Epidemiology and management of *Sclerotinia sclerotiorum* (Lib.) de Bary in kiwifruit (*Actinidia deliciosa* (A. Chev.))

This thesis is presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in Plant Science at
Massey University, Palmerston North,
New Zealand.

Stephen Mark Hoyte
September, 2012March, 2001

Abstract

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson, var. *deliciosa* cv. 'Hayward'), a valuable fruit grown in New Zealand orchards for export, is susceptible to the necrotrophic and cosmopolitan fungus S*clerotinia sclerotiorum* (Lib.) de Bary. Sclerotinia disease causes direct crop loss in the form of diseased fruitlets, scarring of fruit and field rot of fruit. Costs to the industry amounting to an estimated $5 million pa. are incurred through crop loss on vines or during grading and through the purchase and application of fungicide.

This study showed that 19% (range 0–53%) of 3–4 day-old kiwifruit petals were colonised by *S. sclerotiorum* ascospores arising from apothecia that were present within most orchards. Adhering floral tissues (AFT) were present on 38% of fruit 7–8 weeks after anthesis and 11% (range 0–43%) of these were colonised by *S. sclerotiorum*. The incidence of fruit with scarring was significantly higher on fruit with AFT than fruit without AFT. Removal of AFT 10–14 days after anthesis resulted in a 66% reduction in diseased fruitlets and a 85% reduction in fruit with scarring. Adhering floral tissues on fruit were therefore determined to be a major source of secondary spread.

An *in vitro* assay was developed in which freshly detached kiwifruit petals were inoculated with dry *S. sclerotiorum* ascospores in a settling chamber and incubated for 72 h. The number of discrete colonies which formed on selective agar medium, when macerated petal tissue was spread on the agar, effectively quantified petal colonisation. Colonisation of petals significantly increased with increasing flower age. Colonisation of petals incubated under static conditions within saturated salt chambers was highest between 18–27^{o}C and 90–100% relative humidity. Colonisation of petals in dynamically controlled environment chambers was inhibited by incubation in diurnally fluctuating temperature and relative humidity conditions typical of days with <5 mm rainfall during flowering. The minimum, maximum and optimum temperatures for mycelial growth on PDA and percentage germination of ascospores on water agar (5^{o}C, 34^{o}C and 23^{o}C respectively) were similar to those for the rate of colonisation of petals by ascospores at 100% RH.

Symptoms of diseased fruitlets, fruit scarring and field rot were reproduced in field inoculation experiments, provided free moisture was present for at least 9 h. Sclerotia

were extracted from diseased fruitlets and fruit with field rot from three vines, yielding 2.7 and 18.6 sclerotia per unit respectively. Diseased fruitlets produced significantly smaller sclerotia, which had a higher germination rate and produced smaller apothecia, compared with sclerotia from fruit with field rot. External damage on 39% of sclerotia from fruit with field rot and their closer contact with soil micro-organisms during formation and maturation are likely causes for these observed differences in germination rate. Thus, the type of symptom which develops on pistillate vines can affect the potential for ascospore production during subsequent seasons through the production of ecologically distinct populations of sclerotia.

During field studies in 18 orchards, positive relationships were shown between primary inoculum source (apothecial density) and primary infection (incidence of petal colonisation) and between both these factors and the incidence of diseased fruitlets and fruit with scarring. A conceptual model of inoculum production and disease development was constructed. This model highlights the importance of primary colonisation of floral tissues and showed that disease risk can be estimated from the apothecial density or the colonisation of petals by *S. sclerotiorum*. Model structures have also been developed for predicting disease risk and disease incidence. Disease management strategies are discussed, and if implemented, would rationalise decision processes and potentially reduce the costs of controlling sclerotinia. Decision support software could be developed to incorporate such models once they are validated and combined with further research to determine effective control measures. This software could then be integrated into a sclerotinia management system for kiwifruit in New Zealand.

Acknowledgements

I would like to sincerely thank my supervisors and the many friends, colleagues and my family who have given me their support and encouragement throughout this research project and during the compilation of this thesis. Thank you Dr Peter Long (Massey University) for taking on this project and for your advice and assistance from the early planning stages right through to the many valuable comments during the final preparation. Thank you Dr Rob Beresford (MARC, HortResearch) for giving this epidemiology project a home within the Plant Pathogen Ecology and Disease Management Programme and for your sound learned advice and support throughout. Thank you Dr Mark Goodwin (Ruakura, HortResearch) for your vital on-site support, your many questions and encouragement for setting timetables (which I seldom kept to) for reaching the final goal. I am indebted to the New Zealand Kiwifruit Marketing Board (Zespri Group), in particular Bob Martin, for awarding me with their PhD research scholarship that provided my research project with a firm financial base. Many thanks also to HortResearch for the opportunity to further my career and to the Foundation for Science Research and Technology for funding this project.

I especially wish to thank Drs Henry Pak and Phil Elmer for their numerous and invaluable thought provoking discussions over the last four years. There are many who have helped me with practical aspects of my research, for which I am very grateful, Gary Piller, John Meekings, Maree Hart, Maia Melbourne, Rosemary Boyd, and Selwyn Hutchings. Many thanks to my fellow PhiDers: Scrott, Niggel and OllyBali who finished before me and showed me it was all possible. Many thanks to Heather McBrydie for lending so much equipment. Thanks to Sergid for all his welcome distracting emails. Also, thanks to Dave Morgan of University of California for supplying fresh kiwifruit flowers. I am very thankful for the enduring assistance that Barbara Dow has given me for statistical analyses. Thank you Tony Balony for your most valuable last minute proofing and comments. Thanks Mum for your never ending support and belief that I could do it, and for buying me a home computer.

To my partner, Maree, I wish to thank you for the fun times we've had and your endless support you have so freely given to me, I love you (*I luv u 2*). A special HI to my son Jack, born 3-3-00, who has been such a big part of the latter stages in preparing this thesis, you are a wonderful addition to my life.

Table of Contents

Table of figures

Table of tables

Table of plates

Chapter 1: Introduction

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson, var. *deliciosa* cv. 'Hayward') is a perennial deciduous vine grown commercially in New Zealand for its distinctive green-fleshed fruit. *Sclerotinia sclerotiorum* (Lib.) de Bary is a necrotrophic fungal pathogen that is cosmopolitan in distribution and has a wide host range of at least 408 plant species from 278 genera, including many economically important crop plants (Abawi & Grogan, 1979; Boland & Hall, 1994). *S. sclerotiorum* causes disease of kiwifruit fruitlets, scarring of fruit and field rot of fruit, resulting in loss of up to 5% of the New Zealand kiwifruit crop in some seasons (Pennycook, 1985).

Although there are several general descriptions of sclerotinia disease (Pennycook, 1982; Pennycook, 1985; Brook, 1990a; Manning, 1991), there is little data on epidemiology. Effective disease management requires the application of a sound understanding of disease epidemiology (Zadoks & Schein, 1979). The following thesis is a 4 year study of the epidemiology of the *S. sclerotiorum* in kiwifruit and discussion of its implications for disease management.

1.1 Kiwifruit in New Zealand

Kiwifruit cv. 'Hayward', formerly known as Chinese gooseberry, is a large-fruited selection of *A. deliciosa*, a member of the Actinidiaceae indigenous to South East Asia, in particular to the temperate forests of south-western China (Ferguson, 1990b). First introduced to New Zealand as seed from China in 1904, kiwifruit is a deciduous perennial climbing or straggling vine (Ferguson & Bollard, 1990) that has been known by a variety of botanical names, including *A. chinensis* Planch. var. *deliciosa* (A. Chev.) and *A. chinensis* Planch. var. *hispida* C. F. Laing (Ferguson, 1990a). In 1984 the two variants of *A. chinensis*, one with spherical smooth skinned fruit, the other with ovoid or cylindrical fruit with persistent hairs, were placed into separate species (Liang & Ferguson, 1984). The former became *A. chinensis* Planch., and a cultivar selection of this is the commercial variety known as 'Hort16A', newly available in New Zealand and marketed as Zespri Gold™. The latter became *A. deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson, var. *deliciosa*, and is the commercial cultivar 'Hayward' grown widely as an export crop in New Zealand and in many other countries around the world

including Italy, France, Japan, USA (California) and Chile (Ferguson, 1990a; Warrington, 1990).

Early commercial plantings of 'Hayward' kiwifruit were made near Wanganui and later in Bay of Plenty, Auckland and Kerikeri. Plantings were more extensive from 1973 onwards as the export industry rapidly expanded. By 1986 there were almost 17,000 ha across eight regions from Northland to Nelson, including over 9,000 ha in Bay of Plenty (Warrington, 1990). The total area of kiwifruit in New Zealand has stabilised in recent years at about 10,000 ha (Anonymous, 1999a). The first export of kiwifruit from New Zealand was a shipment of 3,500 trays to the United Kingdom in 1953 (Warrington, 1990). From the 1960s the volume of export fruit increased exponentially and by 1986, 28.9 million trays were packed for export. Recently, export production has ranged between 56.2–59.4 million trays with a net revenue between $565 million and $695 million for the years 1997–99 (Anonymous, 1998; Anonymous, 1999a).

New Zealand kiwifruit orchards are planted in blocks of about 0.5–0.75 ha separated by artificial shelter or shelter trees pruned to a height of 6–8 m. Vines are dioecious with staminate and pistillate vines planted in each block to ensure pollination, typically in a ratio ranging from 1:3–1:8 (Goodwin *et al*., 1999). Honey bees (*Apis mellifera* L.) are the predominant insect pollinator and are brought into orchards in large numbers during flowering. Many pistillate kiwifruit vines in New Zealand have been propagated by grafting 'Hayward' scion wood onto seedling 'Bruno' or 'Abbott' rootstock cultivars (Lawes, 1990) or clonal selections such as 'Kaimai' (Lowe *et al*., 1992). There are several staminate clonal selections planted in New Zealand orchards such as 'Matua', 'Tomuri', 'Chieftain', and numerous 'M' series (Hopping, 1981; Alexandra, 1986). The time of flowering, number of flowers per shoot and pollen germination differ slightly between these clonal selections and can impact on their suitability as a pollinator for 'Hayward' vines (Alexandra, 1986).

Vine spacing usually ranges from 2.5–6.0 m between plants and 5.0–6.0 m between rows (Sale & Lyford, 1990). Mature kiwifruit vines are pruned to a single trunk with two permanent main leaders (cordons) supported on post, wire and timber structures ca. 1.8 m off the ground. Vegetative and fruiting canes are trained perpendicularly to the main cordon onto supporting wires and are generally replaced every 1–2 years. The

supporting wires are either on a horizontal plane at the height of the cordon (pergola) or at a range of heights on both sides of the cordon (T-bar) (Sale & Lyford, 1990).

Shoot growth begins during September from axillary buds on 1 year-old canes and occasionally from the main leader (Ferguson, 1990c). Shoot and flower development typically begins 5–10 days earlier on staminate vines than pistillate vines. Shoots can be terminating, when apical growth ceases during the growing season, or non-terminating. Mature leaves are up to 200 mm across, generally heart-shaped and dark green. The lower surface is thickly covered with stellate hairs (Ferguson, 1984).

