cinerea*.

Methods

Nine plants of each of 11 cultivars representing seven species of grain legumes (Table 2.1) were sown and raised in individual Jiffy pots. These were randomised in two 40cmx28cmx12cm plastic trays and grown in a glasshouse under natural light at approximately 20°C for 6 weeks. Another set of plants was prepared in the same way.

One set of plants was inoculated by spraying them to run-off with a 100,000 spores/ml suspension from a 14 day old culture of *B. fabae* isolate F0.5.1 grown on MEA in standard conditions . The suspension was prepared by washing spores from petri dish cultures with distilled water plus a drop of Tween 20, filtering through cheese cloth, and adjusting the concentration with the aid of a haemocytometer. After inoculation the trays with these plants were placed in a dew chamber at 20°C and 100% RH under a 12 hours dark/12 hours incandescent light cycle for 3 days then each plant was scored for presence or absence, and type of, lesions. Plants were scored for sporulation on lesions after a further 2 days in the dew chamber.

The other set of plants was inoculated and scored in the same way but spores of *B. cinerea* isolate C16.0.1 were used. C16.0.1 came from faba bean stubble collected at Kybunga, S.A. Each set of plants was incubated in a separate dew chamber to avoid cross infection.

In a separate experiment seven plants of each of *V. faba* cv Fiord, *V. narbonensis* (Acc. SA 22648), *V. sativa* cv Blanchfleur, *V. sativa* cv Languedoc, *V. benghalensis* cv Popany and *V. villosa* ssp. *dasycarpa* cv Namoi were sown and raised in individual Jiffy pots. Two sets of plants were produced and each set randomised in a plastic tray. These trays of plants were inoculated, one with *B. fabae* isolate F0.5.1 and the other with *B. cinerea* isolate C16.0.1, and incubated as above. The plants in each tray were scored 4 days after inoculation by visually estimating percentage of leaf area affected (%LAA) using ADAS Key No. 4.1.1 as a reference (Anon., 1976).

Results

Host reactions to inoculation with *B. fabae* isolate F0.5.1 and *B. cinerea* isolate C16.0.1 are shown in Table 2.1A and B. Statistical analysis was not possible but some differences in reaction were apparent. Relative susceptibility to both *Botrytis* species was similar, although *B. fabae* was more virulent than *B. cinerea*. All *Vicia* spp. were susceptible whereas the peas and lupin were unaffected. Chickpeas and lentils were variable with some lesion development but were

TABLE 2.1 REACTION OF GRAIN LEGUME SPECIES TO INOCULATION WITH *BOTRYTIS FABAE* AND *B. CINEREA*.

A. *BOTRYTIS FABAE*

TEST PLANTS		NUMBER OF PLANTS WITH SYMPTOMS			
SPECIES	NUMBER PLANTS	NO SYMPTOM	DISCRETE SPOTS	SPREADING LESIONS	SPORES
Pisum sativum					
cv. Alma	9	9	0	0	0
cv. Wirrega	5	5	0	0	0
Lupinus angustifolius					
cv. Danja	8	8	0	0	0
Cicer arietinum					
cv. Tyson	9	4	5	0	5
cv. Opal	9	3	6	0	6
Lens culinaris					
cv. Kye	8	3	4	1	8
cv. Laird	9	8	1	0	7
Vicia sativa					
cv. Languedoc	9	0	0	9	9
cv. Blanchfleur	9	1	0	8	9
V. narbonensis					
Acc. SA 22648	7	0	0	8	9
V.faba					
cv. Fiord	9	0	0	9	9

B. *BOTRYTIS CINEREA*

TEST PLANTS		NUMBER OF PLANTS WITH SYMPTOMS			
SPECIES	NUMBER PLANTS	NO SYMPTOM	DISCRETE SPOTS	SPREADING LESIONS	SPORES
Pisum sativum					
cv. Alma	9	9	0	0	2
cv. Wirrega	5	5	0	0	2
Lupinus angustifolius					
cv. Danja	8	8	0	0	8
Cicer arietinum					
cv. Tyson	9	8	1	0	9
cv. Opal	9	8	1	0	9
Lens culinaris					
cv. Kye	9	0	9	0	9
cv. Laird	9	2	7	0	9
Vicia sativa					
cv. Languedoc	8	0	8	0	8
cv. Blanchfleur	8	0	8	0	8
V. narbonensis					
Acc. SA 22648	9	0	0	9	9
V. faba					
cv. Fiord	8	0	8	0	8

considerably more resistant than the *Vicia* spp. There were no obvious differences between cultivars of each species although lentil cv. Kye may have been more susceptible than cv. Laird to both *Botrytis* isolates. Sporulation, particularly of *B. cinerea*, occurred on plants of all species irrespective of reaction type (Table 2.1B) due to saprophytic development of the fungus under high humidity conditions.

Comparison of disease development (% LAA) on *Vicia* spp. infected with *B. fabae* isolate F0.5.1 and the less virulent *B. cinerea* isolate C16.0.1 are shown in Table 2.2. *V. villosa* ssp *dasycarpa* cv. Namoi and *V. benghalensis* cv. Popany were most resistant to both isolates and significantly less affected than other *Vicia* species. *V. faba* cv. Fiord, *V. narbonensis* Acc. SA 22648 and *V. sativa* cvs. Languedoc and Blanchfleur were all susceptible, with *V. narbonensis* Acc. SA 22648 the most severely affected.

TABLE 2.2. MEAN DISEASE SEVERITY AS PERCENT OF LEAF AREA AFFECTED (%LAA) ON *VICIA* SPP. INOCULATED WITH *BOTRYTIS FABAE* AND *B.CINEREA*

HOST	%LAA (Mean of 7 plants)	
	B. FABAE	*B. CINEREA*
Vicia faba cv.Fiord	12.7	1.1
V. narbonensis Acc. SA 22648	29.0	1.9
V. sativa cv. Blanchfleur	17.5	1.8
V. sativa cv. Languedoc	12.9	1.6
V. villosa ssp *dasycarpa* cv. Namoi	1.3	0.1
V. benghalensis cv. Popany	0.9	0.3
F-Test (One-way ANOVA)	P<0.001	P<0.01
LSD	4.4	1.1

2.2.2 Survival on Stubble and Crop Residue.

Aims

To determine the significance of sclerotia and mycelia in the survival of *B. fabae* and *B. cinerea* in the field over summer.

To determine the source and duration of production of primary inoculum.

Methods

Residue samples from faba bean crops harvested in December, 1986 were collected from 10 sites in South Australia (Table 2.3) during May, 1987. Each sample consisted of approximately 1 kg of stubble and stem residue composed of several sub-samples collected at random across the field. Crop residue (200g) from each site were spread evenly over moist paper towels in five metal boxes (40g/box) and incubated to produce sporulating colonies as described in section 2.1. These were classified as *B. fabae* or *B. cinerea* on the basis of spore size (Harrison, 1983) and were also examined for the presence of sporulating or inactive sclerotia in the host material.

Each site at Bordertown, Strathalbyn, Templers and Hoyleton was sampled again during July 1987, October 1987 and February 1988. Fields at Strathalbyn, Templers and Hoyleton had been cultivated and sown to wheat but considerable bean

residue was still uncovered throughout the crop. The field at Bordertown remained uncultivated throughout 1987. All sites were sampled and material assessed as described above.

A mixture of faba bean crop residues collected from the 10 sites initially surveyed (Table 2.3) was spread outside on bare ground over a $1m^2$ area at the WARI on the 22nd of May, 1987. Six sporulating *B. fabae* colonies with sclerotia present were marked on the 25th May, 1987 and examined every 2 or 3 days throughout the year for evidence of sporulation from mycelial infections and sclerotia.

Results

Total number of *Botrytis* spp. colonies and number with sclerotia in 200g of faba bean residue for each site surveyed in May, 1987 are shown in Table 2.3. The predominant species was *B. fabae* although *B. cinerea* occurred at most sites and exceeded *B. fabae* at Strathalbyn and Kybunga. The number of infections varied considerably between sites but was very high at Bordertown and Struan with 35 and 36 colonies of *B. fabae* /200g bean residue respectively. The majority of infections were not associated with sclerotia which were rare.

The number of active infections of both *B. fabae* and *B. cinerea* in exposed crop residue decreased through the year, although they could still be found at Bordertown as late as February, 1988. The proportion of infections associated with sclerotia tended to increase with later samplings throughout 1987 (Table 2.4).

The dynamics of spore production in crop residue at WARI during 1987 is shown in Figure 2.1. Only mycelial infections produced spores until July when sporulation ceased. Sclerotia began to sporulate in mid-July and continued to do so until October, although sporulation was erratic and ceased during dry periods.

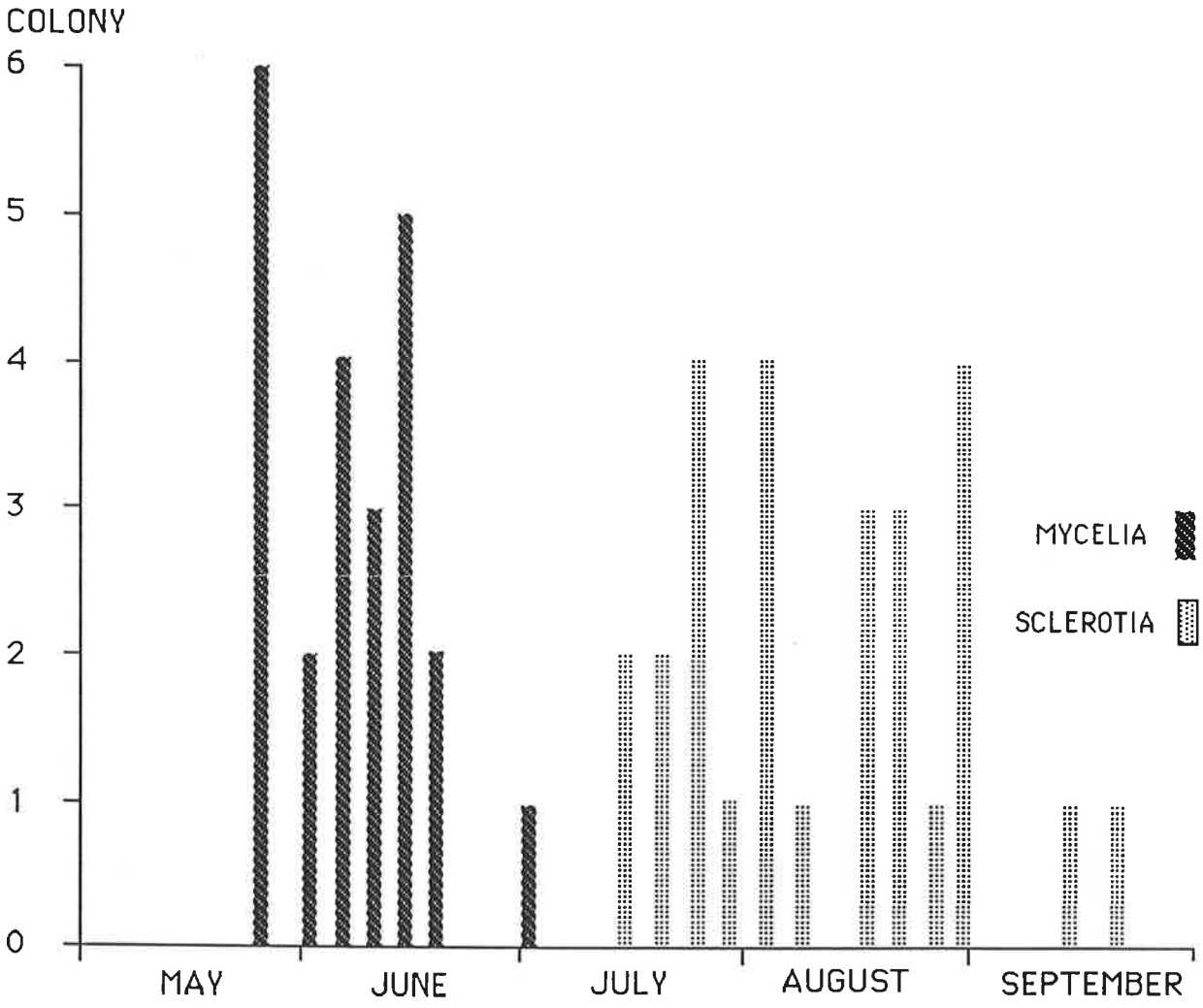
TABLE 2.3 TOTAL NUMBER OF SPORULATING *BOTRYTIS* COLONIES AND NUMBER WITH SCLEROTIA IN 200G OF FABA BEAN RESIDUE COLLECTED FROM VARIOUS SITES IN SA.

