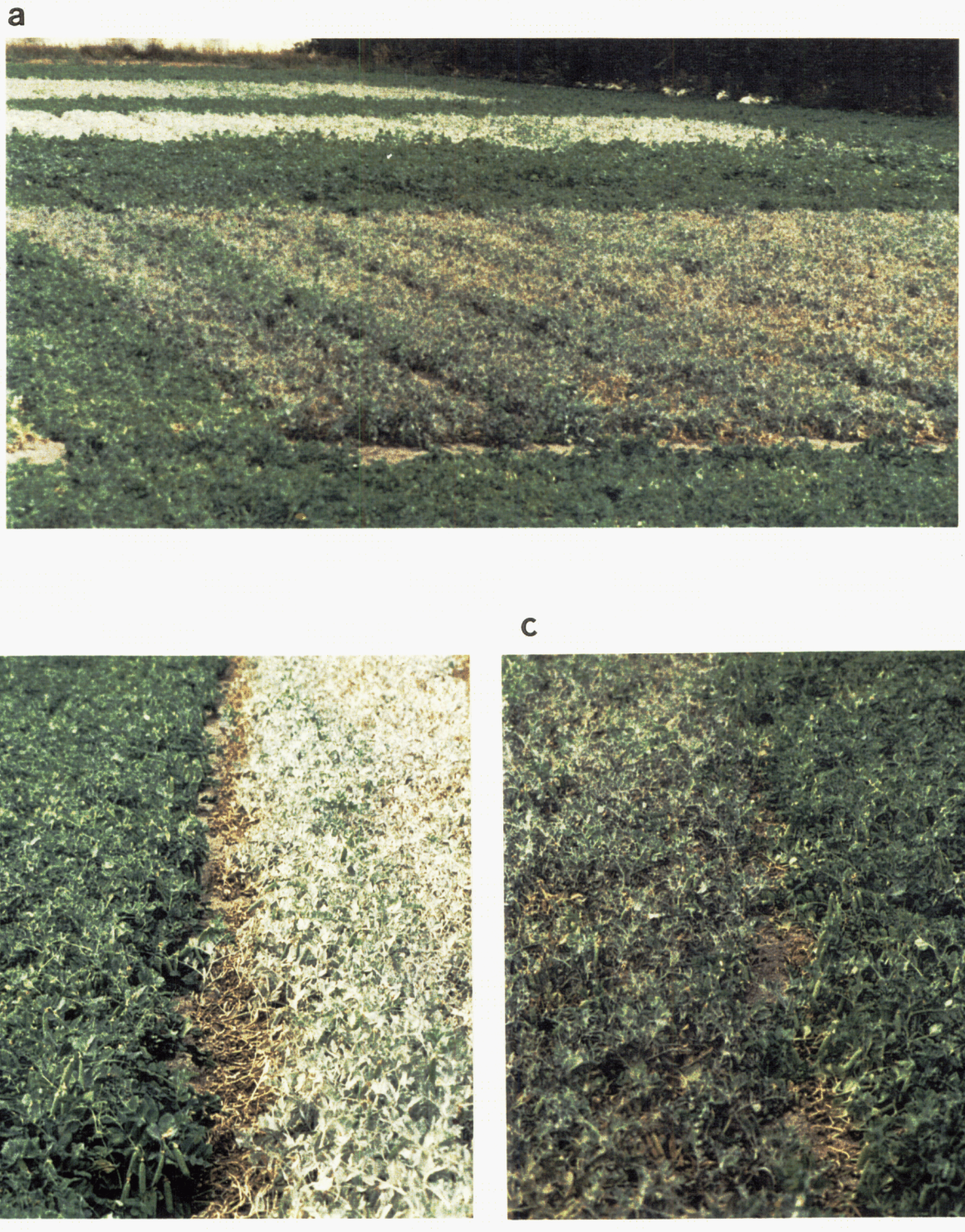

Field expression of quantitative resistance in pea (*Pisum sativum* L.) to *Erysiphe pisi* DC.

a) cultivar Quantum in the foreground, susceptible cultivars Pania and Bolero in the background

b) resistant Trounce (left) and susceptible Pania

c) Quantum (left) and Trounce

Tell me, and I'll forget.

Show me, and I may not remember.

Involve me, and I'll understand.

Native American saying

Expression and detection of quantitative resistance to *Erysiphe pisi* DC. in pea (*Pisum sativum* L.)

A thesis submitted in fulfilment of

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S.L.H. Viljanen-Rollinson

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Expression and detection of quantitative resistance to *Erysiphe pisi* DC. in pea (*Pisum sativum* L.)

by S.L.H. Viljanen-Rollinson

Characteristics of quantitative resistance in pea (*Pisum sativum* L.) to *Erysiphe pisi* DC., the pathogen causing powdery mildew, were investigated. Cultivars and seedlines of pea expressing quantitative resistance to *E. pisi* were identified and evaluated, by measuring the amounts of pathogen present on plant surfaces in field and glasshouse experiments. Disease severity on cv. Quantum was intermediate when compared with that on cv. Bolero (susceptible) and cv. Resal (resistant) in a field experiment. In glasshouse experiments, two groups of cultivars, one with a high degree of resistance and the other with nil to low degrees of resistance to *E. pisi*, were identified. This indicated either that a different mechanism of resistance applied in the two groups, or that there has been no previous selection for intermediate resistance. Several other cultivars expressing quantitative resistance were identified in a field experiment.

Quantitative resistance in Quantum did not affect germination of *E. pisi* conidia, but reduced infection efficiency of conidia on this cultivar compared with cv. Pania (susceptible). Other epidemiological characteristics of quantitative resistance expression in Quantum relative to Pania were a 33% reduction in total conidium production and a 16% increase in time to maximum daily conidium production, both expressed on a colony area basis. In Bolero, the total conidium production was reduced relative to Pania, but the time to maximum spore production on a colony area basis was shorter. There were no differences between the cultivars in pathogen colony size or numbers of haustoria produced by the pathogen. Electron microscope studies suggested that haustoria in Quantum plants were smaller and less lobed than those in Pania plants, and the surface area to volume ratios of the lobes and haustorial bodies were larger in Pania than in Quantum.

The progress in time and spread in space of *E. pisi* was measured in field plots of cultivars Quantum, Pania and Bolero as disease severity (proportion of leaf area infected). Division of leaves (nodes) into three different age groups (young, medium, old) was necessary because of large variability in disease severity within plants. Disease severity on leaves at young nodes was less than 4% until the final assessment at 35 days after inoculation (dai). Exponential disease progress curves were fitted for leaves at medium nodes. Mean disease severity on medium nodes 12 dai was greatest ($P<0.001$) on

Bolero and Pania (9.3 and 6.8% of leaf area infected respectively), and least on Quantum (1.6%). The mean disease relative growth rate was greatest ($P<0.001$) for Quantum, but was delayed compared to Pania and Bolero. Gompertz growth curves were fitted to disease progress data for leaves at old nodes. The asymptote was 78.2% of leaf area infected on Quantum, significantly lower ($P<0.001$) than on Bolero or Pania, which reached 100%. The point of inflection on Quantum occurred 22.8 dai, later ($P<0.001$) than on Pania (18.8 dai) and Bolero (18.3 dai), and the mean disease severity at the point of inflection was 28.8% for Quantum, less ($P<0.001$) than on Pania (38.9%) or Bolero (38.5%). The average daily rates of increase in disease severity did not differ between the cultivars. Disease progress on Quantum was delayed compared with Pania and Bolero. Disease gradients from inoculum foci to 12 m were detected at early stages of the epidemic but the effects of background inoculum and the rate of disease progress were greater than the focus effect. Gradients flattened with time as the disease epidemic intensified, which was evident from the large isopathic rates (between 2.2 and 4.0 m d^{-1}).

Some epidemiological variables expressed in controlled environments (low infection efficiency, low maximum daily spore production and long time to maximum spore production) that characterised quantitative resistance in Quantum were correlated with disease progress and spread in the field. These findings could be utilised in pea breeding programmes to identify parent lines from which quantitatively resistant progeny could be selected.

Keywords: Colony size, conidium germination, conidium production, epidemiology, *Erysiphe pisi* DC., haustorial efficiency, image analysis, image processing, infection efficiency, pea, *Pisum sativum* L., powdery mildew, quantitative resistance, serial sections, spatial and temporal spread, transmission electron microscopy.

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Chapter 1

Introduction and literature review

1.1. General introduction

In many crops, the defence mechanisms against plant pathogens used by breeders constitute resistance. Resistance to plant pathogens is an important attribute because it is easy for growers to use and it reduces the need for other methods of control, especially chemical control. In the past, high levels of resistance have been achieved based on major genes. This kind of resistance, so called vertical resistance (Vanderplank, 1963), has frequently been overcome by new pathogen races and therefore loss of disease control has occurred (Parlevliet, 1993). Current breeding programmes for many crops, especially cereals, are concentrating on other forms of resistance, such as quantitative resistance, which are likely to be more durable than resistance based on major genes (Johnson, 1992; Parlevliet, 1993).

Powdery mildew of pea (*Pisum sativum* L.) is caused by the Ascomycete fungus *Erysiphe pisi* DC. This disease causes problems in pea crops throughout the world (Dixon, 1978). In New Zealand, the disease had been considered of little consequence (Brien *et al.*, 1955; Boesewinkel, 1979) until severe epidemics occurred between 1986 and 1989 (Falloon, McErlich and Scott, 1989). Powdery mildew-resistant cultivars have been grown commercially in New Zealand since 1989 (R.E. Scott, pers. comm.), and the epidemics have not been as severe as earlier. Pea breeding for powdery mildew resistance has often been carried out without proper understanding of the underlying basis of the resistance. This research aims to explore the mechanisms of quantitative resistance using pea powdery mildew as a model for investigation and more general application of epidemiological and structural aspects of host-pathogen interactions.

Relevant literature is reviewed under Sections on the host (1.2.), the pathogen (1.3.), infection processes (1.4.), host-pathogen genetics (1.5.) and quantitative resistance (1.6.). Aims and objectives of the research are described in Section 1.7.

1.2. The host

Pea belongs to the family Leguminosae (Fabaceae) and is grown worldwide as a source of protein, amino acids and carbohydrate. *Pisum* is native to the Mediterranean and the Near East regions but has adapted to a wide range of climates, from subtropical to subarctic cool summer and tropical humid highlands (Sauer, 1993).

In New Zealand, peas have been grown since the commencement of arable agriculture (Jermyn, 1987), and this crop is now the most valuable export grain legume (Hill, 1991). About 30,000 ha of peas are grown annually in New Zealand, of which two thirds is for dry grain and seed production and one third for green pea production (Anon, 1995; Falloon *et al.*, 1993a). Canterbury is the main pea cropping region with nearly 80% of the total New Zealand production of 107,000 tonnes (Anon, 1995). Small areas of organic production are also produced mainly for export to Japanese markets (A.F. McErlich, pers. comm.). Pea cultivars grown for processing are normally sown in Canterbury, New Zealand, between October and December to obtain crops evenly throughout the harvesting season (from December to February). Later sown crops are very likely to be infected with powdery mildew (*E. pisi*) as first disease signs are usually found in late November - early December, and growers are usually compensated by processing companies for growing susceptible cultivars of peas during that period.

1.3. The pathogen

Erysiphe pisi belongs to a family (Erysiphaceae) of obligate parasitic ascomycete fungi. It forms superficial hyphae on the aerial parts of living plants, has large single-celled conidia produced terminally on isolated aerial unbranched conidiophores, and has haustoria in living epidermal cells (Yarwood, 1978), or rarely in mesophyll or palisade layers of their hosts (Braun, 1987). The superficial mycelium and abundant white conidia of the fungus give the diseased hosts the characteristic white, powdery appearance.

1.3.1. Taxonomy

Although Erysiphaceae are widely distributed throughout the world and have been known for some time, their taxonomy is still rather confused. The classification of the genus *Erysiphe* DC., as well as other genera of the same family, has been accomplished by authors adopting either 'broadened' or 'narrowed' concepts of species. Salmon (1900), in the classic monograph of Erysiphaceae, did not recognise biological host specialization and ignored the taxonomic value of the conidial stage, thus

adopting a very wide species concept. He divided *Erysiphe* into eight species and one variety and grouped *E. pisi* (*E. martii*) into *E. polygoni* DC, which had 357 host species in 33 families. Homma (1937) adopted Salmon's system, but recognized more genera and introduced a narrower species concept than Salmon's. Blumer's (1933) monograph of the European powdery mildews became a standard in Europe. He combined morphology with host specialization in delimiting species and therefore came to a narrower species concept. He recognised 15 species within Salmon's *E. polygoni*, and considered *E. pisi* parasitic on *P. sativum* as the correct species concept of pea powdery mildew, as did Junell (1967) three decades later in Sweden. Braun (1987) based the species concept mainly on the morphological differentiations and divided *E. pisi* into two varieties, var. *pisi* and var. *cruchetiana* the latter differing from the former by 'frequently irregularly branched cleistothecial appendages'.

In this research, the currently accepted narrow species concept is adopted. A broad species concept is a disadvantage when working with disease resistance, because such a definition includes several types of the fungi regardless of their host range. However, several of the fungal types are unable to cause disease on a specific host because the pathogen and the host are incompatible (so-called non-host resistance). Furthermore, cleistothecia of the fungus have never been found in New Zealand, so sub-specific delineation cannot be achieved. Thus, the pea powdery mildew pathogen is referred to as *Erysiphe pisi* in this study.

1.3.2. Host range

Adopting the narrow species concept, reports on host range of *E. pisi* vary greatly due to differences in morphological criteria, inoculation techniques, incubation conditions, isolates of the pathogen and sources of host material. Variations may also be due to incorrect identification of hosts, differences in seed sources of the species tested, nutrition of host, or different environments. In addition, every region has its own special flora of powdery mildews (Zheng and Chen, 1981). Further confusion is caused by the fact that several species of powdery mildews may simultaneously attack the same host (Zheng and Chen, 1981).

The following hosts of *E. pisi* have been listed in the literature: (Blumer, 1933; Stavely and Hanson, 1966; Bhardwaj and Singh, 1984; Braun, 1987):

Cajanus spp.
Cicer spp.
Clitoria spp.
Crotolaria spp.
Dorycnium pentaphyllum Scop.
Pisum sativum L.
Lathyrus cicera, L. sativus and *L. tingitanus*

Lens spp.
Lupinus spp.
Macroptilium spp.
Medicago spp.
Vicia spp.
Vigna spp.
Trigonella spp.

In New Zealand, Boesewinkel (1977, 1979) reported *E. pisi* on:

Hebe speciosa (A. Cunn.) Ckn. & Allan (Scrophulariaceae)
Lathyrus odoratus L.
Lathyrus pubescens Hook. & Arn.
Lupinus angustifolius L.
Lupinus argenteus Pursh.
Lupinus polyphyllus Lindl.
Melilotus indica L.
Pisum sativum L.

1.3.3. Disease impact on peas

Morphologic signs of powdery mildew infection are usually apparent before symptoms appear. This is because the powdery mildews are obligate parasites which injure their hosts slowly, and because the mycelium and conidiophores are so conspicuous. *Erysiphe pisi* infects all the green parts of pea plants, ie. stems, leaves, tendrils and pods. Infection in favourable conditions is apparent in about 5 days after inoculation, when small, white fungal colonies appear typically on the upper surfaces of leaves, and coalesce as the disease progresses. Symptoms appear later and include stunting and distortion of leaves, surface necrosis of invaded tissue, a general decline in the growth of the host, yellowing and chlorosis of leaves, and premature leaf fall (Yarwood, 1957). In severe infections, the foliage may wither and occasionally plant death occurs.

Powdery mildew has been most prevalent on crops sown late in spring or on those that mature in late summer. Also seed crops and dry grain types that mature later are more likely to become infected and have larger yield losses than crops sown for fresh pea markets (Falloon and Scott, 1990). Generally 10% yield losses due to pea powdery mildew have been estimated (Dixon, 1978; Mahmood *et al.*, 1983), but yield losses of over 70% have been reported (Gritton and Ebert, 1975; Singh *et al.*, 1978). Glasshouse experiments (Falloon *et al.*, 1993b; 1993c) showed that severe powdery mildew killed young pea plants and adversely affected plant growth. The disease also reduces quality of harvested green pea crops, adversely affecting tenderometer values, flavour and appearance of peas for canning or freezing (Gritton and Ebert, 1975).

1.3.4. Life-cycle

Erysiphe pisi infections are probably initiated by conidia arriving on susceptible hosts from weeds or volunteer plants, from neighbouring crops, or from distant sources. Under favourable conditions germination of conidia start within 1 to 3 h after conidium deposition with the formation of short germ tubes (Singh and Singh, 1983). An appressorium is formed after 6 to 8 h, and secondary hyphae after 24 h. Haustoria are formed after about 30 h. Conidiophore initials develop after 72 h, and conidia are produced after 96 h. Within 4 to 5 days colonies become visible and produce conidia profusely which are then disseminated by wind. The mechanism of survival of the fungus from season to season is uncertain. Cleistothecia have been reported in various parts of the world, but not in New Zealand (Boesewinkel, 1976, 1979; Falloon *et al.*, 1989a). Overwintering in infected seed has been proposed by some authors (Crawford, 1927; Uppal, Patel and Kamat, 1935), but according to Smith (1969) 'it seems unlikely that mycelium borne externally on the seed could remain viable and to assume that the mycelium is borne inside the seed coat presupposes a growth habit unproven for powdery mildews'. Smith (1969, 1970) concluded, after studies of cleistothecia and on alternative hosts, that because powdery mildew of pea occurs late in the season in England (end of July to early August), it is likely that conidia have to spread from warmer areas such as continental Europe.

1.4. The infection process

Before entry into hosts, pathogens are present on host surfaces, and this phase is known as the prepenetration phase. This is followed by penetration, establishment and invasion of hosts. The processes of growth of *E. pisi* on host leaves have been described by Falloon, Sutherland and Hallett (1989).

The external pre-penetration stage of fungal infection includes the arrival of fungal propagules, adhesion to the host, and often there is external growth prior to penetration. Germination of *E. pisi* conidia is affected by temperature, moisture and light. The optimum temperature for conidium germination is about 20° C, with ranges from 10° to 30° C (Singh and Singh, 1983). Powdery mildew conidia are unique in their ability to germinate and infect in the absence of external liquid water, but a moist atmosphere can stimulate germination. Tolerance to low humidity is not uniform throughout the Erysiphales (Yarwood, 1936), which has resulted in many conflicting reports on the subject. There seem to be two broad groups of powdery mildew fungi: one group which germinates both in water and on dry leaf surfaces, the other which germinates poorly in water. *Erysiphe pisi* belongs to the first group (Sivapalan, 1993).

After each *E. pisi* conidium germinates, the resulting germ tube immediately forms an appressorium, a lobed structure close to the conidium. Up to five hyphae then develop from each conidium, with two or three hyphae growing from each end (Falloon *et al.*, 1989b). Hyphae form appressoria at intervals along their lengths. A fine penetration tube arises from each appressorium and pierces the host epidermal cells. The cereal powdery mildew fungi use both chemical (Nicholson *et al.*, 1988; Aist and Bushnell, 1991) and mechanical (Sargent and Gay, 1977; Aist and Bushnell, 1991) means to penetrate host cuticles, as does the grape powdery mildew fungus (Heintz and Blaich, 1990). It is likely that other powdery mildew fungi, including *E. pisi*, penetrate their hosts by the same means.

Only if the fungus enters into a parasitic relationship with its host is infection successful and pathogenesis is initiated. In powdery mildews and rusts the host is considered successfully infected with the development of haustoria. The host-parasite interface formed by biotrophic powdery mildew fungi is a specialized structure involved in the transfer of nutrients from the host to the fungus. An haustorium is enclosed by an invagination of the host plasma membrane, the extrahaustorial membrane (EHM), which is separated from the haustorial body by the extrahaustorial matrix. This is continuous with the host cell plasmalemma but distinct from it both structurally and functionally. A neckband isolates the apoplast around each haustorium so that the transport systems of hosts and pathogen are tightly coupled in series. This structure is termed the haustorial complex (HC; Gil and Gay, 1977), and it is probably involved in recognition and signalling between host and pathogen.

Hyphae of *E. pisi* which successfully establish haustoria continue to grow and branch across the host epidermis until the spread of the infection is stopped or until the plant dies. The factors associated with the infection process are subject to quantitative variation under the control of host, pathogen and environmental factors, as well as genetic factors (Figure 1.1.).

1.5. Genetics of host-pathogen interaction

The host-pathogen interactions at the genetic level are considered in this Section.

1.5.1. Fungal genetics

Phenotypic variation in progeny can result from either sexual or asexual reproduction. In sexual reproduction, variation is due to segregation and recombination of genes during meiotic division of the zygote, a process called hybridization, where two haploid (1N) nuclei unite forming a diploid (2N) nucleus (the zygote). A recombination of genetic factors occurs during subsequent meiotic

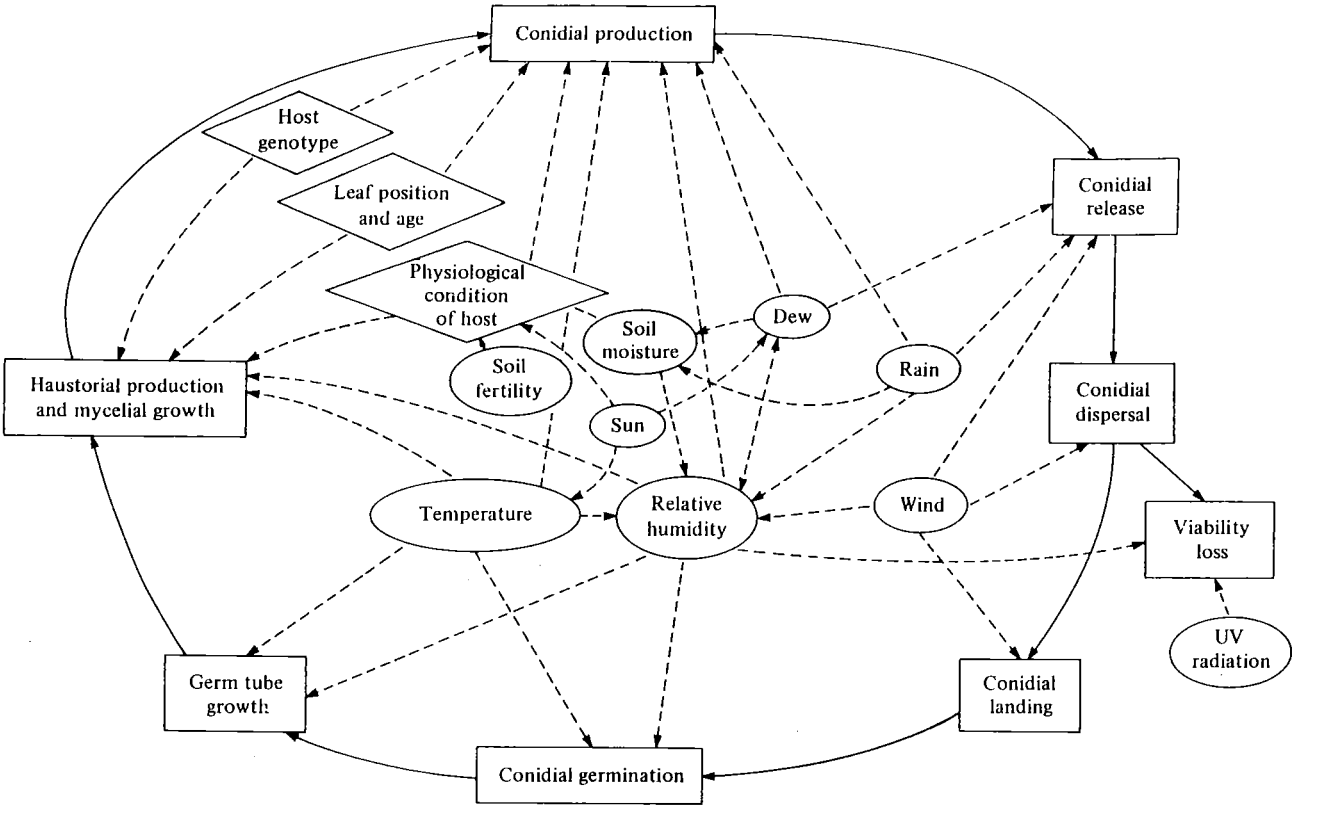


Figure 1.1. A model for *Erysiphe graminis* DC showing interaction of elements during one pathogen generation (○=environmental, ◊=host and □=pathogen effects). There may be host genotypic effects on germination and germ tube growth which are not indicated in this model. Adapted from Manners, 1993, p. 212.

division of the zygote as a result of genetic crossovers. In the fungi, nuclei or gametes often divide mitotically to produce mycelium and spores which result in genetically different groups of homogenous individuals that may produce large populations asexually until the next sexual cycle. Some of the fundamental genetic characteristics of powdery mildews are still not fully understood. Meiosis probably occurs in a manner similar to that in higher organisms, but there is controversy about mitotic events in ascomycetes (Jørgensen, 1988).

In asexual reproduction, frequency and degree of variability are reduced, but variation occurs regularly by mutations and perhaps by reassortment of genetic material contained in the cytoplasm. Mutations occur spontaneously. Most mutant factors are recessive, and in diploid or dikaryotic

organisms, mutations can remain unexpressed until they are combined in a hybrid. Mutations for virulence do not occur more frequently than for any other genetic trait, but given the large number of progeny, a large number of mutants can potentially occur. Planting only a few genetically homogenous varieties of each host crop over large areas increases the incidence of new more virulent mutations. Once a new factor for virulence appears in a mutant, this factor will take part in the sexual or parasexual processes of the pathogen and may produce recombinants possessing virulence quite different in degree or nature from that existing in the parental strains. Cytoplasmic inheritance is the acquisition by the plant or a pathogen, through extrachromosomal inheritance, of the ability to carry out a physiological process which it could not before (Agrios, 1988).

Some specialized mechanisms of genetic variation in fungi are heterokaryosis and parasexualism. Heterokaryosis happens as a result of fertilization or anastomosis, where cells of fungus hyphae or parts of hyphae contain nuclei that are genetically different, but without sexual reproduction. In parasexualism, genetic recombinations can occur within fungal heterokaryons. This comes about by the occasional random fusion of two nuclei and the formation of diploid nuclei. During multiplication, crossing over occurs in a few mitotic divisions, resulting in the appearance of genetic recombinants after the occasional separation of diploid nuclei into their haploid components.

When new physiologic races of a fungal pathogen containing new virulence genes appear in the field, they may have evolved via crossing in the sexual stage, nuclear (chromosomal and gene) reassortment, or a parasexual cycle in the asexual stage, or directly by mutation.

Although no physiologic races of pea powdery mildew have been recorded, it is likely that they occur, because races commonly occur in many powdery mildews, including those on cereals (e.g. Jenkyn and Bainbridge, 1978; Jørgensen, 1994), hops (Royle, 1978) and cucurbits (Sitterly, 1978), and because a breakdown of resistance to *E. pisi* has been reported (Schroeder and Provvidenti, 1965). *Erysiphe pisi* is heterothallic (Smith, 1970), so new races could evolve through recombination in the sexual stage, where the sexual stage occurs. Even though cleistothecia have never been found in New Zealand, it is likely that the sexual stage occurs. The occurrence could be identified by using molecular markers on population diversity (L. Kohn, pers. comm.). In cereals, classification of powdery mildews into physiological races has been done, but due to the geometric increase in the numbers of races, this approach has been abandoned by most researchers and substituted by determination of virulence genes and their frequencies (Jørgensen, 1988). Mutation in *E. graminis* DC f. sp. *hordei* has been seldom reported (Hermansen, 1980, in Jørgensen, 1988), because mutational analysis of obligate plant pathogens is relatively difficult, and because powdery mildews can not be grown on artificial media. Although cleistothecia may be rare, where powdery mildews can persist

in the vegetative state from one year to another, an unrestricted number of selection cycles can act upon individual genotypes of the pathogen (Wolfe and Schwarzbach, 1978).

1.5.2. Host genetics and inheritance of resistance in pea to *E. pisi*

There is an enormous number of plants that are able to be infected by only very few pathogens. Non-host resistance occurs, where a plant is clearly outside the range of hosts that a given parasite may infect. This type of resistance against fungi has been suggested to be multi-component, genetically complex, and parasite non-specific and involves constitutive and induced defences (Heath, 1991). Host-genotype resistance, on the other hand, is commonly parasite-specific and often expressed later in the infection process than nonhost resistance.

The terms 'vertical' and 'horizontal' resistance were introduced by Vanderplank (1963). Vertical resistance occurs when there is a high level of resistance to some races of pathogen and a low level to others, whilst horizontal resistance describes a situation where there are equal levels of resistance to all known races of the pathogen. Vertical resistance is often mono- or oligogenic and horizontal resistance is usually polygenic. Vertical resistance is characterised by the 'boom and bust' cycle where plant breeders have been producing resistant cultivars before pathogen adaptation and, eventually, the loss of resistance, although there are a few examples where resistance based on major genes has been durable (Johnson, 1987).

Limited and conflicting reports on the inheritance of powdery mildew resistance in pea have appeared since the 1940's. A number of genetic studies have been carried out, and all studies have found resistance to *E. pisi* to be inherited as a recessive trait. Using plant material from a remote site in the Andes of Peru, Harland (1948) identified a recessive gene, *er*, that controlled resistance to *E. pisi*. Pierce (1948) found resistance in the cultivar 'Stratagem', but this resistance broke down under field conditions (Schroeder and Provvidenti, 1965). Heringa, Van Norel and Tazelaar (1969) reported from studies with Peruvian and American material that resistance is conditioned by two recessive genes, er_1 and er_2, and other genes may be involved. The er_2 gene was present in two Peruvian lines, but it is not clear whether this gene is the same as that described by Harland (1948). Cousin (1965, in Dixon, 1978) assessed the reaction of about 400 pea cultivars and found that resistance in two cultivars ('Stratagem' and 'Mexique') was dependent upon the same gene. Kumar and Singh (1981) suggested that powdery mildew resistance is conditioned by two recessive genes.

Marx (1971) showed a genetic linkage between the resistance gene *er* and the morphological marker *Gty* and assigned *er* to chromosome 3. He later noted that disease reaction ranged widely depending on the prevailing environmental conditions and also reconsidered his assignment of *er* and *Gty* to

chromosome 3, but did not assign them to a different linkage group (Marx, 1986). Wolko and Weeden (1990) placed *Gty* in linkage group 6 and this was confirmed by Timmerman *et al.*, (1994).

Kalia and Sharma (1988) reported that pea cultivars resistant to powdery mildew contained higher levels of phenolics and phenol-oxidising enzymes than the susceptible cultivars. These compounds may be a useful means of screening segregating populations.

In New Zealand, several pea cultivars resistant to powdery mildew are available, including a locally bred cultivar 'Trounce' (Goulden and Scott, 1993). In addition to resistant pea cultivars protected by plant variety rights, other cultivars are available and traded, and are called cultivars of 'common knowledge' (P. Rhodes, pers. comm.). In Australia, a dry pea cultivar 'Glenroy' is resistant to powdery mildew (Ali, Sharma and Ambrose, 1994). However, Singh and Singh (1988) noted that no cultivar or pea line has been reported to be completely free from powdery mildew in India.

1.5.3. Genetics of host-pathogen interaction

According to Flor's (1942, 1947, 1955) gene-for-gene theory, for each gene conditioning resistance in the host, there is a specific gene conditioning pathogenicity in the parasite. The gene-for-gene relationship has been demonstrated for various host-pathogen systems including fungi, bacteria (Crute, 1985) and viruses (Fraser, 1985). It has been argued that resistance would always follow a gene-for-gene system (Parlevliet, 1981; Ellingboe, 1975), but an alternative view is that in addition to gene-for-gene interactions, there is another type of resistance that is independent of variation in the pathogen (Johnson, 1992).

Robinson (1987) identified various kinds of differential interactions that are a result of a gene-for-gene relationship (as defined by Vanderplank, 1963) as well as many that are not a result of this relationship. A differential interaction means that a series of parasite differentials (physiological races) is necessary to identify any resistance in the host and *vice versa*. Amongst the differential interactions that are not a result of gene-for-gene relationship, Robinson (1987) listed the false differential interaction which is a result of misinterpretation such as interplot interference, the simple change differential interaction which is 'an alteration in the population caused by either selection pressure for a previously rare type, or by selection pressure for a new type produced by mutation' (Robinson, 1987), and the environmental differential interaction which happens when the mechanisms of resistance and/or parasitic ability vary with environmental conditions such as temperature and humidity. In addition, Robinson (1987) identified the polyphyletic differential interaction which occurs as a result of interspecific hybridisation in the host and/or parasite, and the hybridizing parasite differential interaction which happens when the parasite differentials can

hybridize but the host differentials cannot, the hybridising host differential interaction when the host can but parasite cannot, and the immunity differential interaction when neither can.

1.6. Quantitative resistance

The variety of types of genetic control of plant disease resistance has led to a range of terminology adopted by workers in this area. Unfortunately, the criteria for different terms varies, and this has lead to great confusion. The term 'horizontal resistance' is widely but not universally accepted. It is based on pathogen adaptation unlike other terms such as 'partial resistance', 'quantitative resistance', 'slow diseasing', 'incomplete resistance', 'rate-reducing resistance', 'field resistance' and 'general resistance', which are based on the expression of disease. Partial resistance was described as a form of incomplete resistance in which spore production is reduced, even though the host plants are susceptible to infection (Parlevliet, 1979). More recently, partial resistance has been described as 'quantitative resistance based on the additive effects of resistance genes with relatively small effects' (Parlevliet, 1989) and 'characterized by a continuous variation between cultivars ranging from hardly any resistance to fair levels' (Parlevliet, 1992). Quantitative or partial resistance reduces the rate of epidemic development and reduces the severity of the disease (Geiger and Heun, 1989). Some cultivars possess durable resistance which is retained despite large-scale, long-term exposure to the pathogen, under conditions favourable for disease development (Johnson, 1984). Durable resistance is a descriptive term and does not imply anything about underlying causes, including genetic base, and the term does not imply permanent effectiveness (Johnson, 1981). Quantitative resistance may not always be durable, and may depend on major genes (Martin and Ellingboe, 1976; Royer *et al.*, 1984).