Some shoots can form flowers in the leaf axils between nodes 4–15 (Walton & Fowke, 1993), while others remain vegetative (Ferguson, 1990c). The start of anthesis is influenced by the degree of winter chilling and temperature after bud-burst (Morley-Bunker & Salinger, 1987; McPherson *et al*., 1994). The time from leaf bud-burst to anthesis is about 2 months, and from anthesis to harvest is about 150 days (Davidson, 1990). Flowering usually occurs between early-November and mid-December depending on location and altitude and lasts up to 17 days (Hopping, 1982; Davidson, 1990). Hydrogen cyanamide (Hi-cane) is a dormancy breaking chemical used by many orchardists to achieve higher and more uniform bud-break (Henzell, 1986). Hi-cane also reduces the time between bud-burst and anthesis and shortens the duration of flowering (Henzell, 1986; Walton & Fowke, 1993).

Mature pistillate vines typically bear 900–1200 flowers in Bay of Plenty orchards and up to 50% more than this in Hawkes Bay where winter chilling is greater (Cooper & Marshall, 1990). The pistillate inflorescence usually bears a single flower and occasionally two or three flowers (Hopping, 1990). Pistillate flowers are 35–68 mm across and have a superior ovary 7–9 mm long and 5–8 mm wide. They have 31–41 prominent white styles, 166–200 stamens, 3–7 sepals and 5–7 ovate-oblong petals (Figure 1.1 A) (Schmid, 1978; Hopping & Jerram, 1979; Hopping, 1990). The maximum crop load is considered to be ca. 1500 fruit/vine (Lyford, 1981).

Staminate vines produce 3000–4000 flowers, each inflorescence having a terminal flower and two lateral flowers (Alexandra, 1986). Staminate flowers are distinguished from pistillate flowers by the absence of functional styles, a smaller ovary (4–6 mm

length) with no ovules, and fewer stamens (124–182) (Figure 1.1 B) (Palmer-Jones & Clinch, 1974; Brundell, 1975). Pistillate and staminate flower petals are white on opening, but turn yellow/orange as they age, and have a distinct faintly sweet odour (Schmid, 1978). All floral organs possess a thin cuticle (Schmid, 1978).

There are several distinctive development stages used to describe the 1–2 week period before anthesis. 'Calyx splitting' is when the sepals begin to separate and expose the petals and is seen as an indication that anthesis is approaching. 'Smiling' is a stage where the sepals have separated further and the white petals are clearly visible. The 'cup' stage is where anthesis has begun and the petals have opened just enough to expose the internal floral parts. The rate of flower development is largely determined by temperature (Morley-Bunker & Salinger, 1987; Agostini & Habib, 1996). 'Petal-fall' is the drop of petals from flowers and begins as early as 3 days after anthesis (Goodwin & Steven, 1993).

Anthers on pistillate vines initiate dehiscence on the morning of anthesis and the pollen is non-viable. The degree of dehiscence increases each day, up to day 5 (Goodwin, 1986), although complete anther dehiscence has been reported to occur after 3 days (Goodwin & Steven, 1993). The degree of anther dehiscence can be used to reliably determine the number of days since anthesis (M. Goodwin pers. comm.). Stigmatic surfaces remain receptive to pollination for up to 8 days after anthesis (Davidson, 1973) and fertilisation is complete within about 74 h of pollination (Hopping & Jerram, 1979).

For the purpose of this study, flowering is the period from the beginning of anthesis until the end of anther dehiscence within a block of vines. Fruit are considered to have formed ca. 11 days after anthesis, which is the latest point in time when fertilisation may occur, based on the data given above. From the time of petal-fall until 11 days after anthesis the immature fruit is termed a fruitlet.

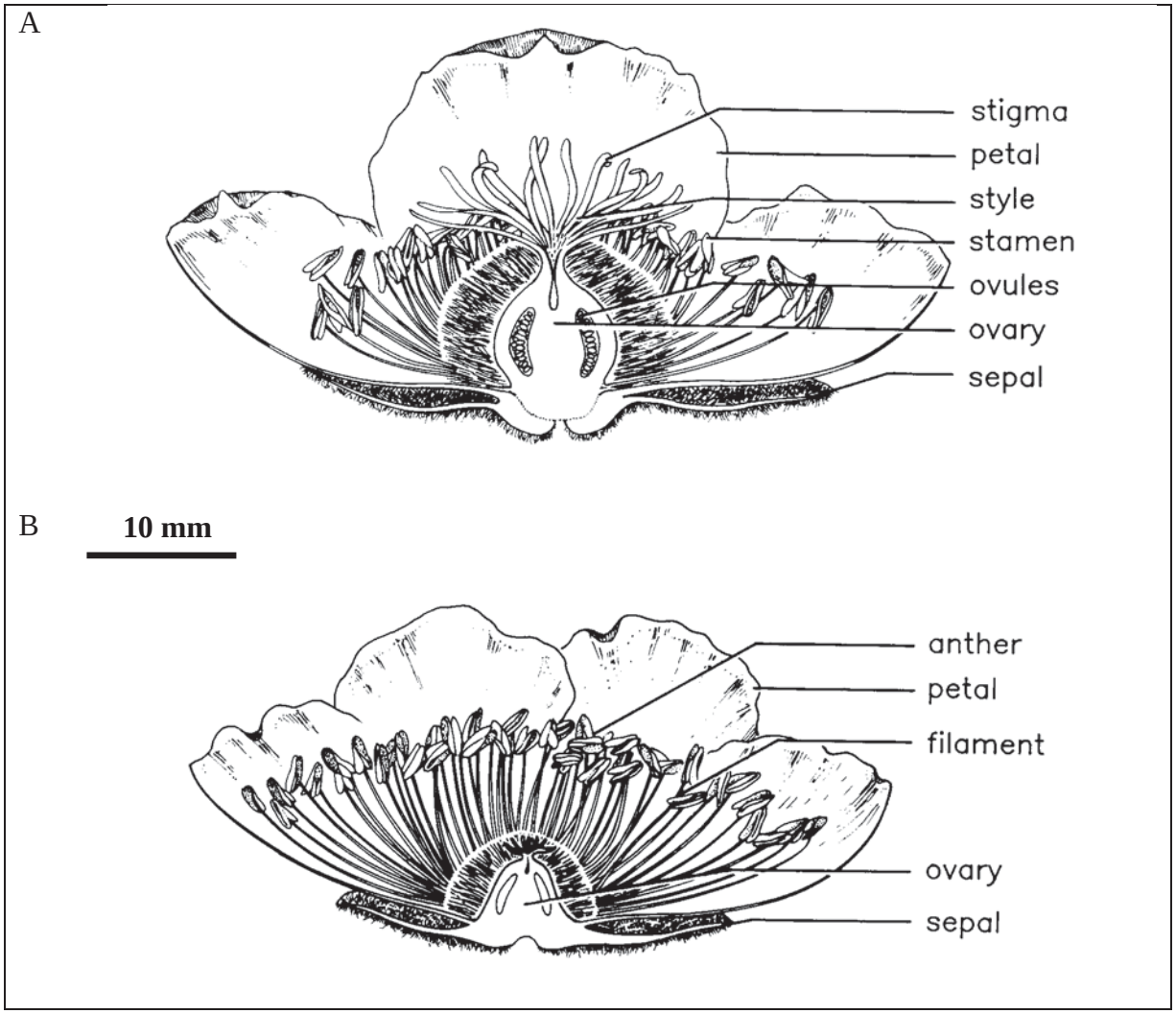


Figure 1.1 Longitudinal section of (A) pistillate flower and (B) staminate flower. (Redrawn after McGregor (1976)).

About two-thirds of the increase in volume and weight of kiwifruit fruit occurs during the first 10 weeks after anthesis and the overall growth curve has been described as double sigmoid (cv. 'Monty') (Hopping, 1976) and triple sigmoid (cv. 'Bruno') (Pratt & Reid, 1974). The latter is suggested to be more accurate (Reid *et al*., 1982). During the first few weeks after anthesis, both cell division and cell enlargement occur, while subsequent increase in fruit size is attributed to cell enlargement in the inner pericarp (Hopping, 1976; Schmid, 1978). The fruit epidermis consists of a cuticle through which extend numerous hairs (Schmid, 1978). Fruit are typically 55–70 mm in length and 40–50 mm in width when mature and weigh 80–120 gm (Beever & Hopkirk, 1990). Fruit size is strongly influenced by the number of seeds, which is affected by pollination (Pyke & Alspach, 1986).

1.2 *Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum (Lib.) de Bary (syn. *Peziza sclerotiorum* Libert, *Sclerotinia libertiana* Fuckel, and *Whetzelinia sclerotiorum* (Lib.) Korf & Dumont) is classified in the Ascomycotina, order Helotiales (Mordue & Holliday, 1976; Holliday, 1992). It is the type species of the genus in the family Sclerotiniaceae, erected by Whetzel (1945) to accommodate inoperculate discomycetes that produce stromata, stipitate apothecia and ellipsoidal ascospores (Kohn, 1979a). *S. sclerotiorum* lacks a conidial anamorph and is assumed to be homothallic, i.e. there is no genetically identifiable sexual incompatibility system and the ascospores are self-fertile (Anderson *et al*., 1992). Although micro-conidia are produced, there is no convincing evidence that they are necessary for apothecial formation (Kohn, 1979b; Willetts & Wong, 1980).

There are three closely related plant pathogenic *Sclerotinia* species, *S. sclerotiorum*, *S. trifoliorum*, and *S. minor*, although only the former of these has been reported from kiwifruit (Pennycook, 1985). A taxonomic key and distinguishing features of these species is presented by Kohn (1979b) and comparative studies of a range of cultural and biochemical criteria has also been reported (Willetts & Wong, 1980; Tariq *et al*., 1985). The main criteria used to distinguish between these species have been host range, growth rate, sclerotia, and dimensions of ascospores and asci (Willetts & Wong, 1980). The considerable variability in these features explains some of the confusion in the past as to their identification and classification (Purdy, 1979). Some authors have included all three in the one species *S. sclerotiorum* (Morrall, 1972; Price & Colhoun, 1975b). The inclusion of apothecium structure and ascospore dimensions more clearly defined this speciation (Kohn, 1979b) and there has for many years been a common acceptance that the three species are distinct.

Within these three *Sclerotinia* species, variation in host specificity, distribution, and virulence has been observed at both inter- and intraspecific levels (Anderson *et al*., 1992). *S. sclerotiorum* shows a high level of intraspecific phenotypic variability (Purdy, 1979; Tourneau, 1979) and although 19 field isolates showed a range in the degree of pathogenicity against 11 host species, this could not be used to divide the isolates into different groups (Price & Colhoun, 1975a). Several distinct clonal lines were identified in Canadian canola (oilseed rape) (*Brassica napus* L.) fields, suggesting field populations of *S. sclerotiorum* are genetically heterogeneous with respect to vegetative

compatibility and genotype and that this may indicate differences in pathogenicity (Kohn *et al*., 1991; Kohli *et al*., 1992). In New Zealand the genetic variability of *S. sclerotiorum* isolates from South Island populations has recently been reported, indicating clonal variations exist that are similar to those in canola (Carpenter *et al*., 1999). It is therefore probable that some degree of genetic variability exists in *S. sclerotiorum* populations in North Island kiwifruit orchards.