ORIGIN	*B. FABAE*		*B. CINEREA*	
	COLONY TOTAL	N° WITH SCLEROTIA	COLONY TOTAL	N° WITH SCLEROTIA
Millicent	10	1	0	0
Struan	36	2	0	0
Bordertown	35	1	6	0
Goolwa	13	0	2	0
Strathalbyn	3	0	16	0
Adelaide (WARI)	0	0	1	0
Templers	5	0	5	1
Hamley Bridge	2	0	0	0
Hoyleton	4	0	0	0
Kybunga	1	0	4	0

TABLE 2.4 TOTAL NUMBER OF SPORULATING *BOTRYTIS* COLONIES AND NUMBER WITH SCLEROTIA IN 200G OF FABA BEAN RESIDUE COLLECTED AT DIFFERENT SAMPLING DATES FROM VARIOUS SITES IN SA.

SAMPLING DATE	BOTRYTIS SPECIES	COLONIES (Total and number with sclerotia)	BORDER-TOWN	STRATH-ALBYN	TEMPLERS	HOYLETON
May 1987	*B. fabae*	Total	35	3	5	4
		Sclerotia	1	0	0	0
	B. cinerea	Total	6	16	5	0
		Sclerotia	0	0	1	0
July 1987	*B. fabae*	Total	13	1	2	2
		Sclerotia	6	0	1	1
	B. cinerea	Total	9	6	0	1
		Sclerotia	4	0	0	1
Oct. 1987	*B. fabae*	Total	7	0	1	0
		Sclerotia	6	0	1	0
	B. cinerea	Total	4	0	0	0
		Sclerotia	4	0	0	0
Feb. 1988	*B. fabae*	Total	2	0	0	0
		Sclerotia	0	0	0	0
	B. cinerea	Total	1	0	0	0
		Sclerotia	1	0	0	0

FIGURE 2.1 Number out of six *R. fabae* colonies sporulating from sclerotia or mycelial infections under field conditions at WARI.



2.3 CONDITIONS FOR INFECTION

Aim

To determine the relationship between temperature, wet period and infection of *V. faba* cv. Fiord leaves by *B. fabae* spores.

Methods

Dry spores from a 14 day old culture of *B. fabae* isolate F0.5.1. grown on MEA in 5cm petri dishes in standard conditions were used to inoculate Fiord plants. The plants were grown in individual 45ml containers for 3 weeks in the glasshouse then cut back to the first leaf pair prior to inoculation. Eleven plants were distributed evenly over the base of a 100cm high by 30cm diameter settling tower and spores introduced at the top by inverting and tapping a single petri dish then replacing the lid of the tower. A 2 minute settling time was allowed before the plants were removed. After removal 10 plants were placed in a plastic container (20 x 12 x 15cm deep) and the remaining plant kept as a control. Six sets of plants were prepared in this way to give a randomised block experiment with six replicates and 10 treatments.

The plants in each container were wetted by spraying with distilled water and the containers sealed and placed in an incubator at the appropriate temperature. This was recorded as time 0 and a single plant was removed from each container after 4, 6, 8, 10, 12, 16, 24, 32, 40 and 48 hours. These

plants were transferred immediately to the glasshouse where they dried in less than 10 minutes. The six control plants were wetted and sealed in a separate container for 48 hours at 20°C to determine maximum potential infection. After all wet period treatments were completed plants were left in the glasshouse for a further 24 hours then assessed for infection by counting the number of spots on the leaves. The above procedure was performed, separately, at temperatures of 5, 10, 15, 20, 25 and 30°C.

The number of infections produced after each wet period at each temperature was expressed as a percentage of the standard control of a 48 hours wet period at 20°C. These data for infection % >0 were used with the multiple linear regression function of Statsview (Apple) to develop and analyse a mathematical model describing infection % as a function of temperature and wet period. A natural logarithm (Ln) transformation was used to make linear the wet period response (McRae *et al*, 1984).

Four Fiord plants, with four or five leaves, in individual 10cm pots were inoculated together in a settling tower with dry spores of *B. fabae* isolate F0.5.1 as described above. The inoculated plants were placed in the field at WARI for 24 hours and temperature and wet period monitored every 30 minutes with a Micropower Data Logger (Tain Electronics Pty Ltd, Melbourne). Pots were placed in a square configuration and the sensors supported at half the plant height in the centre of this square. The temperature sensor was a black plastic coated thermistor shielded from direct sunlight and previously calibrated in the laboratory. The wet period sensor

was an artificial leaf made from an electronic circuit board which distinguished between wet and dry through a change in electrical resistance (Gillespie and Kidd, 1978). It was calibrated for faba bean leaves by comparison with visually observed drying of wetted plants in the laboratory and field. A check was also made during natural dew formation and the sensor was considered accurate to ±15 minutes.

After 24 hours in the field the inoculated plants were placed in a growth chamber at 20°C and < 60% RH for a further 24 hours then the number of lesions on both leaf surfaces counted. The plants were then kept wet for 24 hours at 20°C and lesions recounted to determine the proportion of infections which had occurred in the field conditions.

The above procedure was repeated 43 times between June and November, 1987. Wet period and the average temperature during this time were determined from the data logger output for the 24 hours of exposure for each group of inoculated plants. Where more than one wetting episode occurred these were combined whereas wet periods of less than 2 hours were considered ineffective (see Figure 2.2) and were ignored.

Results

Figure 2.2 shows the response surface produced in the controlled environment experiment. Each point is the mean of six replicates. The optimum temperature for infection was around 15°C, the maximum less than 30°C and the minimum less than 5°C. A minimum of 4 hours wet period was required for infection at 15°C and 20°C and this increased to 16 hours at 5°C and 10 hours at 25°C. Infection % increased with an

increase in wet period at each temperature, but the rate at which this occurred decreased as the temperature deviated from the optimum. Between 10°C and 20°C most infections had been initiated in the first 24 hours but at other temperatures infection development was still low at 48 hours.

A relationship between temperature (T), wet period (W) and infection % (I) for the range of the data is described by Equation 2.1. $R^2 = 0.93$ and all coefficients are significant at $p<0.01$.

EQUATION 2.1 $$I = 34.85T + 89.91(LnW) - 0.85T^2 - 2.69(LnW).T - 404.96$$

The response surface generated from this equation (negative I= 0) is shown in Figure 2.3.

Figure 2.4 shows the percent of infections which were established under the temperature and wet period conditions recorded in the field. Each value is the mean of four plants. The line represents the minimum conditions required for infection as predicted by Equation 2.1. Most wet episodes were caused by dew which produced no infections because of the associated low temperatures. Where infection occurred, wet period and temperature conditions were close to, or above, the predicted minimum requirements.

Comparisons between observed and predicted infection % (I) showed a high level of positive correlation when all data were used (Spearman rank correlation $r = 0.6854$, $p<0.001$). These data, however, are strongly weighted by the frequency of cases for which I=0. Correlation was not significant ($r = 0.5123$, $p=0.19$) when restricted to the observed I>0 data

shown in Table 2.5.

The model tended to underestimate actual infection (Table 2.5) and this may be due to it being based on constant temperature during wet periods. Temperature in the field varies during wet periods and it is likely that this will affect the rate of infection, particularly during long wet episodes. The average temperature during the 23 hours wet period which produced I = 36.3 was 5.1°C but actual temperature varied from a minimum of 1.4°C to a maximum of 10.8°C. Infection may also have been influenced by intermittent wetting, and effective wet periods could have been longer than recorded by the wet period sensor.

The model, however, shows a level of precision which would be useful in determining when temperature and wetness conditions in the field are likely to facilitate *B. fabae* infection of *V. faba* cv. Fiord plants.

FIGURE 2.2 RESPONSE SURFACE SHOWING THE RELATIONSHIP BETWEEN TEMPERATURE (°C), WET PERIOD (H) AND INFECTION (%) FOR *B. FABAE* ISOLATE F0.5.1 ON FIORD. EACH POINT IS THE MEAN OF FOUR REPLICATES.

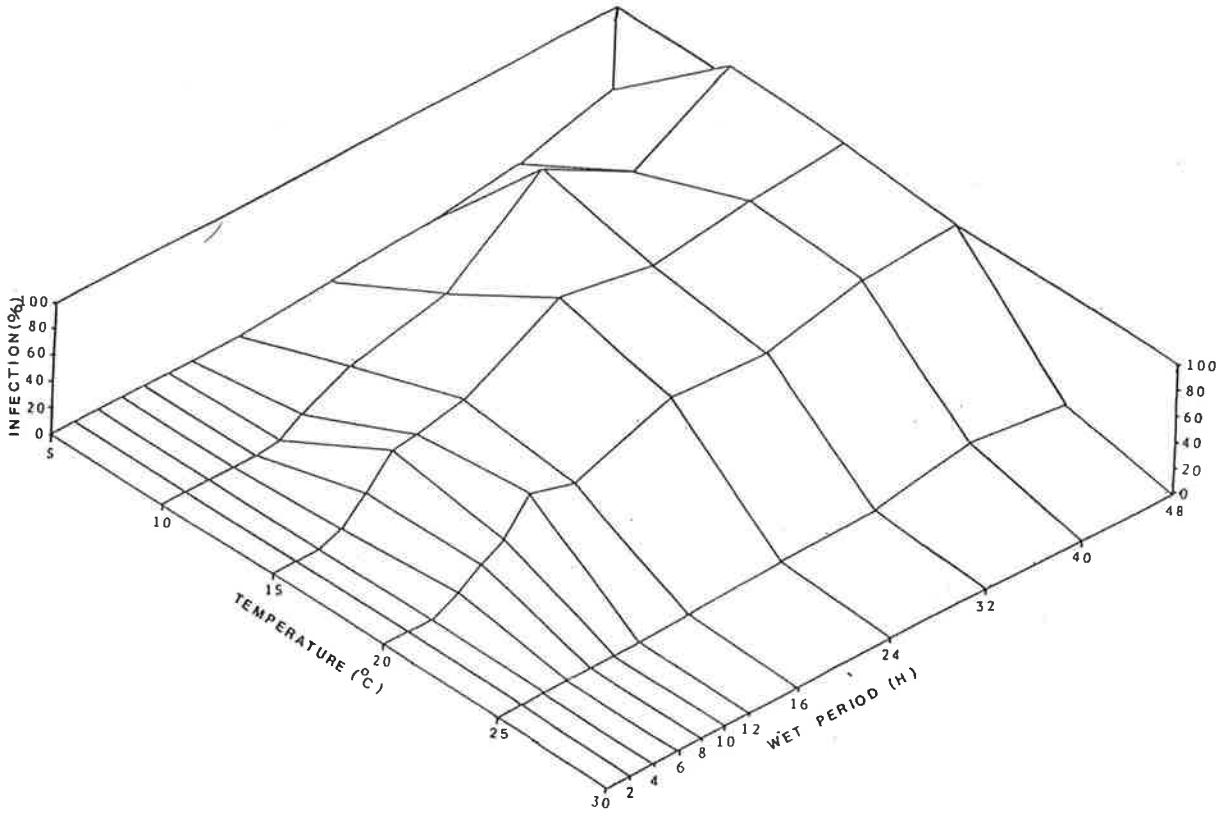


FIGURE 2.3 RESPONSE SURFACE GENERATED FROM THE EQUATION I = 34.84T + 89.91(LnW) - 2.69(Ln).T - 404.96, WHERE I = INFECTION (%), T = TEMPERATURE (°C) AND W = WET PERIOD (H). NEGATIVE I = 0