Throughout this study, the term 'quantitative resistance' is used as it is a slightly broader term than partial resistance, emphasising the continuous nature of variation, and because of the difficulties in assessing the genotypic characteristics from phenotypic characteristics. The definition of quantitative resistance by Geiger and Heun (1989) will be adopted; 'quantitative resistance reduces the rate of epidemic development and reduces the severity of disease, but does not imply anything about the number of genes involved'.

1.6.1. Assessment of and selection for quantitative resistance

Quantitative resistance is evaluated either by a relative measure determining a rank in comparison with a well-known standard cultivar, which is often the most susceptible cultivar available, or by an absolute measure determining a point on a defined scale of continuous variation (Robinson, 1987).

Relative measurements can be done numerically in the laboratory or in the field, measuring components of resistance such as rates of infection and invasion of host tissue, reproduction per unit of host tissue or per unit time, and comparing these with the performance of well-known standards. Relative measurements can also be estimated by measuring the amount of tissue affected by the pathogen, and it is this measure that can be influenced by differences in the level of disease pressure (Beek, 1988; Heun and Geiger, 1989). Absolute measurements involve complex, statistically controlled experiments that determine the loss caused by each kind of parasite in each new cultivar. The measurements can be obtained either in the field or in the laboratory under controlled conditions, and these must be correlated. Quantitative resistance can only be measured accurately in isolated plots, when interplot interference is minimised (Parlevliet and van Ommeren, 1975; Nørgaard Knudsen *et al.*, 1986). Other factors that interfere with the reliable measurement of quantitative resistance are earliness of the cultivar, inoculum density, moment of assessment (optimum when the most susceptible cultivar approaches its maximum assessment score), and plant habit (eg. tallness; Parlevliet, 1989).

Research on quantitative resistance has been unpopular with crop scientists until recently because it is more difficult to study due to the continuous variation, unlike qualitative resistance which is based on Mendelian genetics (Robinson, 1987). Selection for quantitative resistance is often difficult, and adequate screening methods have been developed for very few host-pathogen interactions. Depending on the type of pathogen, breeding methods for disease resistance are very diverse, so there is no single model available (Johnson, 1992). Most commonly, the aim for breeding is to improve several characters simultaneously whilst maintaining others at levels no lower than in previous cultivars (Johnson, 1992). Nevertheless, there are various ways to select for quantitative resistance. The first approach, proposed by Johnson (1978), relies on the transfer of resistance from durably resistant cultivars using pathogen races overcoming the known race-specific genes. The host population can be exposed to a wide race spectrum of the pathogen population and selected, thus selecting some quantitatively resistant lines which are subsequently utilised in the breeding programmes to increase the probability that further new lines will also possess quantitative or durable resistance (Johnson, 1984). The second method, based on the work of Vanderplank (1963) and Robinson (1976), assumes it should be possible to use certain races of the pathogen to overcome all race-specific or vertical resistance in several host cultivars, and then accumulate the horizontal resistance from these in a population from which durably resistant lines may be selected. This system of selection assumes that lines classified as susceptible to a specific pathogen race will not possess any effective genes for race-specific resistance and any residual resistance would be race-non-specific. This assumption is not correct for every host-pathogen interaction, and it is impossible to select the appropriate pathogen race which may not have yet evolved. A third method is based on the assumption that the resistance of some lines may be correlated with quantitative resistance

that is inherited oligo- or poly-genetically, and it is then concluded that any line having these two criteria should be durable (Wolfe, 1993; Simmonds, 1991). This assumption is optimistic.

Different methods of breeding are suitable for different host-pathogen interactions. Parlevliet (1993) proposed that the approach to breeding for quantitative durable resistance may vary with the type of pathogen, and divided pathogens into three groups. Group A consists of pathogens in which new races easily develop and several race-specific resistance genes occur in the corresponding hosts. These pathogens are often specialised, biotrophic or hemiotrophic, airborne or splashborne fungi, for example *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Ericks. & E.Henn., *Puccinia recondita* Roberge ex Desmaz f. sp. *tritici* (Ericks), *Melampsora lini* (Ehrenb.) Desmaz., *Erysiphe graminis* DC f. sp. *hordei* and *Bremia lactucae* Regal. Group B consists of pathogens where a few races are known, where the number of resistance genes described in each host is restricted, and resistance breakdown is uncommon. Pathogens in this group include some fungi (e.g. many f. sp. of *Fusarium oxysporum* Schlechtend.:Fr.) and many viruses. Group C includes pathogens where no races are known and the resistance has been durable so far, for example *Cladosporium cucumerinum* Ellus & Arth. and *Corynespora melonis* in cucumber and *Helminthosporium victoriae* in oats. Breeding for quantitative resistance differs in these three groups. Breeding for quantitative resistance in Group B and C pathogens is relatively straight forward because no races are known and no resistance breakdown is reported (Group C) or where few races exist and resistance breakdown is uncommon (Group B). However, good screening methods that identify small differences in resistance are essential and so far exist for very few host-pathogen interactions. Selection methods for quantitative resistance in Group A pathogens depends on the presence or absence of major resistance genes as described below.

In the segregating populations, the selection for minor genes is even more difficult when most of the genetic variability is non-additive by nature and the heritability under selection is low (Beek, 1988). When both quantitative resistance and race-specific monogenic resistance occur together, plant breeders tend to select for the latter because selection for the former is considered to be too difficult. Also levels of polygenic resistance sometimes do not seem to be sufficient under heavy disease pressure (Parlevliet and Kuiper, 1985). There is an important difference between selecting for quantitative resistance when major genes are absent and when they are present in the host population. Selection in the absence of major gene resistance in the barley - barley leaf rust (caused by *Puccinia hordei* G. Otth.) interaction has been studied by Parlevliet and coworkers. They found that differences in the latent period measured in the glasshouse explained most of the differences in leaf rust resistance observed in the field (Parlevliet and van Ommeren, 1975; Parlevliet, 1975). When selecting for long latent periods in adult plants in the glasshouse, compared plants must be in the same developmental stage because with increasing leaf age the latent period rapidly becomes shorter

(Parlevliet, 1975). Recombination and accumulation of minor genes for a longer latent period was shown to be quite feasible (Parlevliet and Kuiper, 1985). Broers (1989a; 1989b; 1989c) investigating the wheat - wheat leaf rust (caused by *P. recondita* f. sp. *tritici*) interaction, recommended measuring latent period on flag leaves as that gave more reliable results than measurement in the seedling stage. In the field, area under transformed disease progress curve (AUTC) and disease severity were good estimates of quantitative resistance (Broers, 1989b). Disease pressure did not have an effect on cultivar ranking, but differences in cultivar development rate biassed the estimation of resistance. In the glasshouse, quantitative resistance and hypersensitive resistance are easier to distinguish than in the field (Broers, 1989c). The efficiency of the screening methods is influenced by the environment and genotype-environment interaction. Latent period is very sensitive to fluctuations in the environment, especially temperature. Low (12°C) temperature regimes were preferred to distinguish differences in the level of quantitative resistance of wheat to *P. recondita* f. sp. *tritici* (Denissen, 1991). For selection, Broers (1989c) recommended any complex race of this pathogen to neutralize many of the known hypersensitive genes. Selection must be carried out both in the field and in the glasshouse.

To select for quantitative resistance in the presence of major gene resistance, the breeder must first be able to distinguish between the two types of resistance. The major gene resistance in many host-pathogen systems involving biotrophs is often of a hypersensitive type conferring low infection types, which is sometimes difficult to distinguish, especially in the field. Yellow rust of wheat shows a fully continuous spectrum of infection types making it very difficult to use to distinguish quantitative resistance. Parlevliet and van Ommeren (1985) showed that in the field ordinary race-specific major gene resistance can be easily mistaken for non-race-specific effects in quantitative resistance. This so-called Parlevliet effect (Robinson, 1987) occurs when screening many different genetic lines of the host against an uncontrolled mixture of qualitative pathotypes; there may be quantitative differences in the levels of parasitism even though all the hosts are matched. This is because simple (those with few qualitative genes) and common race-specific resistance will be matched with a higher frequency than complex (many qualitative genes) and rare non-race-specific resistances. Interplot interference can also falsely suggest quantitative resistance (Parlevliet, 1992). Vertical resistance can be eliminated during screening for quantitative resistance applying genetic or epidemiological elimination methods as proposed by Robinson (1987).

The measurement of spore production is one the most accurate and least subjective ways of assessing the growth of pathogens and the susceptibility of hosts (Johnson and Taylor, 1976), but it is laborious, and consequently has not been commonly and exclusively used as a selection method in plant breeding. Handling of powdery mildew conidia is more difficult than that of rust spores because powdery mildew conidia are sticky. The problem of collecting spores is overcome either by specially

designed spore samplers or by washing infected leaves with water containing wetting agents (Ward and Manners, 1974). The assessment of spore production is in general similar for both rust and powdery mildew fungi. Various counting methods have been devised: direct counting of spore suspensions in a haemocytometer or Coulter counter (Ward and Manners, 1974), turbidity measurements on suspensions, weighing bulk spore collections, spectrophotometrically using infrared reflectance analysis (Asher *et al.*, 1982; Lind, 1983) and by measuring fungal cell wall sterols (Newton, 1989a, 1990).

Modern molecular techniques will increasingly contribute to plant breeding in the future to permit more precise investigations into the variation and population genetics of various pathogens and the epidemiology of disease. These techniques can be used to localise quantitative resistance genes by mapped markers using restriction fragment-length polymorphism (RFLP). This would identify the number of genes involved and allow the efficiency of marker-sustained selection procedures to be evaluated (Geiger and Heun, 1989).

1.6.2. Effects of plant and leaf age on quantitative resistance

Plant parts that are the same physiological age but are produced at different times in the life of the plant may vary in resistance to plant pathogens. This difference in resistance of leaves with position on the plant is due to the different growth stage or physiological age of the plant (Populer, 1978). Quantitative resistance is often expressed more in adult plants than in seedlings (Aist and Bushnell, 1991). There are several host - pathogen interactions where disease resistance increases with increasing plant age, for example barley and *P. hordei* (Parlevliet, 1975). In *P. hordei*, an increase in quantitative resistance was found with increasing growth stage of barley and was divided into two categories: 1) increase in resistance caused by a difference in leaf tissue; and 2) increase in resistance because quantitative resistance -genes are not, or not fully, expressed at the seedling stage (Broers, 1989b). In the study of infection unit abortions of barley leaf rust, Parlevliet and Kievit (1986) found that seedlings appeared considerably less representative than adult plants for study of the relation between quantitative resistance and the histological parameters of fungal growth in the host tissue from appressoria formation to spore production. The host - pathogen interaction events occurring during and shortly after stoma penetration were of decisive importance in the expression of quantitative resistance (Parlevliet and Kievit, 1986).

Plant parts vary in resistance to a pathogen also with age (Populer, 1978). For example, in barley resistance to *E. graminis* f. sp. *hordei* increased with increasing age of first and second leaves (Nelson, Shiraishi and Oku, 1989). A combination of a high level of adult plant resistance and a high level of seedling resistance may provide a high level of quantitative resistance throughout the entire growing

season (Mastebroek and Balkema-Boomstra, 1991). In rice leaf blast (caused by *Magnaporthe grisea* (Hebert) M.E. Barr, anamorph *Pyricularia oryzae* Cav.), the number of sporulating lesions declined with increasing leaf age (Roumen, 1992). Cultivars with high levels of quantitative resistance showed typical susceptible lesions, but the resistance in leaves rapidly increased with age, and the initial level of susceptibility of new leaves was low (Roumen, Bonman and Parlevliet, 1992). In bean rust (caused by *Uromyces appendiculatus* (Pers.) Unger var. *appendiculatis*, anamorph *Uromyces phaseoli* (Pers.) Wint.), the areas of uredinia, colonies, and secondary uredinia were negatively correlated with leaf age or leaf length at inoculation (Shaik and Steadman, 1989). There has been suggestions that resistance in peas to powdery mildew is dependent on the age of leaf tissue (Cousin, 1964, in Dixon, 1978).

1.6.3. The effect of inoculum density on quantitative resistance

The amount of inoculum is a factor to be taken into account when assessing quantitative resistance. Often very large amounts of inoculum are applied to prevent escapes when assessing cultivars for quantitative resistance, but this tends to reduce or even prevent the expression of small differences (Parlevliet, 1992). Lesion size and spore production is density dependent. In oat powdery mildew, the number of colonies per unit leaf area decreased at levels greater than 1200 spores per cm^2, and there was a reduction in the proportion of spores which established infection when density exceeded 600-700 conidia per cm^2 (Carver and Ingerson-Morris, 1989). In wheat, before sporulation of *P. recondita* f. sp. *tritici* was initiated, colony size was independent of uredium density (7 to 200 uredia per cm^2 leaf area; Baart, Parlevliet and Limburg, 1991). After sporulation started, uredium size was strongly dependent on density. The size of uredia was approximately halved when the uredium density increased from 10 to 150 per cm^2. Urediospore production per uredium decreased with increased uredium density. Thordal-Christensen and Smedegard-Petersen (1988) found that infection efficiency of *E. graminis* f. sp. *hordei* on barley is dependent on inoculum density and is reduced when inoculum density is increased. The reduction in infection efficiency was 3% at 0.2 conidia per mm^2 (=20 conidia per cm^2), and 89% at 6.5 conidia per mm^2. At densities above 20 conidia per mm^2, the reduction was 92% and independent of the inoculum density.

1.6.4. The effects of light and temperature on quantitative resistance

Conflicting reports exist on the effects of temperature and other environmental effects on quantitative resistance. Parlevliet (1975) commented that quantitative resistance is temperature insensitive. Carson and Van Dyke (1994) concluded that in northern leaf blight of maize (caused by *Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs), incubation and latent period were correlated regardless of temperature or light conditions. Quantitative resistance expressed as an increased

latent period appeared a stable trait expressed over a wide range of temperature and light regimes, although high temperatures tended to increase the differences among genotypes. Sporulation interacted with both temperature and light, and was reduced at high temperatures. Other workers reported that quantitative resistance is often environmentally labile with many components interacting individually or pleiotropically with environmental factors. Newton (1989a, 1989b, 1990, 1993) found that infection frequency of *E. graminis* f. sp. *hordei* on barley was always greater at high humidity than at low humidity. One quantitatively resistant cultivar expressed lower infection frequency due to greater resistance to primary germ-tube penetration than susceptible cultivars. He also concluded that the expression of reduced colony size is environmentally dependent. Denissen (1991) found that in leaf rust of wheat, the correlation between infection frequency and latent period was low. There was a significant temperature effect on the latent period, and some temperature - genotype interaction. Greatest differences were at 12°C, and low temperature regimes were recommended to distinguish differences in levels of quantitative resistance.

1.6.5. Structural aspects of quantitative resistance

The extrahaustorial membrane (EHM) surrounding the haustorium is of considerable interest, as it probably regulates the flow of nutrients from the host to the pathogen (Manners, 1989). The EHM is sealed by the neck bands to the fungal wall isolating the haustorial matrix from the leaf apoplast (Gil and Gay, 1977; Manners and Gay, 1977). Transport of nutrients across the EHM may be either by facilitated diffusion due to a high concentration difference between the haustorial plasmalemma and the host cytoplasm, or an active mechanism whereby ionic pumps of the non-invaginated area of the host plasmalemma and of the haustorial plasmalemma may maintain a potential difference at the EHM to actively drive solute transport (Spencer-Phillips and Gay, 1981).

Resistance to *E. graminis* in wheat and barley is expressed as incompletely functioning haustoria (Ellingboe, 1972), but separate genes for resistance condition the mechanisms of resistance. In resistant barley, the development of *E. graminis* f. sp. *hordei* can be retarded or arrested at any stage during infection and colonisation of the host, including prior to haustorium formation, during primary penetration (Johnson, Bushnell and Zeyen, 1979; Kita, Toyoda and Shishiyama, 1981; Koga, Mayama and Shishiyama, 1980) or after formation of a primary haustorium (Wright and Heale, 1988; Aist and Bushnell, 1991). The latter is often associated with host cell hypersensitivity (Wright and Heale, 1988). Some resistance genes condition an intermediate reaction, and resistance is eventually associated with death of host tissue around infection sites and failure of the pathogen to sporulate.

Stumpf and Gay (1989) studied the pea - *E. pisi* interaction, and found that resistant (qualitative) interactions were characterized by the production of relatively few haustoria and by reduced

sporulation. They measured haustorial efficiency by plotting the average total hyphal length against the number of haustoria for each set of data from resistant and susceptible cultivars. They reported that a resistant cultivar had greater length of hyphae per haustorium than a susceptible cultivar, suggesting that haustoria in the resistant cultivar were taking up nutrients at a higher rate than in the susceptible cultivar. Haustoria were produced at a slower rate in the resistant cultivar than in the susceptible cultivar, but the related mycelium in the resistant cultivar maintained a similar rate of hyphal extension. The mechanism of qualitative resistance in *E. pisi* may be different from that of the cereal powdery mildews, because no evidence of chlorosis or necrosis was found and the fungus eventually sporulated (Stumpf and Gay, 1989). They proposed that 'resistance in the resistant cultivar operates at the stages prior to haustorium formation, similarly to that in some non-host and partial resistance systems'.

Haustoria have lobes that protrude from the central body, and in *E. pisi*, the lobes are recurved so that they invest the body instead of projecting directly away from it, unlike lobes of *E. graminis* which are straight and finger-like (Bushnell and Gay, 1978). The distal ends of lobes are in direct contact with the extrahaustorial membrane. Gil and Gay (1977) proposed that these contacts may provide a special pathway between the host and parasite, but no evidence for such contact was found by Manners and Gay (1982a). The haustorial plasmalemma is extensive due to the length of lobes. In *E. pisi* haustoria, the perimeter - area ratios of haustorial walls and lobes were found to be 1.5 - 2.4 greater than the ratio for extrahaustorial membrane measured from two dimensional ultrathin sections (Viljanen-Rollinson, 1991).

Mengden and Nass (1988) used potentiometric cyanine dyes to determine the physiological activity of *E. graminis* f. sp. *hordei* haustoria, correlating the fluorescence in the mitochondria of the fungus with the amount and the type of different sugars fed to the host cells. This technique, in addition to monoclonal antibody and recombination DNA techniques, could be extended to study the efficiency of haustoria in incompatible host-pathogen interactions.

1.7. Aims and objectives of the study

The aims of this research were:

1) to identify the existence of quantitative resistance to *E. pisi* in pea cultivars in the field;
2) to confirm quantitative resistance in glasshouse experiments;
3) to identify the epidemiological basis of quantitative resistance;
4) to examine the structural basis of quantitative resistance;

5) to examine powdery mildew epidemics in the field in cultivars varying in susceptibility to *E. pisi*;
6) to suggest methods of identifying quantitative resistance in parents and selections based on the five points above.

Six objectives of the research project were defined:

1) evaluation of quantitative resistance to *E. pisi* in the cultivar 'Quantum' in the field, measured by the amount of pathogen present on the surface of the host relative to a susceptible cultivar ('Bolero') and to a resistant cultivar ('Resal');
2) to classify different cultivars of peas with varying levels of resistance to *E. pisi*, from very susceptible to practically immune, in the glasshouse, measured by the amount of leaf area affected;
3) to evaluate the effects of quantitative resistance to *E. pisi* in peas on epidemiologically relevant characters by measuring the germination percentage, infection efficiency, length of latent period, and amount of sporulation on whole plants for the pathogen. To detect and quantify effects of leaf and plant age on quantitative resistance.
4) to investigate whether structural differences exist in plants possessing quantitative resistance to *E. pisi* using light, fluorescence and electron microscopy in conjunction with image processing and analysis;
5) to measure epidemic development of *E. pisi* in time and space in field plots of cultivars varying in their susceptibility to the pathogen;
6) to suggest methods to identify quantitative resistance to *E. pisi* in the selection process for breeding, by measuring with epidemiological tests or by structural differences using methods developed in the present research.

In Chapter 2, the existence of quantitative resistance in cultivar Quantum in a field and glasshouse situation is reported. Other cultivars reportedly with quantitative resistance were tested both in a field and glasshouse situation. In Chapter 3, the epidemiological basis of quantitative resistance in Quantum is explored, measuring the proportion of germination of conidia, infection efficiency, and the rate and duration of conidium production, in comparison with two susceptible cultivars. In Chapter 4, haustorial efficiency is measured using transmission electron microscopy and image analysis techniques. In Chapter 5, the spread of powdery mildew epidemics in time and space is examined in the field in cultivars Quantum, Pania and Bolero. In the general discussion (Chapter 6), aspects of quantitative resistance in peas are considered. Suggestions for future research and implications for breeding for powdery mildew-resistant peas are discussed.

Chapter 2

Identification of quantitative resistance to *Erysiphe pisi* in cultivars and seedlines of peas

2.1. Introduction

The first step in studying quantitative resistance in peas was to identify cultivars possessing this type of resistance. Quantitative resistance has, until recently, been generally ignored, mainly because race-specific resistance based on Mendelian genetics has been easier to study. Simmonds (1991) suggested that quantitative resistance has proven to be long lasting, unlike race-specific resistance which has often been characterised by the breakdown of resistance.

Breeding for quantitative resistance may be focussed on the production of lines possessing quantitative resistance, or the identification of parents from which quantitatively resistant progeny will be selected. Different methods depend on whether major gene resistance exists or is absent (Section 1.6.1.). Nevertheless, selection for quantitative resistance is often difficult, and adequate methods to select for this type of resistance have been developed for only a very few host-pathogen interactions.

Powdery mildew resistance in pea is inherited as a recessive trait, and there have been reports of single genes (Harland, 1948; Pierce, 1948; Marx, 1971; Timmerman *et al.*, 1994), two distinct genes (Kumar and Singh, 1981) and two or possibly more genes conditioning the resistance (Heringa, Van Norel and Tazelaar, 1969). The resistance in these and other reports has been either qualitative (Kumar and Singh, 1981; Timmerman *et al.*, 1994) or quantitative (Marx, 1971; Harland, 1948; Cousin, 1965, in Heringa *et al.*, 1969; Heringa *et al.*, 1969).

Quantum, a pea cultivar possessing quantitative resistance to powdery mildew, was identified by discussions with local pea breeders. Quantum was bred by Asgrow Seed Company, Twin Falls, Idaho and is a cross between the cultivars Bolero and Plus, the source of quantitative resistance reportedly being Plus (D. Webster, pers. comm.). Preliminary glasshouse experiments (Appendix I) were also carried out using crude inoculation techniques to apply *E. pisi* conidia to plants of 28 pea cultivars and seedlines. These gave indications of the powdery mildew severity that occurred for a range of pea germplasm, and these results were used to plan the experiments described here.

The experiments reported in this chapter had the following objectives:

1. To confirm quantitative resistance in garden pea (*P. sativum*) cultivar Quantum to *E. pisi* in a field situation compared with a susceptible cultivar Bolero and a resistant cultivar Resal (also known as ZMX7961) by scoring leaf area infected (Experiment 1).

2. To compare the development of pea powdery mildew epidemics in a quantitatively resistant cultivar Quantum when surrounded by differing amounts of inoculum pressure, by planting cultivars with varying levels of susceptibility to the disease. These cultivars were Bolero (susceptible), Quantum, and Resal (resistant; Experiment 1).

3. To identify seedlines additional to Quantum that may possess quantitative resistance to *E. pisi* (Experiments 2 and 3) by inoculating the plants in controlled conditions with a standardised number of conidia, growing the plants in an isolation propagator and measuring the leaf area infected by the pathogen, and in a field experiment (Experiment 4) measuring leaf area infected for different seedlines.

2.2. Materials and methods

2.2.1. Field experiment to confirm quantitative resistance in Quantum and the effect of inoculum pressure on disease development (Experiment 1)

A field experiment was conducted at a Crop & Food Research site at Lincoln on a Templeton silt loam. The area where the field experiment was to be sown was prepared by topdressing with 200 kg ha^{-1} of super phosphate (P:S 9:11). The experimental area was sprayed for weed control with trifluralin (800 g ha^{-1} as Treflan in 300 ℓ water ha^{-1}) one day before sowing and incorporated into the soil by rotary hoe. Cultivars for this experiment were chosen in consultation with local pea breeders, and choices were based on the results of a preliminary laboratory experiment (Appendix I). Seed of the three garden pea cultivars with different levels of resistance was sown on 30 December 1992 with an Oyjord cone seeder, which sows 9 rows 15 cm apart, at the depth of 5 cm and a sowing rate of 130 seeds m^{-2}. A central block (3.75 × 7.0 m) of the quantitatively resistant cultivar Quantum was surrounded by one of three buffer cultivars (Bolero, Quantum or Resal). The buffer width was 1.35 m on all sides making the total plot size 6.45 × 9.7 m. There were four replicates of each treatment in a Latin Square (3 × 3) design. Plots were separated by bare ground 7 m wide in the east-west direction, 5 m wide in the north-south direction and 7.5 m wide in the outside edges. The gaps left by wheel marks (35 cm in width) were rotary hoed and sown by hand. Post-planting, pre-emergence

herbicide terbuthylazine (1000 g ha^{-1} as Gardoprim in 300 ℓ water ha^{-1}) was applied. Post-emergence herbicide treatment was applied on 3 Feb. 1993 and consisted of a mixture cyanazine (1250 g ha^{-1} as Bladex 50 SC) and MCPB (578 g ha^{-1} in 300 ℓ water ha^{-1}). Overhead irrigation was applied when soil moisture deficit reached 50 mm. Seedlings emerged on 11 Jan. 1993 and disease was first noted on 4 Feb. 1993.

A weather station was located at the middle of the experimental area and contained a Psion Organiser II Model LZ64 data logger with four temperature probes which measured:
1) dry bulb temperature in Stevenson screen in a weather box 35 cm above the soil surface
2) wet bulb temperature as above
3) air temperature at the height of 20 cm within the canopy in one of the experimental plots
4) soil temperature at the depth of 10 cm in one of the experimental plots.
Temperature was recorded at 1 h intervals and the data down loaded to a floppy disk with a portable computer at weekly intervals.

Plant populations achieved were assessed on 26 Jan. 1993 when the crop was at the growth stage (Knott, 1987) 103-105 (vegetative, three to five nodes) by counting seedlings in 20 0.1 m^2 quadrats in the surround treatment and in 10 quadrats in the Quantum central areas of each plot. Plant developmental stage (Knott, 1987) was noted each week from 15 Jan. 1993 onwards. Plant developmental stage was also assessed at each disease assessment date in 12 randomly selected plants in each of the surround treatment areas. Disease severity was measured from 18 Feb. 1993 onwards on eight occasions at 4 - 6 d intervals. Ten plants were randomly removed from each of the Quantum central areas and ten plants from each of the surround areas, avoiding the two rows on the edges of the plots. Disease severity (percentage of leaf area infected) for each node of each plant was assessed in the laboratory.

The mean plant populations achieved were analysed by analysis of variance. Disease severity for surround and Quantum areas were analysed for each node by analysis of variance.

2.2.2. Glasshouse assessments to identify quantitative resistance in different seedlines (Experiments 2 and 3)

A collection of *E. pisi* was obtained from a variety of field-grown susceptible cultivars around Lincoln, Canterbury, during 1992-1993 to provide a genetically diverse population. This population was maintained on pea cultivar Pania in a glasshouse unit. Some genetic drift may have occurred during the study period but it was considered to be less problematic than relying on a single-conidium isolate which may not have been pathogenic to all the cultivars tested. Additionally,

powdery mildew conidia are difficult to store by other methods than infecting their host plants. Pania plants were sown every 2 weeks in 18 cm pots and when at growth stage (GS) 105-107 (vegetative, five to seven nodes), they were inoculated by shaking heavily infected plants over them. This technique is commonly used to inoculate powdery mildew fungi (e.g. Stumpf and Gay, 1989). The plants were kept in a glasshouse unit at 22°C (range generally 18 - 26°C, during summer months the temperature sometimes reached 31°C for a short period on some days) and illuminated with fluorescent light to extend daylight to 16 h when necessary. Old, possibly nonviable, conidia were dislodged by air from a Speedivac High Vacuum Pump ED50 (Edwards High Vacuum Ltd, Crawley, England) 24 h before conidium collection. Inoculum was collected from heavily infected leafs into glass vials with the vacuum pump operated cyclone spore collector. The number of conidia deposited per cm^2 leaf area was assessed by placing microscope slides covered with 0.1% water agar on the bottom of the settling tower and counting the number of conidia on ten cross sections on a slide (1.6 × 26.0 mm). Throughout the study, 'leaf' refers to the whole pea leaf, including all pairs of leaflets, and 'leaflet' is used when only the first pair of leaflets was assessed.

Twelve seeds of 15 (Experiment 2) or 32 (Experiment 3) cultivars were germinated in petri dishes lined with a moistened filter paper, placed inside a plastic bag to prevent moisture loss and left in a dark incubator at 20°C for 7 days. Seedlings at growth stage 004 (germination: emergence) with about 1 cm plumule and 5 - 8 cm radicle with secondary rootlets, were planted on 11 Aug. 1994 (Experiment 2) or on 16 Jun. 1995 (Experiment 3) in 13 cm pots filled with a mixture of sterilised washed bark and sand (55:45) mixed with 1.6 kg of lime, 1.0 kg of slow release fertilizer Osmocote, 0.35 kg of super phosphate, 0.45 kg of zeolites, and 0.13 kg calcium nitrate per m^3 of the mixture. Two or three seedlings of each cultivar were planted per pot and there were four replicates arranged in a randomised block design. Eleven days after sowing, plants were thinned to two seedlings per pot and 0.4 g of fertilizer (Nitrophoska, N:P:K:S:Mg 12:5:14:8:1) was added to each pot. The plants were placed in a Burkard isolation plant propagator (Burkard Manufacturing Co Ltd, Woodcock Hill Industrial Estate, Hertfordshire, UK; Jenkyn, Hirst and King, 1973) and Crop & Food isolation plant propagator to prevent infection before inoculation. The propagator was situated in a glasshouse at 22.3°C (range 18.2 - 26.8°C; during summer months the temperature reached 31°C for a short period on some days) with additional illumination to achieve 16 h days. Relative humidity in the plant enclosure of the propagator pots varied between 40 and 100%. When at GS 106 - 107 (vegetative, six to seven nodes) on 31 Aug. 1994 (Experiment 2) or on 11 and 12 Jul. 1995 (Experiment 3), the plants were inoculated in an earthed metal settling tower (height 1.72 m, diameter 0.53 m) with 0.5 mg of *E. pisi* conidia dispensed into a 30 cm long copper pipe through the bottom of the tower. A plastic tube was connected from the copper pipe to a compressed air cylinder, and the conidia were dispersed upwards for 5 sec by compressed air at a pressure of 380 kPa. Conidia were left to settle onto intact plants for 5 min before plants were transferred back to the isolation plant propagator.

This amount of conidia resulted in 10 (7.2 - 13.9) conidia cm^2 on leaf surfaces. Each replicate was inoculated in two (Experiment 2) or four (Experiment 3) batches of plants in randomly selected order. Growth stage and disease severity on leaves was assessed visually 3 weeks after inoculation using a standard disease severity key (Falloon *et al.*, 1995). It was possible to assess disease severity only once because of the difficulties of replacing the transparent polystyrene plant propagator covers, especially on older plants. Three weeks after inoculation was chosen as the assessment date to allow the pathogen to develop on the plants. The results were normalised by logarithmic transformation and analysed by analysis of variance. A non-parametric test was used to confirm the differences between the cultivars.

2.2.3. Field experiment to characterise quantitative resistance in seedlines (Experiment 4)

Seed of lines of garden peas, claimed to quantitatively resistant to *E. pisi*, was obtained from several international seed companies between Sep. and Nov. 1994. A field experiment consisting of 33 seedlines ranging from resistant to susceptible were sown on 22 Dec. 1994 in 50 × 80 cm microplots at the NZ Institute for Crop & Food Research site, Lincoln on a Templeton sandy loam on sand. There were two randomised replicates. Microplots contained 40 seed handsown into five rows, at eight seeds per row at 10 cm spacings. Microplots were separated by 3.2 m in north-south direction and 2.7 m in east-west direction with a resistant cultivar Trounce, sown with an Oyjord cone seeder at sowing rate of 121 seeds m^{-2} to depth of 5 cm and in rows 15 cm apart, and the trial area was rolled the day after sowing. Pre-emergence herbicide terbuthylazine (1000 g ha^{-1} Gardoprim in 300 ℓ water ha^{-1}) was applied 2 days after sowing. Seedlings emerged between 3 and 6 Jan. 1995. Overhead irrigation was applied when soil moisture deficit reached 50 mm (Jamieson *et al*, 1984).

Plots were assessed for powdery mildew on 10 Feb., 11 Mar. and 18 Mar. 1995 measuring whole plot severity using standard disease severity key (Falloon *et al.*, 1995). If plant populations were segregating for response to the pathogen, only the most frequent response was assessed. Mean disease severity data were analysed by analysis of variance, and cultivars were ranked in the order of most susceptible to most resistant based on the disease severity scores on the last assessment date.

2.3. Results

2.3.1. Experiment 1

Disease was first noted in the experimental area on 4 Feb. 1993, after cool temperatures preceding that date (Appendix II), when plants were at a growth stage 104-107 (vegetative, four to seven nodes;

Appendix III). The plant population for the surround treatments was 131 plants m^{-2} (standard error 4.7). There were no differences in plant populations between cultivars and replicates, and no interactions.