There are many common names for the multitude of diseases caused by *S. sclerotiorum* such as: white mold of bean (Abawi & Grogan, 1975), stalk and head rot of sunflower (Purdy, 1979) and cottony rot of vegetables (Roberts & Boothroyd, 1984). The diagnostic symptom of sclerotinia disease is often a watery soft rot of stems, petioles, flowers, and fruit of plants in the field and of fleshy organs in storage. Sclerotinia is one of the most devastating diseases of cultivated plants in cool-temperate climates and subtropical regions (Roberts & Boothroyd, 1984). The common diagnostic signs of the pathogen are the mass of white mycelium giving a cottony appearance and the irregularly shaped black sclerotia that develop within the mycelium.

S. sclerotiorum over-winters as sclerotia produced on or within colonised host tissues. Sclerotia are hardened mycelial structures that have a well differentiated black rind composed of a 2–6 deep layer of dark-walled, globose cells and white medulla free of host tissue remnants (Kohn, 1979b). Sclerotia of *S. sclerotiorum* have not been shown to infect host tissues directly, as is the case with *S. minor* (Hawthorne, 1974). Sclerotia of *Sclerotinia* species can survive for 2–7 years in soil, although viability declines over time (Coley-Smith & Cooke, 1971; Kapoor *et al*., 1987). Survival in soil is affected by factors such as the source of sclerotia (cultured or naturally formed), sclerotial size, depth of burial, soil moisture, micro-organisms, and the production of apothecia (Williams & Western, 1965; Merriman *et al*., 1979; Mitchell & Wheeler, 1990; Ben-Yephet *et al*., 1993). In New Zealand, viability of buried sclerotia has been shown to decline significantly during the first year beneath kiwifruit vines in the Bay of Plenty (Goh & Lyons, 1992b), in vegetable cropping soil near Pukekohe, South Auckland (Alexander & Stewart, 1994), and in Canterbury pastures (Harvey *et al*., 1995).

One to many stipes are formed from sclerotia, usually after a period of cool moist ‘conditioning’ (Phillips, 1987). Stipes are 1–2 mm wide and 3–20 mm long and

differentiate at the tip in response to light to form an apothecial cup 2–10 mm wide (Kohn, 1979b; Singh & Singh, 1987). *S. sclerotiorum* apothecia have an applanate to slightly concave receptacle when young. They are applanate to convex at maturity, often with a central depression and frequently with an undulate margin. Ascospores are uniform in size (10–14 × 4–5 μm) hyaline, binucleate, ellipsoid with a length/width ratio of 2.1–2.4 (Kohn, 1979b). Ascospores are released from the upper surface of the apothecial cup, often in a sudden discharge that propels many thousands of ascospores ca. 10–20 mm from the apothecium, resembling a small puff of smoke and is termed "puffing" (Hartill & Underhill, 1976). This process is considered to give the ascospores a greater chance of escaping the boundary air layer to permit dispersal in air currents (Ingold, 1971).

The dispersal of *S. sclerotiorum* is achieved largely through the production of ascospores which have been shown to be the primary infective propagule in many susceptible annual crops e.g. stem rot of tomato (*Lycopersicon esculentum* Miller) (Purdy & Bardin, 1953), white mold of bean (*Phaseolus vulgaris* L.) (Abawi & Grogan, 1974), leaf blight of sunflower (*Helianthus annuus* L.) (Sedun & Brown, 1987) and pod rot of peas (*Pisum sativum* L.) (Huang & Kokko, 1992). Infection from *S. sclerotiorum* ascospores is usually directly through the cuticle and requires an exogenous food source before ascospores can infect healthy host tissue (Abawi *et al*., 1975). Other studies have shown that dead or senescing tissue, nutrients or injured plant surfaces are necessary for infection by ascospores (Purdy, 1958; Cook *et al*., 1975; Sedun & Brown, 1987). There has been no investigation of ascospore infection of kiwifruit.

Infection hyphae of *S. sclerotiorum* are large (8.8–34.0 μm) and granular in appearance and develop radially from the point of infection and invade host tissues exclusively intercellularly (Lumsden & Dow, 1973). Within 24 h of penetration 'ramifying' hyphae develop and invade dead and dying host tissues both intercellularly and intracellularly (Lumsden & Dow, 1973). Cell component degrading enzymes have been associated with penetration and lesion development e.g. endo- and exo-polygalacturonases, pectin methylesterase, cellulases and hemicellulases (Hancock, 1967; Lumsden, 1969; Lumsden, 1976). Proteolytic enzyme activity has also been associated with cultures and infected tissues (Khare & Bompeix, 1976). Oxalic acid plays a significant role in the colonisation of host tissue through reduction in pH favouring enzyme activity, chelation

of cations that can inhibit certain enzymes, and possible affects on host responses (Maxwell & Lumsden, 1970; Noyes & Hancock, 1981; Dutton & Evans, 1996).

1.3 Sclerotinia Disease Epidemiology

Plant disease epidemics occur when a virulent pathogen population, susceptible host plants and favourable environmental conditions for infection and spread, lead to changes in disease intensity in the host population over time and space (Campbell & Madden, 1990; Kranz, 1990). The development of disease can be separated into phases such as inoculum production and dispersal, infection (the entry of a pathogen into host tissues and the establishment of a parasitic or pathogenic relationship), an incubation period (the time needed for symptoms to develop), and a latent period (the time to complete a generation or to become infectious) (van der Plank, 1963; Zadoks & Schein, 1979; Campbell & Madden, 1990). Diseases can be classed as monocyclic when inoculum comes from a reservoir and generally a single infection cycle occurs during a growing season or polycyclic when several infection cycles form the basis of increase in disease (Zadoks & Schein, 1979). The former is termed a 'simple interest' disease, and the latter a 'compound interest' disease (van der Plank, 1963; Zadoks & Schein, 1979).

The infection cycle of a pathogen often forms a focal point for epidemiological studies and the classification and comparison of diseases (van der Plank, 1963; Zadoks & Schein, 1979). Primary infection occurs when the dispersal propagule of the pathogen infects the host. Secondary spread is any subsequent infection of new host tissues resulting from the mycelium or spores produced by the primary infection, but not directly through lesion expansion (van der Plank, 1963). Jamaux *et al.* (1995) used the term 'primary unit of dissemination' for bean petals, colonised by *S. sclerotiorum* ascospores, that were the source of secondary lesions on leaves.

The appearance of sclerotinia disease symptoms has been linked to the onset of flowering in annual crops such as pea (Huang & Kokko, 1992), bean (Abawi *et al*., 1975; Sutton & Deverall, 1983; Tu, 1989), sunflower (Tourvielle *et al*., 1978; Kondo *et al*., 1988) and several *Brassica* spp. (Gerlagh, 1986; Turkington *et al*., 1991; Jamaux *et al*., 1995). In these crops, infection of flowers by ascospores of *S. sclerotiorum* represents the primary infection stage of an epidemic (Abawi & Grogan, 1975; Huang & Kokko, 1992; Jamaux *et al*., 1995). *S. sclerotiorum* requires a food base such as

colonised floral tissues or other exogenous nutrient sources to infect other healthy host tissue, i.e. for secondary spread to occur (Purdy, 1958; Abawi *et al*., 1975).

Environmental variables such as temperature, relative humidity (RH) and wetness duration have been shown to affect the success and rate of *S. sclerotiorum* infection and colonisation of host tissues by ascospores and/or mycelium (Abawi & Grogan, 1975; Phillips, 1994b; Hannusch & Boland, 1996). In these studies the optimal temperature and wetness duration for infection were shown to be 20–25^{o}C and 48–96 h, respectively. The occurrence of white mold in bean has been linked to the onset of flowering and plant canopy structure and irrigation has been shown to influence disease progress (Blad *et al*., 1978; Weiss *et al*., 1980a).

1.4 Sclerotinia disease of kiwifruit

Sclerotinia was first reported as a pathogen of kiwifruit in 1969 (Dingley, 1969). Ford (1971) considered sclerotinia 'twig blight' not to be a serious problem. Likewise, in 1980 a report of the major causes of imperfections in kiwifruit did not mention fruit loss or fruit damage caused by sclerotinia (Ferguson, 1980). During December of the following year, the first report of significant crop losses (approximately 5% of immature fruit) was recorded in the Te Puke district (Pennycook, 1985). However, there are no industry records of total crop loss caused by sclerotinia.

Sclerotinia rot is the only fungal disease of any significance that affects 'Hayward' kiwifruit fruit while they are still on the vines (Brook, 1990a). Apothecia of *S. sclerotiorum* were first observed in kiwifruit orchards during December 1981 (Pennycook, 1982) and it has been assumed that ascospores are the source of sclerotinia infection in kiwifruit (Pennycook, 1985; Brook, 1990a; Manning, 1991). Subsequently, apothecia have been observed from late September, 6–8 weeks before flowering, to mid-summer (Manning, 1991). It has been stated that apothecia appear in orchards as soil temperature at 10 cm depth reaches 17^{o}C and that they are more common under staminate vines (Manning, 1991), however no data is provided to support this. Hoyte *et al.* (1992) observed that stipe development from sclerotia buried under kiwifruit vines was delayed in late-October under dry soil conditions and that the average development time from emergence of stipes to maturation of apothecia was 8.5 days in November when the mean air temperature was 12.5^{o}C and rainfall was frequent. A long dry period

during January 1982 was also observed to terminate production of apothecia (Pennycook, 1982).

During the growing season, sclerotinia disease affects flowers, shoots and leaves of both staminate and pistillate kiwifruit vines and developing fruit (Pennycook, 1985). Sclerotinia disease of flowers has been referred to as 'sclerotinia rot' (Brook, 1990a), 'blossom blight' (Pennycook, 1985) and 'diseased fruitlets' (Hoyte *et al*., 1992). The latter term is used in this thesis because symptoms appear 10–14 days after flowering and are thus fruitlets rather than flowers. Fruit on vines with soft rot is referred to as 'field rot' and dry lesions on fruit are termed 'scarring' (Pennycook, 1985; Brook, 1990a). Disease on shoots, where girdling causes death of the shoot beyond the lesion, has been termed 'twig blight' (Ford, 1971).

Disease symptoms first appear in kiwifruit orchards in the form of rotting clusters of staminate flowers and pistillate flower buds which turn brown and wither (Pennycook, 1985). Infected pistillate flowers are initially soft but become dry and shrivelled and result in reduced fruit numbers (Pennycook, 1985; Cooper & Marshall, 1990). Lesions can spread during wet conditions to shoots, leaves and immature fruit by direct contact with diseased floral tissues, particularly petals (Pennycook, 1985). Sclerotia developed from 93% of diseased fruitlets and from fruit with field rot (Hoyte *et al*., 1992), although Pennycook (1985) suggested that the major source of sclerotia is from saprophytic growth of *S. sclerotiorum* on weeds and other ground litter.