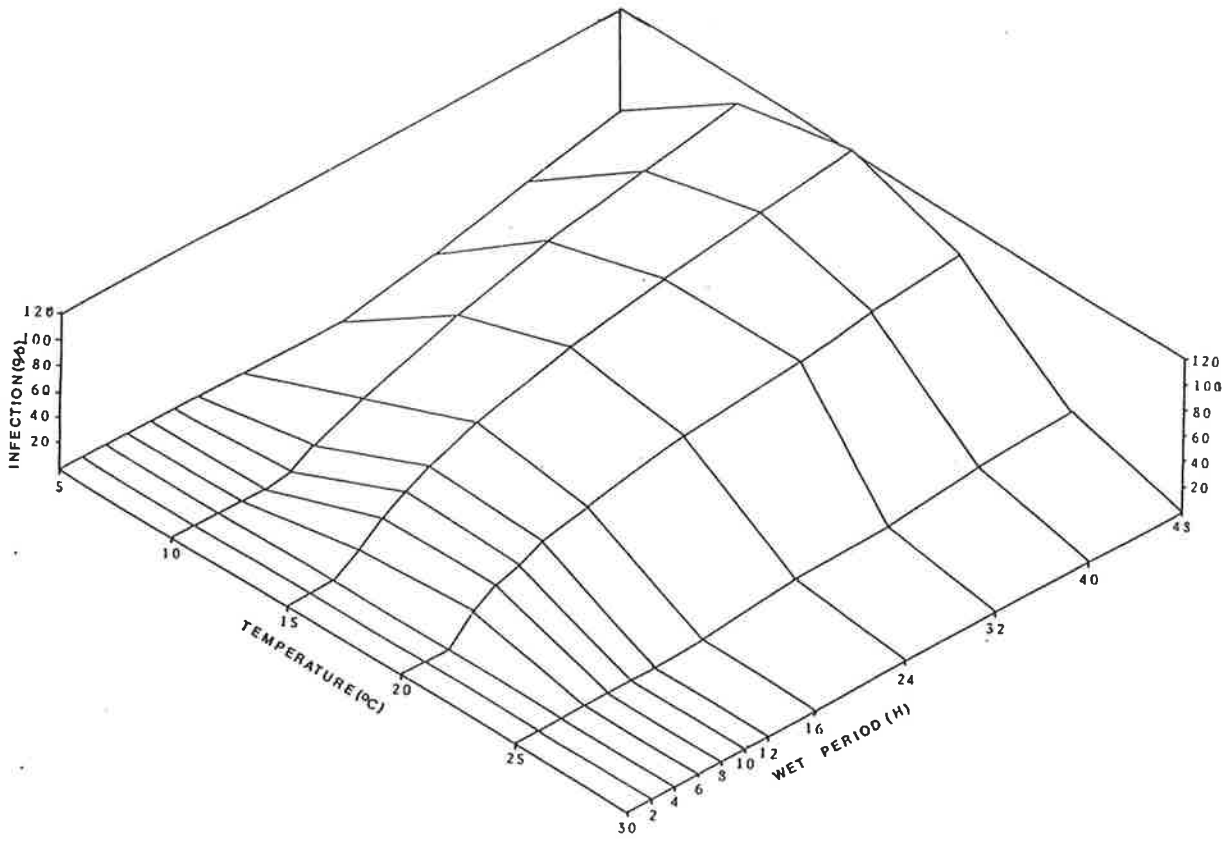
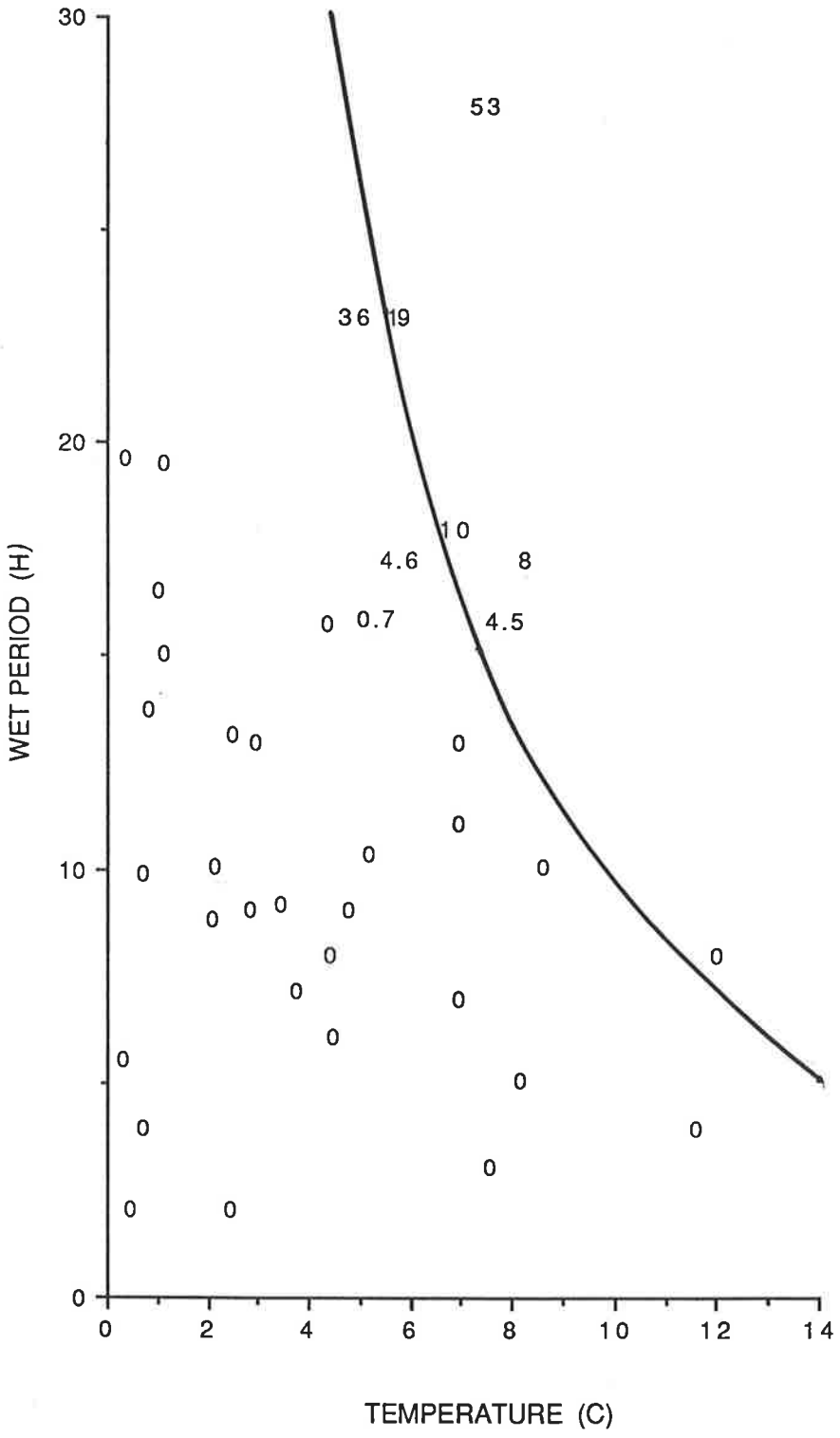


FIGURE 2.4. Effect of temperature and wet period on establishment of infections in the field



FIGURES REPRESENT THE PERCENT OF INFECTIONS THAT ESTABLISHED

LINE REPRESENTS PREDICTED MINIMUM CONDITIONS FOR INFECTION (EQUATION 2.1)

TABLE 2.5 DURATION OF WET PERIOD, AVERAGE TEMPERATURE DURING WET PERIOD AND OBSERVED AND PREDICTED INFECTION % FOR INCIDENCE OF INFECTION GREATER THAN NIL IN FIELD CONDITIONS.

FIELD CONDITIONS		INFECTION %	
AVERAGE TEMPERATURE (°C)	WET PERIOD (hours)	OBSERVED	PREDICTED
7.4	16	4.5	0.7
7.5	28.5	53.3	42.5
5.1	23	36.3	0
6.0	17	4.6	0
5.8	23	18.8	1.7
6.9	18	10.3	1.5
5.6	16	0.7	0
8.3	17	8.0	17.5

2.4 ACCESSION BY ISOLATE INTERACTIONS

2.4.1 Detached Leaf Screening Technique.

The rapid rate of lesion development on faba bean leaves infected with *Botrytis* spp. has enabled simple detached leaf screening techniques to be developed (Mansfield and Deverall, 1974a; El-sherbeeny and Mohamed, 1980; Khalil and Harrison, 1981). These allow several isolates to be screened against a single plant, providing a more economical and efficient use of time, space and host material.

The technique used in this study consisted of placing detached leaflets on two layers of filter paper saturated with distilled water in 9 cm petri dish lids, using the base as a cover. The upper leaf surface was inoculated near the stem end with a 50μl droplet of a 100,000 spore/ml suspension of the test isolate and incubated in standard conditions. In these conditions black/brown lesions appeared below the droplet within 24 hours and spread outward, usually covering the leaflet by 7 days. No discolouration occurred beneath droplets of sterile distilled water and uninoculated leaves remained green and turgid for at least 7 days. Filter papers were kept saturated by the addition of distilled water around the edge of the dish when necessary.

Infected leaves were either scored once for susceptibility at 3 days after inoculation using a 0 - 10 scale (Appendix 1), or scored separately for infection establishment and lesion development. Infection establishment was scored by grading from 0 - 6 (Appendix 1) for the area of blackening/browning under the inoculum droplet after 24 hours.

Lesion development was scored by measuring in mm the distance of spread of discolouration from the inoculum droplet at 5 days. This assessment procedure was based on that of Mansfield and Deverall (1974a) who showed a relationship between leaf discolouration and fungal development.