Powdery mildew severity on nodes 6 - 10 of Quantum plants in inside treatments remained at less than 30% of the leaf area infected, but severity on nodes 11 - 15 reached 85% of leaf area infected (Figure 2.1.). Statistically significant treatment effects occurred for nodes 7, 8 and 10 on which there was more disease ($P<0.05$ and $P<0.001$) on plots surrounded by Quantum than on those surrounded by Resal (Figure 2.1.). On the outside treatments, Resal was free of powdery mildew, and the disease was most severe ($P<0.001$) on Bolero (Figure 2.2.). Disease severity was less ($P<0.05$) on Quantum than on Bolero for nodes 10, 11, and 15.

2.3.2. Experiment 2

There was a clear division into two main levels of susceptibility to *E. pisi* amongst the seedlines tested (Table 2.1.). The mean proportion of leaf area infected on Quantum was 20.3%, which was less ($P<0.05$) than on Pania but not different to Greenfeast or Bolero. The other cultivars tested had less severe disease (1.3% or less).

2.3.3. Experiment 3

A similar division into two main levels of susceptibility occurred in Experiment 3 (Table 2.2.) as in the previous experiment. The mean proportion of leaf area infected with *E. pisi* for Quantum was 25.9%. The lowest percentage of leaf area infected for the higher levels of susceptibility was on Novella (22.5%). The more resistant cultivars had disease severities of 2.2% of leaf area infected or less.

2.3.4. Experiment 4

In the field, Pania was the most susceptible cultivar, based on powdery mildew severity on the last assessment on 18 Mar. 1995 (Table 2.3.). Cultivars that had very low levels of disease in the laboratory, had no disease in the field. The mean disease severity for Quantum was 67% of leaf area infected.

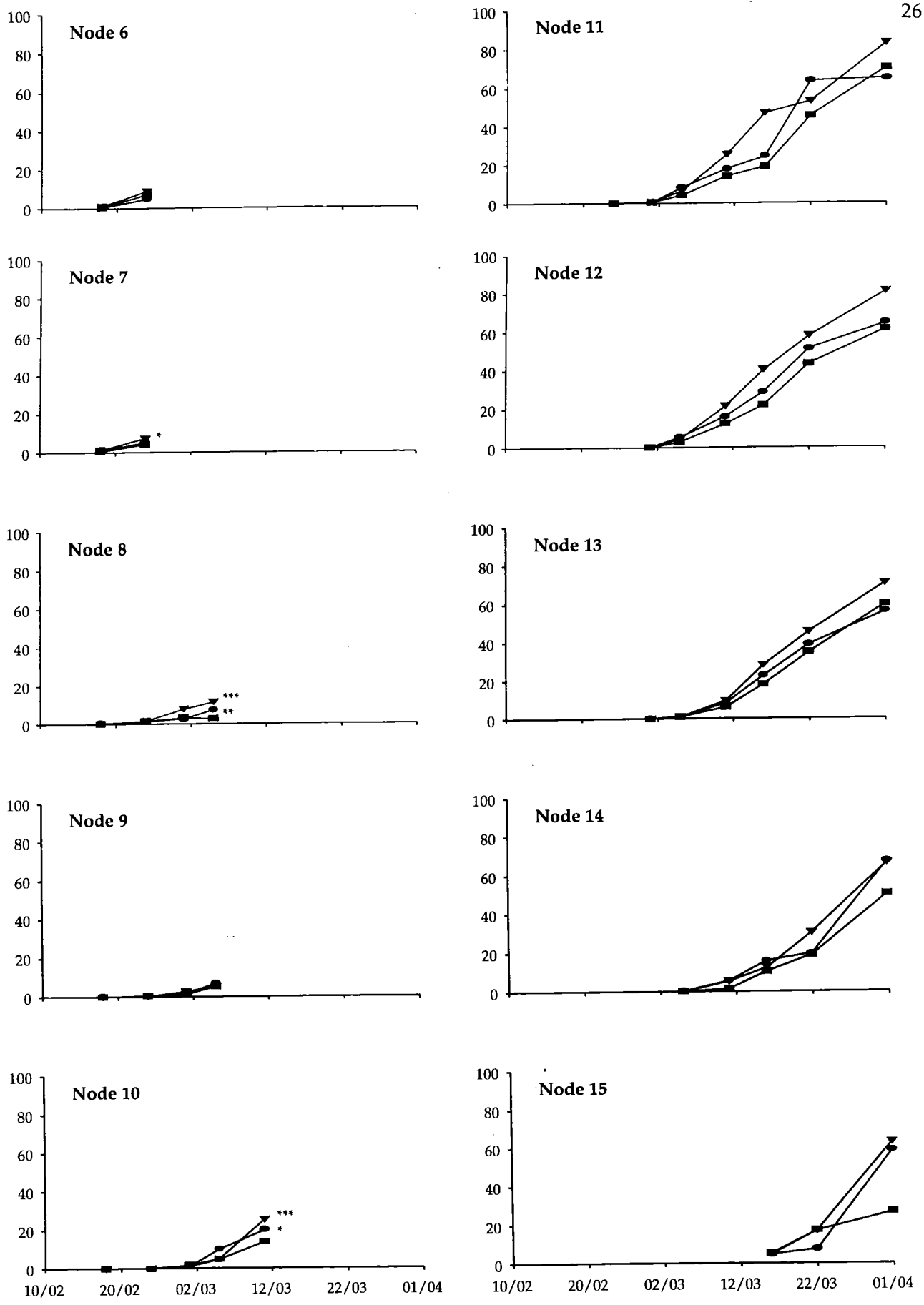


Figure 2.1. Mean disease severity (% leaf area infected) for each inside treatment (Quantum surrounded by Resal = ■, Quantum =▼, Bolero = ●) for nodes 6 - 15 over time. *, ** and *** indicate means different from Resal ($P<0.05$, 0.01 and 0.001 respectively) on the last assessment date.

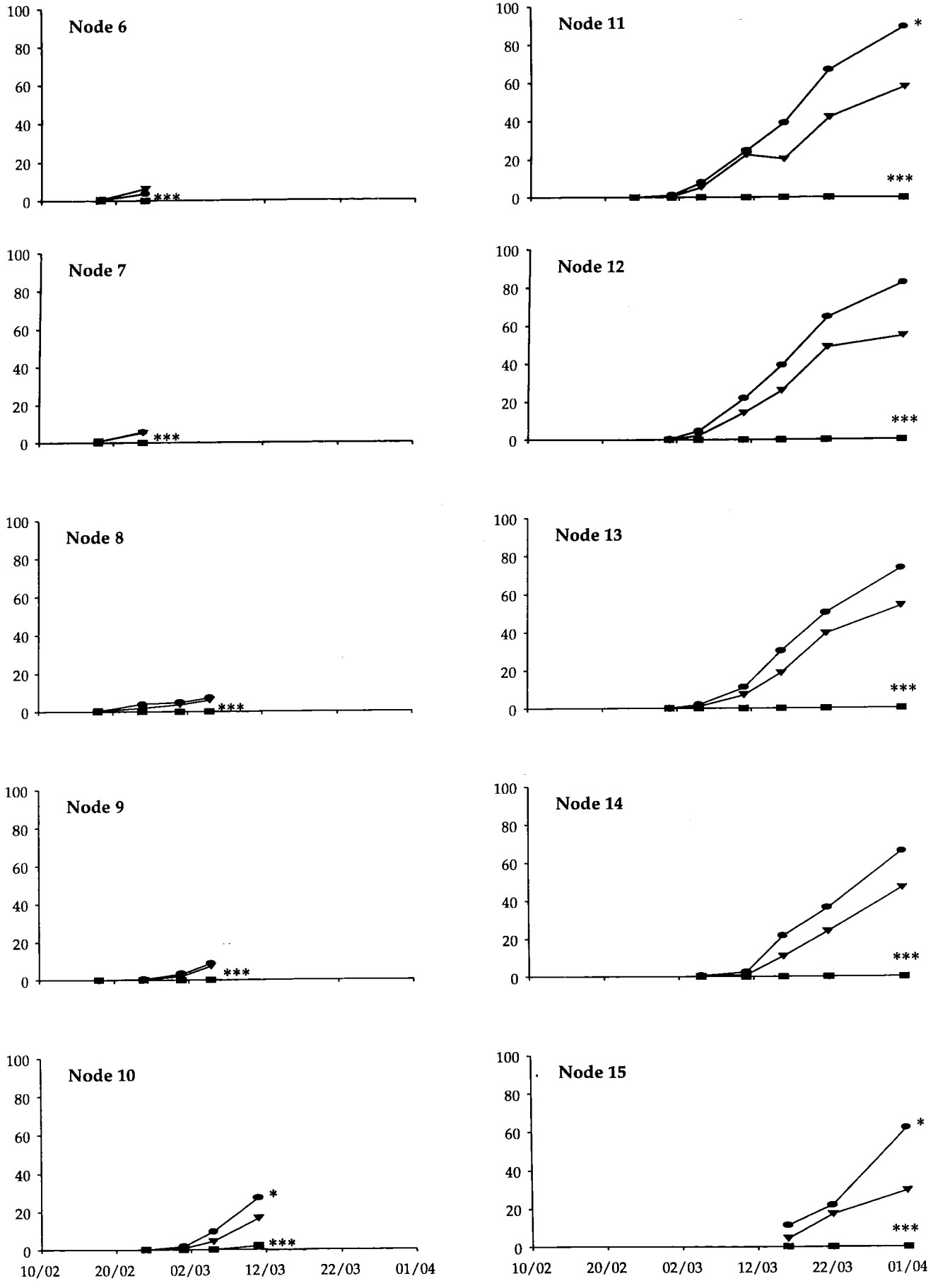


Figure 2.2. The disease severity for each cultivar surrounding Quantum on the outside treatment (Resal = ■, Quantum =▼, Bolero = ●) for nodes 6 - 15 over time. Stars indicate if different from Quantum: * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Table 2.1. Mean proportion of leaf area infected with *E. pisi* 3 weeks after inoculation of cultivars in Experiment 2. Letters designate means that are different ($P \leq 0.05$) using LSD-tests.

Cultivar	Mean % of leaf area infected
Pania	36.2 a
Greenfeast	30.5 ab
Bolero	29.6 ab
Quantum	20.3 b
Tasman	1.3 c
PI 142777	0.4 cd
PI 185183	0.3 d
Bounty	0.3 d
Trounce	0.1 d
Almota	0.1 d
Horizon	0.1 d
PS 010838	0.1 d
Mariner	0.1 d
Resal	0.0 d
PI 201497	0.0 d

Table 2.2. Mean proportion of leaf area infected with *E. pisi* 3 weeks after inoculation of cultivars in Experiment 3. Letters designate means that are different ($P \leq 0.05$) using LSD-tests. * = segregating population.

Cultivar	Mean % of leaf area infected
FR 774	45.8 a
Turbo	35.0 a
C412	34.8 a
Bolero	34.6 a
Pania	32.5 a
Scepter	31.2 a
Plus	27.9 a
Quantum	25.9 a
Vantage	25.2 a
Nomad	24.8 a
Greenfeast	24.5 a
93L10	24.3 a
Novella	22.5 a
Cascadia*	2.2 b
Tasman	1.5 bc
Bounty	1.2 bcd
Almota	1.1 bcde
Florado*	0.8 bcdef
CMG313C	0.7 bcdef
PI 201497	0.7 bcdef
Barbado	0.5 cdef
Aurora	0.5 cdef
Horizon	0.5 cdef
Oregon Giant	0.4 cdef
D17-042	0.4 cdef
Somerset	0.4 cdef
Oregon 605	0.3 cdef
Resal	0.3 cdef
Oregon Sugarpod	0.2 def
Regal 36	0.0 f
D17-201	0.0 f
CMG257C	0.0 f

Table 2.3. Mean percentage of leaf area infected with *E. pisi* for whole plots in the field (Experiment 4). Cultivars are in order of susceptibility according to the last assessment or, when scores are the same, in alphabetic order.

Cultivar	10 Feb.	11 Mar.	18 Mar.
Pania	6.3	100.0	100.0 a[1]
Bolero	6.3	100.0	95.0 ab
FR774[4]	10.0	90.0	91.5 abc
Vantage[4]	7.5	80.0	85.0 bcd
C412[4]	8.8	92.3	82.5 bcde
93L10[4]	7.5	80.0	79.0 cdef
Scepter[6]	3.0	75.0	75.0 defg
Plus[2]	4.3	80.0	73.0 defg
Quantum	7.5	75.0	67.0 fg
Nomad[7]	8.8	75.0	65.0 g
Turbo[2]	5.0	80.0	64.0 g
Almota*	0	0	0
Aurora[7]	0	0	0
Barbado[8]	0	0	0
Bounty	0	0	0
Cascadia[3]*	0	0	0
CMG257C[5]	0	0	0
CMG313C[5]	0	0	0
D17-042[8]	0	0	0
Florado[8]	0	0	0
Horizon	0	0	0
Mariner	0	0	0
Novella[7]	0	0	0
Oregon 605[3]	0	0	0
Oregon Giant[3]	0	0	0
Oregon Sugarpod II[3]	0	0	0
Regal 36[5]	0	0	0
Resal	0	0	0
Somerset[7]	0	0	0
Spartan[6]	0	0	0
Tasman	0	0	0
Trounce	0	0	0

[1] Letters indicate means that are different ($P \leq 0.05$) using LSD-tests. Only cultivars with a disease severity greater than 0 used in this analysis.
[2] Asgrow Seed Company, Twin Falls, Idaho, USA.
[3] J.R. Baggett, Department of Horticulture, Oregon State University, Corvallis, Oregon, USA.
[4] W. Brotherton Seed Co., Inc., Moses Lake, Washington, USA.
[5] Crites-Moscow Growers, Inc., Moscow, Idaho, USA.
[6] Nunhems Seed Corporation, Lewisville, Idaho, USA.
[7] Rogers Seeds Co. Boise, Idaho, USA.
[8] S & G Seeds B.V. Enkhuizen, Holland.
* Segregating population, only the main reaction scored.

2.4. Discussion

There were statistically significant differences in powdery mildew severity between the surround treatment cultivars in Experiment 1. Bolero was the most susceptible and Resal was the most resistant cultivar, whilst disease severity on Quantum was between that of the two other cultivars for most nodes. This is consistent with other reports (R.E. Scott, pers. comm.) suggesting that Quantum has quantitative resistance to *E. pisi*. Although Bolero was the most susceptible cultivar, disease severity on this cultivar did not reach 100% on any of the nodes, indicating that the environmental conditions prevailing during the experiment (Appendix I) were not fully conducive to the development of powdery mildew. In other field experiments disease severity in Bolero always reached 100% (R.E. Scott, pers. comm.).

Development of the disease in the inside plots of Quantum was affected by the surround treatment for nodes 7, 8 and 10 only (Figure 2.1.). Powdery mildew was less severe on inside plots surrounded by Resal than those surrounded by Quantum (nodes 7, 8 and 10) or Bolero (nodes 8 and 10). This and greater disease severity in Bolero than Quantum outside plots suggested that some pathogen adaptation in the plots surrounded by Quantum may have occurred. Disease levels on later-formed nodes were similar between the treatments indicating that *E. pisi* quickly spread within the plots regardless of cultivar surrounding the plot. As there was no disease on Resal, inocula for Quantum inside plots surrounded by Resal must have arrived from other plots or from outside the experimental area.

In the laboratory, quantitative resistance in Quantum was expressed as low disease severity but at a level distinct from the more resistant cultivars (Table 2.1.). In Experiment 3 more cultivars described as quantitatively resistant were included, and the disease severities ranged from 45.8% to 0% but again with two distinctive groups of severity. There was a ten-fold difference in severity between Novella and Cascadia (Table 2.2.), but a continuous range of disease severities in the low resistance group. It is possible that the mechanism of resistance was different in these two groups. The lack of a continuous distribution of disease severity suggested that powdery mildew resistance (qualitative) is conferred by a single recessive gene, or that there had not been selection for intermediate resistance in the past.

Although Experiments 3 and 4 cannot be compared directly due to the slightly different disease assessment methods used, there were similarities in the ranking order of these two experiments. In the field, Pania and Bolero were the most susceptible cultivars followed by FR774, which was the most susceptible cultivar in the glasshouse experiment (Table 2.3.). With the exception of Novella, all cultivars that had disease in the field, were also in the most susceptible group of cultivars in the

glasshouse. The differences in the ranking of these cultivars may have been linked to differences in inoculum pressure between the field and the laboratory situations. Plants for Experiment 3 were inoculated in the settling tower with a standard amount of conidia, but leaves were not supported horizontally. This may have caused some differences in the amount of conidia on different leaves. In addition, some interplot interference in the field was possible whereas in the isolation plant propagator, no migration of conidia from one plant to another was possible.

Based on results from the preliminary experiment (Appendix I), the field experiment (Experiment 1) and from Experiment 2, Quantum had less severe disease than susceptible cultivar Pania. The decision was made to identify components of quantitative resistance in Quantum, compared with Pania, Bolero and, in some cases, the resistant cultivar Resal. The components of quantitative resistance are explored in Chapter 3. Other cultivars possessing quantitative resistance were also identified.

Chapter 3

Epidemiological basis of quantitative resistance in pea plants to *Erysiphe pisi*

3.1. Introduction

Quantitative resistance of agricultural crops to fungal pathogens is characterized by a continuous variation ranging from very low to moderate levels of resistance (Parlevliet, 1992). Quantitative resistance slows epidemic development and reduces the severity of the disease (Geiger and Heun, 1989). Crop cultivars with qualitative resistance, which often is 'vertical' and race-specific, normally have greater resistance but the effectiveness of resistance may be lost through selection of pathogen races with corresponding virulence or aggressiveness (Sections 1.5 and 1.6). Utilising vertical disease resistance has been popular with plant breeders until recently because it was easy to manipulate. The durability of such resistance has in numerous cases been short-lived, however.

There are various ways to breed for quantitative resistance in new plant cultivars (Section 1.6.1.). Selecting quantitative resistance on the basis of symptom expression involves screening plants after their vertical resistance(s) have been eliminated, and only those plants that are susceptible are retained as primary parents in the breeding program (Robinson, 1987). Any resistance that these parents possess is quantitative, which can be increased by recurrent mass selection. This type of selection involves extensive field testing. The disadvantage of this type of testing is the unpredictability of the occurrence of the pathogen, although this can be improved by the introduction of spreader plants and timing of the experiments.

Mechanism-based selection through components of quantitative resistance is only used when the characteristic to be improved is difficult to assess, but another characteristic exists which has higher heritability, is highly correlated to the desired characteristic, and can be measured with the same or lower costs (Parlevliet, 1992). Component selection usually involves complex testing and is therefore often more expensive than selection for symptom expression. It is unlikely that this approach would be used in breeding for quantitative resistance except for a few pathosystems, but it is a worthwhile approach for the development of superior parental material to be used in subsequent breeding programmes. Accumulation of high levels of quantitative resistance in the parental material can produce high dividends through the material that is generated from it, especially when a wide

variety of parental material has been used, and when quantitative resistance has a low heritability (Parlevliet, 1992).

In the mechanism-based approach, the first step towards efficient selection for quantitative resistance is to determine which of the components of quantitative resistance has to be primarily considered. There is no single model available because each host-pathogen interaction is different (Johnson, 1992). A generalised description of the three major components includes reduced infection frequency or density, reduced lesion size or concentration, and reduced propagule production per unit of host tissue over a period of time (Parlevliet, 1992). In most host-pathogen interactions involving biotrophic pathogens, the major epidemiological components of quantitative resistance are infection efficiency (infection frequency), length of the latent period, and rate and duration of sporulation (Parlevliet, 1989), but some components are more important than others in characterising quantitative resistance. It is possible, for many host-pathogen interactions, to recognise one component of quantitative resistance, or a set of components, that adequately represent the quantitative resistance in the field. For example, in barley leaf rust (caused by *P. hordei)*, latent period was the easiest component to assess (Parlevliet and van Ommeren, 1975; Neervort and Parlevliet, 1978). Selection in the seedling stage for increased latent period and decreased infection frequency was effective in selection for quantitative resistance in the field (Parlevliet *et al.*, 1980), but selection in the adult plant stage was even more effective (Parlevliet and Kuiper, 1985; Parlevliet *et al.*, 1985). In the wheat - *P. recondita* f. sp. *tritici* interaction, latent period, infection frequency and uredinia size were important components of quantitative resistance (Denissen, 1993). Rashid (1991) found that in the flax and *M. lini* interaction, incubation period, latent period and sporulation were all important aspects of quantitative resistance.

Latent period has been found to be an important factor of quantitative resistance in most host-pathogen interactions except leaf blast in rice (caused by *M. grisea;* Roumen and de Boef, 1993). In barley powdery mildew (*Erysiphe graminis* f. sp. *hordei*), reduction in infection efficiency made the greatest contribution to quantitative resistance, was the easiest to measure (Asher and Thomas, 1983), and allowed good host differentiation (Heun and Geiger, 1989), but variation of latent period was of only minor importance (Asher and Thomas, 1984).

It is often reported that the components of quantitative resistance are correlated, at least in the fungal pathogens. For example, in barley infected with *E. graminis* f. sp. *hordei*, latent period, infection efficiency, number of pustules per unit leaf area, pustule size, and sporulation, were all positively correlated (Asher and Thomas, 1984; Jones, Sethar and Davies, 1981; Nørgaard Knudsen, 1984). In the wheat *P. recondita* f. sp. *tritici* interaction, Parlevliet (1986) reported a high correlation between infection frequency and latent period. Broers (1989a), however, found that the correlations were not

very high between infection frequency, latent period and the size of uredisori in the spring wheat-leaf rust interaction, especially at the seedling stage. Denissen (1991) also found low correlation between infection frequency and latent period in wheat leaf rust. In the peanut - *Puccinia arachidis* Speg. interaction, the components of quantitative resistance (infection frequency, incubation period, lesion diameter, percentage leaf area damaged and sporulation index) were not fully correlated in many of the genotypes, indicating that some genotypes may have quantitative resistance due to all components whereas others have quantitative resistance due to only some of the components (Mehan *et al.*, 1994). It has not been investigated whether the correlation is a consequence of past selection for quantitative resistance or has an internal but not yet known basis, although correlation of components is not usual in populations that have not been strongly selected for quantitative resistance (Simmonds, 1991).

Quantitative resistance to *E. pisi* in peas has not been fully investigated and few of the factors regulating resistance to this fungus are presently understood. There is some debate even on the existence of the genes controlling the resistance (Section 1.5.2.).

The objectives of this research were to find which of the epidemiological components of quantitative resistance are affected in the *E. pisi* - pea interactions. The following hypotheses were tested: that there is variation in disease severity in pea cultivars Pania, Bolero, Quantum (and Resal). This variation may be expressed by one or a combination of the following which may singly or collectively contribute to quantitative resistance:

1) less germination of conidia,

2) low infection efficiency,

3) extended latent period

4) low rate and/or short period of conidia production, measured in terms of area under curve (AUC) of total conidium production, the amount of daily maximum conidium production (CMAX) and time to the maximum conidium production (TMAX).

Also tested were the effects of the following factors on germination and infection efficiency of conidia:

1) plant age, that the resistance of leaflet tissue changes depending on the growth stage of the plant assessed by comparison of different nodes at the same physiological age,

2) leaflet age, that the resistance of leaflet tissue changes depending on the age of the node.

3.2. Materials and methods

3.2.1. General methods

Pea cultivars used in infection efficiency and conidium production experiments were Pania (susceptible), Bolero (susceptible and similar in growth habit to, and a parent of, Quantum) and Quantum (quantitatively resistant). A resistant cultivar, Resal, was included only in germination experiments because no colonies developed beyond germination on the leaflet surface of that cultivar. Seed was germinated, sown and grown as described previously (Section 2.2.2.). Leaflet age was determined on the basis of one new pair of leaflets emerging every 4.5 d. The same source of inoculum was used as previously and inoculation was carried out as previously (Section 2.2.2.) except that all leaflets were supported horizontally.

3.2.2. Germination of conidia (Experiments 1-6)

Three groups (I, II, III) of experiments were done. Experiments 1-5 were carried out to assess the effect of two plant ages and one (Experiments 1-3) or two leaflet ages (Experiments 4 and 5) on conidium germination. Experiment 6 was carried out to assess the effect of three plant ages and two leaflet ages on germination on the four cultivars (Table 3.1.). Whole plants were inoculated in the settling tower with 6 mg of conidia, giving 120 ± 33 conidia cm^{-2} leaflet area. In Experiment 6 the inoculations were carried out during two successive days doing three inoculations per day by plant

Table 3.1. Details of the three groups of germination experiments. Leaflet age (d) at each node in parenthesis.

	Experiment	Plant GS	Nodes inoculated (leaflet age (d))	No of replicates per cultivar
Group I	1	105	4 (5)	1
	2, 3	105	4 (5)	2
Group II	4, 5	108	4 (15) and 7 (5)	2
Group III	6	105	4 (5)	4
		108	4 (15) and 7 (5)	4
		111	7 (15)	4

age due to the large amount of plants and limited space in the settling tower and constant temperature room. Plants were then transferred into high humidity (above 97%) in a constant temperature room (mean 22.3, range 21.1 - 23.3°C) for 24 h. Germination of the conidia was assessed by removing four leaflet discs(area 0.785 cm^2, diameter 100 mm) from a pair of leaflets with a cork borer. A clearing and staining solution (Keane, Limongiello and Warren, 1988) was placed into a plastic trough (3 × 11 × 3 cm), a filter paper (No 4, Whatman Limited, England) was immersed into the solution so that the opposite end of the paper was lower than the bottom of the trough and the solution was drawn across the filter paper. Leaflet discs were placed onto the filter paper to avoid conidia washing off the discs and left to clear for 7-10 days. Germinated and non-germinated conidia on each leaflet disc were counted by light microscope. In all experiments a single plant was a replicate and four leaflet discs per plant were the sample size. The effects of cultivar, leaflet age and plant age on germination were analysed by analysis of variance within each set of data A - E as illustrated in Figure 3.1. Differences between cultivars were also tested on node 4 (leaflet age 5 days) at GS 105 (vegetative, five nodes). Different sets of data were used for different comparisons.

3.2.3. Infection efficiency (Experiments 7 - 13)

Four groups of infection efficiency experiments were carried out (Table 3.2.). Single plants were replicates and two leaflets per node were the sample size. When plants were at the required growth stage, they were inoculated in the settling tower with 0.5 mg of conidia to receive 10 (range 7.2 - 13.9) conidia cm^{-2}. Plants were incubated in a constant temperature room as described previously (Section 3.2.2.) and then transferred to a glasshouse unit at 21.2°C (range 18.2 - 24.8°C). Number of colonies were counted on each leaflet at the time after inoculation when the final number of sporulating colonies was considered to have been established (i.e. 10 days). A colony was considered established when it was producing secondary hyphae and was visible to the naked eye. Leaflet area was measured with a LI-3000 portable leaf area meter (Li-Cor, Lincoln, Nebraska) or by image analysis. Infection efficiency was calculated as ratio of total number of colonies per cm^2 leaflet area to the total number of conidia deposited per cm^2 leaflet area. Nodes 4 and 5, 7 and 8, and 10 and 11 were grouped for analysis and data were normalised by logarithmic transformation. The effect of cultivar and plant age on infection efficiency was analysed by analysis of variance within each set of data A, D, E and F (Figure 3.2.). The effect of leaflet age was tested within plants at GS 109 (vegetative, nine nodes; B) and 111 (vegetative, 11 nodes; C; Figure 3.2.). Additionally, differences between cultivars were tested on nodes 4 and 5 (leaflet age 1- 5 d) in plants at GS 105 (vegetative, five nodes). Different sets of data were used to test different effects. As plant age had no effect on infection efficiency within the sets of data as described, all data were combined for the overall cultivar effect.

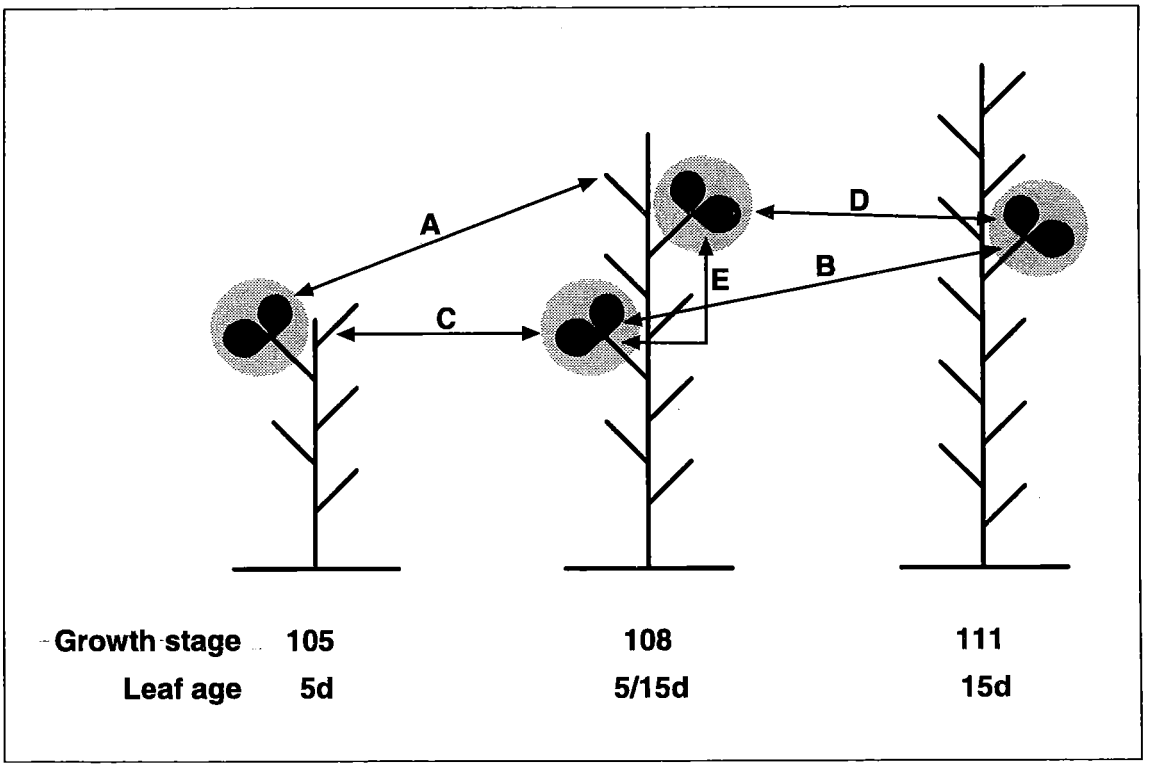


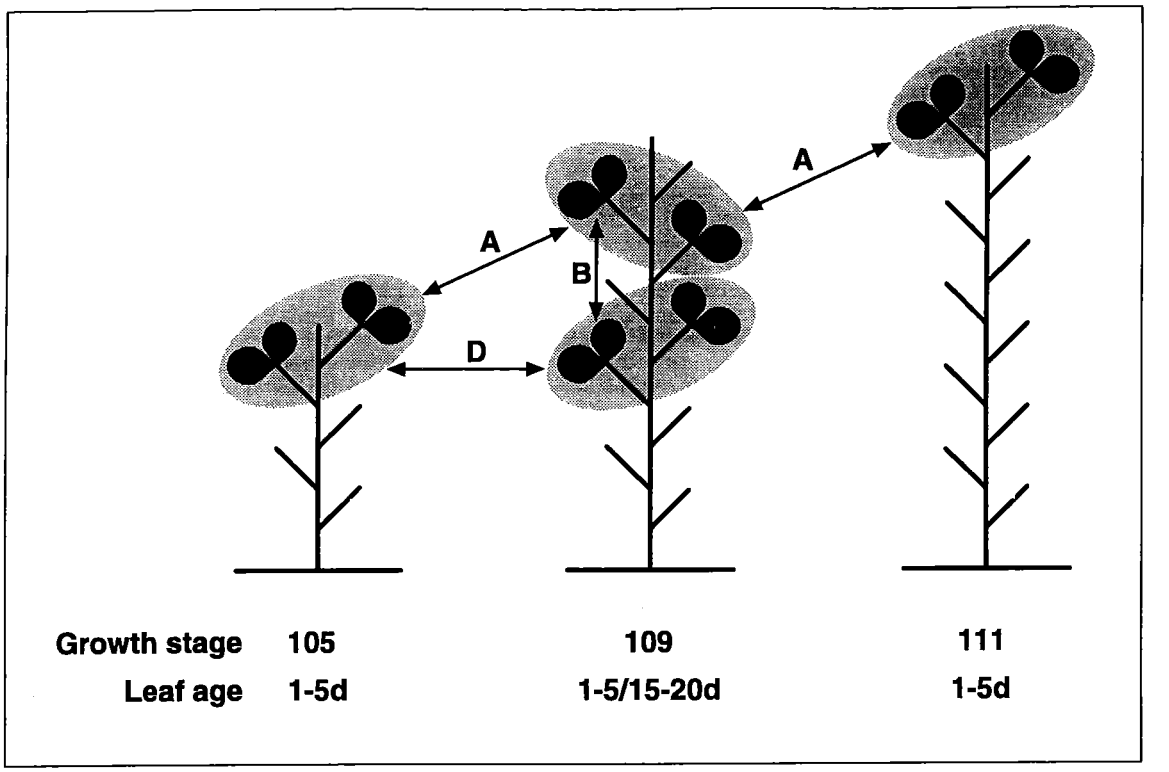
Figure 3.1. Plant age and leaf age comparisons in germination experiments.

The effect of plant age was tested by:
A. Comparison of node 4 (leaf age 5 d) of plants at GS 105 with node 7 of plants at GS 108 (leaf age 5 d).
B. Comparison of node 4 (leaf age 15 d) of plants at GS 108 with node 7 (leaf age 15 d) at GS 111.

The effect of leaf age was tested by:
C. Comparison of node 4 (leaf age 5 d) of plants at GS 105 with node 4 (leaf age 15 d) of plants at GS 108.
D. Comparison of node 7 (leaf age 5 d) of plants at GS 108 with node 7 (leaf age 15 d) of plants at GS 111.
E. Comparison of node 4 (leaf age 15 d) with node 7 (leaf age 5 d) of plants at GS 108.

Table 3.2. Details of the four groups of infection efficiency experiments. Leaflet age (days) at each node in parenthesis.