Symptoms on fruit are most common during December and January, appearing as lesions that initially have a water-soaked appearance and develop into a soft rot (field rot) or scarring on the fruit surface when lesions dry out (Pennycook, 1982; Goh & Lyons, 1992a). Pennycook (1985) states "fruit infection results only if ascospores are deposited at infection sites that are in direct contact with an adequate food base, e.g. senescing stamens and petals, water droplets containing pollen grains or pollen exudate, and if those infection sites remain wet for several hours". However, no data was provided to show that kiwifruit pollen grains or pollen exudates stimulate infection of fruit by *S. sclerotiorum* ascospores. Further, this statement implies that ascospores are deposited just before symptom development on fruit, yet there is no evidence of this.

Infection of fruit by ascospores is also said to occur where touching fruit trap moisture and provide favourable conditions (Pennycook, 1982; Pennycook, 1985). Further, Manning (1991) reported that rainfall to give 72 h continuous surface wetness is required for disease to develop, but provides no supportive data. Goh & Lyons (1992a) reported that the incidence of *S. sclerotiorum* growth from flowers and fruit incubated under high humidity decreased substantially from flowering to the end of January, but no records of apothecial production or of rainfall within the orchards were given.

Although *S. sclerotiorum* is present in other countries that grow kiwifruit, there is only one known report of this pathogen causing disease of kiwifruit flowers and fruit outside New Zealand (in Italy, (Pratella, 1995)).

1.5 Economic significance of fruit loss

The economic significance of sclerotinia disease can vary depending on the initial number of flowers. In districts such as Hawkes Bay, where flower production is high, the occurrence of some diseased fruitlets may be of less importance than in the Bay of Plenty or Kerikeri, where vines produce fewer flowers (Cooper & Marshall, 1990). On the other hand, fruit loss during January and February occurs when orchardists may have already thinned their crops to the desired level and any sclerotinia losses incurred at this stage can reduce export crop.

Scarring on fruit can lead to rejection during grading. Current grading standards require that fruit be rejected if fungal scarring is >100 mm^2 in area and if it detracts from the fruits appearance (Anonymous, 1999c). The proportion of reject fruit resulting from fungal scarring during 1998 from 186 orchards at Waimapu Packhouse was 0.8% (SE ± 0.05, range 0–4%) (W. Young, pers. comm.) and from a further 104 orchards at Centrepac Packhouse was 0.6% (range 0–3%) (P. Mulligan, pers. comm.). These figures are underestimates of total crop loss because they do not include diseased fruitlets, scarred fruit that are thinned off the vines or crop loss from field rot.

Hoyte (1994) reported that the increase in sclerotinia disease in three Bay of Plenty orchards during 1991/92 was greater during December than January. Weekly monitoring of sclerotinia in two Bay of Plenty orchards showed a steady increase in disease incidence in one orchard from late-November to early-April and a sharp

increase during early-February in the other orchard, reaching 14% and 19% of total fruit numbers, respectively (Goh & Lyons, 1992a). These data indicate that actual losses in some orchards from sclerotinia may be considerably higher than the fruit-loss figures from packhouses.

From the perspective of the New Zealand kiwifruit industry and Zespri International, there are three main concerns with regard to sclerotinia disease. First, there are increasing international market demands for fruit that are free of fungicide residues and which have had nil fungicide applications. Second, the development of an environmental management system requires a disease management system that ensures fungicide usage is targeted to medium and high disease risk orchards or blocks. Third, the 2.3 million trays of organic kiwifruit produced during the 1999 season (J. Clendon, pers. comm.) highlights the need for effective disease control strategies that are compatible with Biogro® standards.

Fungicide use in kiwifruit production is exclusively for sclerotinia control. Industry records (E. Harré, pers. comm.) show that during 1998/99 41% of Bay of Plenty and Katikati orchards, from a random sample of 200, used at least one application of Rovral®. In the same season, 22% of orchards from all growing regions (n = 3,250) used Benlate®. The cost of fungicide control for sclerotinia can be estimated by assuming the treated canopy area is proportional to the percentage of orchards that use fungicide. Therefore, 4,100 ha @ \$260 ha^{-1} (Rovral) and 2,200 ha @ \$148 ha^{-1} (Benlate) equals \$1.4 million spent on fungicides. This does not include labour, fuel or maintenance costs or take into account orchards that make two applications of Rovral.

Although there is no reliable industry-wide information on crop loss, the cost of sclerotinia crop losses can be estimated. Assuming that total crop loss is equal to the average of the packhouse data given above, then 0.6–0.8% of 59 million trays is equivalent to 354,000–472,000 trays or \$4.14–5.52 million, calculated at \$11.70/tray mean selling price (Anonymous, 1999a). This equals an estimated annual cost to the industry for disease control and fruit loss of ca. \$5.5–6.9 million per annum. There are further labour costs associated with fruit thinning in orchards and grading in packhouses. This figure might be conservative during a season when conditions are highly favourable for disease, as reported in 1981 by Pennycook (1985).

1.6 Management of sclerotinia disease in kiwifruit

Disease management of sclerotinia largely centres on the application of dicarboximide (iprodione, Rovral) or benzamidazole (benomyl, Benlate) fungicides applied with air-blast sprayers during flowering and early-petal-fall to protect senescing petals and stamens (Pennycook, 1985; Manning, 1991). Rovral can also be applied during fruit development (December–February) if weather conditions favour disease (Pennycook, 1985; Manning, 1991). A maximum of two applications of Rovral are permitted in any one season and Benlate may only be applied before the onset of petal-fall (Walton & Sommerville, 1998). It is recommended that fungicides be applied before wet weather during flowering and be timed to coincide with ascospore deposition on fruit, as it is thought that fruit disease results from establishment of ascospore infection (Pennycook, 1985). The effects of timing of fungicide applications on disease control in relation to stage of flowering or fruit development have not been determined. Although use of these fungicides is claimed to be "extremely effective against sclerotinia" (Pennycook, 1982) there is no published efficacy data.

There are four important consequences associated with fungicide control of sclerotinia. First, the dicarboximide, vinclozolin (Ronilan®), although no longer registered for use, has been shown to significantly reduce seed numbers in fruit of apples, peach and kiwifruit when sprayed during flowering, probably because of effects on pollen grains (Manandhar & Lawes, 1980). As neither Rovral nor Benlate were tested against kiwifruit in their trial, there remains doubt as to any negative affect they may have on pollination or fruit set. Second, if Benlate is applied after the start of petal-fall there are risks of residues on harvested fruit (Walton & Sommerville, 1998). Third, fungicides used for sclerotinia control increase the frequency of fungicide-resistant strains of *Botrytis cinerea* (Pers.) in kiwifruit orchards and result in a higher incidence of sporulation on necrotic leaves and stem-end rot on fruit in storage (Manning *et al*., 1995; Pak *et al*., 1995). Fourth it would be feasible for fungicide-resistant strains of *B. cinerea* to disperse from a kiwifruit orchard and establish in neighbouring glasshouse, vegetable or fruit crops.

Cultural and biological control techniques may in the future play a significant role in disease management as alternatives to fungicides (Zadoks & Schein, 1979). Several cultural control options have been recommended for sclerotinia disease control, based on the assumption that ascospores colonise senescent floral tissues and that these are a

source of disease. This includes the removal of senescent petals and stamens from flowers on pistillate vines with an air-blast sprayer, and early pruning of staminate vines and the disposal of prunings (Pennycook, 1985; Manning, 1991). Disease risk is also said to be reduced by maintaining an open vine canopy to increase air-flow and reduce wetness periods after rainfall (Pennycook, 1985; Manning, 1991). It has been claimed there is a lower risk of sclerotinia disease in T-bar trained orchards than in pergola orchards (Sale & Lyford, 1990), although no explanation is given. There have been no published reports giving quantitative information on any of the above mentioned cultural control strategies for sclerotinia management.

Biological control of sclerotinia in kiwifruit has been attempted using the myco-parasite *Coniothyrium minitans* (Campbell) degrade sclerotia in the soil (A Stewart, pers. comm.), but no efficacy data is available. Application of selected saprophytes to open flowers to reduce primary colonisation of floral tissues by *S. sclerotiorum* has been shown to significantly reduce petal infection and fruit scarring (Elmer *et al*., 1999a), although no commercial product is available.

The overall management of kiwifruit is partially governed by strategies outlined in the KiwiGreen© Manual (Anonymous, 1999b). Sclerotinia disease management receives only a limited mention in this document and orchardists have no reliable basis for making disease management decisions, other than through interpretation of previous disease losses and the current season's weather. Disease incidence is rarely determined by actual measurement and it is difficult to interpret the effect of weather conditions on disease progress without a clear understanding of how environmental variables interact with each of the different stages of the disease cycle. There appears to be no information on the cost-benefits of fungicidal or cultural control strategies.

Epidemiology has much to contribute to plant disease management (Zadoks & Schein, 1979). The objectives of disease forecasting are to achieve efficient allocation of disease management resources, minimise crop losses and reduce the use of pesticides (Fry & Fohner, 1985). With these objectives in mind, there have been several disease forecasting systems developed that are based on relationships between disease risk and inoculum concentrations and/or weather conditions, e.g. black spot of apple (Beresford & Spink, 1992; Tate *et al*., 1996), fire blight of pipfruit (Lightner & Steiner, 1990) and

botrytis leaf blight of onion (*Allium cepa* L.) (Vincelli & Lorbeer, 1989). There may be an opportunity to develop new approaches to sclerotinia disease management in kiwifruit by determining disease risk before flowering and the first application of fungicide (Pak *et al*., 1997).

Modelling of sclerotinia disease risk and disease incidence in kiwifruit may contribute to the understanding of the factors relating to disease progress and provide valuable information for crop management. Systems analysis is a method by which complex situations can be understood and described quantitatively (Analytis, 1980). This can be applied to the development of disease models, strategies for disease management and can be used for prioritising research objectives (Jeffers, 1978).

1.7 Thesis objectives

The overall objective of this study was to understand the factors contributing to sclerotinia epidemics in kiwifruit by:

1. Identifying the pathways of primary infection and secondary spread of *S. sclerotiorum* in kiwifruit.
2. Identifying and quantifying sources of *S. sclerotiorum* inoculum in kiwifruit.
3. Quantifying the affects of environmental variables on colonisation by S. *sclerotiorum* ascospores and mycelium, and on the subsequent development of disease symptoms, in order to understand how weather conditions may affect disease progress.
4. Determining the nature of relationships between *S. sclerotiorum* inoculum and disease.
5. Developing conceptual models that describe the inter-relationships of sclerotinia disease in kiwifruit and propose the structure of a sclerotinia management system.