2.4.2 Comparison With Whole Plant Inoculation.

Aim

To test the effectiveness of using a single detached leaf to identify disease resistance in *V. faba* plants.

Method

Nine *V. faba* accessions, including Fiord and 3 partially resistant lines from ICARDA (Accs. 861, 863 and 969) were grown in 5cm pots in the glasshouse. Each accession was replicated 10 times with a single plant in each pot.

When four fully expanded leaves had developed, one leaflet from the second leaf (from the bottom) was removed from each plant and inoculated as described in 2.4.1 with spores from a 7 day old culture of *B. fabae* isolate F0.5.1. The rest of the plant was inoculated with 2 ml of the same spore suspension by spraying on a rotating turntable. The inoculated whole plants and detached leaves were randomly distributed in a dew chamber at 100% RH and incubated in standard conditions. The detached leaves were scored for infection establishment and lesion development at 1 and 5 days after inoculation as described in 2.4.1 whereas the whole

plants were scored at 4 days for % leaf area affected (%LAA) (Anon., 1976).

Results

Comparisons between scores obtained from detached leaves at 1 and 5 days, and whole plants at 4 days all showed significant positive correlations (Table 2.6).

Each method identified Acc. 861 as significantly ($p = 0.05$) more resistant than Fiord (Table 2.7) with Acc. 969 also ranking highly. Both times of scoring for the detached leaf test identified Acc. 407 as significantly more susceptible than Fiord and this accession also ranked last in the whole plant test.

These results showed that the detached leaf test was equivalent to, and as effective as, whole plant inoculation in detecting differences in disease resistance.

TABLE 2.6 CORRELATION BETWEEN DISEASE DEVELOPMENT ON DETACHED LEAVES AND WHOLE PLANTS.

CORRELATION	COEFFICIENT	P
Whole plant x Detached leaf (Day 1)	0.3778	0.001
Whole plant x Detached leaf (Day 5)	0.2775	0.012
Detached leaf (Day 1) x Detached leaf (Day 5)	0.5583	0.000

TABLE 2.7 DISEASE DEVELOPMENT ON DETACHED LEAVES AND WHOLE PLANTS OF *V. FABA* ACCESSIONS.

ACCESSION	DETACHED LEAF DAY 1	DETACHED LEAF DAY 5	WHOLE PLANT
FIORD	3.9[A]	2.7[B]	20.7[C]
77	4.4	4.6	21.3
407	5.0	5.4	26.3
588	3.6	6.1	21.3
834	3.5	5.2	21.0
861	1.2	0.6	12.0
863	3.3	3.7	16.2
930	3.9	4.3	18.7
969	2.9	2.1	8.0
LSD	1.0	1.9	7.5
P	0.05	0.05	0.05

A = blackening under the inoculum droplet (see Appendix 1)
B = spread of lesion from the inoculum droplet (mm)
C = % leaf area affected

2.4.3 Screening the WARI *V. faba* Collection

Aim

To identify *V. faba* accessions with resistance to the *B. fabae* reference isolate F0.5.1.

Methods

At the time of screening, 946 *V. faba* accessions, each representing a population sample, were listed as held in storage at 4°C at WARI. Many, however, were missing while others were unavailable because of low seed stocks. Nine other introductions selected at ICARDA for resistance to chocolate spot or Ascochyta blight (*Ascochyta fabae*) were in quarantine and these were screened separately, but using the same procedure, when seed became available.

Four seeds from an accession were planted in the same 10cm pot and grown in a glasshouse until four fully expanded leaves had developed. The second leaf from the bottom was then removed and each of the two leaflets inoculated with the *B. fabae* reference culture, F0.5.1, as described in 2.4.1. Where less than three seeds germinated the test was either repeated if sufficient seed were available, or that accession omitted. Accessions were sown in batches of approximately 60 at weekly intervals. Germination and plant development was usually even but some late developing accessions were transferred to the next batch for inoculation.

Inoculum cultures were maintained on 2% MEA and subcultured regularly to produce sufficient 7 day old sporulating cultures to inoculate each batch of detached leaves. Detached leaves from Fiord plants were inoculated with each batch of accessions to check the pathogenicity of the inoculum.

Each leaflet was scored as described in 2.4.1 for both infection establishment at 24 hours and lesion spread at 5 days to detect any plants resistant to infection establishment but susceptible to lesion development, or *vice versa* (Tivoli *et al*, 1986). The mean scores of each accession were used to classify it as resistant (R), moderately resistant (MR) or susceptible (S) to infection establishment (R= 0-1; MR= 2-3; S= 4-6) and lesion development (R= <3mm; MR= 3-5mm; S= >5mm).

After all available accessions had been screened once, those with a R or MR score were tested again using the same procedure.

Results

A total of 546 accessions was screened against isolate F0.5.1 for resistance to infection establishment and lesion development (Appendix 2). Ten accessions (Accs. 77, 588, 834, 861, 930, 968, 969, 972, 973 and 974) were scored as moderately resistant (MR) for both infection establishment and lesion development. These included the ICARDA lines BPL 710 (Accs. 861 and 969), BPL 261 (Acc. 968), ILB 3025 (Acc.973), ILB 3026 (Acc.974) and ILB 2282 (Acc. 972). All other accessions tested were susceptible, with no evidence of different reaction types for infection establishment and

lesion development.

There was variation in reaction between individual plants in many accessions which was expected since most were not genetically uniform lines. It is, therefore, possible that resistant plants could be found in some susceptible lines with more rigorous screening. The limited sample (3-4 plants) tested from each accession , however, was enough to identify any lines, such as the ICARDA introductions above, with a high proportion of resistant plants.

2.4.4 Screening Against *B. fabae* and *B. cinerea* Isolates.

Aims

To identify races of *B. fabae* and *B. cinerea* different to *B. fabae* isolate F0.5.1, and determine their origin and distribution.

To assess the relative importance of *B. fabae* and *B. cinerea* in chocolate spot development, and identify *V. faba* accessions with the best resistance to these pathogens.

Methods

Fiord and nine accessions moderately resistant to *B. fabae* isolate F0.5.1 (see 2.4.3) were used to test the pathogenicity of a range of *B. fabae* and *B. cinerea* isolates. Accession 861, which is the same as the later introduction Acc. 969, was omitted. Five plants of an accession were grown in the same 30cm pot in the glasshouse and supported by a wire

frame. Single leaflets from each plant were harvested for detached leaf tests as required. One set of plants was sufficient for all isolates.

Each of the *B. fabae* and *B. cinerea* isolates stored on PDA slopes (see 2.1.1) was subcultured on 2% Faba bean Dextrose Agar (FDA) (Hanounik, 1986) and incubated in standard conditions. Sporulation was more successful on FDA than MEA but many isolates still failed to sporulate or produce sufficient spores for use in inoculation tests. Overall a total of 20 *B. fabae* and 23 *B. cinerea* isolates (Appendix 3) covering most areas of faba bean production in southern Australia were used. A culture of the reference isolate, F0.5.1, was also maintained and subcultured on FDA to produce fresh inoculum for each test.

One detached leaflet from each faba bean plant was inoculated near the stem end with the test isolate as described in 2.4.1. The leaflets were taken from a similar position on older leaves of each plant so that each isolate was tested against 10 different accessions with five replications. Each leaflet was also inoculated near the tip with the reference isolate F0.5.1 for disease reaction comparisons. This also served as a control against differences which may be caused by leaf age and position on the plant (Heilbronn and Harrison, 1989). Preliminary observations had shown that infection establishment and lesion development were not affected by position on the leaf surface. After inoculation the petri dishes with detached leaflets were randomised and incubated in standard conditions.

Each leaflet was scored 3 days after inoculation on a 0-10 scale (Appendix 1) for lesion development at the test and

control (F0.5.1) inoculation sites. Mean scores were used to rank accessions for resistance to the test and control isolates. Each control/test pair was analysed separately.

Under a conservative approach evidence for a different race would only be accepted if an isolate significantly affected the relative reaction of accessions compared to the control. Differences in overall disease severity between test and control isolates without changes in accession ranking were attributed to a difference in "aggressiveness" between these isolates (Caten, 1987).

Results

Reactions of each of the *V. faba* accessions to each *B. fabae* and *B. cinerea* isolate are tabulated in Appendix 3. A direct comparison of scores for the control and test isolate on each accession was not possible because of the influence of aggressiveness. The mean score and LSD (P= 0.05) for each accession were therefore determined separately for the test isolate and the control isolate using a one-way ANOVA. In this method evidence of races was only accepted if the score for an accession was significantly higher than Fiord for the test isolate and significantly lower for the control isolate, or *vice versa*. Since there were no cases where this occurred it was concluded that no new races were found.

Mean scores on each accession for the control (F0.5.1) and all test isolates of *B. fabae* and *B. cinerea* are shown in Table 2.8. Accessions 969 and 968 were significantly more resistant than Fiord for isolates of both *Botrytis* species, including the control F0.5.1. Accessions 834 and 973 tended to be more resistant, and accession 77 more susceptible than Fiord although these differences were not always significant.

The distribution frequencies for disease score as the mean of the 10 accessions (Appendix 3) are shown for each *B. fabae* and *B. cinerea* isolate in Figure 2.5 B and C. Each score category contains all scores in that range (e.g. scores from 1.0 to 1.9 are in category 1). Disease development was usually less for *B. cinerea* isolates but there was an overlap in the range of mean scores, with *B. fabae* varying from 2-9 and *B. cinerea* from 0-4. Some of the variation in each species may have been due to inherent differences in degree of pathogenicity between individual isolates (i.e aggressiveness (Caten, 1987)). Distribution of mean scores from each test for the *B. fabae* reference isolate, F0.5.1 (Figure 2.5 A), however, suggests variability within the technique. This is supported by a significant positive correlation ($r=0.71$, $p<.001$) between these scores and those of the *B. fabae* test isolates. This correlation was also positive, but not significant, with *B. cinerea* isolate means ($r=0.30$, $p=0.16$). There was no relationship between pathogenicity and source or origin of isolates (Appendix 3).

These results showed that Accession 969 was most resistant to chocolate spot irrespective of *Botrytis* spp. and source and origin of inoculum. No races different to the reference isolate F0.5.1 were found among the 43 isolates tested but there was some evidence for differences in aggressiveness. The most pathogenic *B. cinerea* isolates produced disease symptoms within the range of *B. fabae* and may contribute significantly to chocolate spot in the field.

TABLE 2.8. COMPARISON OF MEANS OF ALL *B. FABAE* AND *B. CINEREA* ISOLATES, AND THE *B. FABAE* REFERENCE ISOLATE F0.5.1, FOR DISEASE SCORE ON 10 *V. FABA* ACCESSIONS.