	Experiment	Plant GS	Nodes inoculated (leaflet age)	No of replicates per cultivar
Group I	7, 8	105	4,5 (1-5)	2
Group II	9	109	4,5 (15-20); 7,8 (1-5)	2
Group III	10, 11, 12	105	4,5 (1-5)	2
		109	4,5 (15-20); 7,8 (1-5)	2
Group IV	13	105	4,5 (1-5)	4
		111	4,5 (25-30); 10,11 (1-5)	4
		114	10,11 (15-20)	4



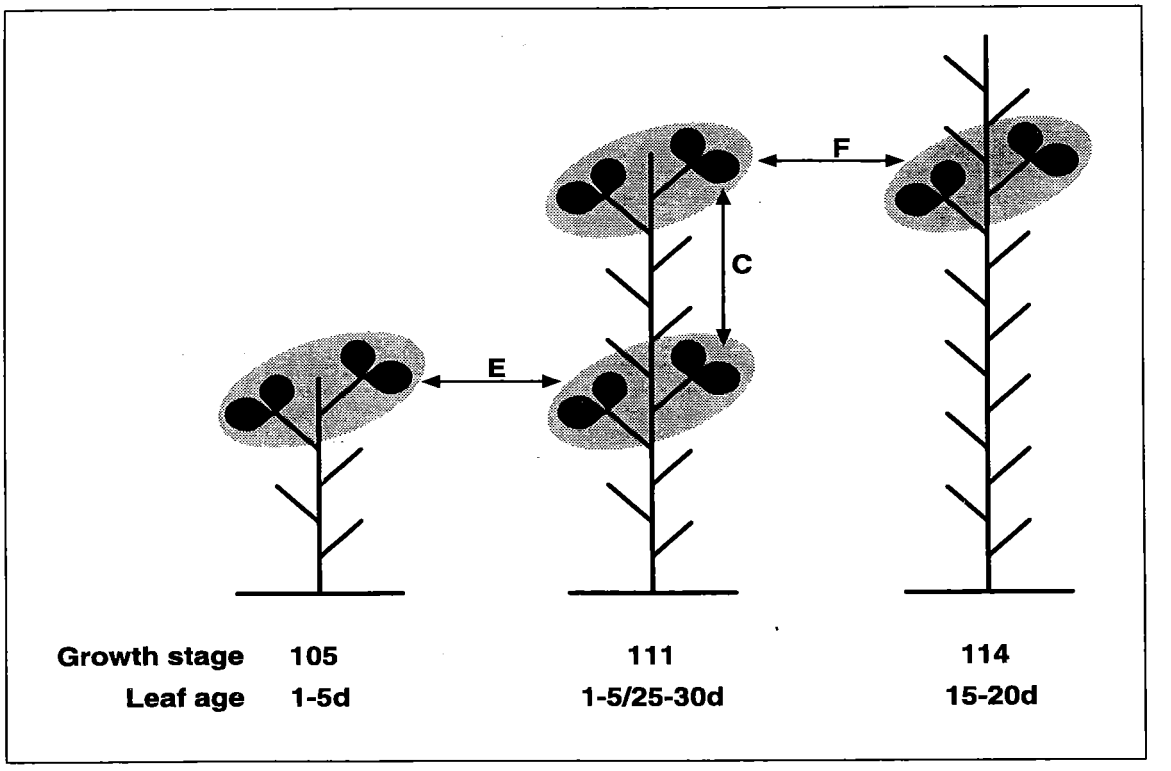


Figure 3.2. Plant and leaf age comparisons in infection efficiency experiments.
A. Nodes 4 and 5 of plants at GS 105 were compared with nodes 7 and 8 of plants at GS 109 and with nodes 10 and 11 of plants at GS 111 (leaf age 1 - 5 d).
B. The effect of leaf age was tested within plants at GS 109 by comparing nodes 4 and 5 (leaf age 15 - 20 d) with nodes 7 and 8 (leaf age 1 - 5 d).
C. The effect of leaf age was tested within plants at GS 111 by comparing nodes 4 and 5 (leaf age 25 - 30 d) with nodes 10 and 11 (leaf age 1 - 5 d).
D. Nodes 4 and 5 of plants at GS 105 (leaf age 1 - 5 d) was compared with nodes 4 and 5 of plants at GS 109 (leaf age 1 - 5 d).
E. Nodes 4 and 5 (leaf age 1 - 5 d) of plants at GS 105 were compared with nodes 4 and 5 (leaf age 25 - 30 d) of plants at GS 111.
F. Nodes 10 and 11 (leaf age 1 - 5 d) of plants at GS 111 were compared with nodes 10 and 11 (leaf age 15 - 20 d) of plants at GS 114.

3.2.4. Conidium production and latent period (Experiments 14 - 16)

Three experiments were conducted (Experiments 14, 15 and 16). The experiments were designed to test the effect of environmental conditions on latent period and conidium production (Experiment 14) or on conidium production only (Experiment 15 and 16) on nodes 6, 7 and 8 on cultivars Quantum, Bolero and Pania. The experimental design of Experiment 14 was a three (temperature) × three (cultivar) × three (node position) factorial design with a sample unit of three plants, and three replicates per treatment combination. The experimental design of Experiments 15 and 16 was two (temperature) × three (cultivar) × three (node position) factorial with a sample unit of three plants and four replicates per treatment combination.

Seeds for each experiment were germinated on moistened filter paper in petri plates, planted 7 days later and grown in the isolation plant propagator (Section 2.2.2.) until plants were at growth stage 107-108 (vegetative, seven to eight nodes). The plants were transferred to controlled environment cabinets for acclimatization at 14°C (± 2°C) day, 6°C (± 1.5°C) night gradually increasing the daytime temperature to 20°C (± 2°C) over a period of 3 days to avoid plant damage from the low light situation. Light levels in cabinets were between 220 and 260 μE m^{-2} s^{-1} supplied by daylight-incandescent and fluorescent lights. This light level is between 10 and 15% of light intensity (1700 - 1900 μE m^{-2} s^{-1}) at noon in Canterbury on a cloudless day (Bungard, 1996), but approximately 25% of sunlight over a 16 h day. Whole plants were inoculated in three batches of 27 plants each over 3 days (Experiment 14) or four batches of 24 plants each (Experiments 15 and 16) over 4 days due to the limitations of space in the constant temperature room. Each batch was divided into three (Experiment 14) or four (Experiments 15 and 16) inoculations due to limitations of space in the settling tower. From each inoculation, one plant per cultivar was assigned to each temperature treatment to avoid confounding inoculation effects with treatment effects. The amount of inoculum was 0.35 mg producing 7 (± 2) conidia per cm^2 to get approximately one infection per cm^2 leaflet area. Plants were incubated in a constant temperature room at 22.3°C (21.1 - 23.3°C) for 24 h and then transferred back to the controlled environment cabinets. The effect of the following three environmental conditions on conidium production and latent period were tested:

1) daytime temperature 13°C (actual mean 13.1, range 10.9 - 14.5°C) and night temperature 6°C (mean 5.8, range 4.5 - 6.9°C);
2) daytime temperature 19°C (mean 18.8, range 16.5 - 21.5°C) and night temperature 11°C (mean 10.9, range 9.2 - 13.5°C);
3) daytime temperature 23°C (mean 23.0, range 20.2 - 26.2°C) and night temperature 15°C (mean 15.2, range 14.2 - 16.2°C).

The actual temperatures were somewhat different from those aimed at; 13/5, 18/10 and 23/15°C day/night respectively. The effect of the lowest temperature on conidium production was not tested in Experiments 15 and 16 as it was not conducive to the pathogen. Day/night cycle was 16/8 h.

Conidium collection started 5 days after inoculation (dai). Conidia from each of the three node positions of the three sample unit plants for each cultivar were collected daily between 7.00 and 9.00 h into glass vials (diameter 5 mm, length 50 mm) with a cyclone spore collector attached to a vacuum pump (Section 3.2.1.). This was continued until leaflets of the particular node position senesced (60 dai at 13°C, up to 42 dai at 18° and 23°C). Care was taken not to damage the leaflet surface with the spore collector and not to disturb the leaflets and lose any conidia produced. Numbers of conidia were estimated by adding 0.5 ml of 0.1% Tween-80 solution into each vial, mixing the conidia with the solution in a vortex mixer for 1 min and counting conidia in six liquid samples from each vial with a haemocytometer. The same procedure was followed in Experiment 15 and 16 with the exception that node 7 in Experiment 15 and all nodes in Experiment 16, were collected daily as in Experiment 14, bulked together, and counted once every 3 days except during peak production as a time-saving measure. The length of latent period in Experiment 14 was the number of days from inoculation until the first day when conidia were seen, less 12 h to account for conidia that were formed between the sampling times.

The number of replicates in Experiment 15 was reduced when powdery mildew colonies on the plants in two of the four controlled environment cabinets failed to show any disease signs, or only a few colonies, 5 days after inoculation. The reason for apparent fungitoxicity or unfavourable environmental conditions for the development of the fungus in the two cabinets was not identified. Data collection in the remaining two cabinets was carried out as planned until leaflets senesced. These two faulty cabinets were not used in Experiment 16.

Disease severity (%) was measured on nodes 6, 7 and 8 with the aid of a disease key (Falloon *et al.*, 1995) every day after colonies first became visible to the naked eye. The number of colonies on these nodes was counted 10 d (20 d in the lowest temperature) after inoculation. At the same time, the size (cm^2) of the leaflets was estimated with the aid of paper leaflet models of known area. The colony area (cm^2) was calculated from the percentage of leaf area infected and the leaf size. Conidium production was expressed on a colony area and leaflet area basis. Three variables, total conidium production expressed as the area under the curve for conidium production (AUC), the maximum number of conidia was produced per day (CMAX) and the number of days to CMAX (TMAX), were used to characterise conidium production over time. The data for both AUC and CMAX were normalised by logarithmic transformations prior to statistical analysis. Analysis of variance was used to compare AUC, CMAX and TMAX values between cultivars, temperatures and node positions.

All interactions were illustrated in a graph-form, although it was recognised that there were no values between 6, 7 and 8 nodes.

3.3. Results

3.3.1. Germination of conidia

There were no differences (P=0.290) in germination of *E. pisi* conidia on leaflets of the different pea cultivars. Mean germination on Pania was 73.6%, Bolero 70.3%, Quantum 73.3% and Resal 69.1% ($LSD_{0.05}$ = 5.54). Germination of conidia was greater on young leaflets than on older leaflets; more conidia germinated on 5 d old leaflets than on 15 d old leaflets of plants at all growth stages tested (Table 3.3. C-E). Plant growth stage did not affect conidium germination (Table 3.3. A and B).

Table 3.3. Mean germination percentages of *E. pisi* conidia on leaflets of intact pea plants of different ages, and on leaflets of different ages.

Plant GS	A[1]	B
105	79.1	-
108	76.5	60.7
111	-	58.3
P-value	0.112	0.343
$LSD_{0.05}$	2.16	5.15

Leaflet age (days)	C	D	E
5 d	79.8 a[2]	68.2 a	70.0 a
15 d	69.7 b	58.3 b	63.4 b
P-value	<0.001	<0.001	<0.001
$LSD_{0.05}$	2.64	4.13	4.53

[1] Capital letters refer to the comparisons made in the Figure 3.1.
[2] Lower-case letters indicate means that are different ($P \leq 0.05$) using LSD-tests.

3.3.2. Infection efficiency

Infection efficiency on Pania (19.4%) was greater (P=0.004) than on Quantum (12.9%; Table 3.4.). Plant age (A) and leaflet age (B, C), or a combination of these (D, E, F) had no effect on infection efficiency (Table 3.4.).

Table 3.4. Mean percent of infection efficiency for *E. pisi* conidia on plants of different pea cultivars of different ages, and on different aged leaflets.

Cultivar	% infection efficiency (mean of all experiments)	Plant GS	A[1]
Pania	19.4[2] a[3] (18.2-20.5)[4]	105	12.6 (10.6-15.1)
Bolero	15.6 ab (14.4-16.8)	109	15.8 (12.6-19.7)
Quantum	12.9 b (11.8-14.4)	111	14.1 (11.6-17.2)
P-value	0.004	P-value	0.313

Leaflet age	B GS 109	C Growth stage 111
1-5 d	21.5 (15.6-29.8)	14.9 (11.9-18.0)
15-20 d	15.7 (11.4-21.7)	-
25-30 d	-	15.1 (12.3-17.9)
P-value	0.162	0.929

D		E		F	
GS 105, leaflet age 1-5 d	9.3 (5.6-15.5)	GS 105, leaflet age 1-5 d	19.9 (14.9-24.8)	GS 111, leaflet age 1-5 d	15.1 (10.6-19.6)
GS 109, leaflet age 15-20 d	11.4 (7.3-17.7)	GS 111, leaflet age 25-30 d	14.0 (8.7-19.3)	GS 114, leaflet age 15-20 d	18.2 (13.8-22.7)
P-value	0.524	P-value	0.114	P-value	0.929

[1] Capital letters refer to Figure 3.2.
[2] Means have been back transformed from logarithmic values
[3] Lower-case letters indicate means that are different ($P \leq 0.05$) using LSD-tests. (LSD-value is not shown as means have been back transformed from logarithmic values.)
[4] 95% confidence intervals.

3.3.3. Latent period and conidium production

Leaflets on nodes 6 and 7 on Quantum plants were larger (P=0.004) than Pania or Bolero leaflets for the same nodes (Figure 3.3), and therefore supported more colonies than Bolero or Pania leaflets. For this reason it was necessary to express all results on a per leaflet area (cm^{-2}) as well as a per colony area basis. Size of leaflets on node 8 did not differ between the cultivars (Figure 3.3.).

The latent periods did not differ (P=0.449) between the cultivars, but latent period decreased (P<0.001) with increased temperature (Table 3.5.). Latent period was longer (P=0.020) on node 8 than on nodes 6 or 7. There was no interaction between cultivar and temperature, node and cultivar or node and temperature (Table 3.5.).

Both cultivar and temperature affected all variables relating to *E. pisi* conidium production (Table 3.6.). Node position explained some of the variation, and on some occasions there were interactions between the main effects (Table 3.6.).

The mean total conidium production (AUC) for Bolero was less than for Pania, and intermediate in Quantum (Table 3.7.). The differences between cultivars decreased when conidium production was expressed on per colony area or per leaflet area bases, although the order or the statistical significance of the differences between cultivars did not change.

Total conidium production was greatest at 23°C compared with 19 or 13°C (Table 3.7.). Expressing conidium production per colony or per leaflet area did not change the statistical significance of the temperature effect. Cultivars behaved differently at the different temperatures; Pania and Bolero were very similar in their response, but Quantum appeared to have a narrower temperature range of conidium production per colony area than Pania or Bolero. Quantum produced fewer conidia per colony area at 23°C than Pania (Figure 3.4).

Total conidium production was lowest (P<0.001) on leaflets from node 6 (oldest node) and increased with node position (Table 3.7.) There were no differences (P=0.909) between nodes when the conidium production was expressed per colony area. However, conidium production was lowest (P=0.001) at node 6 when expressed per leaflet area. Conidium production increased (P=0.003) with increasing node position for each cultivar except for node 8 on Quantum (Figure 3.5.). Bolero had the lowest AUC for each node, and the differences between Bolero and the other two cultivars were greatest for nodes 6 and 7 (Figure 3.5.). AUC per leaflet area followed a similar pattern to that for AUC (Figure 3.6.).

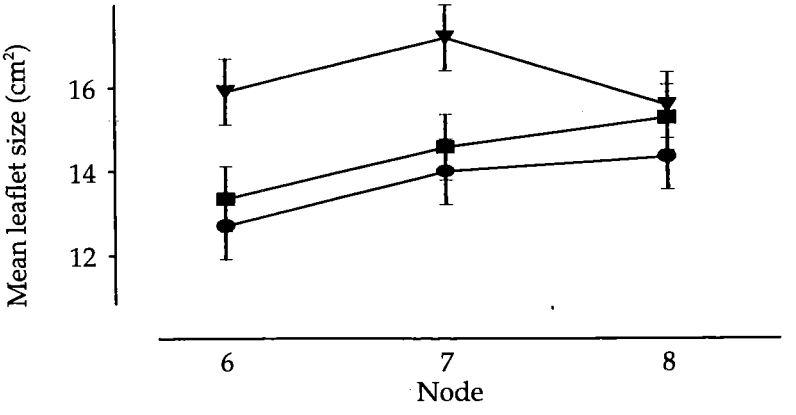


Figure 3.3. Mean leaflet size at three nodes for three pea cultivars (Pania = ■, Bolero = ●, Quantum = ▼). Bars in this and following figures represent 95 % confidence intervals.

Table 3.5. Mean latent periods (days) for *E. pisi* conidia on pea leaflets from different nodes on plants, at different temperatures and on different cultivars.

Node position		Temperature (°C)		Cultivar	
6	8.2 a[3]	13	10.5 a	Pania	8.2
7	8.2 a	19	8.1 b	Bolero	8.7
8	8.9 b	23	6.8 b	Quantum	8.5
P-value	0.029	P-value	<0.001	P-value	0.464
$LSD_{0.05}$	0.56	$LSD_{0.05}$	1.40	$LSD_{0.05}$	1.40
Interaction P-values					
Cultivar * temperature	0.540				
Node * cultivar	0.937				
Node * temperature	0.250				

[1] Letters indicate means that are different ($P \leq 0.05$) using LSD-tests.

Table 3.6. Probability values from analysis of variance to assess effects of cultivar, temperature and node position on parameters of conidium production in *E. pisi*.

	AUC[1]	AUCc[2]	AUCl[3]	CMAX[4]	CMAXc[5]	CMAXl[6]	TMAX[7]	TMAXc[8]	TMAXl[9]
Cultivar	≤0.001	0.001	≤0.001	0.002	≤0.001	≤0.001	0.073	≤0.001	0.005
Temperature	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001
Cultivar * temperature	0.229	0.029	0.096	0.330	0.062	0.075	0.336	0.006	0.216
Node	≤0.001	0.909	0.001	≤0.001	0.095	0.100	0.003	0.091	0.023
Node * cultivar	0.003	0.432	0.006	0.007	0.53	0.003	0.350	0.003	0.004
Node * temperature	0.076	≤0.001	0.015	0.263	0.026	0.604	0.036	0.057	0.027

[1] area under curve of total conidium production.
[2] area under curve per colony area.
[3] area under curve per leaflet area.
[4] maximum conidium production per day.
[5] maximum conidium production per day per colony area.
[6] maximum conidium production per day per leaflet area.
[7] time to CMAX.
[8] time to CMAXc.
[9] time to CMAXl.

Table 3.7. Mean numbers of *E. pisi* conidia produced on leaflets of different pea cultivars, at different temperatures, and on different nodes, expressed as area under curve for total conidium production (AUC), conidium production per colony area (AUCc) and conidium production per leaflet area (AUCl).

	AUC ($\times 10^3$)	AUCc ($\times 10^3$) (conidia cm^{-2} colony area)	AUCl ($\times 10^3$) (conidia cm^{-2} leaflet area)
		Cultivar	
Pania	1380.6[1] a[2] (921.1-2069.1)[3]	44.8 a (38.9-51.5)	19.9 a (15.4-25.7)
Bolero	676.0 b (451.1-1013.2)	36.2 b (31.4-41.6)	11.8 b (9.1-15.2)
Quantum	1125.8 ab (751.2-1687.2)	40.0 ab (34.7-46.0)	14.5 ab (11.2-18.7)
		Temperature	
13°C	360.5 a (165.2-568.7)	20.5 a (16.6-25.4)	4.6 a (3.1-6.7)
19°C	1384.7 b (969.1-1978.5)	44.7 b (39.5-50.6)	21.2 b (16.9-26.6)
23°C	2473.1 c (1730.9-3533.7)	68.7 c (60.7-77.7)	35.2 c (28.0-44.1)
		Node position	
6	1069.8 a (1001.8-1142.4)	45.3 (43.3-47.3)	18.8 a (17.8-19.9)
7	1515.1 b (1418.8-1617.9)	49.1 (47.0-51.3)	22.7 b (21.5-24.1)
8	1664.4 c (1558.7-1777.4)	49.4 (47.3-51.6)	24.0 b (22.7-25.4)

[1] Means have been back transformed from logarithmic values.
[2] Letters indicate means within cultivars, temperatures or nodes that are different ($P \leq 0.05$) using LSD-tests.
[3] 95% confidence intervals.

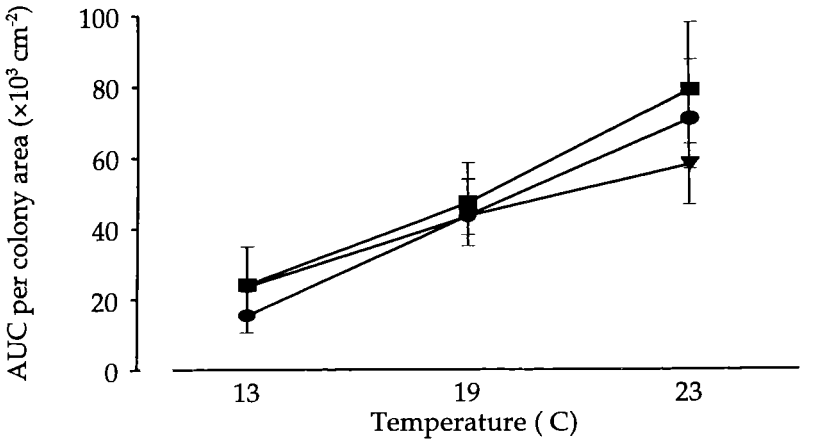


Figure 3.4. Mean AUC per colony area for numbers of *E. pisi* conidia produced at three temperatures on leaflets of three pea cultivars (Pania = ■, Bolero = ●, Quantum = ▼).

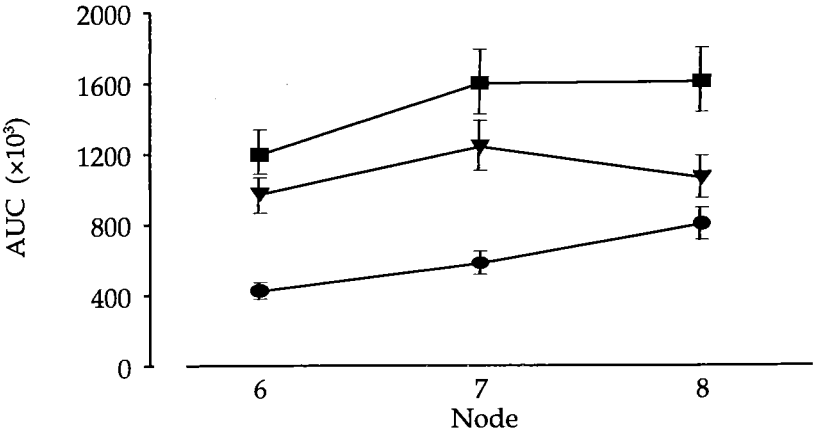


Figure 3.5. Mean AUC for numbers of *E. pisi* conidia produced on leaflets at three nodes of three pea cultivars (Pania = ■, Bolero =●, Quantum = ▼).

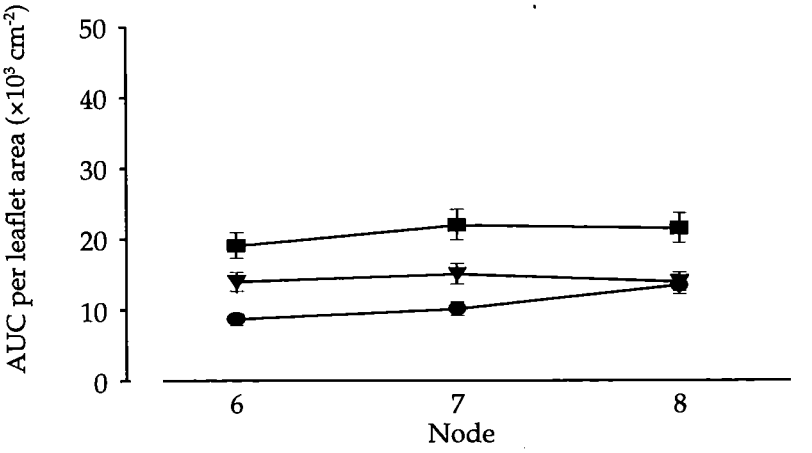


Figure 3.6. Mean AUC per leaflet area for numbers of *E. pisi* conidia produced on leaflets at three nodes of three pea cultivars (Pania = ■, Bolero = ●, Quantum = ▼).

There was a temperature × node interaction (P<0.001) for conidium production per colony area (Figure 3.7.); at 13°C, node 6 produced more conidia per colony area than nodes 7 or 8. At 19°C node 7 produced more conidia than 6 or 8, and at 23°C node 6 produced fewer conidia than 7 or 8. Conidium production per leaflet area (Figure 3.8.) followed the same pattern except that there were no differences between the nodes at 13°C.

The mean maximum conidium production per day was also greatest in Pania, intermediate in Quantum and least in Bolero (Table 3.8.). When CMAX was expressed per colony area, Quantum had lowest (P<0.001) CMAX and it was significantly different from CMAX on Pania. CMAX expressed as per leaflet area was greatest (P<0.001) in Pania, values for Quantum and Bolero did not differ. Maximum conidium production per day was greatest (P<0.001) at 23°C and least at 13°C (Table 3.8.). Adjusting CMAX for colony area or leaflet area did not change the order or significance of the effect of temperature. Maximum conidium production was lowest (P<0.001) on node 6 but when CMAX was expressed per colony and leaflet areas, the nodes did not differ. CMAX on node 8 on Quantum was lower than that on node 7 (Figure 3.9.), and CMAX per leaflet area on node 8 on Quantum was lower than for Bolero (Figure 3.10.). There was a node × temperature interaction for CMAX per colony area; at 13°C CMAXc was higher on node 6 than nodes 7 or 8, but at 19° and 23°C it was lower on node 6 than on node 7 (Figure 3.11.).

Mean time to maximum conidium production did not differ (P=0.073) between the cultivars, but when TMAX was adjusted for colony area, Quantum had longest (P<0.001) TMAXc (21.5 d; Table 3.9.). When TMAX was adjusted for leaflet area, Quantum had a longer TMAXl (25.0 d) than Pania (21.4 d). TMAX was longest (P<0.001) at 13°C (28.6 d) , but did not differ between 19°C (20.0 d) and 23°C (17.8 d; Table 3.9.). Expressing TMAX per colony area did not change the order or the significance of this result, but adjusting for leaflet area increased the differences between the temperatures. Quantum had longer TMAXc than Pania or Bolero at 13°C, but this was not the case at the other temperatures (Figure 3.12.). At 13°C, TMAXc of Bolero was shorter than for Pania or Quantum, but at 19° or 23°C TMAXc did not differ between the cultivars (Figure 3.12.). TMAX was shortest (P=0.003) on node 7 and longest on node 8 (Table 3.9.). There were no differences between nodes for TMAX per colony area. Although analysis of variance showed a difference (P=0.023) for TMAX per leaflet area (Table 3.6.), LSD values did not show this. TMAXc was longer (P=0.003) for node 8 on Quantum than for Pania or Bolero, and shortest for node 6 on Bolero (Figure 3.13.). TMAXl was again longer (P=0.004) for node 8 on Quantum than on the other cultivars, but TMAXl was the longest for node 6 on Bolero than on the other cultivars (Figure 3.14.). Node had no effect on TMAX at 19° and 23°C, but TMAX on node 8 at 13°C was longer than for the other nodes (Figure 3.15.). When TMAX was adjusted for leaflet area, both nodes 6 and 8 had longer TMAXs than node 7 at 13°C, but not at the other temperatures (Figure 3.16.).

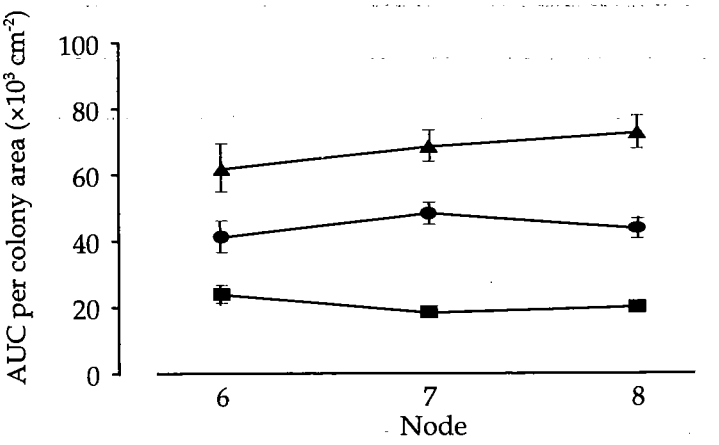


Figure 3.7. Mean AUC per colony area for numbers of *E. pisi* conidia produced on leaflets at three nodes of pea plants at three temperatures (13°C = ■, 19°C = ●, 23°C = ▲).

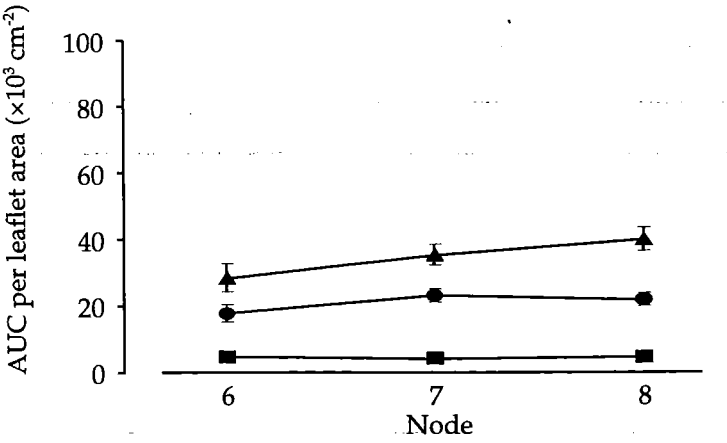


Figure 3.8. Mean AUC per leaflet area for numbers of *E. pisi* conidia produced on leaflets at three nodes of pea plants at three temperatures (13°C = ■, 19°C = ●, 23°C = ▲).

Table 3.8. Mean daily maximum numbers of *E. pisi* conidia (CMAX), maximum numbers per colony area (CMAXc) and maximum numbers per leaflet area (CMAXl), on leaflets of different pea cultivars at different temperatures and on different nodes.

	CMAX ($\times 10^3$)	CMAXc ($\times 10^3$) (conidia cm^{-2} colony area)	CMAXl ($\times 10^3$) (conidia cm^{-2} leaflet area)
		Cultivar	
Pania	197.0[1] a[2] (131.0-269.2)[3]	4.9 a (4.2-5.7)	2.1 a (1.6-2.7)
Bolero	108.7 b (72.3-163.4)	4.1 ab (3.5-4.8)	1.4 b (1.1-1.8)
Quantum	152.1 ab (101.1-228.7)	3.3 b (2.9-3.9)	1.4 b (1.1-1.8)
		Temperature	
13°C	29.9 a (16.0-55.7)	1.5 a (1.2-1.9)	0.3 a (0.2-0.4)
19°C	250.4 b (174.8-358.9)	5.0 b (4.3-5.7)	2.4 b (1.9-2.9)
23°C	435.4 c (303.8-623.9)	8.9 c (7.7-10.2)	5.4 c (4.3-6.7)
		Node position	
6	181.1 a (168.7-194.5)	5.4 (5.1-5.7)	2.4 (2.2-2.6)
7	242.6 b (225.9-260.4)	5.8 (5.4-6.1)	2.8 (2.6-3.0)
8	256.3 b (238.7-275.21)	5.4 (5.1-5.7)	2.8 (2.6-3.0)

[1] Means have been back transformed from logarithmic values.
[2] Letters indicate means that are different ($P \leq 0.05$) using LSD-tests.
[3] 95% confidence intervals.

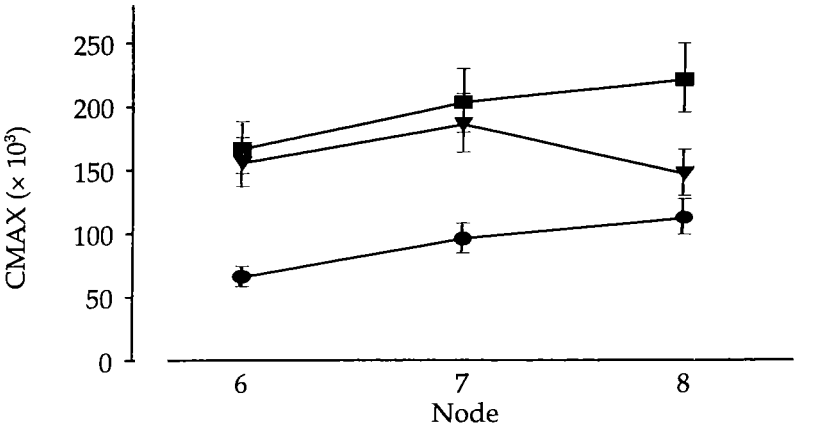


Figure 3.9. Mean maximum numbers of *E. pisi* conidia produced per day (CMAX) on leaflets at three nodes of three pea cultivars (Pania = ■, Bolero = ●, Quantum = ▼).

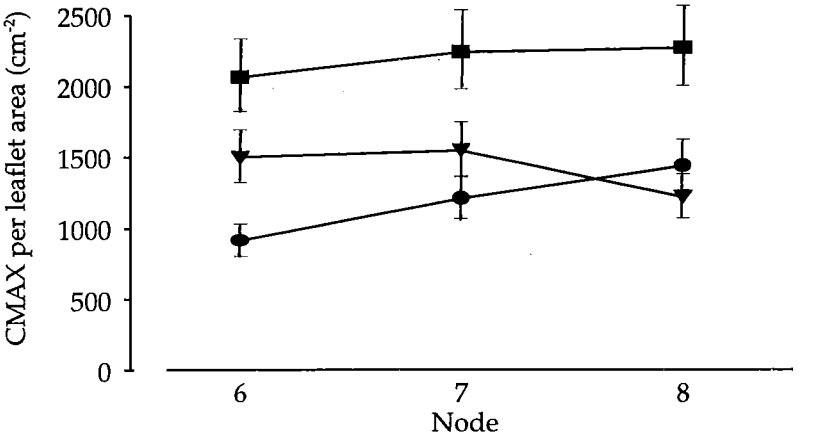


Figure 3.10. Mean maximum numbers of *E. pisi* conidia produced per day (CMAX) per leaflet area on leaflets at three nodes of three pea cultivars (Pania = ■, Bolero = ●, Quantum = ▼).