1.8 Thesis outline

The thesis starts with descriptions of the signs and symptoms of sclerotinia disease in kiwifruit and investigates the occurrence of *S. sclerotiorum* within different floral tissues (Chapter 2). The role of adhering floral tissues during disease development is investigated to establish a link between primary infection and secondary spread

(Chapter 3). Chapter 4 describes the development of a detached petal assay and the set-up of static and dynamic controlled environmental chambers. The work in Chapter 5 utilises the controlled environmental chambers to investigate the affects of flower age and a range of temperature and relative humidity conditions on the infection and colonisation of detached petals by ascospores (*in vitro*). Chapter 6 then investigates the effects of misting duration on the development of disease symptoms following inoculation of flowers and fruit on kiwifruit vines (*in vivo*). Sources of inoculum and inoculum-disease relationships are quantified and discussed in Chapter 7. Chapter 8 discusses the overall findings of this research project and its significance for sclerotinia disease management in New Zealand kiwifruit. The potential to develop a disease risk prediction system and a disease model are also discussed.

Chapter 2: The disease cycle - signs and symptoms

2.1 Introduction

Although general descriptions of sclerotinia disease in kiwifruit have been provided by Pennycook (1982; 1985) and Manning (1991), there are very few experimental reports. Goh & Lyons (1992a; 1992b) investigated incidence of disease and the degradation of sclerotia in two Bay of Plenty orchards. Hoyte *et al.* (1992) studied the number and size distribution of sclerotia produced from diseased fruitlets and fruit and the pattern of apothecial production from culture-grown sclerotia buried under kiwifruit vines. Spore trapping data suggests ascospores can be present through most of the growing season (Hoyte, 1994) and disease losses in three orchards were reported to be greater during December than January (Hoyte, 1996). Recently, positive relationships were shown between the density of apothecia beneath kiwifruit vines and the incidence of *S. sclerotiorum* in petals and diseased fruit (Pak *et al*., 1997).

Many aspects of the life cycle of *S. sclerotiorum* in kiwifruit are either not clearly understood or have not been documented. For example, floral tissues have been suggested to be a source of infection on developing fruit, but the occurrence of these tissues on fruit beyond petal-fall has not been quantified. It is not known whether ascospores colonise floral tissues just before symptom appearance on fruit or primarily during flowering. Further, it is unknown whether ascospores directly infect fruit.

2.2 Objectives

To determine the likely pathways of disease development of sclerotinia in kiwifruit by:

- observing and describing the range of sclerotinia disease symptoms in orchards and by confirming the presence of *S. sclerotiorum* apothecia as a source of primary inoculum.
- determining if floral tissues are colonised by *S. sclerotiorum* during flowering and interpreting the likely importance of this in disease development.

2.2 Materials and methods

2.2.1 Identity of apothecia

Nine Bay of Plenty orchards (orchards 1, 2, 3, 4, 6, 8, 9, 11, and 12, Appendix 1) and three Waikato orchards (orchards 22, 23 and 24, Appendix 1) were selected to give a spread of locations within these regions. A single block from each orchard was visited once a month from November 1996 to January 1997. Searches for apothecia were carried out by carefully examining approximately 2 m^2 of soil surface under each of 10 vines equally distributed across each block. Up to four apothecia were collected from each orchard at each visit. Apothecia were washed separately three times in sterile distilled water (SDW) and placed onto Gibco potato dextrose agar (PDA, Life Technologies) and lightly crushed with a sterile glass rod. Colony growth and the presence of sclerotia was observed after 5 and 12 days incubation at 20^{o}C in the dark to determine whether the apothecia were of *S. sclerotiorum*.

2.2.2 Disease signs and symptoms

In the same 10 vines of each orchard block, leaves, shoots, flowers, petals and fruit were searched for sclerotinia disease symptoms for up to 2 minutes per vine. This was carried out during mid-flowering, petal-fall and when fruit were 30–40 mm in length. Although the incidence of symptoms within vines was not quantified, selected diseased material was returned to the laboratory for isolation. Up to five diseased fruitlets from each orchard were surface-sterilised by the following method:

Step

1 Floral tissues were placed into a wooden frame (240 × 320 mm) covered with a plastic coated gauze (2 mm^2), that closed like a book and could hold 80–100 petals.
2 The whole wooden frame enclosing the tissues was submerged for 30 seconds in a plastic tray (300 × 500 mm) containing 2.6 ℓ of drum ethanol.
3 The frame was removed, drained for a few seconds and shaken to remove excess liquid.
4 The frame was rinsed for 15 seconds by submerging in a sink filled with tap water.
5 The frame was then submerged for 2.5 minutes in a second tray containing 2.6 ℓ of 1% sodium hypochlorite amended with 0.1% Tween80 and 0.1% acetic acid. These additives reduced surface tension and increased the available chlorine.

6 Repeat step 3 once and step 4 twice.

7 The frame was rinsed for 30 seconds in a third plastic tray containing 2.6 ℓ of SDW. The SDW was replaced after every third batch.

8 Tissue samples were allowed to drain for at least 1 minute.

This surface-sterilisation method was used throughout this study. From each diseased fruitlet one adhering petal was placed on a Petri dish of PDA and the ovary was aseptically cut in half and placed on a separate PDA Petri dish. A 100 mm^2 section was cut from the margin of 20 necrotic leaf lesions, surface-sterilised as above and placed singly on PDA Petri dishes. All Petri dishes were incubated on the laboratory bench and checked for fungal growth after 5 and 12 days.

Attention was also paid to symptoms similar to sclerotinia, but which may have arisen from other causes. Rainfall data was acquired from weather stations at the Te Puke and Waikato Research Orchards (orchards 17 and 20, respectively, Appendix 1). A photographic record was made of commonly occurring sclerotinia disease symptoms and signs of the pathogen.

2.2.3 Recovery of *S. sclerotiorum* from floral tissues

Five that were 3–4 days-old (i.e. 3–4 days since anthesis) were removed from each of 10 pistillate vines at the start of petal-fall, prior to any application of fungicides, in each of two Waikato orchards (22 and 23) on 28 November and 2 December 1996, respectively. Flowers of this age were identified by having 50–80% anther dehiscence. These orchards had a moderately high incidence of sclerotinia petal infection (38% and 26% *S. sclerotiorum* respectively, section 7.4.1). Each flower was dissected with flame-sterilised forceps and scalpel to separate the pedicel, sepals, petals, stamens and ovary. The pedicel, sepals and pistils were kept whole (n = 50) while two petals and two clusters of approximately 30–50 stamens were sub-sampled from each flower (n = 100). These dissected tissues were surface-sterilised by the method described in section 2.2.2.

Tissue samples were placed on PDA with flame-sterilised forceps. The two petals from each flower were placed onto opposite sides of the same Petri dish, as were the two clusters of stamens from each flower. Other floral tissues were placed singly in the centre of each Petri dish. After 5–12 days incubation at room temperature fungal

colonies were identified to genus where possible. This was achieved by observation of culture characteristics and conidiophores with a stereomicroscope (40–80×), and occasionally conidia with a compound microscope (200×) and with reference to mycological descriptions (Kohn, 1979b; Sutton, 1980). Analysis of variance (ANOVA) was performed using Genstat™ (Lawes Agricultural Trust) to determine differences in the incidence of *S. sclerotiorum* from different floral tissues.

2.3 Results

2.3.1 Observations and identity of apothecia

Apothecia of *S. sclerotiorum* were observed under kiwifruit vines during November and December 1996, but not during January 1997. The ease with which apothecia could be found varied between orchards with none being found in two orchards. Apothecia were usually observed singly or in groups of up to five, although on one occasion more than 40 apothecia were observed under a single vine in orchard 22 (Plate 2.1). Apothecia were applanate to slightly concave, pale apricot/beige and 2–6 mm in diameter. They were always within 10 mm of the soil surface and developed from 1–2 mm wide dark brown stipes. Older apothecia were paler and often convex with an undulate margin. On two occasions apothecia were observed emerging from sclerotia within the remains of fruit with field rot that had over-wintered on the orchard floor (Plate 2.2).

Discharge of ascospores was observed on three occasions when apothecia were disturbed. This had the appearance of a small puff of pale smoke rising 20–30 mm above the apothecial disc and was in keeping with descriptions of ‘puffing’ (Hartill & Underhill, 1976; Pennycook, 1985).

Plate 2.1 Group of ca. 45 apothecia of *S. sclerotiorum* beneath a pistillate kiwifruit vine at orchard 22.

Plate 2.2 Three apothecia emerging from the remains of a fruit with field rot that had formed sclerotia internally during the previous season.

In total, 34 apothecia were collected from 10 orchards. The cultures derived from all of these apothecia had white mycelium that covered the Petri dish within 4 days and formed irregular patches of slightly raised aerial mycelium. All cultures had formed black sclerotia ca. 3–10 mm in length after 12 days, mostly at the colony margin and occasionally scattered throughout the colony (Plate 2.3). There were no conidiophores or conidia present after 3 weeks, confirming that cultures were not *B. cinerea*.

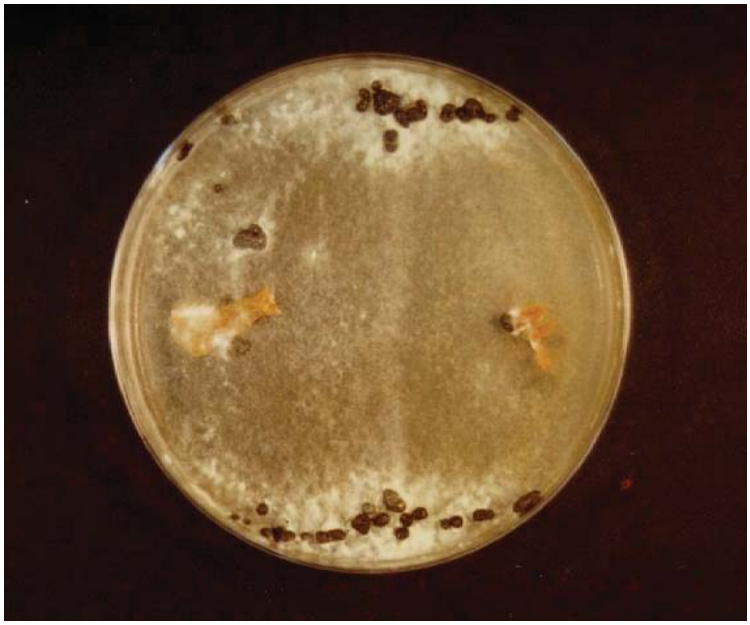


Plate 2.3 Culture of *S. sclerotiorum* grown on PDA, isolated from two kiwifruit petals, showing white mycelium and black sclerotia near colony margin.

2.3.2 Descriptions of disease

2.3.2.1 Disease of flowers and fruitlets

Sclerotinia disease symptoms were observed in all orchards except one in which no apothecia were found. The first symptoms observed of infection by *S. sclerotiorum* were soft and limp petals on staminate and pistillate flowers. These were orange/yellow with a water-soaked appearance and a greyish tinge with a fine 'skin' of white fungal mycelium on the petal surface visible when touched (Plate 2.4 A and B). Petals with discrete lesions ≤5 mm diameter were seen, however the cause of these lesions was not determined by isolation. There were no disease symptoms observed on kiwifruit flower buds, other than that attributable to bacterial blossom blight symptoms (Young *et al*., 1988).