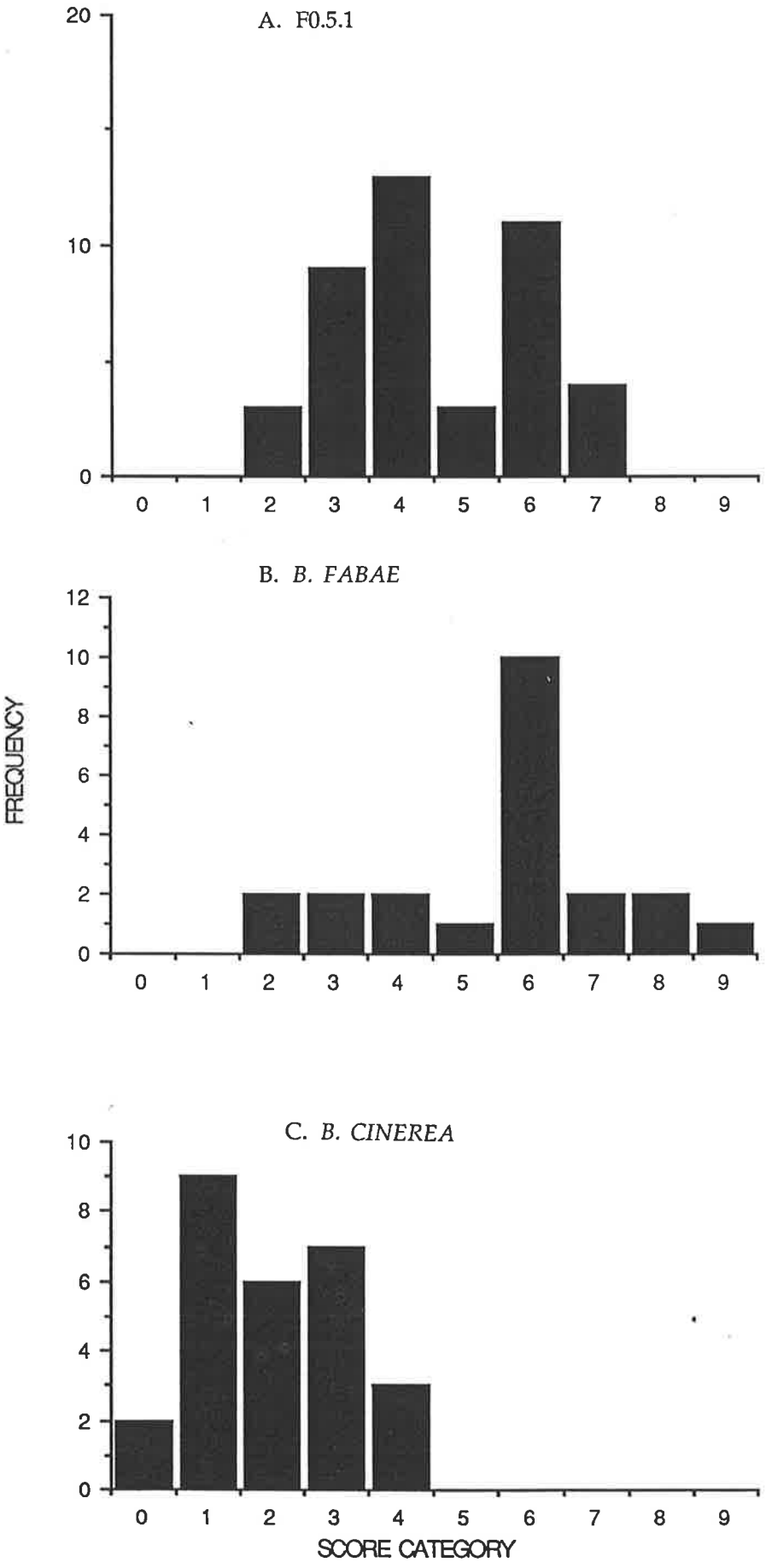
ACCESSION	DISEASE SCORE		
	B. FABAE (MEAN OF 20 ISOLATES)	*B. CINEREA* (MEAN OF 23 ISOLATES)	ISOLATE F0.5.1 (MEAN OF 43 TESTS)
FIORD	6.3	3.4	5.2
77	6.6	3.5	5.7
586	6.0	3.2	5.3
834	5.6 *	2.3 *	5.0
930	6.4	2.8	5.6
968	5.5 *	2.0 *	4.5 *
969	4.9 *	1.0 *	3.8 *
972	6.3	3.1	5.4
973	6.0	2.4 *	4.8 *
974	5.7	2.4 *	5.3
MEAN	5.9	2.6	5.1
LSD (0.05)	0.6	0.6	0.3

SCORES FOR INDIVIDUAL ISOLATES ON EACH ACCESSION WERE THE MEANS OF FIVE REPLICATES (APPENDIX 3) SCORED USING THE 0-10 SCALE FOR DETACHED LEAVES (APPENDIX 1).

THE REFERENCE ISOLATE F0.5.1 WAS USED WITH EACH TEST GIVING A TOTAL OF 43 REPLICATES.

* = DISEASE SCORE SIGNIFICANTLY LESS THAN FIORD.

FIGURE 2.5 DISTRIBUTION OF MEAN SCORES FROM EACH SCREENING TEST FOR *B .FABAE* CONTROL ISOLATE FO.5.1 ,AND *B. FABAE* AND *B. CINEREA* ISOLATES.



2.4.5 Disease Development in the Field

Aim

To compare selected *V. faba* accessions for resistance to *Botrytis* species in field conditions in South Australia.

Methods

Seed from Fiord and each of the nine accessions used in section 2.4.4 were sown in single row plots replicated three times inside an insect proof enclosure at Northfield Research Laboratories. Each plot consisted of five seeds sown in a 75cm single row and each replicate was separated by a row of Fiord. The trial was sown in May and relied on natural rainfall until late spring when this was supplemented with irrigation from overhead sprinklers. A mixture of *Botrytis* infected bean stubble collected from the mid-north and south-east of South Australia was distributed over the trial after sowing as a source of chocolate spot inoculum.

Plots (5mx2m) of Fiord and accessions 834, 968, 969, 973 and 974 were sown at 20 plants/m^2 in four replicates in the field at Struan Research Centre in the south-east of South Australia. The trial was sown in June and relied on natural rainfall and inoculum for crop and disease development.

Both trials were scored for chocolate spot at the stage when several mature pods had formed on Fiord. Each plot was scored for disease development on a 0 - 9 scale (Appendix 1) and the means for each accession determined. The % LAA for each accession at Struan was also measured by visually

assessing 10 randomly selected stems in each plot with the aid of ADAS Key No. 4.1.1 (Anon., 1976). The *Botrytis* spp. infecting diseased leaves collected from each trial were identified after sporulation had been induced in a humid chamber.

Results

Comparison of means for disease development on each accession at Northfield and Struan is shown Table 2.9. Only *B. fabae* was isolated from infected leaves collected at each site. Disease development was greatest at Struan with severity on accessions ranging from the beginning of the aggressive phase (6) to numerous dropped leaves (8). These scores, however, were not directly correlated with % LAA which averaged 12.8% at this site. Disease severity at Northfield ranged from some chocolate spot on some plants (2) to very numerous chocolate spots (5) but did not reach the aggressive phase.

Accessions 968 and 969 were most resistant at both sites with other accessions showing no significant difference from Fiord when assessed for disease development on a 0 - 9 scale. These results were similar to those obtained from glasshouse screening of detached leaves as described in section 2.4.4. A quantative measurement of disease (% LAA), which is likely to be a more direct indicator of yield loss, identified accession 969 as the best source of field resistance.

TABLE 2.9 COMPARISON OF MEANS FOR CHOCOLATE SPOT DEVELOPMENT ON ACCESSIONS AT TWO FIELD SITES IN S.A.

ACCESSION	DISEASE SCORE		% LAA
	NORTHFIELD (MEAN OF 3 PLOTS)	STRUAN (MEAN OF 4 PLOTS)	STRUAN (MEAN OF 4 PLOTS)
FIORD	4.3	7.0	10.9
77	4.7	-	-
588	4.7	-	-
834	3.7	6.8	18.8
930	4.3	-	-
968	2.0 *	6.0 *	11.2
969	2.3 *	6.5	6.7
972	4.7	-	-
973	4.3	7.3	16.3
974	3.7	6.8	12.6
MEAN	3.9	6.7	12.8
LSD (0.05)	1.5	0.8	5.4

DISEASE SCORE WAS ASSESSED FOR EACH PLOT ON A 0-9 SCALE USING THE FIELD SCORING KEY (APPENDIX 1).

%LAA WAS ASSESSED FOR EACH PLOT AS THE MEAN % LEAF AREA AFFECTED ON 10 RANDOMLY SELECTED STEMS.

* = SIGNIFICANTLY LESS DISEASE THAN FIORD.

- = NOT TESTED

CHAPTER THREE DISCUSSION AND CONCLUSION

3.1 DISCUSSION

3.1.1 Summer Survival and Source of Inoculum

Mycelial infections on crop residues were shown to be the most important mechanism for summer survival of *Botrytis* spp. and the main source of primary inoculum. Most colonies, however, are exhausted during the cropping season with few surviving longer than 12 months. Sclerotia become more prominent later in the season and may be important in longer term survival. Their long term viability is unknown but they could act as a source of inoculum in a field over several years and cause inoculum build-up with frequent cropping of faba beans.

Some control of chocolate spot may be possible by avoiding exposure to primary inoculum. A break of at least one year, and as long as possible, should be maintained between faba bean crops in a field to avoid contact with infectious stubble and sclerotia. This already occurs on most farms where faba beans are grown in rotation with other crops. Crops should also be sited as far away as possible from stubble to reduce contact with airborne inoculum. It may be possible to reduce inoculum by incorporating or harvesting stubble and the effect of this needs further investigation. Airborne dispersal of *Botrytis* spores over long distances, however, makes it unlikely that adequate control can be achieved by inoculum avoidance and other control strategies will also be necessary.

Host range studies showed that *V. sativa* cvs. Languedoc and Blanchfleur are as susceptible to *B. fabae* and *B. cinerea* as *V. faba* cv. Fiord. They should, therefore, be considered as equivalent to faba beans in rotations and cropping practices aimed at minimising chocolate spot inoculum survival and disease development. Other vetch species (*V. villosa* ssp. *dasycarpa* cv. Namoi and *V. benghalensis* cv. Popany) are resistant and would be more suitable than *V. sativa* for vetch production in rotations with faba beans, or in areas where chocolate spot is prevalent. Narbon beans (*V. narbonensis*) are being developed in South Australia as an alternative to faba beans in low rainfall areas. These studies showed that they also have a potential for development of severe chocolate spot although resistant genotypes have been reported (Tivoli *et al*, 1986). A chocolate spot screening program should, therefore, be undertaken as part of their development.

The non-aggressive phase of infection could be produced by *B. fabae* and *B. cinerea* on other grain legume species, with varying levels of susceptibility. This was, however, under ideal conditions with very high inoculum rates and it is unlikely that these species would be significant hosts under natural conditions.

3.1.2 Conditions for Infection

The optimum temperature for infection of Fiord leaves with *B. fabae* isolate F0.5.1 was calculated at 18.5°C, which is similar to estimates from other studies (Harrison, 1988). The minimum wet period for infection establishment increased from 4 hours at the optimum to more than 24 hours at

temperatures less than 5°C (Figure 2.4). The temperature and duration of dew periods in bean crops would usually be too low for infection establishment and the major environmental influence on infection is frequency and duration of rainfall.