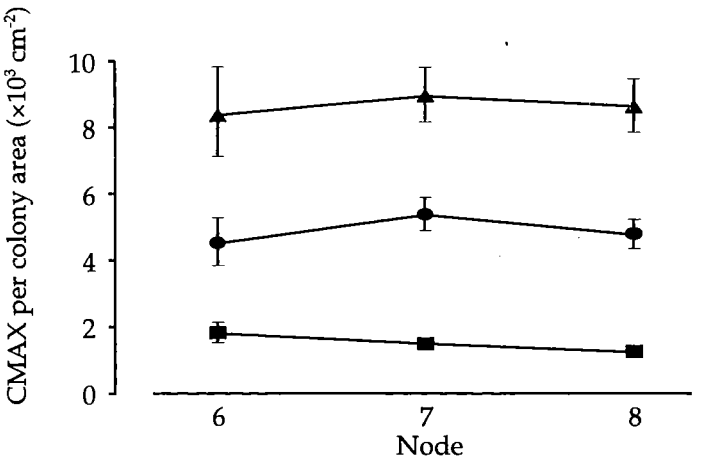


Figure 3.11. Mean maximum numbers of *E. pisi* conidia produced per day (CMAX) per colony area on leaflets at three nodes of pea plants at three temperatures (13°C = ■, 19°C = ●, 23°C = ▲).

Table 3.9. Mean time (days) to CMAX (TMAX), to CMAXc (TMAXc) and to CMAXl (TMAXl), on leaflets of three pea cultivars at different temperatures and on three nodes.

	TMAX (days)	TMAXc (days)	TMAXl (days)
		Cultivar	
Pania	20.9 (19.0-22.8)[1]	18.0 a[2] (16.5-19.6)	21.4 a (19.3-23.4)
Bolero	22.3 (20.4-24.2)	16.4 a (14.9-18.0)	24.0 ab (21.9-26.1)
Quantum	23.2 (21.3-25.1)	21.5 b (20.0-23.0)	25.0 b (22.9-27.0)
		Temperature	
13°C	28.6 a (25.7-31.5)	23.6 a (21.3-26.0)	30.8 a (27.6-33.9)
19°C	20.0 b (18.3-21.6)	16.7 b (15.3-18.1)	21.2 b (19.4-23.0)
23°C	17.8 b (16.2-19.5)	15.6 b (14.3-17.0)	18.3 c (16.5-20.1)
		Node position	
6	19.1 ab (18.3-19.8)	16.6 (15.9-17.4)	20.9 (20.1-21.8)
7	19.0 a (18.3-19.7)	16.4 (15.6-17.1)	20.3 (19.4-21.1)
8	20.0 b (19.2-20.7)	17.3 (16.6-18.1)	21.0 (20.1-21.8)

[1] 95% confidence intervals.

[2] Letters indicate means within cultivars, temperatures or nodes that are different ($P \leq 0.05$) using LSD-tests.

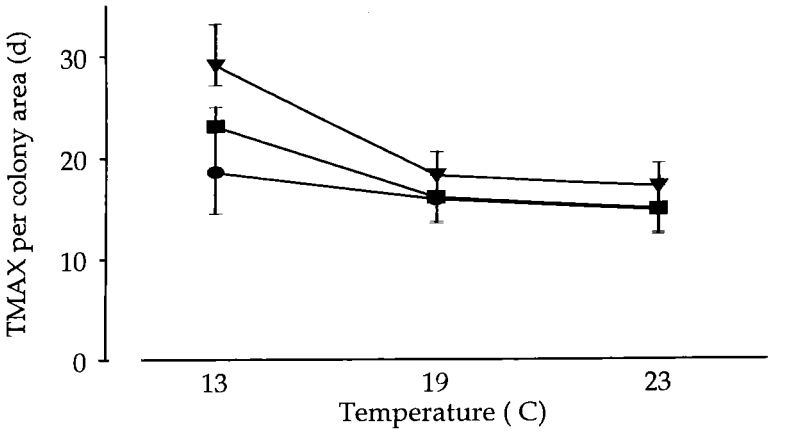


Figure 3.12. Mean time to maximum *E. pisi* conidium production per day (TMAX) per colony area at three temperatures on leaflets of three pea cultivars (Pania = ■, Bolero = ●, Quantum = ▼).

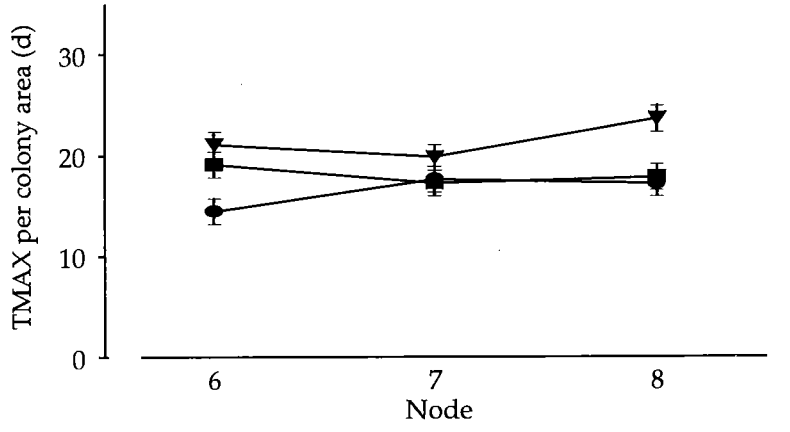


Figure 3.13. Mean time to maximum *E. pisi* conidium production per day (TMAX) per colony area on leaflets at three nodes of three pea cultivars (Pania = ■, Bolero = ●, Quantum = ▼).

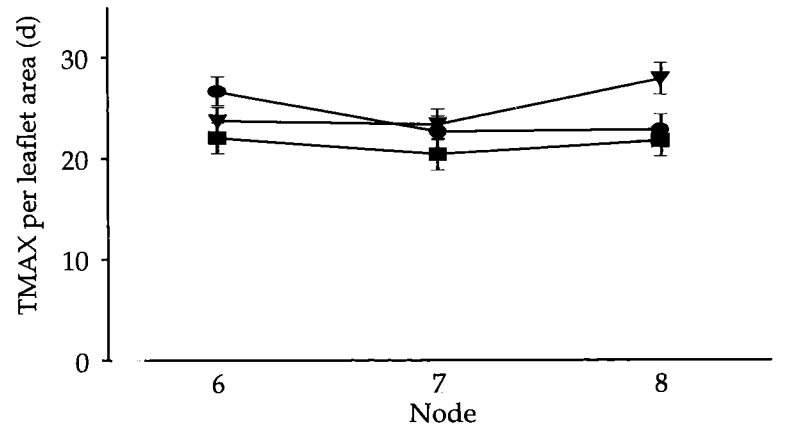


Figure 3.14. Mean time to maximum *E. pisi* conidium production per day (TMAX) per leaflet area on leaflets at three nodes of three pea cultivars (Pania = ■, Bolero = ●, Quantum = ▼).

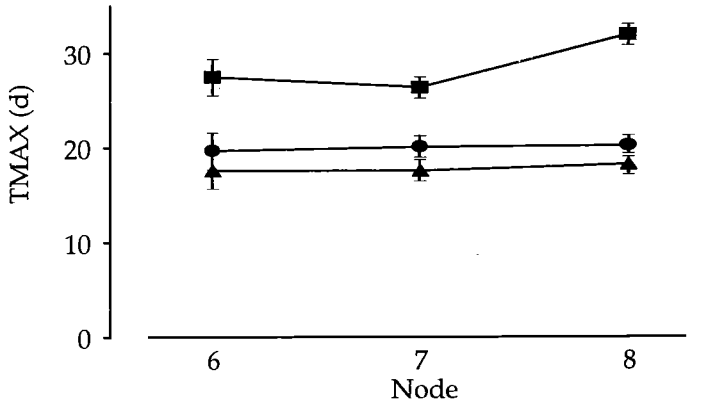


Figure 3.15. Mean time to maximum *E. pisi* conidium production per day (TMAX) on leaflets at three nodes on pea plants at three temperatures (13°C = ■, 19°C = ●, 23°C = ▲).

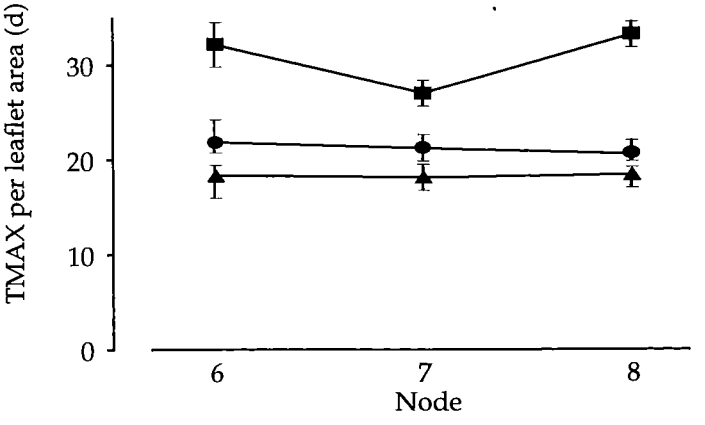


Figure 3.16. Mean time to maximum *E. pisi* conidium production per day (TMAX) per leaflet area on leaflets at three nodes of pea plants at three temperatures (13°C = ■, 19°C = ●, 23°C = ▲).

3.4. Discussion

A number of epidemiological factors were measured that may characterise quantitative resistance to *E. pisi* in the pea cultivar Quantum, by making comparisons with two cultivars (Bolero and Pania) that are susceptible to the pathogen. The study measured conidium germination, infection efficiency, latent period and the rate and duration of conidium production for the pathogen on the cultivars, and, by making cultivar comparisons, has enabled quantitative resistance in Quantum to be characterised in epidemiological terms. Some evidence was also found for reduced conidium production on Bolero compared with Pania.

3.4.1. Conidium germination

The proportion of conidium germination was not an important factor for characterising quantitative resistance in Quantum. Host genotype affects germination in some host-pathogen interactions, but not others. Host genotype was reported not to affect germination of *E. pisi* conidia on peas (Singh and Singh, 1983), *Erysiphe graminis* DC. f. sp. *avenae* conidia on oat (Douglas, Sherwood and Lukezic, 1984; Carver and Adaigbe, 1990), or E. *graminis* f. sp. *hordei* on barley (Wright and Heale, 1984). However, Mukhopadhyaya and Russell (1979) found significant differences in germination of *Erysiphe betae* (Vañha)Weltzien conidia on sugar beets with variable levels of resistance. In other fungi causing downy mildew, germination percentage of *B. lactucae* spores was reduced on a field resistant lettuce cultivar compared with other cultivars (Lebeda and Reinink, 1991). Other studies have indicated that germination of *Erysiphe* conidia is rarely influenced by external factors other than environmental conditions. Conidia can germinate on nonhost plants, as demonstrated by Johnson, Bushnell and Zeyen (1982) who found that on seedling leaves of barley, oats, wheat and rye, and on other Graminae, conidia of *E. graminis* f. sp. *hordei* produced normal short germ tubes. Gay, Martin and Ball (1985) demonstrated that some conidia germinated even when in contact with various toxic chemicals. Sivapalan (1993) studied germination of powdery mildew conidia from 50 different hosts belonging to 25 different families, either on or in water or on the appropriate host leaves. One group germinated in water at levels comparable to that on the leaf surfaces, while a second group germinated poorly in water. Conidia of *E. pisi* and *E. graminis* f. sp. *hordei* retained their ability to grow normally on leaves after a period on or in water although colony growth was reduced. De Waard (1971) reported germination of several powdery mildew conidia, including *E. pisi*, on cellulose membranes laid on agar.

In the present study, leaflet age affected germination, with more conidia germinating on young leaflets than on old leaflets. Several studies have compared *E. graminis* conidium germination on seedling and adult plant leaves of cereals. Carver and Adaigbe (1990) found that more *E. graminis*

f. sp. *avenae* conidia germinated on young than on old leaves of oats, and on adult than on seedling leaves, although these effects were small in absolute terms. Others have found the reverse applying where more conidia germinated on adult than on seedlings leaves of barley (Ayres and Woolacott, 1980; Russell, Andrews and Bishop, 1975) and oats (Douglas, Sherwood and Lukezic, 1984).

3.4.2. Infection efficiency

Infection efficiency of *E. pisi* was shown to be 34% less on Quantum than in Pania in the present study. Other studies indicate that infection efficiency is an important component of quantitative resistance. In oats, reduced infection efficiency of *E. graminis* f. sp. *avenae* conidia was found to contribute to quantitative resistance (Jones, 1978). Reduction in colony number per leaf area was also shown to be a component of quantitative resistance in the barley - *E. graminis* f. sp. *hordei* interaction (Asher and Thomas, 1983; Heun, 1986; Newton, 1990). Nørgaard Knudsen (1984) found that infection efficiency of *E. graminis* f. sp. *hordei* varied by a factor of 2.8 for the most susceptible cultivar of barley compared with the most resistant cultivar. In contrast to these studies, Raju and Anilkumar (1990) found that on cowpea, *E. polygoni* colony numbers per leaflet were not a good indication of resistance level, especially at later stages of colony development when colonies in susceptible genotypes coalesced.

The effect of leaflet age on germination of conidia was not evident in infection efficiency experiments in the present study, a result that contrasts with those from some other infection efficiency studies. Asher and Thomas (1983) showed that a reduction in *E. graminis* f. sp. *hordei* colony number contributed to quantitative resistance in barley, and the fifth and sixth leaves were identified as optimal for such studies. In spring wheat, a large growth stage effect on infection efficiency of *Puccinia.* f. sp. *tritici* was demonstrated by Broers (1989b). Roumen (1992) reported a decline in the number of sporulating *Pyricularia oryzae* Cavara lesions per cm^2 rice leaf area with increase of leaf age in all genotypes.

3.4.3. Latent period

Effects on latent period did not appear to be an important characteristic of quantitative resistance in Quantum. The differences between cultivars may have been too small to be detected in this type of experiment, where the start of the conidium production was examined only once per day and not as the proportion of colonies producing conidia. Latent period is sometimes measured as the number of days when 50% of colonies are producing conidia, and may provide a more accurate measurement. In most host-pathogen interactions, latent period has been found to be an important component of quantitative resistance although the differences have also been small. Latent period

has been found to contribute to quantitative resistance in oats to *E. graminis* f. sp. *avenae* (Jones, 1978), in barley to *P. hordei* (Parlevliet and van Ommeren, 1975; Neervoort and Parlevliet, 1978), in wheat to *P. recondita* f. sp. *tritici* (Lee and Shaner, 1985; Broers, 1989b; Denissen, 1993), in flax and *M. lini* (Rashid, 1991), and in peanut to *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton (Aquino *et al.*, 1995). However, Roumen and de Boef (1993) reported that for rice and *P. oryzae*, latent period was not an important component of quantitative resistance, as this parameter varied only slightly between treatments, with a maximum difference of 8 h between cultivars. Asher and Thomas (1984) reported that in barley, *E. graminis* f. sp. *hordei* colony growth rate appeared to be retarded in the early stages of development, and this persisted to give an extended latent period although variation of latent period was of only minor importance and unlikely to contribute to resistance (Asher and Thomas, 1984). Latent periods are likely to be longer in the field compared with controlled environment conditions because of lower and more variable temperatures in the field (Nørgaard Knudsen, 1984). Therefore, even minor differences in latent period frequently found under controlled environment conditions could be an important component of quantitative resistance as found by many studies.

In the present study about 2 day differences were found in latent period in the different temperatures treatments, but there were no temperature - cultivar interactions (Table 3.5.). Denissen (1991) found a significant temperature effect on latent period and a significant temperature-genotype interaction for *P. recondita* f. sp. *tritici* on wheat. Latent periods of the relatively resistant genotypes were most sensitive to temperature; the range of latent period was highest at the lowest temperature (12°C) and therefore lower temperature regimes were preferred to distinguish differences in the level of quantitative resistance (Denissen, 1991). Asher and Thomas (1984) postulated that latent period of powdery mildew on barley was greater at lower temperatures, and the range of values obtained was greater at 5°C than at 10°C. Carson and van Dyke (1994) found that in northern leaf blight of maize (caused by *E. turcicum*) incubation and latent period were correlated regardless of environmental conditions, although higher temperatures tended to increase the differences among genotypes.

3.4.4. Conidium production

Total conidium production in Quantum was reduced by almost 20% and by 50% in Bolero compared with that of Pania. The reduction was about 10% and 20% respectively when adjusted for colony area, and 25% and 40% respectively when adjusted for leaflet area. The differences in conidium production on colony and leaflet area bases are attributable to reduced infection efficiency on Quantum compared with Pania. Reduced conidium production due to quantitative resistance has also been found in *E. graminis* -infected barley (Asher, 1982; Asher and Thomas, 1984; Nørgaard

Knudsen, 1984), wheat (Shaner, 1973; Rouse *et al.*, 1980, Nass *et al.*, 1981) and oats (Jones, 1978), and in the cowpea - *E. polygoni* interaction (Raju and Anilkumar, 1990).

The difference in total conidium production between Quantum and Bolero may have been caused by the differences in leaflet size, as Quantum leaflets were larger than those of Pania or Bolero for nodes 6 and 7. Larger leaflets may support greater conidium production than smaller leaflets, so that when conidium production is corrected per unit leaflet area, the differences between the cultivars remain. Pustule size has been found to affect *E. graminis* f. sp. *tritici* conidium production in wheat. Shaner (1973) found variation in pustule area and density of conidial chain; bigger pustules, called Class 3, produced up to 13 times more conidia than smaller pustules (Class 1), and Class 0 pustules produced no conidia.

The maximum conidium production per day in Quantum was reduced by over 20% and by 40% in Bolero when compared with Pania. The reductions were over 30% and 16% respectively when adjusted for colony areas, and 33% for both cultivars when adjusted for leaflet areas. The difference in time to maximum conidium production between Quantum and Pania was approximately 10%. Time to maximum spore production per day provided an epidemiologically similar method of assessing delayed spore production to latent period. This study has indicated that Quantum had a narrower range of conidium production per colony area at the different temperatures than the other cultivars (Figure 3.4.). Time to maximum conidium production at 13°C was longer on Quantum than on the other cultivars, but no such difference occurred at 19° or 23°C (Figure 3.12.).

There appeared to be differences in conidium production measured on different nodes (Tables 3.7 - 3.9). However, no differences between the nodes were found when the components of conidium production were corrected for colony area. Therefore, all differences in total conidium production that were found between the different nodes were mainly a result of different leaflet sizes.

Temperature had a significant effect on all aspects of conidium production. Total conidium production per colony area increased 2.15 times and CMAX per colony area increased three-fold for every 5°C increase in temperature (Table 3.7.). There were no differences in TMAX per colony area between 19 and 23°C, but at 13°C TMAX per colony area was 1.5 times of that at the higher temperatures. Ward and Manners (1974) found sporulation was greatest at 20°C for *E. graminis* f. sp. *hordei*, optimum relative humidity for sporulation was 100%, but light intensity and photoperiod had little effect on sporulation.

Conidium production experiments in the present study were not designed to test specifically for leaflet age effects, although the difference in age between node positions 6 and 8 was approximately

10 days. In the barley - *E. graminis* f. sp. *hordei* interaction, fifth and sixth leaves were optimal in quantitative resistance studies as later produced leaves were too resistant (Asher and Thomas, 1984).

3.4.5. Conclusions

Reductions in infection efficiency and maximum conidium production per colony area, and increases in time to maximum conidium production are important epidemiological factors that characterise quantitative resistance in the pea cultivar Quantum compared with the susceptible cultivar Pania. Germination percentage of conidia or latent period did not differ between the cultivars.

The differences between Quantum and Pania and Bolero and Pania were -34% and -20% in infection efficiency, -33% and -16% in maximum number of conidia produced per day (CMAX) per colony area, and -16% and +9% in time to CMAX (TMAX) per colony area, respectively. Collectively these differences between Quantum and Pania amount to a substantial effect. Bolero is one of the parent lines of Quantum, and although quantitative resistance in Quantum is reported to be inherited from Plus (D. Webster, Asgrow Seed Company, pers. comm.), it was evident that Bolero also had some resistance to the disease, but that this differed from the quantitative resistance observed in Quantum. The total conidium production per colony area was less in Bolero than in Quantum, but the conidia on Bolero were produced faster and in a shorter time period than on Quantum.

Attempting to breed cultivars for characteristics which would reduce germination of *E. pisi* conidia has little potential on the present evidence, although more cultivars would need to be tested. Other factors affecting germination such as thickened cuticle, the presence of toxins in the cuticle, cell wall or sap (Ayres and Woolacott, 1980), or rapid deposition of silica in host cell walls in response to germ tube contact (Carver, Zeyen and Ahlstrand, 1987) might be better strategies in plants for disease avoidance. There is potential to breed pea cultivars for other factors contributing to quantitative resistance, however. These include decreased infection efficiency and reduced conidium production. Measuring conidium production is very labourious, therefore it is unlikely that this component would be used to produce quantitatively resistant lines. The costs in time may be worthwhile in production of parental material, however. Before this is possible, suitable screening procedures for the components must be available, and providing information for developing these has been the purpose of this study. Other factors, such as heritability of quantitative resistance and the amount of variation shown by the component (Parlevliet, 1992), also need to be considered.

Careful inoculation and disease assessment is essential for characterisation of quantitative resistance. Number of conidia per cm^2 must be optimised for the host differentiation and uniform inoculation

is extremely important. The germinability of conidia is also important and needs to be standardised. Heun (1986) found that small environmental variations changed the success of inoculation and allowed differentiation; in some situations it differentiated resistant genotypes, in other situations the differences between susceptible genotypes were more obvious. Measurement and analysis of small differences in quantitative resistance will always be difficult, however.

Chapter 4

Morphological characteristics of *Erysiphe pisi* in susceptible and quantitatively resistant pea plants

4.1. Introduction

Infection of host plants by powdery mildew fungi includes the development of haustoria, complex host-pathogen interfaces, which enables the flow of nutrients from host to pathogen. Each haustorium is contained in an invagination of the infected host cell plasmalemma (the extrahaustorial membrane; EHM) separated from the haustorial body by the extrahaustorial matrix and sealed by the neck band to the fungal wall isolating the matrix from the leaf apoplast (Bushnell and Gay, 1978; Manners and Gay, 1983). Direct evidence for the role of haustoria in nutrient uptake of *E. pisi* was obtained by Manners and Gay (1978), using a technique that isolated haustorial complexes. This has permitted direct access to the interface between host and fungus for biochemical analysis of haustorial metabolites (Manners and Gay, 1978; 1980; 1982a) and *in vitro* studies of the permeation of solutes (Manners and Gay, 1982b; Gay and Manners, 1987).

The efficiency of nutrient uptake from host cells through haustoria may be a reflection of the degree of host resistance. Resistance in barley to *E. graminis* can be expressed at any stage during the infection process (Section 1.6.5.), including prior to or after haustorium formation (Johnson, Bushnell and Zeyen, 1979; Wright and Heale, 1988). In cereal powdery mildews, race-specific resistance is usually associated with hypersensitive responses (Aist and Bushnell, 1991). However, these rarely occur with quantitative resistance. This form of resistance is most often expressed as reactions at the time of formation of papillae (Asher and Thomas, 1983; Clifford, Carver and Roderick, 1985; Wei *et al.*, 1994), although other mechanisms have been suggested (Carver, 1986). These include prevention of appressorium development to stimulate localised host responses, prevention of penetration beyond localised host responses, and death of epidermal cells. It is likely that quantitative resistance can be expressed at any stage of pathogen development on hosts due to multiple resistance mechanisms which individually or collectively act at the different stages (Aist and Bushnell, 1991). Quantitative resistance to *E. graminis* on cereals is often dependent on host plant or leaf physiological age; adult plant leaves are often more resistant than those on seedlings (Carver and Carr, 1977; Asher and Thomas, 1983; Wright and Heale, 1984).

Haustorial efficiency as a determinant of colony growth has been studied in some host-pathogen interactions. Carver and Carr (1978) found that haustoria of *E. graminis* f. sp. *avenae* on oats varied in their efficiency as measured by the mean total length of mycelia produced by each haustorium, but the efficiency was greatest in the most susceptible host, Manod. Stumpf and Gay (1989) reported that, in resistant pea cultivars, each *E. pisi* haustorium supported a greater total hyphal length than in a susceptible cultivar, but hyphal growth rates were very similar on both types of cultivars. In wheat, *P. recondita* colonies in quantitatively resistant cultivars were smaller than colonies in susceptible cultivars (Lee and Shaner, 1984), and in barley cultivars with quantitative resistance genes, the growth and developmental rate of *P. hordei* colonies was reduced (Niks and Kuiper, 1983).

The numbers of haustoria formed in quantitatively resistant cultivars has been reported to be reduced in several host-pathogen interactions. In studies of pea - *E. pisi* interactions, Stumpf and Gay (1989) found that resistant interactions were characterized by the production of relatively few haustoria and by reduced sporulation. *Puccinia recondita* colonies on wheat cultivars possessing quantitative resistance had fewer haustorial mother cells than colonies on susceptible cultivars (Lee and Shaner, 1984).

Haustoria may be smaller in hosts possessing quantitative resistance than in susceptible hosts. This is suspected to limit nutrient supply to colonies (Clifford *et* al., 1985). Carver and Carr (1978) found that in oat species varying in the degree of resistance, primary haustoria of *E. graminis* f. sp. *avenae* were smaller (measured by their length) than on susceptible species. Haustorial lobes contributed most to the total haustorial surface area, while only about 10% of surface area was contributed by the haustorial body. The surface area to volume ratio of haustoria is of particular interest, since this may be associated with reduced nutrient intake in incompatible interactions. Viljanen-Rollinson (1991) found that the perimeter to area ratios of haustorial walls and lobes of *E. pisi* haustoria were 1.5 - 2.4 greater than the same ratios for extrahaustorial membranes.

The objective of experiments reported in this chapter was to assess *E. pisi* haustorial efficiency in the quantitatively resistant cultivar Quantum by:

1) measuring the size of individual *E. pisi* colonies formed on leaflets of different age on intact plants of different growth stages 8 d after inoculation, and comparing these to colonies on the susceptible cultivars Pania and Bolero.

2) measuring numbers of *E. pisi* haustoria per colony area on leaflets of different ages on intact plants of different growth stages 8 d after inoculation, and comparing these to those on Pania and Bolero.

3) measuring surface area to volume ratios of Quantum haustoria compared with Pania haustoria using transmission electron microscopy with image analysis and processing techniques.

4.2. Materials and methods

4.2.1. Size of *E. pisi* colonies on different cultivars (Experiments 1-5)

Five experiments were carried out to measure *E. pisi* colony sizes on three pea cultivars at two growth stages and two leaflet ages (Table 4.1.). Plants of Pania, Bolero and Quantum were grown as described in Section 2.2.2. When at the required growth stage, plants were inoculated in the settling tower with 0.5 mg of conidia to receive 10 (range 7.2 - 13.9) conidia cm^{-2} of leaflet surface (Section 3.2.3.). Plants were incubated and grown for 8 d after inoculation as described previously (Section 3.2.3). Colony size of five individual colonies on leaflets at each inoculated node was determined by measuring the colony diameter with a vernier calliper under a stereo microscope (Experiments 1, 2 and 3) or tracing each colony with a VideoPro 32 (version 2.51) chromatic colour image analyser (Leading Edge Pty Ltd, Bedford Park, Australia; Experiments 4 and 5) and measuring the area of each colony. The mean colony areas were analysed by analysis of variance comparing cultivars in Experiments 1 and 2, and leaflet and plant age in addition to cultivar effects in Experiments 3, 4 and 5.

Table 4.1. Description of plants used in Experiments 1 to 5 , where *E. pisi* colony size was measured.

Experiment	Plant growth stage	Nodes inoculated	Leaflet age (d)	Plants per cultivar
1,2	105	4 and 5	1-5	2
3,4,5	105	4 and 5	1-5	1
	108	4 and 7	15 and 5	1

4.2.2. Numbers of haustoria

An experiment was conducted to assess numbers of haustoria in different cultivars by measuring the number of *E. pisi* haustoria close to the centre of colonies on leaflets 7 days after inoculation. Plants of Pania, Bolero and Quantum were grown as described in Section 3.2.1. When they were at growth stage 106 (vegetative, six nodes) or 203 (reproductive, first fully open flower, 12 nodes), nodes 4 and 5 in young plants (leaflet age 1-5 d), and nodes 4 and 5 (leaflet age 30-35 d) and 10 and 11 (leaflet age 1-5 d) in older plants, were inoculated and incubated as described previously (Section 3.2.3.). Seven days after inoculation, three leaflet pieces (approximately 1 cm^2), each containing a complete *E. pisi*

colony, were cut from each inoculated leaflet, and processed as described by Rohringer *et al.* (1977). An Olympus BH-2 microscope equipped with a BH2-RFC reflected light fluorescence attachment and an Ushio USH-102D mercury burner, a DM455 dichroic mirror, BP440 exciter filter and Y475 barrier filter, was used to locate centres of colonies. The number of haustoria were counted by light microscopy from five fields of view of each colony close to the colony centre using a ocular grid (quadrat size 4000 μm^2) and a SPlan ×40 objective. Light microscopy distinguished haustoria better than reflected light fluorescence. Data were pooled for nodes 4 and 5, and 10 and 11 and analysed by analysis of variance.

4.2.3. Transmission electron microscopy and image processing and analysis

Plants of Bolero, Pania and Quantum were grown as described in Section 3.2.1. When they were at growth stage 106 (vegetative, six nodes), 109 (vegetative, nine nodes) or 203 (reproductive, first fully open flower, 12 nodes), leaflets at node 4 (GS 106 and 109) or node 7 (GS 106 and 203) were inoculated and grown as described previously (Section 3.2.3.). There were seven plants per cultivar for each growth stage. For TEM studies, inoculated leaflets were collected 6, 12, 24, 36, 48, 72 h and 7 d after inoculation and submerged in 0.025 M phosphate buffer (pH 7.23). Three pieces (about 2 mm^2) were cut from each leaflet and fixed in 5% glutaraldehyde in phosphate buffer overnight under vacuum (50.8 cm mercury). Segments were then washed in phosphate buffer three times for 15 min each, post-fixed in osmium tetroxide (2% in water) for 2 h, and then dehydrated in graded (20, 50, 70, 90, 100%) acetone series (15 min each point). The samples were then embedded in a graded Araldite PY303 epoxy resin (Ciba-Geigy) and acetone series (50/50, 75/25, 100/0), and left at 4 °C to rotate overnight. Samples were then covered with fresh resin, left at 4°C to rotate for 6-7 h, covered again with fresh resin, and polymerised overnight at 65°C.

Embedded leaflet pieces were cut into 4 μm thick sections with a pyrometer, stained and checked for the presence of haustoria by light microscopy. No haustoria were located in leaflet samples incubated for 6, 12 or 24 h, several haustoria were located in 36 and 72 h samples, and many were located in the 7 d samples. Leaflets at node 4 of young plants and node 7 of old plants (leaflet age for both samples was 1-5 d) were chosen for TEM examination, as they contained the greatest numbers of haustoria. Initially, 20-40 serial sections 220-250 nm in thickness were cut from each leaflet piece with a Reichert OM U4 ultramicrotome with a diamond or glass knife. The sections were stained with 2% aqueous uranyl acetate for 12 min and lead citrate for 2 min. Sections were viewed with a Zeiss 902 transmission electron microscope at 80 kV accelerating voltage to locate haustoria. Between five and ten haustoria of each cultivar and node were examined, and haustoria from leaflets at node 4 were chosen for further processing. Cross section images of serial sections of five haustoria of Quantum from two separate *E. pisi* colonies were recorded on S-VHS videotape

with a Panasonic AG 7355 video recorder using a video camera (MIT SIT 66 PART SIT-66 (625/50) with an intensified silicon diode tube (Resolution 550 lines centre, 350 corners) permanently attached to the TEM. Two of the Pania haustoria were so large that they did not fit in the field of view of the video camera, and these were also photographed with a camera attached to the TEM onto a black and white film (negative size 100 × 80 mm). Three average sized haustoria, based on examination of a total of 15-20 haustoria, were chosen for surface area and volume calculations. Images of sections of haustoria from video tape images or black and white negatives were digitised with a VideoPro 32 (version 2.51) image analyser. Extrahaustorial membranes were traced on the digitised images, and areas and perimeters were measured in pixels and later calibrated for size. Haustorial bodies and lobes were either traced in a similar manner as the EHM, or thresholded automatically depending on the quality of images, and the areas and perimeters were measured. Volumes and surface areas were calculated by multiplying the sum of each by mean section thickness (0.24 μm). Means, standard error of means and P-values based on t-tests were calculated for the cultivar means.

4.3. Results

4.3.1. Colony size

There were no differences in colony size between the cultivars for leaflets at nodes 4 and 5 (P=0.991) or at node 7 (P=0.675). However, the average colony area in plants at GS 109 was larger (P=0.028) on leaflets at node 7 (27.4 mm^2) than on leaflets at nodes 4 and 5 (20.8 mm^2).

4.3.2. Numbers of haustoria

There were no differences (P=0.82) between the cultivars in overall numbers of haustoria per colony area. More haustoria (P=0.027) were present in plants at GS 203 on nodes 10 and 11 (3.6 haustoria per 1000 μm^2 of colony area) than on nodes 4 and 5 (2.4 haustoria per 1000 μm^2, SEM=0.23).