On some staminate vines, secondary spread of *S. sclerotiorum* among adjacent flowers resulted in clusters of flowers becoming blighted, often with visible white cottony mycelial growth. On some pistillate flowers the whole flower fell off the vine when they were gently touched during assessments, leaving a green pedicel. These flowers had a petal collapsed with soft rot and a small soft white/brown lesion, <5 mm across, extending from the base of this petal into the adjacent ovary tissue and sepals in contact with the diseased petal. These lesions were approaching or had reached the point at

which the pedicel attached to the flower. Lesions were also observed to occur where an over extended sepal had made close contact with the base of the pedicel (Plate 2.5).

A B

Plate 2.4 A and B. Soft, limp kiwifruit petals with a water-soaked appearance on (A) pistillate flowers and (B) staminate flowers.

Plate 2.5 Secondary spread of *S. sclerotiorum* from a diseased sepal that has over-extended and made close contact with the base of the pedicel, causing pedicel blight.

Diseased fruitlets (referred to as blossom blight by Pennycook (1985)) were first observed on pistillate vines ca. 10–14 days after anthesis. These were light brown and had a partially or completely dry brown pedicel (Plate 2.6). The ovary was ca. 10–12 mm in length. All diseased fruitlets seen in orchards had one or more diseased petals

and a cluster of stamens remaining attached. Several small white tufts (<1 mm) could be seen on the surface of some of these attached petals. Examination with a binocular microscope (20×) confirmed that they were tufts of mycelium protruding from the petal. Forty eight diseased fruitlets were sampled for isolation and all of the petals and ovary tissues gave rise to colony growth of *S. sclerotiorum*. Pure colony growth of *S. sclerotiorum* was present on 86% of the petals and 79% of the ovaries.

Diseased fruitlets were initially soft and easy to squash between finger and thumb, but several weeks after flowering were dry and firm. Some diseased fruitlets were observed still attached to vines during December and January. Sclerotia were found within internal cavities when diseased fruitlets were broken apart. Production of sclerotia from diseased fruitlets is reported in Chapter 7. On two occasions a single sclerotium was observed on individual petals on the ground beneath pistillate vines during petal-fall.

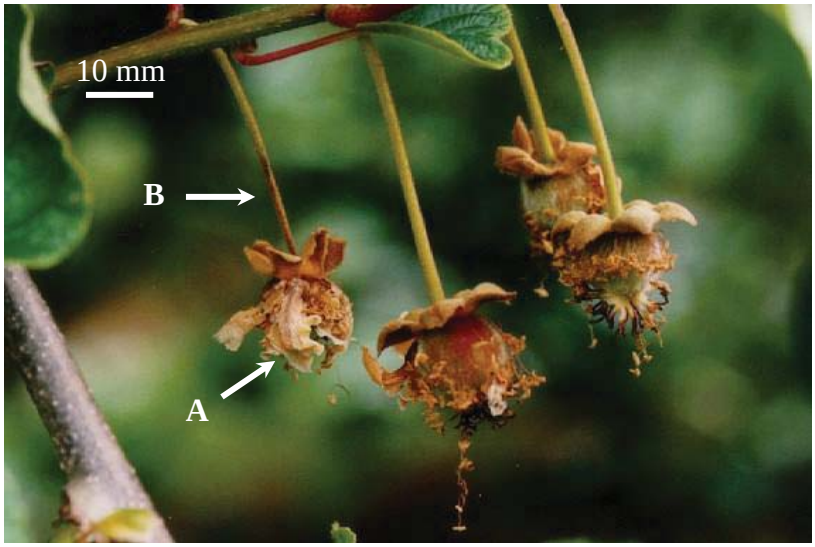


Plate 2.6 Three healthy fruitlets (right) 10 days after anthesis and a diseased fruitlet with adhering petal (A) and stamens, note the brown and slightly shrivelled lesion on the lower half of the pedicel (B). The colour of healthy fruit is variable, including the red/brown and green shown here.

2.3.2.2 Disease of leaves, shoots and canes

White/brown necrotic lesions were observed on leaves in eight orchards. Most lesions had petals, stamens or a diseased fruitlet (Plate 2.7 A) adhering to the leaf surface near the centre of the lesions which ranged in size from 1–20 cm^2. Sclerotia were sometimes present on dead leaves collected from the ground (Plates 2.7 B). *B. cinerea* sporulation

was seen on some necrotic lesions on leaves. Sixteen of the 20 sections of necrotic lesions placed on PDA produced *S. sclerotiorum* colony growth; five of these also produced *B. cinerea* colony growth. The remaining necrotic leaf samples produced a range of fungi including, *B. cinerea* and species of *Phoma* and *Phomopsis*, but these were not quantified. Lesions were also observed on shoots and canes, either where flowers directly contacted adjacent shoots or from lesions spreading from the base of pedicels. Typically, these lesions were a white to tan colour with purple/brown margins and occasionally formed sclerotia.

A B

Plate 2.7 A and B. (A) Necrotic lesion on kiwifruit leaf with adhering diseased fruitlet on the abaxial surface, (B) kiwifruit leaf collected from the ground, colonised by *S. sclerotiorum*, mature sclerotia (MS) and sclerotial initial (SI).

2.3.2.3 Diseased fruit

Sclerotinia disease of fruit was observed from 2 weeks after petal-fall to late February. The earliest symptoms were small brown patches 1–4 mm^2 in area where floral tissues were touching or in close proximity to the fruit surface during early-December. During late-December and January, more obvious scarring symptoms occurred which varied considerably in area and depth of penetration into the fruit. They were often small and superficial, approximately 6–200 mm^2 in area and a tan/brown colour, sometimes with a corky or callus appearance (Plate 2.8 A). These minor lesions had missing fruit hairs or

fruit hairs left in clusters with bare patches of epidermis in-between. Other diseased fruit had lesions which were approximately 100–600 mm^2 and up to 10 mm deep, where infection had penetrated into the outer pericarp (Plate 2.8 B). Scarring symptoms on fruit occurred near the top shoulder and on the sides and lower parts of fruit. Fruit with scarring symptoms remained attached to the vine and sclerotia were rarely present on the fruit surface.

A B

Plate 2.8 A and B. (A) Superficial scarring lesion on fruit with a corky appearance to the skin and (B) severe scarring lesion on fruit penetrating into pericarp.

In addition to scarring symptoms, dry and shrivelled whole fruit ca. 15–30 mm in length were seen hanging on vines or fallen to the ground during December and January. These shrivelled fruit, and fruit with scarring symptoms, frequently still had floral tissues attached to the fruit. These floral tissues consisted of one or more petals and clusters of stamens which had not shed from the fruit after petal-fall and are termed ‘adhering floral tissues’ (AFT) in this study (Plate 2.9 A). Lesions were also seen on fruit that were in contact with diseased fruitlets, necrotic lesions on leaves or other diseased fruit (Plate 2.9 B). Occasionally mycelium was seen connecting between AFT and the fruit surface at the centre of a developing lesion (Plate 2.11 A).

Field rot was observed on fruit on two occasions (20 December 1996 and 6 January 1997) in three orchards (12, 22 and 23). There had been over 30 mm rainfall during the 7 days before each of these dates. On 20 December 1996, 3 cm long fruit were observed with soft rot spreading down the length of the fruit (Plate 2.10), similar in appearance to stem-end rot of stored harvested kiwifruit caused by *B. cinerea*.

A B

Plate 2.9 A and B. (A) Adhering floral tissues on healthy 3–4 week old fruit and (B) large field rot lesion developing on a fruit that has contacted an adjacent diseased fruit.

Plate 2.10 Healthy fruit (left) and sclerotinia field rot on 3 cm long fruit (cross-section on right).

A B

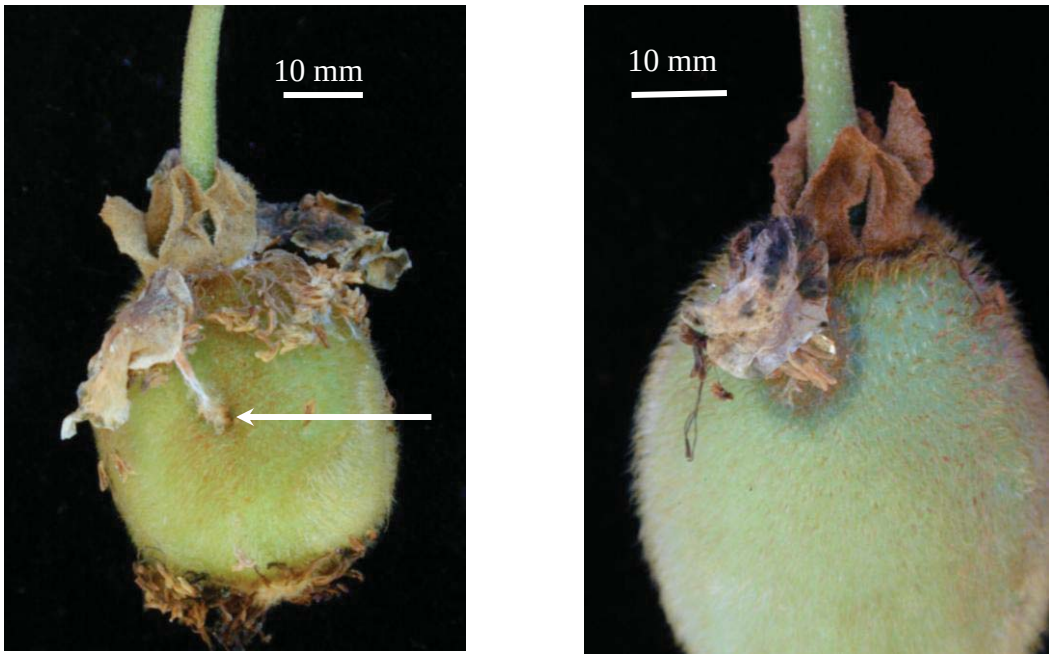


Plate 2.11 A and B. (A) Fruit with early stages of infection showing white mycelium between adhering floral tissues and fruit surface, 'brown speckle' symptom (arrow) and (B) field rot lesion on fruit directly beneath adhering floral tissues, note exudation droplet and pale green lesion margin.

Some lesions on fruit with field rot were small (<10 mm diameter) with a soft pale green centre and a 2–3 mm wide green margin, with little or no surface mycelium (Plate 2.11 B). Larger lesions had a similar water-soaked appearance and green margin. Fruit with field rot that had fallen to the ground often had numerous sclerotia (Plate 2.12). Some fruit without obvious field rot symptoms dropped from vines when touched. Close examination of these revealed small lesions, ca. 3–7 mm across, extending from the base of AFT towards the base of the pedicel, similar to the lesions on flowers that fell when touched (see above).

Plate 2.12 Dried and shrivelled remains of a fruit with field rot and many black sclerotia.