Most rainfall in southern Australia occurs during winter but disease development would be slow because of the cold temperatures. Conditions most suitable for disease development are, therefore, extended wet periods during late autumn and spring. This reflects the pattern of chocolate spot epidemics in southern Australia which are usually associated with early sown crops and/or wet spring conditions. Primary inoculum levels would be high under wet conditions early in the season (see 2.2.2) producing many primary infections which would predispose plants to rapid disease development in the spring. Conditions become ideal for chocolate spot development during wet periods in spring through retention of moisture in the crop canopy and warm temperatures. There is also sporulation and build up of secondary inoculum on dropped flowers and leaves, and possible enhancement of spore infectivity through nutrients from pollen (Chou and Preece, 1968).

This study indicates that crops in areas of South Australia with a high frequency of rainfall in September and October (e.g. lower south-east) will be at most risk from chocolate spot. This relationship should be developed further to produce disease risk maps for different bean producing areas to aid farmers in managing chocolate spot disease.

Present evidence suggests that resistant varieties alone will not be sufficient to give optimum disease control (see 2.4.5). For best effect, however, fungicides should be applied early in disease development and only when the potential for

severe disease is high. It would, therefore, be useful to have a predictive model for disease development and the relationship in Equation 2.1 could be useful in its development. Other factors important in disease development, such as lesion growth and inoculum production, would also have to be considered. The relationship in Equation 2.1 will also be important in determining optimum conditions for use in disease screening and other infection studies.

3.1.3 Accession by Isolate Interactions

A detached leaf technique was developed which was as effective as whole plant inoculations in assessing disease resistance. This enabled a faster screening turnover to be achieved through a reduction in resource requirements and the ability to score several isolates against a single plant. The technique was used to identify *V. faba* accessions with partial resistance to a *B. fabae* reference isolate, and to screen these accessions against other isolates of *B. fabae* and *B. cinerea* collected from the field.

The most resistant accessions were those derived from the ICARDA lines BPL 710 (Acc. 969) and BPL 261 (Acc. 968). This confirmed that the sources of resistance considered most effective in other countries were also most effective in southern Australia. It is possible that accessions susceptible to the reference isolate but resistant to other isolates could occur and not be detected in the screening procedure which did not examine all isolate by accession combinations. Such accessions, however, would be susceptible in the field and not as useful as BPL 710 and BPL 261 for resistant breeding.

Resistant plants might also be found in some accessions which were classified as susceptible if large numbers of plants were screened.

It is unclear whether variation in disease severity between tests was due to differences in aggressiveness between isolates, or was an artefact of the screening system. The results, however, show that *B. fabae* isolates are usually more pathogenic than *B. cinerea* isolates. The former species is, therefore, considered to be the major causal organism of chocolate spot on faba beans although *B. cinerea* could be a significant contributor in some cases. Accessions resistant to *B. fabae* were also resistant to *B. cinerea* and screening against *B. fabae* only would be sufficient in a breeding program. *B. cinerea*, however, may need to be considered separately in chemical control programs since fungicide resistant strains of this species are known (Harrison, 1984 and 1988).

BPL 261 is reported to be resistant in Africa but susceptible in Europe (Hanounik and Maliha, 1986) indicating differences in *B. fabae* pathotypes between these regions. Its partial resistance in this study suggests that the *B. fabae* population in Australia originated from the Mediterranean region rather than western Europe. It is, however, also possible that a *B. fabae* race virulent on BPL 261 has developed in Europe only and occurs in conjunction with a more widespread race avirulent on BPL 710 and BPL 261. To date there have been no reports in the literature to support this and until the racial structure of *B. fabae* has been resolved no conclusion can be made about the origin of *B. fabae* on faba beans in Australia.

Glasshouse screening studies and field disease nurseries produced no evidence for different pathotypes of either *B. fabae* or *B. cinerea* and identified BPL 710 and BPL 261 as potential sources of resistance to chocolate spot in Australia. They are, however, only partially resistant and the effect of these levels of resistance on disease development in the field needs to be investigated further. It is likely that other control methods will also be needed to give optimum disease control.

3.2 CONCLUSIONS

1. The biology of the host/pathogen system for chocolate spot in southern Australia is similar to that described in other countries and reviewed in Chapter 1. Significant factors in its epidemiology in Australia are:

   a. The main causal organism is *B. fabae*. *B. cinerea* can also produce chocolate spot but is usually less virulent.

   b. *B. fabae* inoculum oversummers predominantly as mycelial infections on stubble and crop residue.

   c. *V. sativa* and *V. narbonensis* are alternate hosts for *B. fabae* and can be as susceptible as *V. faba* varieties. Other grain legume species are not significantly affected by *B. fabae*.

d. The optimum temperature for infection is near 18°C, with a minimum wet period of 4 hours, making wet spring conditions most suitable for development of severe epidemics.

e. The *B. fabae* population in South Australia consists of one race. Some WARI accessions have partial resistance to this race with the best being Accs. 969 and 968 derived from the ICARDA resistant genotypes BPL 710 and BPL 261 respectively.

2. Entry of inoculum into a crop and disease development in the crop cannot be prevented because spores are airborne and there is a lack of highly resistant varieties. This makes complete control of chocolate spot unlikely. It should, however, be possible to improve current levels of disease control through the integration of factors identified in this study. These include:

a. Improved genetic resistance by utilising Accs. 969 and 968 in breeding programs.

b. Reduction in primary inoculum through incorporation, harvesting or grazing of stubbles.

c. Avoidance of inoculum through breaks between consecutive bean crops and siting new crops far away from infective stubble.

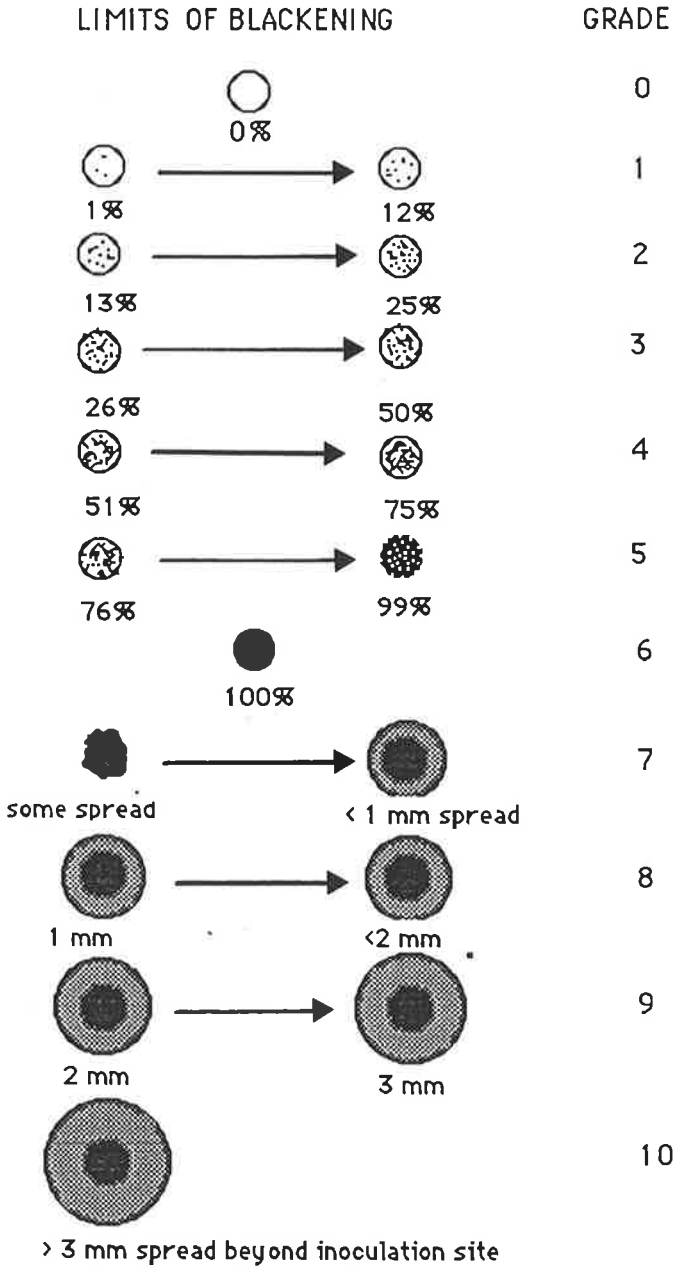
d. Avoiding the use of other susceptible *Vicia* species in rotations with faba beans.

e. Development of disease risk models to allow more effective use of fungicides.

3. Further research is needed to relate disease severity , plant growth stage and yield loss for current and future, more resistant varieties. This will enable control of disease in crops to be optimised by using disease resistance supplemented by fungicides as appropriate.

APPENDIX 1. DISEASE ASSESSMENT KEYS FOR CHOCOLATE SPOT

A. DETACHED LEAF ASSESSMENT (MANSFIELD AND DEVERALL 1974a)



B. FIELD ASSESSMENT

0 NO NOTATION

1 NO SYMPTOM

2 SOME CHOCOLATE SPOT ON SOME PLANTS

3 SOME CHOCOLATE SPOT ON ALL PLANTS

4 NUMEROUS CHOCOLATE SPOTS

5 VERY NUMEROUS CHOCOLATE SPOTS

6 BEGINNING OF "AGGRESSIVE PHASE"

7 COALESCENT SPOTS, SOME DROPPED LEAFLETS.

8 WIDE SPREAD SPOTS, NUMEROUS DROPPED LEAFLETS

9 NO MORE LEAFLETS

APPENDIX 2. DISEASE RESISTANCE REACTIONS OF ACCESSIONS FROM THE WARI *V. FABA* COLLECTION TO *B. FABAE* ISOLATE F0.5.1

ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION	
	INF.	DEV.		INF.	DEV.		INF.	DEV.		INF.	DEV.
2	S	S	3	S	S	4	S	S	5	S	S
8	S	S	9	S	S	10	S	S	11	S	S
12	S	S	13	S	S	14	S	S	15	S	S
17	S	S	18	S	S	20	S	S	21	S	S
25	S	S	26	S	S	28	S	S	29	S	S
30	S	S	31	S	S	32	S	S	35	S	S
36	S	S	37	S	S	38	S	S	39	S	S
40	S	S	41	S	S	42	S	S	43	S	S
44	S	S	45	S	S	47	S	S	49	S	S
51	S	S	52	S	S	55	S	S	56	S	S
57	S	S	58	S	S	59	S	S	60	S	S
62	S	S	63	S	S	64	S	S	65	S	S
67	S	S	68	S	S	71	S	S	72	S	S
74	S	S	75	S	S	76	S	S	77	MR	MR
78	S	S	82	S	S	83	S	S	84	S	S
85	S	S	86	S	S	87	S	S	88	S	S
90	S	S	91	S	S	93	S	S	94	S	S
98	S	S	101	S	S	105	S	S	107	S	S
108	S	S	110	S	S	112	S	S	113	S	S
114	S	S	115	S	S	118	S	S	119	S	S
120	S	S	125	S	S	126	S	S	127	S	S
128	S	S	129	S	S	131	S	S	132	S	S
133	S	S	135	S	S	136	S	S	138	S	S
139	S	S	140	S	S	143	S	S	144	S	S
145	S	S	146	S	S	147	S	S	148	S	S
149	S	S	150	S	S	155	S	S	156	S	S
159	S	S	162	S	S	163	S	S	164	S	S
168	S	S	169	S	S	170	S	S	171	S	S
173	S	S	174	S	S	176	S	S	178	S	S
179	S	S	180	S	S	181	S	S	183	S	S
185	S	S	187	S	S	189	S	S	190	S	S
191	S	S	193	S	S	195	S	S	196	S	S
197	S	S	198	S	S	199	S	S	200	S	S
201	S	S	202	S	S	204	S	S	206	S	S
207	S	S	209	S	S	211	S	S	212	S	S

ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION	
	INF.	DEV.		INF.	DEV.		INF.	DEV.		INF.	DEV.
215	S	S	219	S	S	221	S	S	222	S	S
225	S	S	226	S	S	228	S	S	229	S	S
230	S	S	234	S	S	235	S	S	236	S	S
239	S	S	241	S	S	242	S	S	245	S	S
246	S	S	249	S	S	250	S	S	251	S	S
252	S	S	253	S	S	254	S	S	255	S	S
257	S	S	258	S	S	259	S	S	260	S	S
261	S	S	263	S	S	264	S	S	265	S	S
267	S	S	268	S	S	274	S	S	280	S	S
281	S	S	283	S	S	286	S	S	287	S	S
288	S	S	289	S	S	294	S	S	296	S	S
298	S	S	301	S	S	302	S	S	304	S	S
305	S	S	306	S	S	311	S	S	316	S	S
318	S	S	319	S	S	320	S	S	324	S	S
325	S	S	326	S	S	330	S	S	331	S	S
332	S	S	336	S	S	338	S	S	341	S	S
345	S	S	353	S	S	359	S	S	360	S	S
370	S	S	371	S	S	372	S	S	373	S	S
374	S	S	377	S	S	378	S	S	381	S	S
382	S	S	385	S	S	387	S	S	388	S	S
389	S	S	390	S	S	391	S	S	392	S	S
393	S	S	395	S	S	396	S	S	397	S	S
398	S	S	399	S	S	401	S	S	402	S	S
403	S	S	405	S	S	406	S	S	408	S	S
411	S	S	413	S	S	414	S	S	417	S	S
418	S	S	419	S	S	420	S	S	428	S	S
434	S	S	435	S	S	440	S	S	441	S	S
442	S	S	443	S	S	444	S	S	446	S	S
448	S	S	449	S	S	450	S	S	451	S	S
452	S	S	453	S	S	454	S	S	455	S	S
456	S	S	457	S	S	459	S	S	460	S	S
461	S	S	462	S	S	464	S	S	467	S	S
469	S	S	477	S	S	478	S	S	488	S	S
489	S	S	494	S	S	502	S	S	504	S	S
507	S	S	510	S	S	512	S	S	513	S	S
519	S	S	521	S	S	523	S	S	524	S	S
526	S	S	528	S	S	530	S	S	533	S	S

ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION	
	INF.	DEV.		INF.	DEV.		INF.	DEV.		INF.	DEV.
536	S	S	537	S	S	540	S	S	541	S	S
544	S	S	546	S	S	547	S	S	550	S	S
551	S	S	555	S	S	556	S	S	557	S	S
558	S	S	561	S	S	562	S	S	563	S	S
564	S	S	566	S	S	567	S	S	568	S	S
569	S	S	570	S	S	571	S	S	573	S	S
574	S	S	579	S	S	580	S	S	583	S	S
584	S	S	585	S	S	588	MR	MR	589	S	S
590	S	S	591	S	S	592	S	S	593	S	S
594	S	S	595	S	S	596	S	S	597	S	S
599	S	S	600	S	S	601	S	S	603	S	S
604	S	S	605	S	S	606	S	S	607	S	S
608	S	S	615	S	S	618	S	S	624	S	S
625	S	S	629	S	S	632	S	S	633	S	S
637	S	S	638	S	S	640	S	S	641	S	S
643	S	S	645	S	S	647	S	S	648	S	S
651	S	S	652	S	S	653	S	S	654	S	S
655	S	S	656	S	S	657	S	S	658	S	S
659	S	S	662	S	S	665	S	S	667	S	S
670	S	S	677	S	S	681	S	S	682	S	S
684	S	S	685	S	S	686	S	S	687	S	S
688	S	S	689	S	S	690	S	S	693	S	S
696	S	S	698	S	S	700	S	S	703	S	S
704	S	S	705	S	S	709	S	S	711	S	S
713	S	S	714	S	S	715	S	S	716	S	S
717	S	S	718	S	S	719	S	S	720	S	S
721	S	S	724	S	S	725	S	S	726	S	S
729	S	S	730	S	S	731	S	S	732	S	S
733	S	S	734	S	S	736	S	S	742	S	S
743	S	S	745	S	S	747	S	S	748	S	S
750	S	S	751	S	S	752	S	S	754	S	S
755	S	S	756	S	S	757	S	S	758	S	S
759	S	S	761	S	S	762	S	S	763	S	S
764	S	S	765	S	S	772	S	S	784	S	S
786	S	S	787	S	S	788	S	S	789	S	S
790	S	S	792	S	S	793	S	S	794	S	S
796	S	S	797	S	S	798	S	S	803	S	S

ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION		ACCESSION NUMBER	DISEASE REACTION	
	INF.	DEV.		INF.	DEV.		INF.	DEV.		INF.	DEV.
805	S	S	808	S	S	810	S	S	811	S	S
812	S	S	813	S	S	814	S	S	815	S	S
816	S	S	818	S	S	819	S	S	821	S	S
822	S	S	823	S	S	824	S	S	825	S	S
826	S	S	827	S	S	831	S	S	833	S	S
834	MR	MR	835	S	S	836	S	S	837	S	S
838	S	S	840	S	S	845	S	S	846	S	S
850	S	S	853	S	S	856	S	S	857	S	S
861	MR	MR	862	S	S	863	S	S	865	S	S
866	S	S	867	S	S	868	S	S	869	S	S
870	S	S	874	S	S	875	S	S	876	S	S
877	S	S	881	S	S	884	S	S	885	S	S
886	S	S	889	S	S	890	S	S	891	S	S
892	S	S	893	S	S	894	S	S	895	S	S
896	S	S	897	S	S	898	S	S	899	S	S
900	S	S	903	S	S	904	S	S	905	S	S
909	S	S	910	S	S	911	S	S	913	S	S
914	S	S	915	S	S	917	S	S	918	S	S
919	S	S	920	S	S	921	S	S	922	S	S
923	S	S	924	S	S	925	S	S	926	S	S
927	S	S	928	S	S	929	S	S	930	MR	MR
931	S	S	932	S	S	933	S	S	934	S	S
935	S	S	936	S	S	937	S	S	938	S	S
939	S	S	940	S	S	943	S	S	944	S	S
945	S	S	946	S	S	968	MR	MR	969	MR	MR
970	S	S	971	S	S	972	MR	MR	973	MR	MR
974	MR	MR	975	S	S	976	S	S			

INF. = ESTABLISHMENT OF INFECTION. (MR = 2-3; S = 4-6 on disease assessment key for detached leaves (Appendix 1))

DEV. = SPREAD OF LESION FROM INOCULATION SITE. (MR = 3-5mm; S = > 5mm)

MODERATELY RESISTANT ACCESSIONS ARE SHADED.

APPENDIX 3. REACTION OF 10 *V. FABA* ACCESSIONS TO *B. FABAE* AND *B. CINEREA* ISOLATES.

B. FABAE

TEST ISOLATE	ORIGIN	LOCALITY	INOCULUM	ACCESSIONS										MEAN SCORE	LSD P=0.05
				FIORD	77	588	834	930	968	969	972	973	974		
F2.0.1	BEAN LEAF	BORDERTOWN S.A.	T	6.6	6.4	6.6	6.3	6.6	6.4	3.6[a]	6.6	4.4[a]	7.0	6.0	1.7
			C	6.7	6.7	6.2	6.3	7.0	6.6	5.2	6.5	5.5	7.2	6.4	NS
F6.0.1	BEAN STUBBLE	STRUAN S.A.	T	9.8	9.7	9.4	9.3	9.8	8.5	9.8	9.3	9.5	9.6	9.5	NS
			C	6.8	7.0	7.0	7.0	7.2	5.8	6.3	6.8	6.2	7.2	6.7	NS
F9.0.1	BEAN STUBBLE	BORDERTOWN S.A.	T	2.8	5.7[b]	1.6	1.8	3.2	2.0	0.8[a]	3.3	2.8	3.2	2.8	2.0
			C	3.0	6.2[b]	3.0	2.4	4.6	2.4	0.8	3.8	3.2	3.0	3.3	2.7
F13.0.1	BEAN STUBBLE	GOOLWA S.A.	T	4.5	5.3	3.4	4.6	5.4	3.0	1.0[a]	4.7	3.0	3.6	3.8	2.2
			C	3.0	4.0	3.0	2.8	3.6	2.0	0.8[a]	3.3	2.0	3.6	2.8	1.4
F14.0.1	BEAN STUBBLE	GOOLWA S.A.	T	7.8	7.3	7.4	6.8	7.4	5.6[a]	5.0[a]	6.7	7.7	7.2	6.9	1.8
			C	4.7	4.8	4.8	4.4	6.2	4.0	2.0[a]	4.3	4.7	4.2	4.4	1.5
F15.0.1	BEAN STUBBLE	STRATHALBYN S.A.	T	6.0	7.7	3.4[a]	4.4	8.2	3.6	4.2	6.5	5.0	3.6	5.3	2.4
			C	3.3	4.2	1.0[a]	2.0[a]	4.6[b]	1.0[a]	0.7[a]	2.5	2.0	1.8	2.3	1.1
F22.0.1	BEAN STUBBLE	HOYLETON S.A.	T	3.8	4.0	2.8	2.8	2.2	3.0	1.2[a]	3.8	3.0	2.2	2.8	1.6
			C	3.7	4.5	5.0	4.4	4.8	3.4	1.5[a]	4.5	4.5	4.6	4.1	1.9
F25.0.1	BEAN STUBBLE	KYBUNGA S.A.	T	6.7	7.2	7.0	5.8	6.4	6.2	4.3[a]	6.5	5.3[a]	6.0	6.1	1.2
			C	6.3	7.2	7.0	5.8	6.6	5.6	5.3[a]	6.0	5.2[a]	5.8	6.1	1.0
F34.0.1	BEAN STUBBLE	MILLICENT S.A.	T	4.2	4.2	4.0	2.6	4.8	4.3	4.2	4.7	4.4	4.6	4.2	NS
			C	4.0	3.8	4.4	3.4	5.4	4.0	3.4	5.0	3.6	4.6	4.1	NS
F37.0.1	BEAN SEED	PRICE S.A.	T	7.5	7.5	7.8	7.6	6.3	4.0[a]	7.0	7.7	7.7	6.6	7.0	1.6
			C	7.0	7.3	7.5	6.8	7.8[b]	6.6	7.0	7.3	7.7	7.2	7.2	0.7
F38.0.1	BEAN LEAF	ADELAIDE S.A.	T	5.4	6.8	6.8	6.0	6.6	6.3	4.6	6.3	6.4	6.6	6.2	NS
			C	4.4	4.8	4.4	3.6	4.6	3.3	3.2	3.7	4.2	4.0	4.1	NS
F43.0.1	BEAN STEM	BORDERTOWN S.A.	T	7.0	4.6[a]	5.8	5.2	7.0	7.2	4.6[a]	7.2	7.8	4.6[a]	6.1	2.4
			C	5.8	3.6[a]	5.2	4.2[a]	4.2[a]	4.0[a]	2.4[a]	5.6	2.8[a]	4.0[a]	4.2	0.8
F45.0.1	BEAN LEAF	BOOL LAGOON S.A.	T	4.4	3.8	2.0	4.4	4.0	5.6	2.8	4.4	4.2	3.8	3.9	NS
			C	4.6	3.8	4.0	4.6	4.6	3.8	1.8[a]	4.4	3.8	3.6	3.9	1.6
F49.0.1	BEAN LEAF	FURNER S.A.	T	7.4	7.8	7.0	7.2	6.0	7.3	7.4	7.0	7.2	7.4	7.2	NS
			C	5.2	5.8	3.0	4.4	4.8	3.3	4.0	5.0	4.2	5.6	4.6	NS
F50.0.1	BEAN LEAF	FURNER S.A.	T	7.4	7.0	6.0	5.6	5.8	6.0	6.2	7.0	5.8	6.8	6.3	NS
			C	4.4	5.2	3.2	3.8	4.0	2.7	2.4	5.3	3.8	5.6	4.0	NS
F51.0.1	BEAN LEAF	STRATHALBYN S.A.	T	8.6	10.0	9.4	7.2	9.6	6.8[a]	9.6	9.2	9.6	9.2	8.9	1.7
			C	7.4	7.0	8.2	7.4	7.6	7.2	7.0	7.2	7.0	7.6	7.4	NS
F53.0.1	BEAN POD	WONWONDAH VIC.	T	8.6	8.0	9.4	6.6	7.6	8.4	5.8[a]	8.0	8.2	8.8	7.9	2.1
			C	7.6	7.8	7.6	7.7	7.2	7.8	7.0[a]	7.2	7.4	8.0	7.5	0.6
F54.0.1	BEAN LEAF	BORDERTOWN S.A.	T	6.0	6.2	7.0	6.6	6.2	6.3	5.6	5.3	6.2	6.6	6.2	NS
			C	3.6	3.8	4.6	4.2	4.0	4.3	2.4[a]	4.0	3.2	3.4	3.7	0.9
F56.0.1	BEAN POD	OWEN S.A.	T	8.4	7.8	8.0	7.2	8.0	7.2	6.6	7.4	7.4	3.6	7.2	NS
			C	7.4	7.2	7.4	7.0	7.4	7.0	7.2	7.2	7.2	7.6	7.3	NS
F59.0.1	BEAN FLOWER	TARLEE S.A.	T	4.0	5.2	5.2	4.6	6.2[b]	2.6	3.0	4.3	3.6	3.8	4.3	1.7
			C	3.4	5.0	4.8	4.8	6.2[b]	2.7	3.0	4.3	3.6	3.4	4.2	1.7