4.3.3. Size of haustoria

Haustoria from leaflets of Pania plants were larger and contained more lobes than those from Quantum plants (Figure 4.1. and 4.2.). This was confirmed by greater (P=0.019) surface area to volume ratio for the haustorial plasmalemmae for haustoria from Pania than for those from Quantum (Table 4.2.). The surface area to volume ratio is dependent on the complexity and number of lobes, so this alone does not clearly indicate the degree of lobing. The ratios of total surface area of haustorial plasmalemmae to the surface area of EHM for Pania haustoria were more than twice

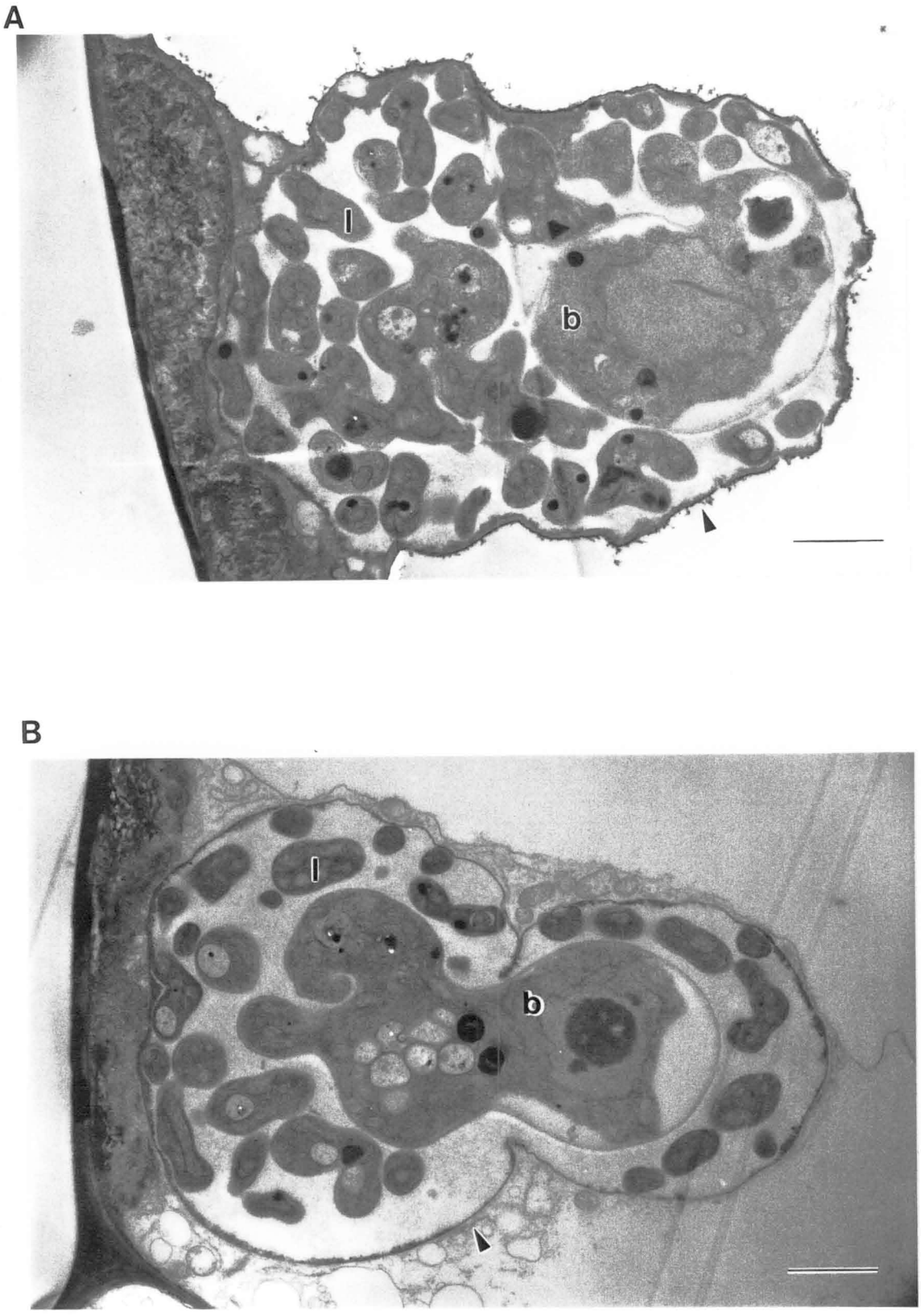


Figure 4.1. Haustorium number 2 (A) and 3 (B) from leaves of Pania plants. Each haustorium has numerous lobes (l), a haustorial body (b) and an extrahaustorial membrane (arrow). Bar=5μm.

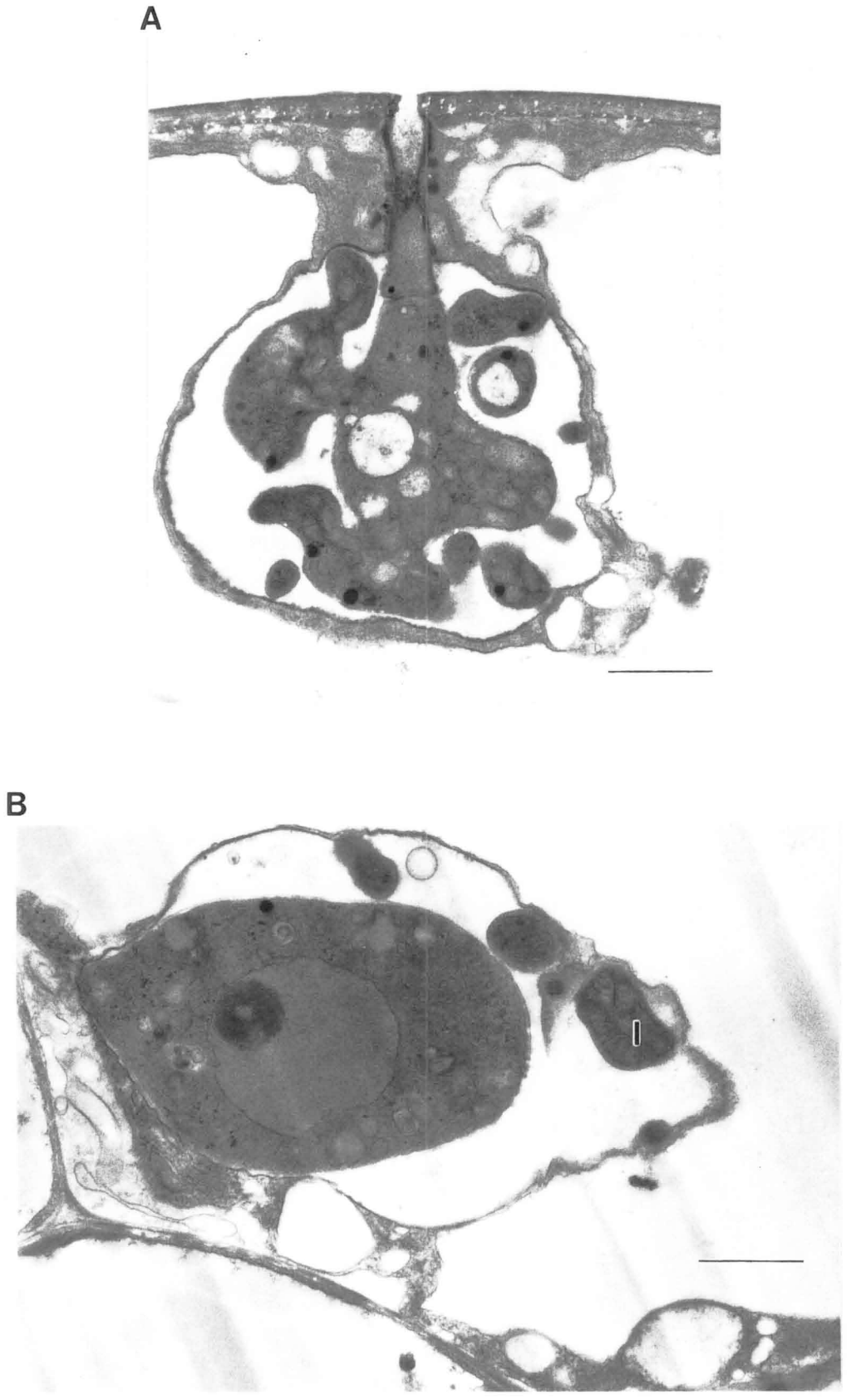


Figure 4.2. Haustorium number 2 (A) and 3 (B) from leaves of Quantum plants. A few lobes (l) can be seen. Bar=2μm.

Table 4.2. Total volumes and surface areas of *E. pisi* haustoria from Pania and Quantum plants measured from transmission electron micrographs and video taped images.

	Haus	EHM			Haustorial plasmalemma			
Cultivar	no[1]	vol (μm^3)	SA[2] (μm^3)	SA/vol	vol (μm^3)	SA (μm^3)	SA/vol	H/EHM[3]
Pania	1	985	397	0.40	694	909	1.31	2.28
	2	2191	749	0.34	1515	2162	1.43	2.89
	3	2215	830	0.37	1236	2381	1.93	2.87
Mean		1797	659	0.37	1148	1817	1.56	2.68
SEM		406	133	0.017	241	459	0.190	0.200
Quantum	1	740	341	0.46	483	508	1.05	1.49
	2	785	398	0.51	434	460	1.06	1.16
	3	662	356	0.54	500	352	0.70	0.99
Mean		729	365	0.50	472	440	0.94	1.23
SEM		35.9	17.1	0.023	19.8	46.1	0.118	0.147
P-value[4]		0.12	0.16	0.019	0.11	0.096	0.069	0.0097

[1] Haustoria number
[2] SA = surface area
[3] H/EHM = surface area to volume ratio of the haustorial plasmalemma divided by the surface area to volume ratio of the EHM
[4] P-value for unpooled variances between cultivar means using t-tests.

(P=0.0097) those from Quantum. This ratio is affected by the relative volume of the haustorial body and lobes to that of the EHM. For example, the proportion of the volume of the EHM on Pania haustorium 3 was the same (56%) as that from Quantum haustorium 2, so these two haustoria could be considered similar and compared. The haustorial plasmalemma to EHM ratio from this Pania haustorium was still nearly 2.5 times that from the Quantum haustorium.

4.4. Discussion

Quantitative resistance to *E. pisi* in Quantum was expressed mainly as reduced sporulation and a longer time to maximum production of conidia when compared with the susceptible cultivar Pania (Chapter 3). This reduction was at least partly due to a reduced number of conidia that successfully initiated infections. It is likely that other factors were involved, such as the efficiency of haustoria. Efficiency of haustoria in Quantum, Pania and Bolero was evaluated by measuring the total area of five individual colonies per cultivar and the number of haustoria produced in different leaflet positions and on different aged plants, and assessing the size and the degree of lobing in haustoria based on a TEM study. Colony size, measured on 8 d old colonies, was not an important aspect of quantitative resistance in Quantum compared with Bolero and Pania in these experiments. Measuring colony area provides a two-dimensional picture of a three-dimensional system, so the effect of sporulation is also required as an indication of quantitative resistance. This was shown to be reduced in cultivar Quantum relative to Pania (Chapter 3). Whipps *et al.*, (1980) demonstrated that the sporulating area of pustules of *P. hordei* in barley was much reduced in the quantitatively resistant cv. Peruvian, although many of these colonies were as large or even larger than in a susceptible cv. Gold. Therefore sporulating colony area does not necessarily relate to amount of mycelial tissue.

In the present study, numbers of haustoria per unit area did not correlate with cultivar resistance to *E. pisi*. However, there was an indication that the haustorial plasmalemma to EHM ratio was greater in Pania than in Quantum. The extent of haustorial plasmalemmae may be an indication of the efficiency of nutrient uptake by haustoria. The EHM has been shown to lack ATPase activity which is believed to be essential for efficient uptake of nutrients (Spencer-Phillips and Gay, 1981). The haustorial plasmalemma has significant ATPase activity and this maintains a potential difference at the EHM to actively drive solute transport. The haustorial body itself has been found to be of limited importance as a nutrient absorbing organ. In *E. graminis* haustoria on oats, the haustorial body was only about 10% of the total surface area of the haustorial plasmalemma (Carver and Carr, 1978). In this study, the haustorial body appeared to contribute more of the total haustorial plasmalemmae on haustoria of Quantum than on Pania, mainly because of less lobing in Quantum haustoria. There

are, however, some differences between *E. graminis* and *E. pisi* haustoria; the lobes of *E. graminis* haustoria are finger-like as opposed to *E. pisi* haustoria which are recurved.

Haustorial function is affected by the number, size and efficiency of haustoria (Carver and Carr, 1978), but the mechanism for this is not clear. In powdery mildews, the efficiency of transport of nutrients from symplast to leaflet apoplast and to epidermal symplast varies between the genotypes (Clifford *et al.*, 1985). It is possible that a less utilisable or less mobile nutrient is produced by some genotypes. Restriction of haustorial development may result from several factors not evident in the first 7-8 d after inoculation, and for this reason no difference in the size of colonies were detected. Carver and Carr (1978) found that quantitative resistance to *E. graminis* in the oat cv. Maldwyn was not necessarily detectable in the earlier haustorial generations, but became amplified through each subsequent generation of haustoria. The tertiary generation of haustoria was most affected. This is presumably because small differences in relative susceptibility of hosts act continuously during the development of the pathogen, and become compounded as the infection progresses (Clifford *et al.*, 1985). Quantitative resistance in cv. Maldwyn was expressed by reduced penetration of host cells and consequent prevention of haustorium formation, and restriction in haustorium size and efficiency resulting in lower infection frequency and slower colony development and reduced and delayed sporulation. Evidence presented here suggests that quantitative resistance in Quantum operates in a similar way.

Restricted haustorial formation is often associated with the presence of papillae (Aist and Bushnell, 1991). The importance of papillae in the present study was not clear, and no penetration sites were observed where haustoria were not formed because of papilla formation. It is possible that papilla formation is not a characteristic of quantitative resistance. Stumpf and Gay (1989) found that necrosis of leaves was not commonly associated with *E. pisi* resistance in the pea cv. JI 1049, but papillae were found adjacent to A bands of the haustorial neck walls. The neck bands did not have close associations with invaginated host plasmalemmae, and B bands alone seemed to be responsible for nutrient flow. Greater haustorial efficiency was found in the resistant cultivar JI 1049. Resistance operated through the inhibition of infection structure formation, including haustorium formation. This was similar to quantitative resistance to *P. hordei* found in barley (Niks, 1986). In cv. Vada, fewer *P. hordei* haustoria were produced than in a susceptible cv. L94. A long latent period and slow mycelium growth were largely due to 'faulty' haustorium formation, especially during the first few days of the infection. No differences in haustorium size or shape were found.

There was an indication that haustorial size may correlate with quantitative resistance in Quantum. However, care must to be taken to interpret the results of surface area to volume ratios, because the number of lobes also indicates the age of haustoria (Gil and Gay, 1977), and the sample size in this

experiment was small. The technique for size analysis was very labourious and time-consuming, and it is possible that large haustoria also existed in Quantum but that they were not detected in the samples that were examined. More sampling may be needed and a less labourious method for investigating the surface area to volume ratios would be useful. It is possible that confocal laser microscopy (Kwon, Wells and Hoch, 1993) could be used for this purpose. Images acquired digitally, and reconfigured in various ways could be used to detect spatial organisation of the haustoria. Section thickness would be critical, as a section less than 0.3 μm is required to ascertain differences between haustorial lobes.

Chapter 5

Spatial and temporal spread of *Erysiphe pisi* in field grown pea

5.1. Introduction

Plant epidemics of polycyclic pathogens such as powdery mildews frequently start from foci of infection. A focus is a patch of crop with disease limited in space and time, which tends to influence the pattern of further transmission of the disease (Anon, 1953). A travelling wave of a polycyclic epidemic (Minogue and Fry, 1983a; 1983b) can be visualised as a disease profile that moves through space with constant velocity without changing its shape, and proceeds at constant rate during a limited period and then diminishes usually at the end of the season (Zadoks and van den Bosch, 1994). Three orders of epidemics have been described depending on the size, complexity and time scale of focus expansion by Zadoks and van den Bosch (1994). A zero-order epidemic usually starts with a single successful propagule and results in a primary focus of 0.5 to 2 m in diameter after several monocycles. Good examples are when new races of wheat rusts appear in previously resistant cultivars. When the primary focus expands over a large area from one field to a diameter of about 100 km, but during one growing season, it is called a first-order epidemic. For example, the cereal rusts and some powdery mildews have been recorded as 1st-order epidemics. A second-order epidemic spreads over a large area (several thousand km) during a certain number of years, as has happened with late blight of potato (*Phytophthora infestans* (Mont.) de Bary) and powdery mildew of grape (*Uncinula necator* (Schwein.) Burrill). Second-order epidemics are not restricted to fungal diseases, but also occur with other plant pathogens, insects and vertebrates.

Modelling plant pathogen epidemics is frequently used as a tool to determine rates of epidemics and to estimate initial and future disease in crops. Such models usually have up to three objectives: description, prediction and explanation of disease epidemics (Hau, 1988). Spatial distribution of fungal plant pathogens is determined by components of the disease cycle such as survival, source of primary inoculum, and mode and amount of inoculum dissemination. Genotypes with quantitative resistance can exhibit lower levels of disease by reducing infection efficiency, lengthening the latent period or reducing conidium production of the pathogen (Section 1.6.). One or more of these components can also reduce the disease progress (temporal increase) and/or spread (spatial increase) in the field. Modelling of spatial (Aquino *et al.*, 1995; Luke and Berger, 1982; Steffenson and Webster, 1992) and both spatial and temporal (Headrick and Pataky, 1988;

MacKenzie, 1976) spread of fungal plant pathogens has been used as a tool to differentiate host genotypes in their resistance to pathogens.

Vanderplank (1963) was one of the first to note that in the early stages of an epidemic, the development of disease usually follows an exponential curve, expressed as

$$x_t = x_0 e^{rt}, \quad (1)$$

where x_0 is the amount of disease (x) at zero time (t), e is the base of natural logarithms and r is the rate of disease progress. The slope of the curve is determined by the multiplication rate of the pathogen under the prevailing conditions, and the position of the curve is determined by the amount of pathogen present at any particular time. This increase in disease in the early stages of an epidemic is the logarithmic or exponential stage, and later there is usually a levelling off in epidemic development. The principle reason for the decline in disease spread is the reduction in the amount of tissue available for infection, which results from the tissue already infected not being capable of contributing to an increase in infected area. When the proportion of healthy tissue ($1 - x$) approaches 0, then the disease progress curve begins to develop an S -shaped curve.

Temporal and spatial models have been used to describe polycyclic plant disease epidemics.

5.1.1. Temporal models

Several models, including logistic, Gompertz models and the Weibull function, have been proposed to describe the progress of polycyclic diseases over time, but the logistic and the Gompertz equations have been used most widely (Berger, 1981). Disease progress curves are linearised to determine the rate of the epidemic, and to estimate initial and future disease (Berger, 1981).

The logistic model for disease progress was described by Vanderplank (1963):

$$\text{logit}(y) = \ln(y\,(1-y)^{-1}) \quad (2)$$

where y=disease proportion in the range $0<y<1$. The logistic curve is sigmoid and symmetrical about its central point of inflection. When the daily increase of disease has a skewed distribution, the transformed values are nonlinear. The logistically transformed curves usually have steep slopes at $y<0.05$, linearisation for the range $0.05<y<0.6$, and values that fall below the general slopes when $y>0.6$. The initial increase of logistically transformed curves is very rapid (Berger, 1975; 1977). The logistic transformation has severe limitations for many disease progress curves that are asymmetrical.

The Gompertz model is a model for increasing growth:

$$y = \exp(-B * \exp(-kt)) \quad (3)$$

The k parameter of this model corresponds to the apparent infection rate (r) of the logistic equation. The integrated curve is sigmoid but asymmetrical about its point of inflection. The plot of the derivate is skewed to the right. Berger (1981) found the Gompertz model superior to the logistic model in linearising 113 disease progress curves.

A third model for disease progress is **the Weibull function** (Pennypacker *et al.*, 1980; Thal, Campbell and Madden, 1984), described as:

$$y = a(1-\exp(-(t/b)^c) \quad (4)$$

where y = disease proportion; t = time and $t > a$, $b > 0$, $c > 0$, is more flexible and simple than the other two equations in situations where the final disease level is not known, with small data sets and when estimated parameters are highly correlated.

5.1.2. Spatial models

Gregory's power function is a popular model to explain disease spread in spatial terms:

$$y=ax^b, \quad (5)$$

in which y is the proportion of disease at x units of distance from the source, a is the value of y at x=1, and b is the rate of change in y with the change in x (b is an estimate of the slope of the spatial gradient and it is usually negative; Gregory, 1968).

Gregory's model does not predict a finite number of infections at the source, and it was modified by Mundt and Leonard (1985) to

$$y=a(x'+c) \quad (6)$$

in which a is the number of infections per unit area at 1-c units of distance from the source, x' is the distance from the centre of the source to the centre of a receptor, and c is a truncation factor that provides for a finite y-intercept when x'+0. This modification allows for a gradient curve with a

finite y-intercept while maintaining a shape similar to that provided by the original model by Gregory. Lambert, Villareal and MacKenzie (1980) described gradient curves with variable shapes.

Berger and Luke (1979) and Luke and Berger (1982) regarded the use of disease gradients alone as an unreliable method of differentiating cultivar resistance and proposed the use of isopathic rates in conjunction with infection rates, r (from the logistic model) and k (from the Gompertz model). Kiyosawa and Shiyomi's (1972) model of spore dispersal in multiline cultivars has been used to study interplot interference (Paysour and Fry, 1983) and it has been incorporated into equations that describe the spatial and temporal increase of disease simultaneously (Jeger, 1983). This latter approach uses analytical advances to investigate the qualitative behaviour of plant disease. The analytical approach has also been used to explain focus expansion in space and time as a travelling wave theory, where an epidemic spreads as a wave travelling at constant speed (van den Bosch, Zadoks and Metz, 1988a; 1988b). The temporal and spatial increase of disease has been further developed and checked for relevance with epidemics of *P. infestans* in potato crops by Kosman and Levy (1994). Kosman and Levy (1994) based their model on the assumption that the main factor influencing disease progress is not the total diseased tissue but the infectious tissue producing inocula, and the progress of an epidemic depends on the change in size of the infectious area.

Analytical models are used to analyse epidemics on theoretical bases alone and do not take into account effects of external variables, such as changing environmental conditions. Simulation models do, to a certain extent, consider external variables (Hau, 1990).

5.1.3. Objectives of the study

The objective of this study was to define the progress and spread of powdery mildew on two susceptible and one quantitatively resistant pea cultivar in time and space, to confirm the slow-mildewing in Quantum and to test collectively whether the components of resistance found in laboratory conditions occur and differentiate the cultivars in the field.

5.2. Materials and methods

5.2.1. Crop culture

A field experiment was conducted at the New Zealand Institute for Crop & Food Research site, Lincoln, on Templeton sandy loam on sand, with a small area of Wakanui sandy loam on the eastern side of the 60 × 150 m site. Three pea cultivars were planted: Pania (susceptible to powdery

mildew), Bolero (susceptible) and Quantum (quantitatively resistant), and the plots were separated and surrounded with a resistant cultivar Trounce to reduce the effects of interplot interference. Two 19.2 × 22.8 m plots per cultivar were sown on 22 December 1994 with an Oyjord cone seeder at sowing rate of 121 seeds m^{-2} to depth of about 5 cm and in rows 15 cm apart, and the trial area was rolled the day after sowing. The plots were separated by 10 m (east-west direction) or 12 m (north-south direction) areas of Trounce, and buffered by 20 m of Trounce at the north and south end of the site, and 5.5 m in the east and west ends of the site. The pre-emergence herbicide terbuthylazine (100.0 g ha^{-1} Gardoprim in 300 ℓ water ha^{-1}) was applied two days after sowing. Seedlings emerged between 3 and 6 Jan. 1995. Overhead irrigation was applied when required according to standard practices (N. Gourley, pers. comm.).

5.2.2. Inoculation

Pania plants were sown in 18 cm pots (two plants per pot) and grown in a glasshouse unit until growth stage 106 (vegetative, six nodes). The plants were then inoculated with conidia of *E. pisi* from the collection described previously (Section 2.2.2) by shaking heavily infected plants over them. The plants were kept in the glasshouse unit at 22/18°C day/night temperatures until 1 d before the inoculation date, when they were placed in a cold frame for acclimatization. Leaves of the source plants were heavily infected with powdery mildew. Plots were inoculated by planting six source plants in the middle of each plot (Figure 5.1.) on 18 Jan. 1995, when the plants in the field were at growth stage 105 (vegetative, five nodes). This date was designated as day 0. A second inoculation was carried out a week later by the same method. These source plants remained in the plots for the duration of the experiment.

5.2.3. Characterisation of disease in time and space

The plots were sampled from 22 Feb. 1995 in eight directions and at distances of 1.5, 3, 6, 9 and 12 m (1.5 m not included in assessment 1) from the point of inoculation in the middle of each plot (Figure 5.1.). At 3 - 5 d intervals, thereafter each node of five plants (not same plants at each time) at each direction and distance point was assessed for powdery mildew severity on a scale from 0 - 100% of leaf area infected using a disease severity key (Falloon *et al.*, 1995) as a guide. A total of 185 (145 in the first assessment) plants per plot were assessed at each assessment. Assessments were continued for 35 d after inoculation (dai).

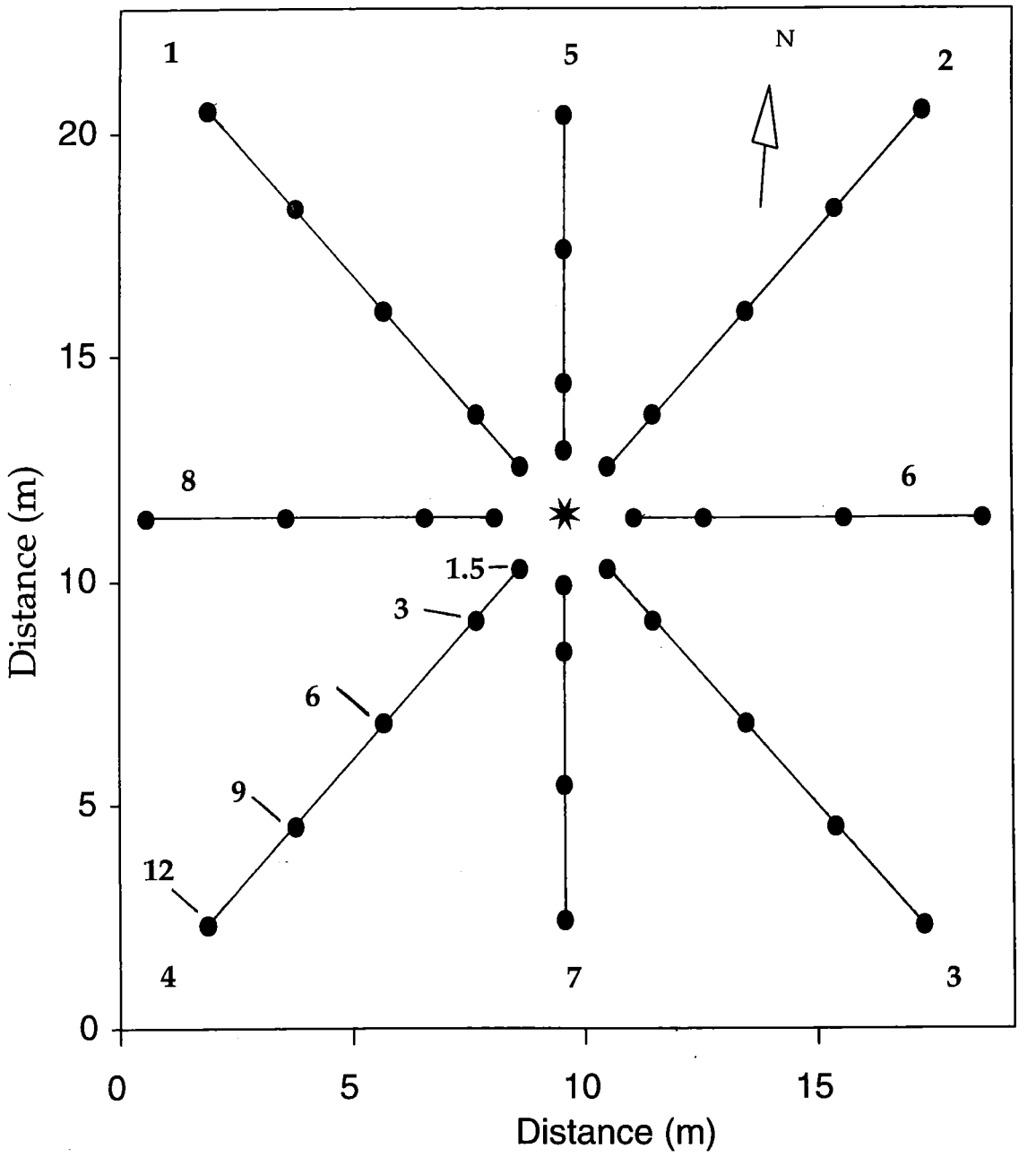


Figure 5.1. Diagram of one field plot, illustrating the eight directions (1-8) and four or five distances (●) in each direction where five plants were assessed for disease severity. Distance (m) from the source (✷) is described for direction 4. The open arrow points to true north.

Initially, disease severities for each distance and direction for each cultivar, replicate and assessment date, were plotted as three-dimensional points to visualise disease progress and for evidence of disease gradients in each plot. The nodes of plants were grouped into young, medium and old nodes, after initial statistical analysis combining all nodes together failed to describe the disease progress due to variations in disease severity within plants. The number of nodes included in each group depended on the growth stage of the crop and varied between assessment dates. The three last formed nodes were termed 'young', three early formed nodes above those that had senesced completely were designated 'old', and anything in between, usually two or three nodes, were designated 'medium'. In the first assessment only the oldest two nodes were designated old because there were some plants that had fewer than seven nodes. In the last two assessments, the two youngest nodes on each plant were designated young, because plants were producing nodes at a slower rate than previously. This division into three groups of nodes allowed comparisons between nodes of similar physiological age rather than nodes at fixed position on plants, at least until new nodes were no longer produced.

To determine whether there was a directional gradient, the data were analysed by analysis of variance by cultivar. In all three cultivars in all node age groups, orientation of the gradient was not a significant source of variation. Data from all eight directions were therefore merged for subsequent analysis.

The disease progress on young nodes was graphed, and the differences between cultivars at the last assessment date were analysed by analysis of variance. An exponential equation (severity = $e^{(a + b(\text{time}))}$)was fitted for the medium nodes. This model was chosen because the early stages of epidemics often follow exponential patterns (Vanderplank, 1963). Assessment date 1 was set as time = 0 when fitting the curves. Disease levels at 12 dai and relative growth rates for each cultivar and distance were analysed by analysis of variance.

Disease gradients for old nodes were determined by graphing the logarithm of severity by $\log_{10}$ of distance (m) from focus for each cultivar for each assessment date. The differences between cultivars for distance 12 m from foci were investigated by analysis of variance for 15 and 20 dai. The Gompertz equation (severity = $c \times \exp(-d \times \exp(-k \times \text{time}))$ was fitted for the old nodes. This equation was chosen because of the severe limitations of the logistic curve for disease progress curves that are asymmetrical and for it's superiority over the logistic model for many diseases (Berger, 1981). For example, Luke and Berger (1982) found that when oat crown rust (*Puccinia coronata* Cda. f. sp. *avenae* Fraser & Led.) severity was low, small increases in disease caused great increases in the logistic rate (r) compared to the changes in the Gompertz rate (k). They concluded that Gompertz transformation was more consistent than logistic transformation at detecting slow

rusting because there was less variation in k values than r values among replications, distances from infection foci and rating periods. Assessment date 1 was set as time = 0 when fitting the Gompertz curves. For each cultivar and distance, asymptote, time and level of point of inflection, and the rate k were analysed by analysis of variance.

Isopathic rates (Berger and Luke, 1979) for old nodes, based on actual data of each cultivar, were calculated by plotting the distance (m) from foci by the number of days needed for 40% disease severity at the central focus (0 m) to reach the same severity at 1.5, 3, 6, 9 and 12 m from the focus. Severity of 40% was chosen because it was close to point of inflection for Pania and Bolero. Slopes of isopathic rates were compared between the cultivars by analysis of variance.

Data of hourly rainfall, temperature (soil and air at different depths) and wind speed and direction were obtained from the Broadfield H32642 weather station, located 800 m from the field trial site.

5.3. Results

Data for wind direction during the period of the experiment are summarised in Figure 5.2. During the time from inoculation of the plots (18 Jan. 1995) to the last assessment (22 Feb. 1995), over a third of the winds were from the north-northeast, about a quarter from south-southeast and about 10% from south-southwest, north-northwest and north-northeast (Figure 5.2.). Data for the mean, minimum and maximum temperatures are summarised in Appendix IV.

Disease severity was initially graphed as a 3-dimensional response surface for each assessment, replicate and cultivar for different groups of nodes. Disease severity within the plots was variable, and there was no strong visual evidence for directional gradients.

Disease severity on the young nodes was constant at less than 4% until the last assessment (Figure 5.3.), when disease severity on Bolero reached 51%, on Pania 35% and on Quantum 21%. The disease severity was significantly different ($P<0.001$) between the cultivars 35 dai. No attempt was made to fit curves to these data since only one or two points could be used for curve fitting.