2.3.2.4 Other sclerotinia like symptoms

Flower buds and open flowers were observed which had symptoms characteristic of bacterial blossom blight caused by *Pseudomonas savastanoi* (Young *et al*., 1988). On buds this included chocolate brown, slightly sunken lesions on sepals and open cracked lesions <1 mm wide at the base of sepals. The latter was often associated with moist brown rot of the internal floral parts, particularly the stamens. Flowers were observed that had brown and stunted stamens and stunted pistils (Plate 2.13). Severely affected buds and flowers often failed to open, had moist and sticky internal floral parts and readily dropped from the vines leaving a green pedicel. These did not have discrete lesions, as occurred when flowers aborted because of sclerotinia (section 2.3.2.1).

Dry dark-brown shrivelled flowers were observed on canes and shoots broken by wind damage during flowering or petal-fall. These were different to sclerotinia diseased fruitlets in appearance because the ovary tissue was only 5–8 mm in length, they were very hard, had no internal cavities in which sclerotia had formed, and they did not have petals or stamens attached.

Plate 2.13 Kiwifruit flower with bacterial blossom blight symptoms, showing browning and stunting of stamens and pistils.

There were two symptoms on fruit that were similar to, but distinguishable from sclerotinia scarring symptoms. First, damage caused by fruit rubbing together or against shoots or leaves, produced circular or oval lesions with a slightly roughened often cracked appearance, ranging in colour from green/brown to dark brown and black. Second, leaf roller caterpillar damage appeared as scarring on fruit that had a well-defined scalloped margin and occasionally webbing and/or larvae or pupae present in close proximity to the point of damage (A. Tomkins, pers. comm.).

2.3.3 Recovery of *S. sclerotiorum* from floral tissues

Small lesions were observed during dissection of flowers that extended from the base of petals and stamens into the base of the ovary and sepals. *S. sclerotiorum* was recovered from 70% and 80% of flowers from orchards 22 and 23, respectively. This included recovery of *S. sclerotiorum* from all types of floral tissue sampled from orchard 22, but there was no *S. sclerotiorum* recovered from pistils and pedicels from orchard 23 (Figure 2.1). There was a significantly ($P<0.05$) higher incidence of *S. sclerotiorum* isolated from petals and stamens than from other tissues for each orchard (Figure 2.1).

Species of *Cladosporium*, *Phoma* and *Phomopsis*, and *Epicoccum purpurescens* were isolated from 15–51% of floral tissue samples from each orchard (Figure 2.2). *B.*

cinerea was isolated from 31% of floral tissues from orchard 23, but from only 3% of floral tissues from orchard 22. A further four genera (*Fusarium*, *Alternaria*, *Penicillium* and *Fusicoccum*), plus several unidentified cultures were isolated less frequently from flowers (0–17%) (Figure 2.2).

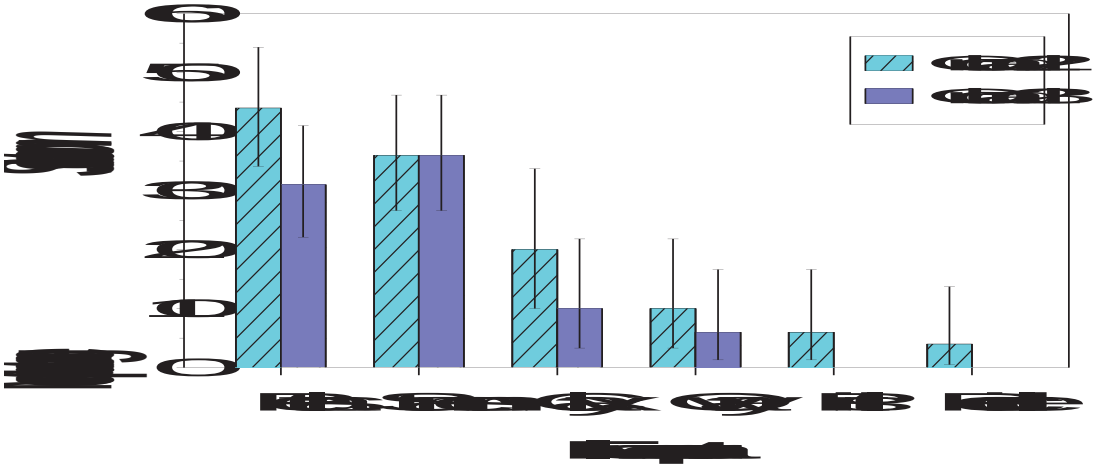


Figure 2.1 Mean percentage of *S. sclerotiorum* recovered from petals and stamens (n=100) and other floral tissues (n = 50) dissected from 3–4 day-old flowers in two orchards during November 1996. Bars are 95% confidence intervals.

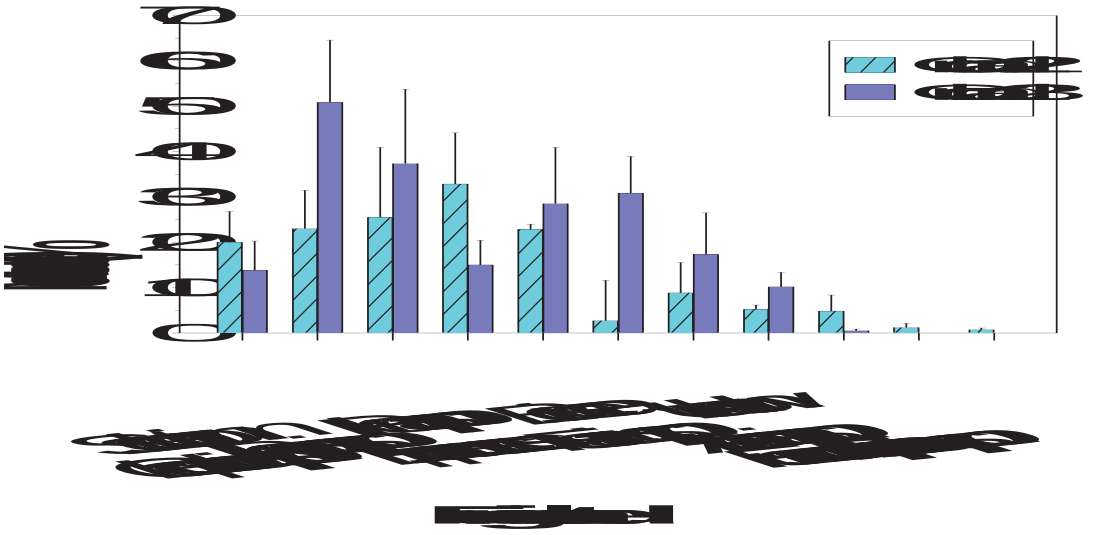


Figure 2.2 Mean percentage of fungi recovered from floral tissues dissected from 3–4 day-old flowers in two orchards during November 1996. Data is the mean of six tissue types and bars are standard errors.

2.4 Discussion

Although detailed descriptions of apothecial morphology were not made, the apothecia observed in kiwifruit orchards and the cultures which grew from the 34 apothecia placed on PDA were consistent with descriptions of *S. sclerotiorum* (Kohn, 1979b; Tariq *et al*., 1985) (section 1.2). Apothecia of *B. cinerea*, a common pathogen in kiwifruit, are similar to *S. sclerotiorum* apothecia but have not been reported in kiwifruit orchards, and several reviews of *Botrytis* spp. suggest that apothecial production is uncommon (Coley-Smith *et al*., 1980; Harrison, 1988; Brook, 1990b). It was therefore concluded that all apothecia observed were likely to be of *S. sclerotiorum*.

The presence of apothecia in groups (Plate 2.1) suggests that some degree of aggregation of apothecia occurs in kiwifruit orchards. Aggregation of *S. sclerotiorum* apothecia has been shown to affect the distribution of disease in bean and soybean (*Glycine max* (L.) Merr.) (Boland & Hall, 1988a; 1988b) and may also affect the distribution of airborne inoculum and/or disease in kiwifruit. Aggregation of apothecia may have resulted from aggregation of disease and sclerotial production or be caused by factors affecting the localised survival and germination of sclerotia (Coley-Smith & Cooke, 1971; Boland & Hall, 1988a). Apothecial density beneath kiwifruit vines is investigated in Chapter 7.

Symptoms of sclerotinia disease were observed in 11 of the 12 orchards during 1996/97. Pak *et al.* (1997) recorded sclerotinia disease in 16 out of 18 orchards studied during a survey from the same growing regions during the same season. Only a single block from each orchard was used in this study and that of Pak *et al.* (1997), therefore no conclusions can be reached regarding the distribution of sclerotinia disease between individual blocks within orchards.

Sclerotinia disease symptoms on fruitlets, leaves, shoots, and scarring and field rot of fruit were similar to those described by Pennycook (1982; 1985). However, 'dry withered buds', considered by Pennycook (1985) to be caused by *S. sclerotiorum*, rather than *Pseudomonas savastanoi* which is the causal organism bacterial bud rot (Young *et al*., 1997), were not observed during this study. It is unclear how common this disease symptom might be in kiwifruit orchards because Pennycook (1985) did not report on the incidence of sclerotinia diseased buds. It is possible that both *S. sclerotiorum* and *P.*

savastanoi were involved in the dry withered bud symptoms he describes, making correct identification of the causal pathogen difficult.

There are several observations in this chapter that suggest petals and stamens are the primary site of infection by *S. sclerotiorum* ascospores in kiwifruit. First, surface-sterilised petals and stamens without symptoms sampled from dissected 3–4 day-old flowers had the highest incidence of *S. sclerotiorum* compared with other floral tissues, suggesting these tissues are readily colonised by ascospores. Second, all of the 48 petals sampled from diseased fruitlets were colonised by *S. sclerotiorum*, the majority of these producing pure colony growth. Third, on flowers that dropped from vines when touched, small soft lesions extended from the base of petals which were collapsed with soft rot and that had remained attached to the flower. It cannot be determined from these observations whether both petals and stamens are colonised directly by *S. sclerotiorum* ascospores or if infection transfers from one to the other via mycelial spread.

The wide range of fungi other than *S. sclerotiorum* that were isolated from 3–4 day-old floral tissues suggests there is a high level of microbial competition on these non-permanent floral tissues while they are still attached to the flowers. *S. sclerotiorum* appears to be capable of establishing infections within these tissues despite this.

The lesions on flowers that dropped from vines when touched are clearly the early stages of the colonisation of the ovary and sepal tissues. Although the progress of these lesions was not monitored, colonisation of the permanent flower parts would presumably take place during the subsequent days, had they not been dislodged. The size of the ovary on diseased fruitlets is 1.5–2 times larger than the ovary of freshly opened flowers (Hopping, 1990). The time required for a doubling in size of the ovary corresponds to the appearance of diseased fruitlets ca. 7–14 days after anthesis.

The source of *S. sclerotiorum* inoculum leading to necrotic lesions on leaves may have been the floral tissues observed adhering to these lesions, as was suggested by Pennycook (1985). It was not unlikely that fungi such as *B. cinerea*, *Phoma* sp. and *Phomopsis* sp. were present within these necrotic lesions because floral tissues were colonised by several fungal genera (Figure 2.2).