B. CINEREA

TEST ISOLATE	ORIGIN	LOCALITY	INOCULUM	ACCESSIONS										MEAN SCORE	LSD P=0.05
				FIORD	77	588	834	930	968	969	972	973	974		
C1.0.1	BEAN STUBBLE	STRATHALBYN S.A.	T	2.0	3.2	1.2	1.5	2.0	0.8	0.3[a]	3.2	2.3	2.2	1.9	1.7
			C	6.7	6.5	6.8	7.0	7.0	6.4	5.7	7.0	6.7	7.0	6.6	NS
C4.0.1	BEAN STUBBLE	BORDERTOWN S.A.	T	1.0	3.6[b]	1.0	0.8	1.4	1.6	0.5	0.8	2.4	0.8	1.4	1.9
			C	1.8	4.2	4.0	2.8	5.2	2.4	0.8	3.8	2.8	2.4	3.0	NS
C6.0.1	BEAN STUBBLE	GOOLWA S.A.	T	0.3	2.2[b]	0.0	0.2	1.6	1.8	0.0	1.5	1.2	0.2	0.9	1.6
			C	2.5	3.7	2.2	1.2	2.8	1.6	0.8[a]	3.5	2.0	2.8	2.3	1.7
C7.0.1	BEAN STUBBLE	GOOLWA S.A.	T	2.0	4.5[b]	3.6	2.3	2.6	0.8	0.3[a]	2.5	1.3	1.2	2.1	1.7
			C	7.2	7.5	7.8	7.0	5.6	6.0	5.7	7.0	6.0	6.6	6.6	NS
C11.0.1	BEAN STUBBLE	TEMPLERS S.A.	T	1.0	1.2	1.8[b]	1.0	1.6	0.8	0.3	1.0	1.7	1.4	1.2	0.8
			C	5.8	5.0	4.8	5.0	5.6	2.4[a]	1.8[a]	4.5	4.2[a]	4.8	4.4	1.6
C15.0.1	BEAN STUBBLE	KYBUNGA S.A.	T	5.8	4.2	4.2	2.6[a]	2.2[a]	3.4[a]	0.7[a]	4.3	2.7[a]	4.4	3.5	1.8
			C	5.5	6.7[b]	5.0	5.4	6.0	5.0	5.5	5.3	4.5[a]	6.6[b]	5.5	0.9
C16.0.1	BEAN STUBBLE	KYBUNGA S.A.	T	6.0	4.3	5.4	2.0[a]	2.4[a]	2.8[a]	0.8[a]	4.2	4.0	2.6[a]	3.5	2.1
			C	5.6	6.7[b]	6.8[b]	5.4	5.2	4.6[a]	5.2	5.8	5.2	6.2	5.7	1.0
C17.0.1	BEAN SEED	MILANG S.A.	T	7.0	5.0	5.6	3.6[a]	5.4	3.4[a]	1.7[a]	4.7[a]	2.5[a]	2.6[a]	4.1	2.3
			C	7.0	6.8	6.6	6.4	7.2	6.2	6.2	6.7	7.0	6.4	6.7	NS
C18.0.1	CHICKPEA SEED	? N.S.W.	T	3.5	4.0	4.6	3.8	4.2	1.2	0.7[a]	1.5	1.7	1.2	2.6	2.6
			C	5.7	8.0	6.2	6.2	6.2	6.4	5.8	7.0	6.3	6.8	6.5	NS
C19.0.1	BEAN SEED	BARUNGA S.A.	T	5.0	2.8	5.6	4.0	3.4	3.4	0.7[a]	4.3	1.5[a]	1.8	3.2	2.4
			C	7.2	7.0	7.0	6.6	6.4	7.2	3.2[a]	7.3	6.5	6.8	6.5	1.2
C23.0.1	BEAN SEED	FREELING S.A.	T	5.2	2.5[a]	4.4	3.8[a]	2.4[a]	0.8[a]	1.5[a]	3.8[a]	0.7[a]	0.8[a]	2.6	1.2
			C	7.3	7.0	6.6	6.6	7.2	6.8	5.7	7.0	7.0	7.0	6.8	NS
C24.0.1	BEAN SEED	YANKALLILA S.A.	T	6.0	5.2	5.4	3.4[a]	5.4	3.6[a]	0.8[a]	5.7	1.8[a]	2.2[a]	3.9	2.4
			C	6.5	7.8	6.6	6.4	7.2	7.4	5.2[a]	7.3	6.5	6.4	6.7	1.2
C25.0.1	CHICKPEA SEED	FREELING S.A.	T	5.3	4.8	4.5	2.5[a]	3.6	2.4[a]	1.3[a]	4.5	3.5	4.4	3.7	2.2
			C	6.0	7.0	5.5	4.5	6.8	2.2[a]	4.5	6.0	5.8	5.4	5.4	2.1
C36.0.1	BEAN FLOWER	TEMPLERS S.A.	T	3.6	3.6	3.2	3.6	2.0	1.7[a]	1.0[a]	1.7[a]	3.4	4.0	2.9	1.8
			C	5.2	5.4	4.8	5.8	5.8	4.0	3.2[a]	5.3	4.6	4.0	4.8	1.4
C37.0.1	BEAN FLOWER	TEMPLERS S.A.	T	0.6	1.4	1.6	1.2	0.6	0.0	0.0	1.7	2.2	0.8	1.0	NS
			C	5.0	5.2	5.8	4.8	5.4	4.7	2.8	3.7	3.0	4.0	4.5	2.0
C38.0.1	BEAN LEAF	MORTLOCK S.A.	T	2.6	4.6	3.6	4.0	5.0	3.3	1.8	3.3	4.4	4.4	3.7	NS
			C	4.8	6.2	5.4	5.8	6.0	3.3	2.8[a]	3.7	3.8	4.2	4.7	1.7
C39.0.1	BEAN LEAF	TEMPLERS S.A.	T	1.0	3.2	1.6	1.4	1.0	1.0	0.4	2.3	1.6	2.2	1.6	NS
			C	5.2	5.8	5.6	4.6	5.2	3.3[a]	3.4[a]	5.0	3.0	4.0	4.5	1.8

TEST ISOLATE	ORIGIN	LOCALITY	INOCULUM	ACCESSIONS										MEAN SCORE	LSD P=0.05
				FIORD	77	588	834	930	968	969	972	973	974		
C42.0.1	BEAN SEED	NARACOORTE S.A.	T	5.6	5.0	3.8[a]	2.8[a]	4.2[a]	3.0[a]	3.4[a]	5.7	4.4	4.4	4.2	1.6
			C	4.2	4.4	3.8	3.4	3.4	3.0	3.0	5.3	4.0	3.6	3.8	NS
C43.0.1	PEA SEED	GAWLER S.A.	T	4.8	4.6	4.0	2.2[a]	4.0	3.0[a]	3.4[a]	4.0	3.8	4.4	3.8	1.3
			C	3.4	4.0	3.6	2.4	3.4	4.0	2.2	2.7	2.8	3.0	3.1	NS
C44.0.1	LENTIL SEED	KAPUNDA S.A.	T	4.6	4.2	3.8	2.8	2.8	3.3	3.2	4.3	4.0	4.4	3.7	1.3
			C	3.6	4.2	3.8	3.2	3.4	2.3	2.2	4.0	3.8	3.6	3.4	1.1
C45.0.1	LUPIN SEED	COLEBATCH S.A.	T	2.6	2.0	1.8	1.2	1.8	0.7	0.8	2.7	2.4	1.6	1.8	NS
			C	3.4	4.6	2.4	1.8[a]	3.0	1.7[a]	2.8	3.7	3.4	3.4	3.0	1.3
C46.0.1	LUPIN SEED	MUNDULLA S.A.	T	1.2	2.4	2.0	0.4	1.4	0.0	0.0	1.3	1.0	1.6	1.2	1.3
			C	3.6	4.0	3.2	1.8[a]	3.6	4.0	2.2	3.7	4.2	3.2	3.3	1.5
C47.0.1	BEAN SEED	STRATHALBYN S.A.	T	0.8	2.2[b]	1.4	1.5	2.8[b]	2.2	0.5	1.2	1.3	1.2	1.5	1.3
			C	4.8	6.7	5.4	6.5	5.8	6.8	3.5	7.5[b]	6.3	7.4	6.0	2.2

T = TEST ISOLATE C = *B. FABAE* REFERENCE CULTURE F0.5.1

a = SIGNIFICANTLY LOWER THAN FIORD b = SIGNIFICANTLY HIGHER THAN FIORD

NS = NO SIGNIFICANT DIFFERENCE BETWEEN ACCESSIONS

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