Good fits for the medium nodes (Figure 5.4) were obtained fitting exponential curves $y = e^{(a + b(time))}$ to the data, where y = disease severity, a = constant and b = rate of disease. Distance from focus was not an important factor of disease progress on medium nodes (Appendix V), and all distances were combined for analysis (Figure 5.4.). Models used for exponential growth curves were improved slightly by correcting for the distance effect. However, since gradient effects were small, this had

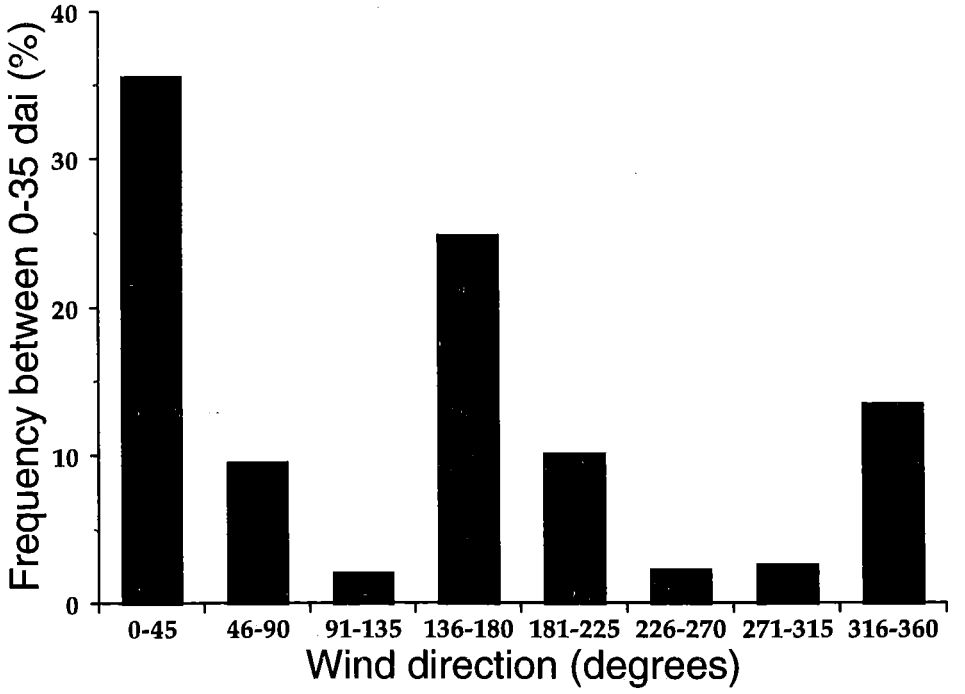


Figure 5.2. Frequency (% of total) of wind from each direction (degrees) from 18 Jan. 1995 to 22 Feb. 1995 (0 to 35 days after inoculation dai) at Lincoln. Source: Broadfield Weather Station.

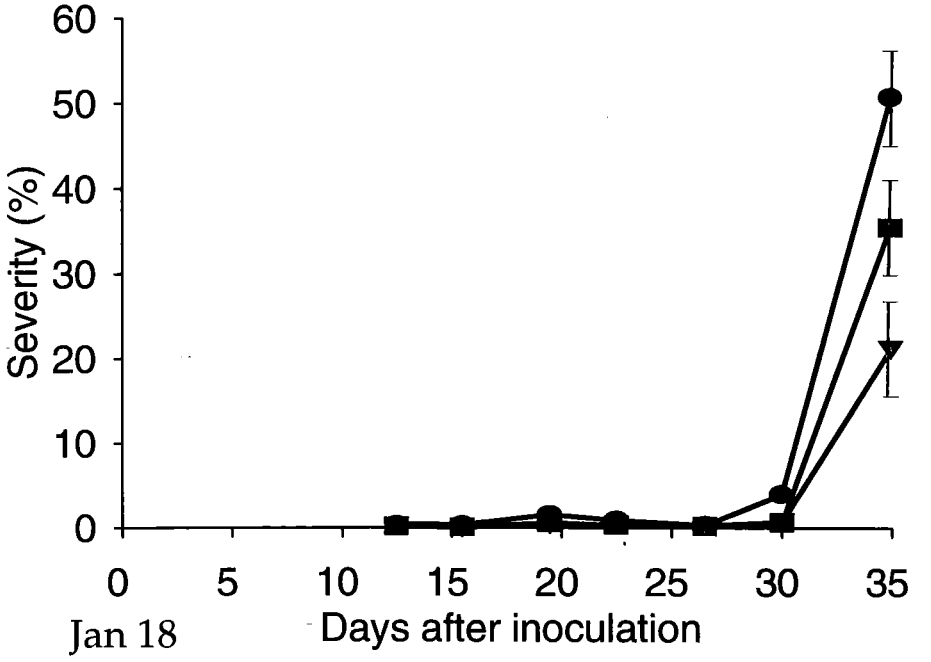
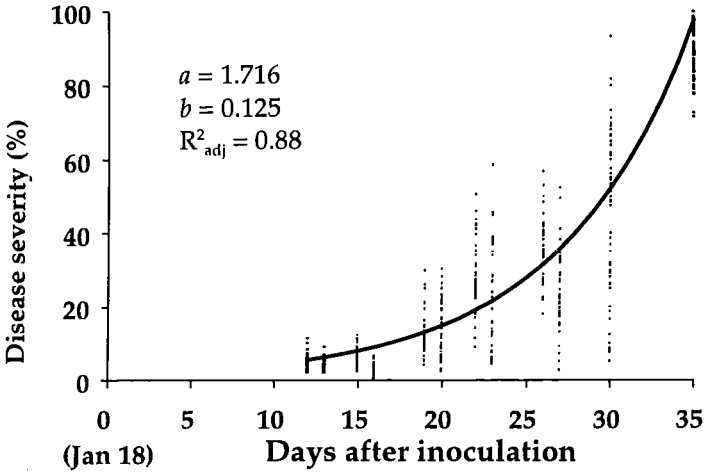
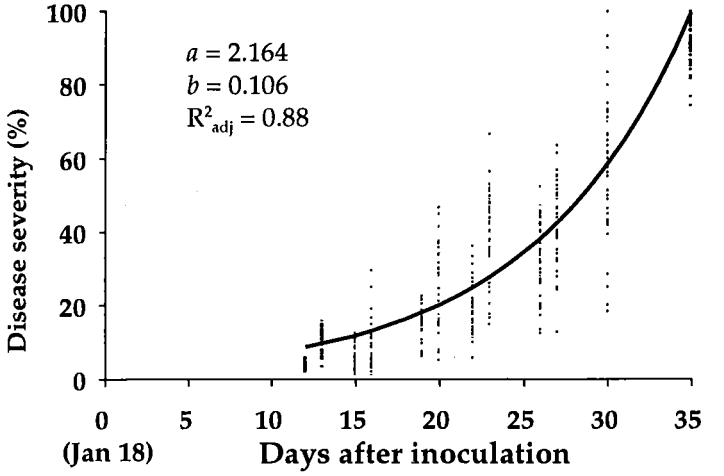


Figure 5.3. Mean disease severity over time (days after inoculation) for young nodes of Pania (■), Bolero (●) and Quantum (▼). Bars represent 95% confidence intervals.

Pania



Bolero



Quantum

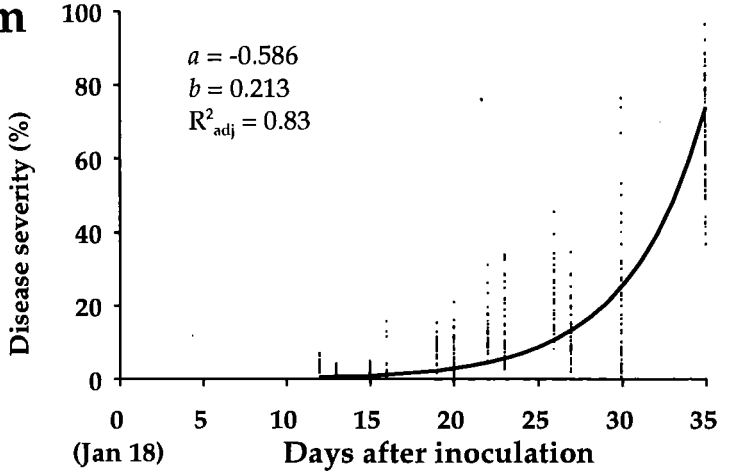


Figure 5.4. Mean disease severities fitted to exponential growth curves for medium aged nodes on Pania, Bolero and Quantum. Actual data points are shown. *a* = disease level at time=0, *b* = relative growth rate.

little effect and was considered an unnecessary complication in the analysis. The calculated mean disease level at time = 0 dai (*a*) for Pania and Bolero were 1.7% and 2.2% respectively, but for Quantum 0.6% (Figure 5.4.). Mean disease severity 12 dai was significantly (P<0.001) lower in Quantum (1.6%) than in Pania (6.8%) or Bolero (9.3%; Table 5.1). Mean relative growth rate was highest for Quantum (0.21), significantly different (P<0.001) from Pania (0.13) and Bolero (0.11; Table 5.1), although mean disease severity on Quantum only reached 68% compared with 91% for Pania and 94% for Bolero (Figure 5.4.). There were no differences in disease severity 15 dai (P=0.24) or relative growth rate (P=0.767) at different distances from the sources. There were no statistically significant interactions (Table 5.1.).

Some indication of disease gradients was observed from the three-dimensional curves (Appendix V and VI) and this was tested for old nodes only. Significant (P=0.021, 0.001 and 0.019 respectively) gradients for each cultivar (Pania, Bolero and Quantum) were observed 15 dai, but there were no differences (P=0.653) between the cultivars (Figure 5.5.). Gradients were also observed for each cultivar 20 dai, and Quantum had a steeper (P=0.004) gradient than Bolero (Figure 5.5.). No gradients were found at later assessment dates (not shown).

The Gompertz curve $y=c\times\exp(-d\times\exp(-k\times\text{time}))$ where y=disease severity, c=constant, d=constant and k=infection rate, was used to describe disease progress on old nodes. This provided good fits for the data (Figure 5.6.) so no other curves were fitted. Models used for growth curves could have been improved slightly by correcting for the distance effect, but since gradient effects were small in total disease severity, this was considered an unnecessary complication in analysis. The asymptotes (*c*) were greater (P<0.001) for Pania and Bolero (105.6% and 104.7% respectively) than for Quantum (78.2%; Table 5.2., Figure 5.6.). The estimated plateau exceeded 100% for Pania and Bolero because the sampling ceased at 35 dai after which all leaves had senesced so the full form of the sigmoid curve was not present in the data. Points of inflection occurred 18.8 and 18.3 dai for Pania and Bolero respectively, but not until 22.8 dai (P<0.001) for Quantum (Table 5.2.). The level of disease at the point of inflection for Quantum was at 28.8% leaf area infected, lower (P<0.001) than for Pania or Bolero at 38.9 and 38.5% respectively (Table 5.2). The rate value *k* was not different (P=0.654) on the different cultivars (Table 5.2.). The time of the point of inflection increased (P=0.001) with distance. At the source of infection the point of inflection was 17.7 days and this increased to 21.6 d at 12 m from the source of infection (Table 5.2.). The rate of disease progress was greatest (P=0.025) at the source of infection and greater (P=0.025) than for distances beyond 3 m from the source (Table 5.2.). There were no significant interactions between distance and cultivar (Table 5.2).

Table 5.1. Mean disease severities (% leaf area infected) at the first assessment date (12 dai) and mean relative growth rates for medium nodes of three cultivars and for six distances in field plots.

		Disease severity at assessment 1 (%)	Relative growth rate
Cultivar	Pania	6.81 a[1]	0.125 a
	Bolero	9.28 a	0.106 a
	Quantum	1.60 b	0.213 b
	P-value	<0.001	<0.001
	SEM[2]	0.908	0.0158
Distance	0	8.79	0.128
	1.5	6.16	0.134
	3	5.52	0.149
	6	5.42	0.148
	9	5.21	0.154
	12	4.28	0.174
	P-value	0.240	0.767
	SEM	1.285	0.0224
Cultivar*distance interaction P-value		0.99	0.99

[1] Letters indicate means that are different ($P \leq 0.05$) using LSD-tests.
[2] Standard error of the mean

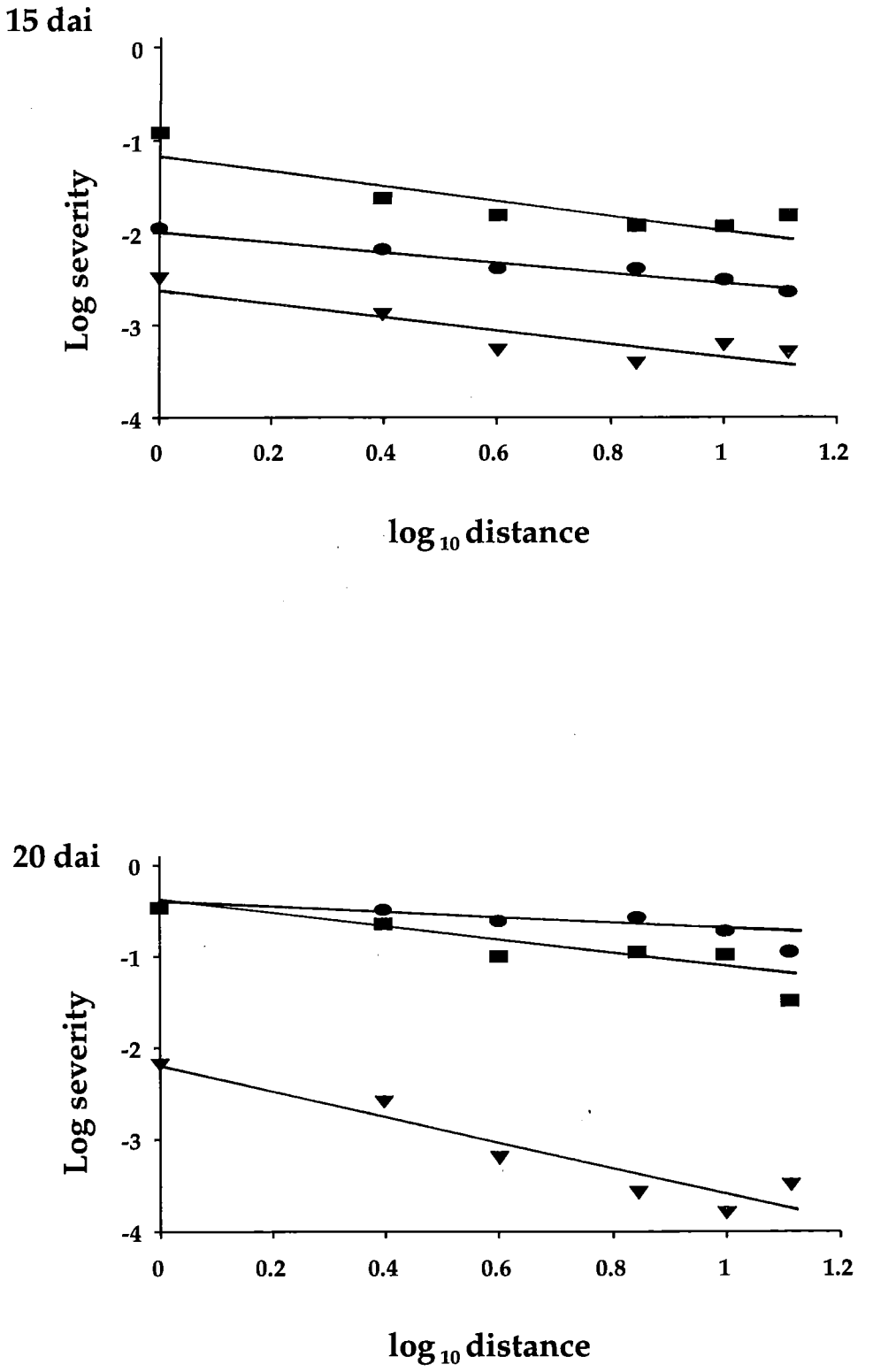
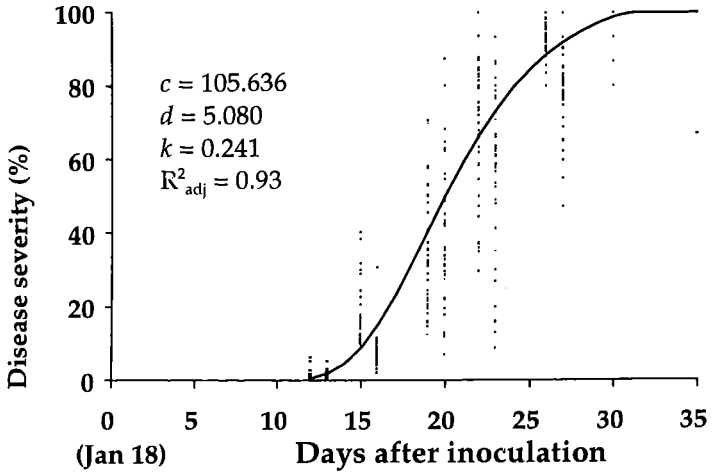
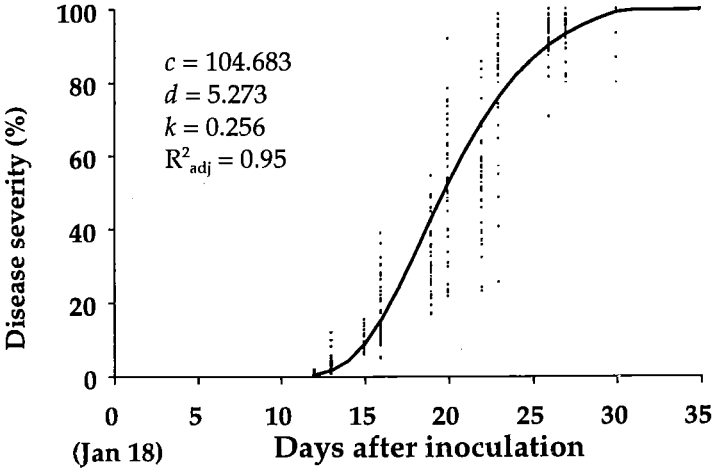


Figure 5.5. Graphs of logit mean disease severity by $\log_{10}$ distance (m) for Pania (■), Bolero (●) and Quantum (▼) 15 and 20 d after inoculation .

Pania



Bolero



Quantum

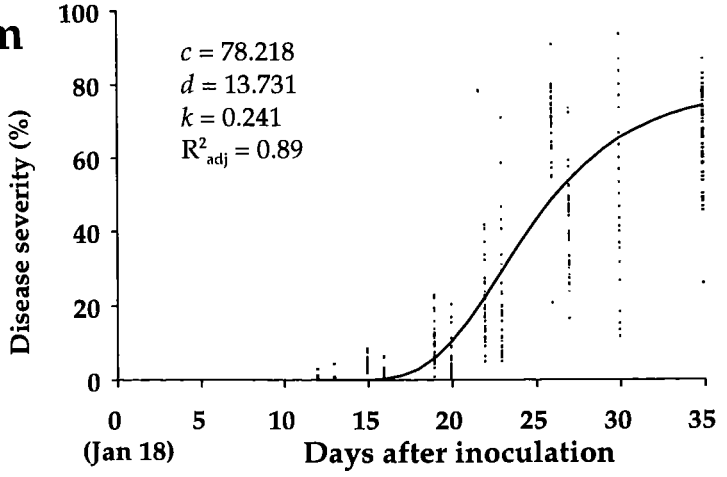


Figure 5.6. Mean disease severities over time, fitted to Gompertz equations for old nodes on Pania, Bolero and Quantum. Actual data points are shown. c = asymptote, d = constant, k = rate of disease progress.

Table 5.2. The asymptotes, points of inflection (time and level) and *k*-values for old nodes for three cultivars and six distances in field plots.

		Asymptote	Point of inflection (time d)	Point of inflection (level %)	*k*
Cv.	Pania	105.6 a[1]	18.8 a	38.9 a	0.241
	Bolero	104.7 a	18.3 a	38.5 a	0.256
	Quantum	78.2 b	22.8 b	28.8 b	0.241
	P-value	<0.001	<0.001	<0.001	0.654
	SEM[2]	1.55	0.41	0.57	0.0129
Distance	0	95.4	17.7 a	35.1	0.304 a
	1.5	93.2	19.1 ab	34.3	0.259 ab
	3	95.4	19.9 bc	35.1	0.235 b
	6	96.2	20.4 bcd	35.4	0.242 b
	9	97.8	21.1 cd	36.0	0.231 b
	12	99.1	21.6 d	36.5	0.207 b
	P-value	0.489	0.001	0.489	0.025
	SEM	2.19	0.58	0.804	0.018
Cultivar*distance interaction P-value		0.76	0.98	0.76	0.19

[1] Letters indicate means that are different ($P \leq 0.05$) using LSD-tests.
[2] Standard error of the mean

The outward spread of powdery mildew to the level of 40% severity from 1.5 m to 12 m from foci was calculated by isopathic rates, which varied between 2.23 m d^{-1} (Pania) and 4.04 m d^{-1} (Bolero) but were not different between cultivars (Figure 5.7.).

5.4. Discussion

Direction from the infection focus was not an important factor of disease progress in this experiment. The main winds in Canterbury during January and February are from the northeast, southwest and northwest. The winds during this experiment (Figure 5.2.) probably distributed the inoculum evenly in the plots, especially since some turbulence would have occurred.

The division of nodes into young, medium and old node categories was required because of considerable variability in disease severity between nodes within plants at each time point. Severities ranging from 0 to 100% were recorded when using a mean disease severity within whole plants, even though reasonable fits to progress curves were achieved. It was clear that a significant proportion of this variation could be explained by node age.

Young nodes had very little (less than 4% of total leaf area infected) disease until the last assessment. Low disease levels indicated that young nodes were produced at a greater rate than the disease could infect them. At the last assessment date, plants were near full maturity and either no new nodes were being formed or they were formed at a much slower rate than previously. For this reason the disease severity in the young nodes at the last assessment was greater than during active growth of plants.

At medium nodes, the disease severity on Quantum was lower than on Pania or Bolero for all assessment dates (Table 5.1., Figure 5.4.), but the relative growth rate was the highest for Quantum. This was mainly caused by the large increase in disease severity between assessment 6 (30 dai) and 7 (35 dai). This may be a reflection of the mean temperature, which increased on 16 and 17 Feb. 1995 (Appendix IV) and may have delayed increase in disease severity about five days later. At that time, the disease levels in both Bolero and Pania were already close to 100% so disease development on Quantum was affected most by the weather conditions. Disease epidemics are dependent on various complex factors (see Figure 1.1) and analytic models assume that most parameters are constant, a situation that only occurs in an unchanging environment (Hau, 1990).

The observed exponential disease progress on medium nodes suggested that the amount of leaf tissue was not a limiting factor. Vanderplank (1963) noted that in the early stages of an epidemic,

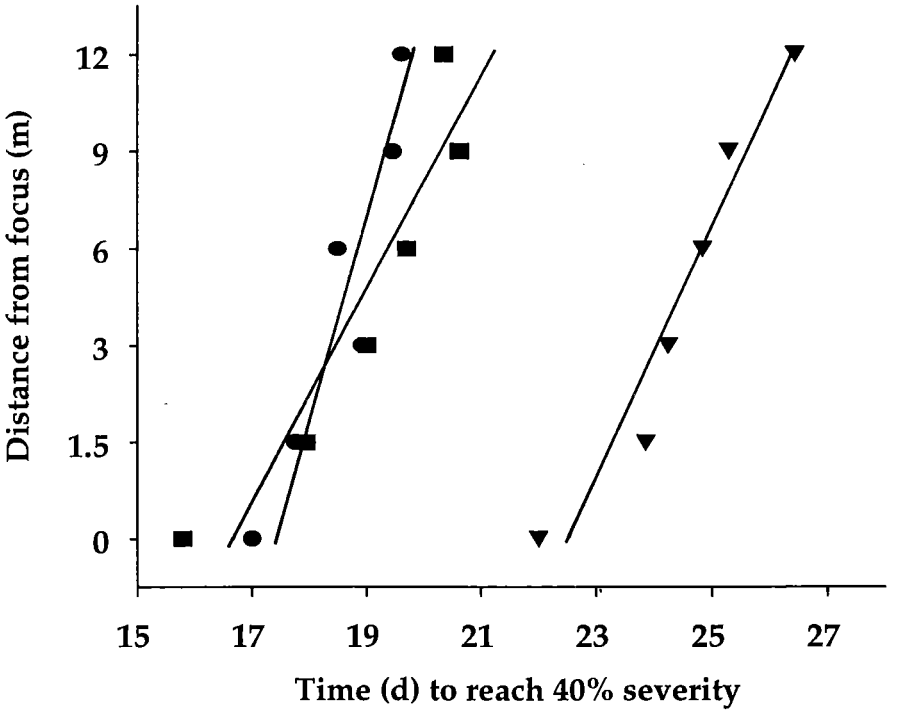


Figure 5.7. Isopathic rates, expressed by number of days needed for disease severity to reach 40% leaf area infected at 0 - 12 m from the focus of infection for Pania (■, $R^2 = 0.77$), Bolero (●, $R^2 = 0.79$) and Quantum (▼, $R^2 = 0.88$).

the development of disease usually follows an exponential curve, but there is a levelling off later. This was demonstrated by the disease progress on old nodes, which followed a sigmoid pattern (Figure 5.6.). On old nodes, disease progress on Quantum lagged behind Bolero and Pania and reached a plateau at less than 80% compared with Pania or Bolero which reached 100%. The lower disease severity in Quantum may be a reflection of the grouping of the nodes into young, medium and old. Severe powdery mildew infections speed the senescence of individual leaves, as has been observed in the glasshouse. As Bolero and Pania leaves on older nodes were heavily infected and senesced earlier than those on Quantum, the physiological age of what was defined as old nodes in Pania and Bolero plants was greater than in Quantum.

In the old nodes, the point of inflection occurred at a later date for Quantum and at a lower level than for Pania or Bolero (Table 5.2) but rate as indicated by the k value from the Gompertz curve was not different between the cultivars. The intensification of powdery mildew in time (k) was between 0.2 and 0.5 for the different cultivars. This is similar to the rate in rusts, which was reported to be between 0.3 and 0.6 (Berger and Luke, 1979; MacKenzie, 1976; Luke and Berger, 1982; Vanderplank, 1963). The outward spread of powdery mildew was delayed on Quantum compared with Pania or Bolero, but once epidemics started, the rates did not differ between the cultivars (Figure 5.7.). It would be extremely valuable if the present quantitative resistance in Quantum could be incorporated to a new line with reduced relative growth rate, improving the overall resistance.

The division of nodes into different age groups was a useful method of reducing the variability within plants. This does not imply that the disease development in the crop was discontinuous, however. It does imply the decreasing availability of leaf tissue for infection which results from the proportion of tissue already infected, and not available for infection (Vanderplank, 1963).

Although some evidence for disease gradients was observed (Figure 5.5., Table 5.2.), it was clear that other foci in plots appeared. Gradients flattened very quickly, and this was not surprising considering the fast rate of disease progress as expressed by the isopathic rate (Figure 5.7.). Additional foci were caused by interplot interference and the spread of disease within and between the plots by wind and during assessment of disease severity. Interplot interference is dependent on the gradient steepness and can be reduced by choosing square plots, increasing the plot size and spacing between the plots (Paysour and Fry, 1983). In the present experiment, disease assessment was not possible without entering the plots, but footpaths formed into crops have been used elsewhere in efforts to reduce foot traffic effects on disease spread (Vloutoglou, Fitt and Lucas, 1995). Although the plot size was large compared to those used by other workers for rusts and powdery mildews (Berger and Luke, 1979; Luke and Berger, 1982; MacKenzie, 1976; Subba Rao, Berggren and Snow, 1990) it may have not been large enough to detect the gradients.

Some background inoculum may have entered the plots from surrounding crops either prior to or during the early part of the epidemic, and influenced the disease gradients. There was some evidence of external inoculum entering the plots, which appeared as peaks in the 3-dimensional response surface early in the epidemic. Powdery mildew conidia can travel considerable distances (Butt, 1978; Pedgley, 1986) and become airborne at wind speeds as low as 0.42 m s^{-1} (Hammett and Manners, 1974). A small area of peas, some susceptible, was grown at the south end of the experimental area and even though more than 30 m of buffer separated these peas from the experimental area, some inoculum from this area may have caused additional foci to form in the plots. Inocula may have also arrived from further afield. An earlier sowing date would have reduced the sources of inocula from outside the experimental area, but the risk of unfavourable weather conditions for the development of powdery mildew epidemics is greater in earlier sowings.

Gradients flattened with time (Appendix V and VI). Several workers have reported that gradients commonly flatten with the intensification of the epidemic in time due to secondary spread of polycyclic diseases with short monocycles (MacKenzie, 1976; Gregory, 1968; Vanderplank, 1963). Disease gradients have been found to be unreliable for differentiating cultivar resistance in wheat and *P. graminis* f. sp. *tritici* (MacKenzie, 1976), wheat and *P. recondita* (Subba Rao *et al.*, 1990), oat and *Puccinia coronata* f. sp. *avenae* (Berger and Luke, 1979; Luke and Berger, 1982) and maize and *Puccinia sorghi* Schwein (Headrick and Pataky, 1988) because gradients are affected by some components of resistance but not others. However, in monocyclic diseases spread by rain-splash, such as in soybean stem canker (caused by *Diaporthe phaseolorum* (Cke. & Ell.) var. *caulivora* Athow & Caldwell), the use of disease gradients was found to indicate greater levels of cultivar resistance (Damicone, Snow and Berggren, 1990).

Based on the present study, the major effect of quantitative resistance to powdery mildew in Quantum was expressed as delayed epidemic development and lower disease severity than in Pania or Bolero. The disease progress on old or medium aged leaves, and during the early part of the epidemic, gave best differentiation between cultivars varying in susceptibility to powdery mildew. It is possible that a reduction in plot size would not adversely affect the results if gradients were not examined, so that plant breeders could test large numbers of cultivars in small areas for parental selection.

Chapter 6

General discussion

6.1. Introduction

Utilising disease resistance is an important strategy for combatting the harmful effects of plant pathogens. Disease resistance is cost-effective, easy for farmers to use, and environmentally more acceptable than reliance on pesticides (Hogenboom, 1993). Major gene race-specific disease resistance has been widely used in agriculture because of the high degree of resistance that is achieved and the ease of gene transfer and selection, but breakdown of resistance by the selection of new pathogen races in many host-pathogen interactions has meant that this type of resistance is short-lived (Parlevliet, 1992; Wolfe and Schwarzbach, 1978). Quantitative resistance reduces the rate of epidemic development in crops and therefore the severity of disease (Geiger and Heun, 1989), and is often a more durable (Johnson, 1984) alternative to race-specific disease resistance.

Pea cultivars resistant to *E. pisi*, the cause of powdery mildew, have been bred and selected in New Zealand (Goulden and Scott, 1993) since severe epidemics occurred in the late 1980s (Falloon *et al.*, 1989a), but these cultivars probably possess single, major gene resistance. There have been no reports of the occurrence of races in *E. pisi*, although it is highly likely that they occur, and a breakdown of resistance to this pathogen has been recorded elsewhere (Schroeder and Provvidenti, 1965).

This study was undertaken to identify quantitative resistance to *E. pisi* in pea seedlines and cultivars. Epidemiological and structural aspects of quantitative resistance were examined both in a glasshouse and in field experiments, with a cultivar possessing quantitative resistance, and comparing this cultivar with susceptible and resistant cultivars. A field trial was also conducted to ascertain spatial and temporal spread of an *E. pisi* epidemic in three cultivars varying in resistance to *E. pisi*. This information was used to recommend strategies for identification of quantitative resistance in parents and for selection of this type of resistance in pea breeding programmes.

6.2. Identification of quantitative resistance in cultivars and seedlines

A preliminary glasshouse study was undertaken to assess susceptibility of cultivars and seedlines to *E. pisi* and to choose cultivars for further experiments. Disease severity on cv. Quantum was less than on fully susceptible cultivars, but distinct from the more resistant cultivars (Appendix I). Quantitative resistance reduces pathogen growth in the field under normal growing conditions (Parlevliet, 1992). Disease severity in the field on cv. Quantum was intermediate between cvs Bolero and Resal (Figure 2.2.). The effect of inoculum pressure was tested by surrounding areas of Quantum with cultivars varying in susceptibility to *E. pisi.* Disease severity was greater in areas surrounded by cvs Bolero and Quantum than in areas surrounded by a resistant cultivar Resal, but only early in the epidemic on some nodes of individual plants (Figure 2.1.). This suggests that *E. pisi* conidia are easily transported from further afield and that the epidemic quickly spreads within plots.

Further experiments were carried out in an isolation plant propagator and in field plots to assess disease severities on cultivars reputedly possessing quantitative resistance. Two distinct groups of cultivar response to *E. pisi* were found, suggesting that the mechanism of resistance was different in the two groups and/or that there had been no previous selection for intermediate resistance. Cultivars that were in the low disease severity group in laboratory conditions, had no disease in the field. There was a continuous range of susceptibility within the susceptible group in the field, suggesting that quantitative resistance operated in that group.

The implications of these experiments were that cultivars and seedlines possessing quantitative resistance exist, and these could be used as parents in breeding programmes for production of quantitatively resistant cultivars. This led to using cv. Quantum and susceptible cvs Pania and Bolero to assess whether simple variables related to epidemic progress, and/or structural interactions could be assessed in controlled environment conditions to identify the mechanism of quantitative resistance and to be used to select suitable parents or progeny in breeding programmes.

6.3. Epidemiological aspects of quantitative resistance

Three main components of the infection process which may affect epidemic development and final disease severity are infection efficiency, rate of colonisation, and propagule production by the colonies (Parlevliet, 1992). In order to select parents for quantitative resistance, it may be possible to measure one of these components that satisfactorily represents this type of resistance in the field, at least with some host-pathogen interactions. Experiments in controlled conditions were carried out to assess whether these components were important in the *E. pisi* - pea interaction.

The major epidemiological components of quantitative resistance in Quantum when compared with Pania were reduced infection efficiency, reduced maximum conidium production per day, and increased time to maximum conidium production. Fewer infections per unit area (Table 3.4.) caused less leaf area to be infected and therefore lower maximum daily conidium production. Reduction in the number of colonies has also been an important component of quantitative resistance to *E. graminis* in oats (Jones, 1978) and barley (Asher and Thomas, 1983; Heun, 1986; Newton, 1990).

The reproductive potential of pathogens is of great importance in the spread of epidemics, and a high propagule production per unit area of host tissue over a period of time (as short as possible), measured by latent period and the amount and rate of conidium production, is essential for rapid infection of new host tissue (Parlevliet, 1992). The maximum daily conidium production per unit colony area was reduced (Table 3.8.) and it took 3.5 d longer (Table 3.9.) to achieve in Quantum than in Pania. Time to maximum daily conidium production was a better measure of delayed conidium production than latent period (Table 3.5.), because, in this study, latent period measured the number of days when the first conidium was produced on each leaf, and did not consider the proportion of colonies producing conidia at that time. A long latent period has been found to be an important component of quantitative resistance in many host-pathogen interactions, including leaf rust on barley (Parlevliet and van Ommeren, 1975; Neervort and Parlevliet, 1978) and wheat (Denissen, 1993). However, it was less important in rice blast (caused by *M. grisea*; Roumen and de Boef, 1993) and in barley powdery mildew (*E. graminis* f. sp. *hordei*; Asher and Thomas, 1984).

The total conidium production per unit colony or leaflet area was not significantly affected (Table 3.7.), due in part to the strong interaction between conidium production per colony and colony density. This has also been demonstrated for the wheat/*P. recondita* f. sp. *tritici* (Baart *et al.*, 1991) and oat/*E. graminis* f. sp. *avenae* interactions (Carver and Ingerson-Morris, 1989). It was important to assess the amount of conidium production per colony area, because the larger leaves on Quantum (Figure 3.3.) supported more total conidium production.

Bolero also showed quantitative resistance in laboratory experiments, but in different components to Quantum. Bolero had a reduced rate of colony expansion and produced fewer conidia per unit colony area than Pania (Table 3.7), but the maximum daily conidium production did not differ from Pania (Table 3.8.). The most important difference between Quantum and Bolero was a shorter time to maximum daily conidium production in Bolero (5 days less) than in Quantum (Table 3.9.). The conidia that are produced early in an epidemic are often more important in furthering the epidemic than those produced later because it is these conidia that start the next monocycle (Parlevliet, 1992). However, after a few monocycles, the number of conidia produced will become the limiting factor in furthering the epidemic rather than the time to production.

The differences between Quantum and susceptible cultivars observed in controlled conditions became more evident in the field (Chapter 5). Quantum had considerably less disease, measured as a proportion of leaf area infected, and a delayed rate of disease progress compared with Pania or Bolero, and this was consistent for leaves on all parts of plants (Figure 5.3., 5.4. and 5.6.). It was not clear why disease severity on Quantum never reached the same proportion as that on Pania and Bolero, although it was suspected that leaf and plant senescence occurred before the disease severity reached 100%.

The quantitative resistance observed on Bolero in controlled conditions was not present in the field. The main reason for this was probably the shorter period to maximum daily conidium production in Bolero than in Quantum, which supports the theory that conidia produced early in the epidemic are more important than those produced later. It is also possible that the quantitative resistance in Bolero observed in controlled conditions is conditioned by a quantitative, major, race-specific gene (or genes) that lose effectiveness in the field when exposed to a diverse pathogen population. The effects of vertical (Vanderplank, 1963) resistance can sometimes be quantitative, and this has been observed commonly with rusts and powdery mildews (Robinson, 1987). Another possibility is that Bolero possesses some quantitative resistance characteristics, but the effects of these were negated by the short time to maximum conidium production in that cultivar.

Leaves were separated into those occurring on young, medium and old nodes due to variability in disease severity within the plants. An exponential growth curve for the mean disease severity was fitted for medium nodes, and a Gompertz curve was fitted for the old nodes. Some distance gradients from the source of infection foci were evident in the field experiment, but these were small compared to the total disease severities in the plots (Figure 5.5., Appendix V and VI). Gradients were not observed late in the epidemics, and this was explained by the fast rate of disease progress as expressed by the isopathic rate (Figure 5.7.), and possibly by inoculum introduced from external sources.

Possible effects on conidium germination was not an important aspect of quantitative resistance in Quantum (Section 3.3.1.). This finding is consistent with other studies of *E. pisi* conidia on peas (Singh and Singh, 1983), and *E. graminis* on cereals (Carver and Adaigbe, 1990; Douglas, Sherwood and Lukezic, 1984; Wright and Heale, 1984), but some reports have indicated that host genotype affects germination of *Erysiphe* spp. conidia (Mukhopadhyay and Russell, 1979). Germination of conidia is affected more by environmental conditions, such as humidity and temperature, than by the host genotype (Carver and Adaigbe, 1990; Manners and Hossain, 1963).

6.4. Structural aspects of quantitative resistance

Fungi causing powdery mildew diseases are biotrophic, which form haustoria, complex host-pathogen interfaces, which enable the flow of nutrients from host to pathogen (Manners and Gay, 1978). Haustorial function is affected by the number, size and efficiency of haustoria. The efficiency can be measured in terms of the amount of mycelium or the size of colonies produced. Haustorial efficiency and frequency were measured on Quantum, Pania and Bolero (Chapter 4). The genotype did not affect colony size or the frequency of haustoria. Electron microscope studies suggested that haustoria in leaves of Pania plants were larger by volume, and contained more lobes, than haustoria on leaves of Quantum. The mean ratio of surface area of haustorial plasmalemma to the surface area of extrahaustorial membrane was 2.68 for haustoria in leaves of Pania plants and 1.23 for haustoria in Quantum leaves. This indicates a greater degree of lobing in Pania haustoria than in Quantum haustoria, and possibly greater uptake of nutrients. Although the sample size in this experiment was rather limited due to the laborious techniques used, and the degree of lobing has elsewhere been correlated with age of haustoria (Gil and Gay, 1977), it nevertheless suggested that the size of haustoria could affect the amount of uptake of nutrients to the fungus. This in turn could affect the amount of sporulation, and ultimately the expression of quantitative resistance. This aspect should be investigated further.

6.5. Effects of leaf and plant age on quantitative resistance

Plant parts vary in resistance to a pathogen with age, and with physiological age of the plant (Populer, 1978). Quantitative resistance is often expressed more in adult plants than in seedlings (Aist and Bushnell, 1991). The effects of leaf and plant age on germination, infection efficiency, colony size, and the number of haustoria per unit colony area were investigated in this study. There were no interactions between host genotype and plant or leaf age in any of the components tested. This suggests that quantitative resistance in peas to *E. pisi* is not correlated to the age of leaves or plants. However, differences in some of the components were found on all genotypes. Plant age did not affect any of the components tested, but resistance increased with increasing leaf age when measured in terms of germination of conidia (Table 3.3.), size of colonies (Section 4.3.1.) and numbers of haustoria (Section 4.3.2.). There were more germinating conidia, more haustoria, and the colonies were larger in the most recently emerged leaflets than in leaflets between 15 and 30 d old. Resistance also increased with leaf age in barley powdery mildew (*E. graminis* f. sp. *hordei*; Nelson *et al.*, 1989), in rice leaf blast (*M. grisea*; Roumen, 1992) and bean rust (*U. appendiculatus*; Shaik and Steadman, 1989). Aked and Hall (1993) suggested that 6th leaf pairs of peas did not form as many or as large

cells as the 7th leaf pairs, or that there was expansion of the intercellular volume of the leaves as they aged. This could account for some of the observed differences in the present study.

Plant form and plant growth habit has to be taken into consideration when assessing quantitative resistance, especially in the field where quantitative resistance may be wrongly attributed to the differences in the time cultivars have been exposed to pathogens. Resistance is often overestimated in late developing cultivars (Parlevliet, 1992). For this reason, the time of assessment is also important. The differences in quantitative resistance between cultivars may not be distinguished or may be underestimated if assessment is carried out too early. The most appropriate time to assess quantitative resistance is when the most susceptible cultivar approaches the maximum assessment score (Parlevliet, 1992). This study has confirmed that in the cultivars tested this occurred 28-30 dai on older leaves, but the point of inflection was detectable even earlier (Table 5.2.). This information could be used to reduce sampling times in field assessments to identify quantitative resistance.

Quantitative resistance is also affected by plant habit, such as tallness and density of crop (Parlevliet, 1992). Plant habit was not taken into consideration in the conidium production experiments carried out in the present study. Questions such as how quickly new leaves were formed, or do the size and duration of leaves also affect the final number of leaves produced, were not considered, and these also warrant further investigation.

6.6. Effects of environment on the expression of quantitative resistance

Environmental effects on quantitative resistance were tested in the conidium production experiments. Cultivar × temperature interactions occurred for the mean total conidium production per colony area (Figure 3.4.), and for the time to maximum daily conidium production per colony area (Figure 3.12.). Mean total conidium production on Quantum was not affected by the highest temperature tested, whereas there was a temperature effect on total conidium production on Pania and Bolero. However, the effect on time to maximum daily conidium production could be distinguished better at 13°C than at 19 or 23°C. Low temperatures (12°C) have also been recommended for distinguishing differences in quantitative resistance between genotypes of wheat to leaf rust (*P. recondita* f. sp. *tritici*; Denissen, 1991).

6.7. Applications to breeding for quantitative resistance to *E. pisi* in peas

The present study has identified that the most important components of quantitative resistance in Quantum were infection efficiency, maximum daily conidium production and time to maximum conidium production, and possibly structural differences in haustoria. The measurement of infection efficiency was relatively easy and less labour intensive than the measurement of conidium production, and could, therefore, be applied to a greater number of genotypes to be tested. It is unlikely that component selection would be used in selection for quantitative resistance due to costs in time and labour, but these measures could be used in the identification of superior parental material. Investment in good parental material can be worthwhile, especially if a large number of potential parent lines is available and if the resistance proves durable. Other factors determining whether this approach is appropriate are the heritability of quantitative resistance in the field, the amount of variation shown by the component, heritability of the component, and costs associated with the selection (Parlevliet, 1992). It is potentially more efficient to match a parent with different (and many) components of quantitative resistance and then select for the component or total expression of quantitative resistance, than to cross lines randomly when many of the crosses may be of similar genotypes, and therefore may contain the same component of quantitative resistance instead of accumulating several. In many host-pathogen interactions, components have not been identified, which this study has achieved that for *E. pisi* and pea interaction.

Selecting for improved levels of quantitative resistance alone is not appropriate. The aim of plant breeding is to improve several characters simultaneously to produce genotypes with good agronomic value of no lower quality than previously (Johnson, 1992).

6.8. Conclusions and suggestions for future work

In the present study, quantitative resistance was confirmed in Quantum in glasshouse and in field experiments (Chapter 2), other cultivars and breeding lines possibly possessing quantitative resistance were identified (Chapter 2), some epidemiological (Chapter 3) and structural (Chapter 4) components of quantitative resistance in Quantum were identified (Chapter 3), and these were related to epidemic development in the field (Chapter 5). The following questions remain:

Is reduced infection efficiency, reduced maximum daily conidium production, and a longer time to daily maximum conidium production stable and heritable? Genetic studies are needed to assess the heritability of these components. Are there additional components of quantitative resistance in other

cultivars that were not detected in Quantum? More cultivars and seedlines (identified in Chapter 2) need to be tested to identify other components.

Do races of *E. pisi* exist in New Zealand, and is resistance in presently cultivated cultivars likely to 'break down'? Molecular markers could be used to define whether sexual reproduction occurs and whether races of *E. pisi* exist.

Is it possible to assess conidium production in a less labourious and time-consuming way? Image analysis techniques suggested by Kampmann and Hansen (1994) could be employed to assess conidium production on different cultivars.

Is it possible to use molecular markers to identify specific genes linked to characters of quantitative resistance, eliminating labourious testing of components? Appropriate techniques are in the process of development and molecular markers could be used in the near future.

How important is efficiency of haustoria in quantitative resistance? Less labourious methods, such as confocal laser microscopy (Kwon *et al.*, 1993) or the use of potentiometric cyanine dyes (Mengden and Nass, 1988) are required to assess efficiency of haustoria.

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Appendix I

Preliminary experiment: identifying quantitative resistance in seedlines and cultivars in a glasshouse

Seed of 24 seedlines of garden peas were sown into 14 cm pots (two per pot; three pots of each seedline) in a mixture of sterilised washed bark and sand (55:45) and placed in a glasshouse unit at 22°C (± 4°C) with additional illumination to achieve 16 h day. When seedlings were at GS 106 - 107 (vegetative, 6-7 nodes) on 19 Mar. 1993, the plants were inoculated by shaking pea plants heavily infected with *E. pisi* over them. The severity of powdery mildew at each node was assessed 1, 2 and 3 weeks after inoculation using a disease severity key (Falloon *et al.*, 1995). The experiment was repeated with 12 cultivars, three of which were not used in the first experiment. Data from the two experiments were pooled for analysis of variance, and cultivars were ranked in order from the most susceptible to most resistant according to disease severity at the last assessment.

Tere, Pania and Piri were the most susceptible cultivars and Tripod was the most resistant cultivar (Table i). The range of disease severities was more even in this experiment than in Experiments 2 and 3 (Section 2.3.2. and 2.3.3.). This was probably because of movement of conidia between plants, as the plants were in a glasshouse unit rather than the plant propagator, which prevented drift of conidia between plants. Disease severity on Quantum was 21.3 % at the last assessment date, and this was a third of the disease severity on Tere. Disease severity on Novella II was the closest to disease severity on Quantum, but this was somewhat misleading as the population was segregating into totally resistant or fully susceptible plants. Novella II was therefore considered an unsuitable candidate for quantitative resistance without further breeding. PI 142777 had disease severity of 10.5 %, but this cultivar was not agronomically suitable cultivar for further field testing, because it has very long internodes.

Table i. Mean powdery mildew disease severity (proportion of leaf area infected) for whole plants of 28 cultivars assessed 7, 14 and 21 days after inoculation (dai).

Cultivar	7 dai	14 dai	21 dai
Tere	21.9	65.0	63.9 a[3]
Pania	12.6	59.9	63.0 ab
Piri	15.7	58.8	61.7 abc
Greenfeast	23.3	43.9	53.3 abcd
Bolero[2]	5.4	16.0	44.5 de
79467	10.7	45.4	38.4 ef
Quantum[2]	3.3	14.5	21.3 g
Novella II*	2.8	10.8	14.7 gh
PI 142777	3.6	7.3	10.5 ghi
Somerset	3.1	5.3	9.6 ghi
PI 201497	3.5	5.3	8.4 ghi
Bounty	4.7	6.3	8.2 ghi
Tasman	1.6	6.5	7.5 ghi
Mariner	2.7	5.1	6.9 ghi
Headliner	2.8	3.9	5.9 hi
Almota	5.2	4.2	5.8 hi
Parlay	3.7	5.7	5.6 hi
Spartan	1.0	6.6	5.5 hi
Horizon	1.6	3.3	4.7 hi
Trounce	1.5	3.9	4.5 hi
PI 185183	2.7	3.6	3.5 hi
Sounder	0.7	4.1	3.5 hi
87/19L	3.0	3.0	3.1 hi
HTPMRPVRR	1.1	1.6	2.2 hi
Resal[2]	0.2	0.7	2.0 hi
OSU-65	0.3	1.5	1.9 hi
87/18	0	0.6	1.3 hi
Tripod	0.4	0.7	0.6 I

[1] segregating population
[2] included in the second experiment only
[3] Lowercase letters indicate means that are different ($P \leq 0.05$) using LSD-tests.

Appendix II

Weather summaries for Experiment 1 (Chapter 2)

Weather summaries for the experimental site during the field experiment (10 Jan. - 6 Apr. 1993). Canopy temperature was measured 20 cm above the soil level within the canopy. Soil temperature (Tsoil10) was measured at the depth of 10 cm below soil level in one of the experimental plots. Relative humidity (RH) was calculated from dry and wet bulb temperatures measured in a Stevenson screen 35 cm above the soil level in the centre of the experimental area. Rainfall and evapotranspiration (ET) values were measured at Broadfield weather station situated 500 m from the experimental site.

		Canopy						
	Date	Tmax	Tmin	Tmean	Tsoil10	RH	Rain	ET
Jan.	10	26.7	9.2	18.1	16.3	55.7	0.0	5.3
	11	22.3	12.0	17.8	19.1	59.8	0.0	6.5
	12	25.4	8.8	16.8	18.1	60.7	0.0	5.4
	13	16.1	11.2	13.5	17.6	75.8	1.2	2.4
	14	17.8	10.6	13.5	17.3	79.3	0.0	4.0
	15	24.8	13.3	17.5	18.8	61.2	0.0	5.0
	16	18.1	10.5	13.7	17.6	79.6	3.6	3.5
	17	25.2	10.3	18.8	19.1	58.6	0.0	7.6
	18	32.7	19.9	25.8	23.6	42.3	1.0	8.0
	19	19.8	13.5	16.0	19.8	70.2	0.0	3.0
	20	31.1	11.9	20.1	21.8	53.6	0.0	7.7
	21	22.0	13.2	17.8	20.8	65.2	0.0	6.0
	22	22.3	10.9	17.2	21.3	63.5	1.2	5.4
	23	18.5	6.2	13.1	19.4	71.9	0.2	4.2
	24	20.8	11.8	15.5	19.7	69.7	0.0	5.0
	25	21.8	12.4	17.5	17.5	66.1	6.2	3.9
	26	20.9	9.6	14.0	18.4	78.9	8.2	3.7
	27	16.3	6.3	11.8	15.4	78.7	0.0	3.5
	28	19.4	10.3	13.7	17.7	73.6	0.0	5.8
	29	20.4	10.8	15.3	15.7	79.2	0.0	6.5
	30	21.6	7.0	12.9	16.0	87.1	6.6	2.1
	31	17.6	4.3	11.0	14.2	71.6	0.0	4.0
Totals and means		21.9	10.6	16.0	18.4	68.3	28.2	108.5
Long term (1975-1991) means for January		22.6	11.4	17.0			50.3	

	Date	Tmax	Tmin	Tmean	Tsoil10	RH	Rain	ET
Feb.	1	20.4	4.0	10.8	14.1	75.8	3.4	3.8
	2	15.5	4.0	9.8	7.3	71.2	0.0	4.4
	3	18.5	3.9	10.4	13.8	72.4	0.0	3.8
	4	23.2	10.4	17.1	15.3	55.9	0.0	6.2
	5	22.4	11.7	16.8	17.5	62.4	0.0	5.6
	6	20.2	2.6	12.8	16.9	67.8	0.0	4.3
	7	19.8	9.4	14.6	17.6	76.2	0.0	5.5
	8	31.6	11.8	18.4	19.3	72.5	0.0	6.2
	9	21.6	12.8	16.3	19.8	73.4	0.0	3.4
	10	18.3	11.6	14.2	18.1	79.9	24.4	1.5
	11	15.0	10.7	12.4	15.9	93.8	1.6	1.5
	12	19.7	11.6	14.9	16.7	86.3	0.0	3.9
	13	20.9	11.4	15.6	17.1	86.0	3.2	4.2
	14	14.4	6.8	11.1	15.3	80.4	0.0	1.5
	15	18.4	3.0	11.4	15.2	77.8	0.0	4.0
	16	18.2	5.9	13.0	15.2	76.3	0.0	5.1
	17	19.6	12.2	15.4	17.2	71.7	0.0	5.6
	18	20.2	8.2	14.7	17.6	81.5	0.0	4.4
	19	20.9	7.7	14.8	17.5	78.3	0.4	3.6
	20	15.3	11.9	14.0	16.7	94.3	10.8	1.8
	21	20.1	14.7	16.3	17.1	95.9	0.0	3.0
	22	19.4	14.9	16.5	17.5	86.8	0.2	2.9
	23	19.4	13.7	16.1	17.3	86.2	0.0	3.3
	24	19.1	15.2	16.8	17.7	91.8	0.0	4.1
	25	30.4	15.1	21.5	18.9	67.2	0.0	5.2
	26	23.9	10.8	17.8	19.4	75.6	0.0	4.3
	27	22.6	9.7	16.7	19.5	80.0	0.0	3.6
	28	20.0	14.9	16.5	19.1	82.2	0.0	3.4
Totals and means		20.3	10.0	14.9	16.8	78.6	44.0	110.1
Long term means for February		21.7	11	16.3			51.3	

	Date	Tmax	Tmin	Tmean	Tsoil10	RH	Rain	ET
Mar.	1	27.4	14.0	19.4	19.7	76.0	0.0	5.5
	2	25.2	14.8	19.3	19.1	68.5	0.2	3.0
	3	23.6	16.0	18.5	18.5	66.7	0.6	4.0
	4	23.5	11.5	17.0	17.6	60.3	0.0	4.4
	5	23.9	10.6	16.2	17.9	74.0	0.0	4.6
	6	16.2	8.8	12.5	16.7	81.8	2.4	1.7
	7	17.5	5.7	11.9	15.3	75.0	0.0	2.6
	8	18.1	7.8	12.5	15.6	68.2	0.0	2.7
	9	17.1	9.4	12.5	15.7	73.9	0.0	2.4
	10	16.8	11.4	13.4	15.8	67.5	0.0	3.6
	11	22.9	12.4	16.9	16.7	63.2	0.0	4.9
	12	17.2	7.2	14.4	16.9	67.9	0.0	1.2
	13	19.7	4.8	12.1	15.6	77.6	0.0	4.2
	14	21.3	4.6	12.7	16.1	77.9	0.0	3.9
	15	19.0	5.3	12.6	15.9	80.9	0.0	4.3
	16	23.2	12.9	15.9	16.9	81.1	0.0	3.4
	17	24.2	11.1	16.2	17.2	70.1	7.8	3.3
	18	13.9	6.7	10.4	14.4	72.7	6.2	3.4
	19	15.5	6.8	11.3	13.0	79.4	0.0	3.2
	20	16.2	9.9	12.0	13.9	83.4	0.2	2.4
	21	13.6	5.0	9.0	12.4	87.2	8.4	-0.2
	22	13.3	4.7	9.0	9.9	66.0	0.0	2.8
	23	16.2	2.7	10.3	11.5	80.3	0.0	2.9
	24	17.9	5.0	11.9	13.0	81.8	0.0	3.1
	25	13.8	6.8	10.2	12.5	93.1	2.8	0.9
	26	20.2	6.6	12.0	12.8	77.3	0.0	2.9
	27	18.9	3.6	11.3	13.2	78.5	0.0	-2.4
	28	13.3	3.0	8.5	11.9	78.6	0.0	1.1
	29	15.7	8.9	11.3	12.7	75.5	0.8	3.4
	30	15.6	12.1	13.0	13.4	89.5	5.6	1.9
	31	13.6	9.8	11.8	13.4	97.9	27.6	0.4
Totals and means		18.5	8.4	13.1	15.0	76.5	62.6	85.5
Long term means for March		20.1	9.9	15.0			58.9	

	Date	Tmax	Tmin	Tmean	Tsoil10	RH
Apr.	1	14.2	8.6	10.4	12.1	81.4
	2	14.8	4.8	10.3	11.2	79.9
	3	16.2	7.4	12.4	12.1	86.1
	4	21.9	12.2	15.6	13.6	80.2
	5	21.7	10.7	15.8	14.1	72.6
	6	20.9	7.2	12.9	13.7	83.6

Appendix III

Mean number of nodes and growth stage in Experiment 1 (Chapter 2)

Mean numbers of nodes, and growth stages for plants in Experiment 1 (Chapter 2) at various dates during the growing season.

Date	Mean number of nodes	Growth stage	
15 Jan.	1.8	Germination Vegetative	004 101 - 103
22 Jan.	3.3	Vegetative	102 - 104
29 Jan.	4.5	Vegetative	103 - 106
4 Feb.	5.7	Vegetative	104 - 107
12 Feb.	8.1	Vegetative	105 - 110, 201
18 Feb.	10.2	Enclosed bud Bud emergence	201 - 203
24 Feb.	11.8	Open flower Immature pod	202 - 204
1 Mar.	13.1	Immature pod Flat pod	203 - 205
5 Mar.	13.5	Flat pod	203 - 206
11 Mar.	13.9	Pod swell Pod fill	204 - 207
16 Mar.	14.5	Pod fill	204 - 208
22 Mar.	14.1	Pod fill Wrinkled pod	205 - 208
1 Apr.	14.3	Wrinkled pod	205 - 209

Appendix IV

Temperature data for field experiment (Chapter 5)

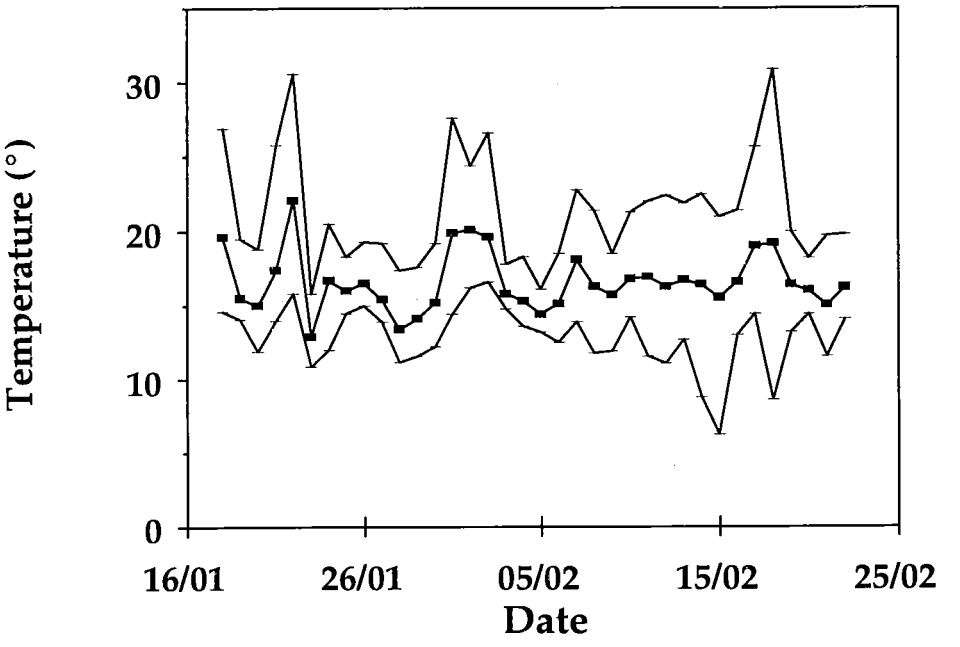

Figure i. Daily mean, minimum and maximum temperatures for the duration (18 Jan. - 22 Feb. 1995) from inoculation to the last disease severity assessment of field experiment (Chapter 5).

Appendix V

Mean disease severity in time and by distance for medium nodes on three pea cultivars in the field

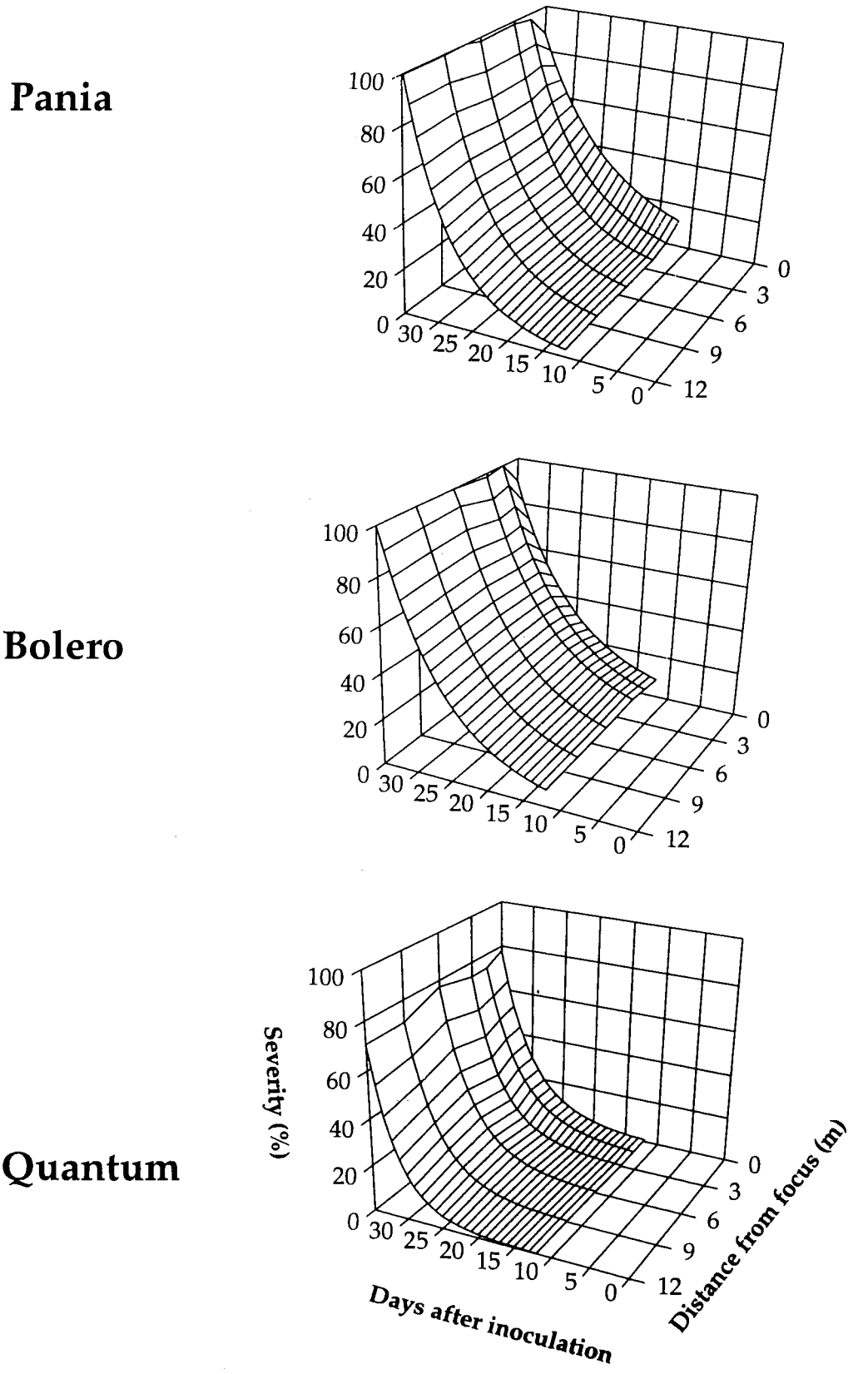

Figure ii. Disease severity of medium nodes in time and by distance for Pania, Bolero and Quantum as response surfaces. Data used are from fitted curves.

Appendix VI

Mean disease severities in time and by distance for old nodes on three pea cultivars in the field

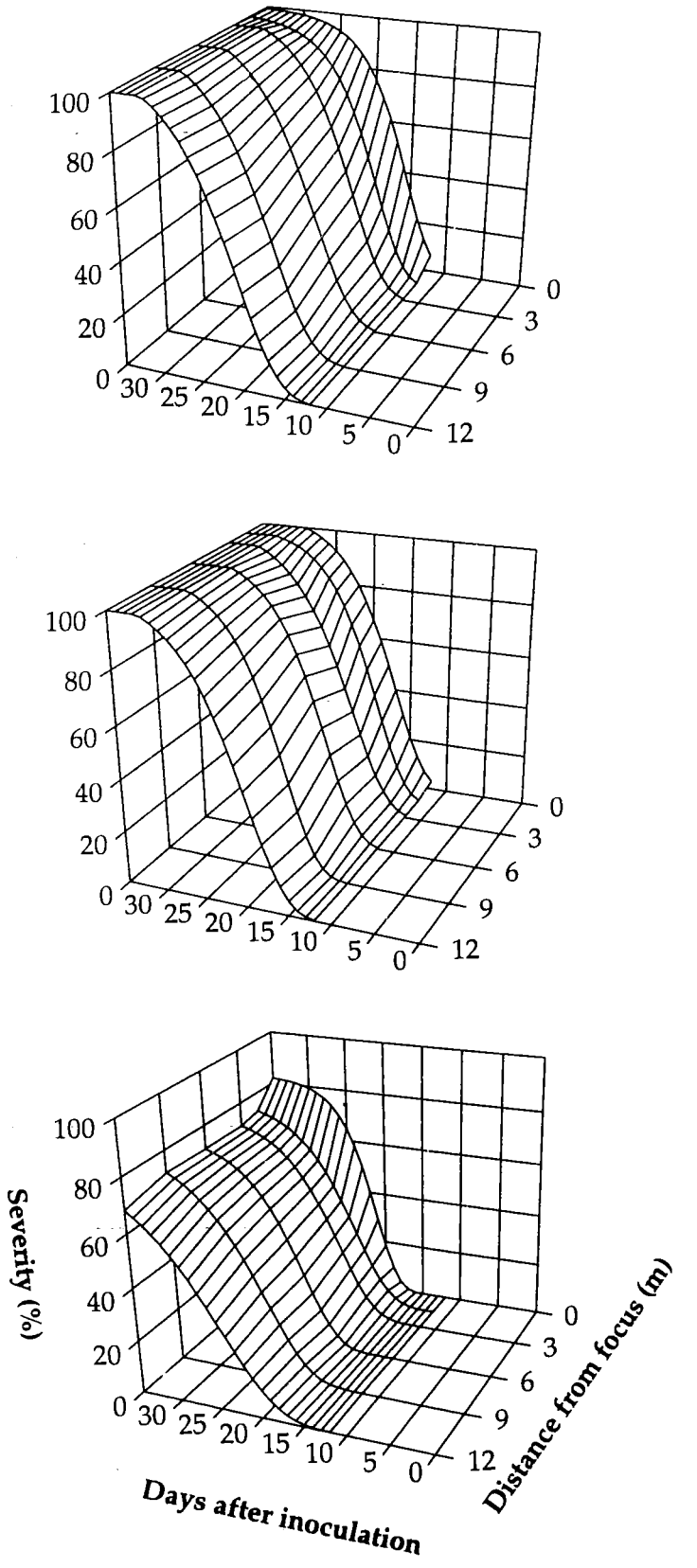

Figure iii. Mean disease severities for old nodes over time and by distance for Pania, Bolero and Quantum as response surfaces. Data used are from fitted curves.