Sclerotia developed on many diseased host tissues, including: fruitlets, staminate flower clusters, individual pistillate petals, lesions on shoots, fruit with scarring and field rot, and on several weed species beneath vines. This suggests there are several sources of over-wintering inoculum. Quantification of sclerotial production from diseased fruitlets and fruit with field rot is described in Chapter 7.

Fruit scarring symptoms were observed that were similar to those described and illustrated by Pennycook (1985) and Manning (1991). Small brown patches on fruit surfaces directly adjacent to floral tissues that remained attached to fruit were observed during December. These have not been described previously and it is possible that these lesions represent the early stages of fruit infection. Monitoring of symptom development on individual fruit would be necessary to confirm this.

Scarring symptoms on fruit were often small and superficial, while other lesions penetrated the outer pericarp causing a depression in the fruit surface. Fruit with field rot tended to drop from vines and rot on the ground. Pennycook (1985) suggests the main reason for the formation of scarring symptoms, as opposed to field rot, is that favourable conditions leading to infection are followed by dry weather, however these 'favourable conditions' were not defined. It is possible that the initial infection of fruit only occurs within the epidermal layers and that dry conditions may halt the expansion of this lesion into deeper cell layers. More advanced lesions are likely to result from infections spreading into the outer pericarp tissues leading to sunken scarring lesions by the time lesion expansion ceases. Experiments in Chapter 6 test the hypothesis that misting duration affects the type and severity of fruit disease.

Adhering floral tissues, necrotic lesions on leaves or diseased fruitlets were often in close proximity to scarring lesions on fruit. This is in agreement with descriptions given by Pennycook (1982) and Brook (1990a). Pennycook (1985) suggests fruit infection occurs when ascospores are deposited at infection sites that are in direct contact with a food base, such as adhering floral tissues. Yet, it is also possible that ascospores infect and colonise these floral tissues primarily during flowering and that fruit disease develops through mycelial infection whenever conditions are favourable. In addition, although Brook (1990a) states that "ascospores of *S. sclerotiorum* do not

invade fruits directly", this has not been proven experimentally and remains a possible infection pathway.

The following is a summary of the various distinguishable classes of sclerotinia disease symptoms observed in 'Hayward' kiwifruit in the Waikato and Bay of Plenty in New Zealand:

'**Petal blight**'- soft rotting of the flower petals visible ca. 5–10 days after anthesis (Plate 2.4 A and B).

'**Flower blight**' - observed on staminate vines during flowering as rotting clusters of flowers, frequently with white cottony mycelium (Pennycook, 1985) Staminate_Flower_Blight. Flower blight of pistillate vines was visible as small (<5 mm) lesions in the region between the base of the petal, the sepals and basal area of the ovary. Flower blight on pistillate vines, described by Pennycook (1985) as blossom blight and distinct from bacterial blossom blight, appeared ca. 10–14 days after anthesis, and is referred to in this thesis as 'diseased fruitlets'.

'**Diseased fruitlets**' - although soft during formation, these are dried fruitlets that form on pistillate vines ca. 10–14 days after anthesis and remain attached to the vine for several weeks or months before falling to the ground (Plate 2.6) Diseased_Fruitlet. The ovary portion of these is ca. 9–12 mm in length and the pedicel is typically completely brown. They always have adhering petals and stamens which are heavily colonised by *S. sclerotiorum*. Sclerotia are often present within internal cavities and externally.

'**Pedicel blight**' - white/brown lesion with purple/brown margin on pedicel, often near its base resulting from contact with an over-extended diseased sepal (Plate 2.5) Pedicel_Blight.

'Leaf and shoot blight' - necrotic white/brown lesions can develop on leaves, usually through contact with diseased petals during petal-fall and occasionally contact with diseased fruitlets (Plate 2.7 A) Leaf_Blight. Necrotic lesions on shoots and canes can develop where secondary spread has extended beyond the base of the pedicel in staminate vines or where other diseased tissues make contact.

'**Fruit scarring**' - this is a dry scar-like lesion on fruit that remain attached to vines. They are variable in area and severity, ranging from superficial patches ≤200 mm^2 (Plate 2.8 A) to larger areas that do not noticeably affect the fruit shape Fruit_scarring.

More severe scarring lesions develop that penetrate 2–10 mm into the fruit surface (Plate 2.7 B).

'**Brown speckle**' - small chocolate brown lesions ≤ 3 mm diameter observed mainly near the fruit shoulder in close proximity to AFT within a few weeks of flowering (Plate 2.11 A) Brown_speckle.

'**Field rot**' - soft rot of fleshy fruit that appear from ca. 2 weeks after anthesis until late February. Field rot can appear as a tide mark of soft rot progressing down the length of the fruit (Plate 2.10) Field_rot. Other lesion often spread from the shoulder of the fruit where AFT are present (Plate 2.11 B) Field_rot_AFT. Some of these may remain on vines and form a dry-shrivelled diseased fruit (a continuum of diseased fruitlets), although most fruit with field rot fall from vines within several days of infection. Field rots also develop during January and February, when spreading lesions form on large fruit (ca. 30–50 mm length), which readily fall from vines and can form numerous sclerotia.

Weed blight – an often localised secondary epidemic of sclerotinia on various weed spp., particularly originating from vine prunings laying beneath staminate vines.

These observations of sclerotinia in kiwifruit have more clearly defined the symptomology of this disease. They have also provided valuable experience in identifying sclerotinia symptoms and assisted in the design of field and laboratory experiments. The potential importance of petals as a site for ascospore infection and the role that floral tissues play in disease progress is investigated in Chapter 3.

Chapter 3: The role of adhering floral tissues in disease progress

3.1 Introduction

Floral tissues colonised by *S. sclerotiorum* ascospores are utilised as a food base from which subsequent disease develops in adjacent healthy tissues in several annual field crops, such as pea (Huang & Kokko, 1992), bean (Cook *et al*., 1975; Sutton & Deverall, 1983), sunflower (Kondo *et al*., 1988), and canola (Gugel & Morrall, 1986). This disease pathway is essential as *S. sclerotiorum* ascospores cannot directly infect healthy host tissues without external nutrients (Abawi & Grogan, 1975; Purdy, 1979; Tu, 1989).

Observations from Chapter 2 suggested that a close association exists between the presence of adhering floral tissues (AFT) on fruit and the development of scarring and field rot in kiwifruit. This is in agreement with previous observations that fruit disease is often centred on floral tissues that have remained on fruit (Sale, 1981; Pennycook, 1985; Manning, 1991). However, these reports do not quantify the occurrence of AFT on fruit, the role of ascospores as primary inoculum, nor did they confirm the relationship between AFT and development of fruit disease.

Isolations from surface-sterilised kiwifruit flower parts collected during flowering showed that *S. sclerotiorum* was more abundant within petals and stamens than in other floral tissues (section 2.3.3). Diseased fruitlets appeared 7–14 days after anthesis and fruit disease can occur from this time until late February. *S. sclerotiorum* mycelium has been shown to remain viable in diseased stems of dry bean, sunflower and canola for at least 4 months under field conditions (Huang & Kozub, 1993). *S. sclerotiorum* mycelium might survive within AFT on kiwifruit fruit. The hypothesis tested in this chapter is that *S. sclerotiorum* within AFT acts as a source of inoculum for secondary spread into permanent flower parts and fruit.

3.2 Objectives

To investigate the role of AFT in sclerotinia disease by determining:

- the pattern of stamen- and petal-fall in two orchards.
- if fruit disease is more prevalent on fruit with AFT.

- whether the removal of AFT soon after petal-fall reduces disease incidence on fruit.
- the range of fungi which colonise petals on intact flowers and AFT on fruit, and whether colonisation of petals by *S. sclerotiorum* causes them to adhere to developing fruit.
- whether *S. sclerotiorum* mycelium remains viable within kiwifruit petals in the orchard environment.

3.3 Survey of adhering floral tissues on fruit

3.3.1 Materials and methods

Two Katikati orchards were selected that had been treated with Hi-cane, but which had different vine training systems (orchards 2 and 4, Appendix 1). Two 0.5 m^2 areas of canopy were marked with coloured tape in each of 10 pistillate vines from two blocks in each orchard. Each of these vines were separated by at least two buffer vines. The two canopy areas were 0.3–1.0 m ('inner') and 1.3–2.0 m ('outer') away from the main cordon and on opposite sides. The 'inner' canopy area was located on the East side of odd numbered vines and on the West side of even numbered vines. Each canopy area contained 25–30 fruit.

The incidence and category of AFT was recorded on 20 fruit selected at random within each canopy area on six occasions during a 7 week period, starting 10 days after the start of anthesis (19 November 1996–6 January 1997). The category of AFT on fruit was determined by the number of petals (0, 1, 2, 3^+) and stamens (0–3, 4–10, 11–30 and 30^+) present. Whether stamens were grouped together, uniformly spread or clustered together directly beneath the petal(s) was also recorded.

In seven other Bay of Plenty orchards and three Waikato orchards (section 2.2.1) the incidence and category of AFT was determined on one occasion 7 to 8 weeks after petal-fall. This corresponded to the last assessment at the two Katikati orchards. The category of AFT was scored on a random sample of 20 fruit on each of 10 vines from one block in each orchard. Genstat was used to analyse the proportions of fruit with AFT using generalised linear models with logit link and binomial errors.

3.3.2 Results

Within the T-bar block (orchard 2) the 'inner' and 'outer' canopy areas had a similar percentage ($P>0.05$) of fruit with different categories of AFT. In the pergola block (orchard 4) there were significantly ($P<0.01$) more fruit with 'no AFT' and significantly fewer ($P<0.01$) fruit with 'stamens only' in the 'outer' canopy area compared with the 'inner' canopy area, from 7 December to 6 January. Other than this there were no significant differences between the two canopy areas or between the two orchards, therefore the data was pooled.

Less than 30% of fruit had three or more petals on 19 November, indicating that petal-fall was well advanced in each orchard (Figure 3.1). The percentage of fruit with two or more adhering petals decreased significantly ($P<0.001$) with time in both orchards. The percentage of fruit with one adhering petal remained at a similar level, ranging from 21–25% (Figure 3.1). From 30 November onwards 60–94% of fruit with adhering petals also had adhering stamens that were grouped together and clustered in close association with the petals.

The percentage of fruit with 'stamens only' increased significantly ($P<0.001$) between 19 and 30 November and then declined steadily (Figure 3.1). Of these, 11%, 38% and 51% had 4–10, 11–30 and 30^{+} stamens, respectively, on 23 November. However on 6 January 79%, 13% and 8%, had 4–10, 11–30 and 30^{+} stamens, respectively. The percentage of fruit with 'no AFT' increased significantly between 30 November and 6 January (Figure 3.1).

The percentage of fruit with different categories of AFT from the 10 orchards was similar to the 6 January assessment in the two Katikati orchards (Figure 3.1 and 3.2), although there was considerable spread in the orchard means e.g. 'petal = 1' ranged from 12–33%.

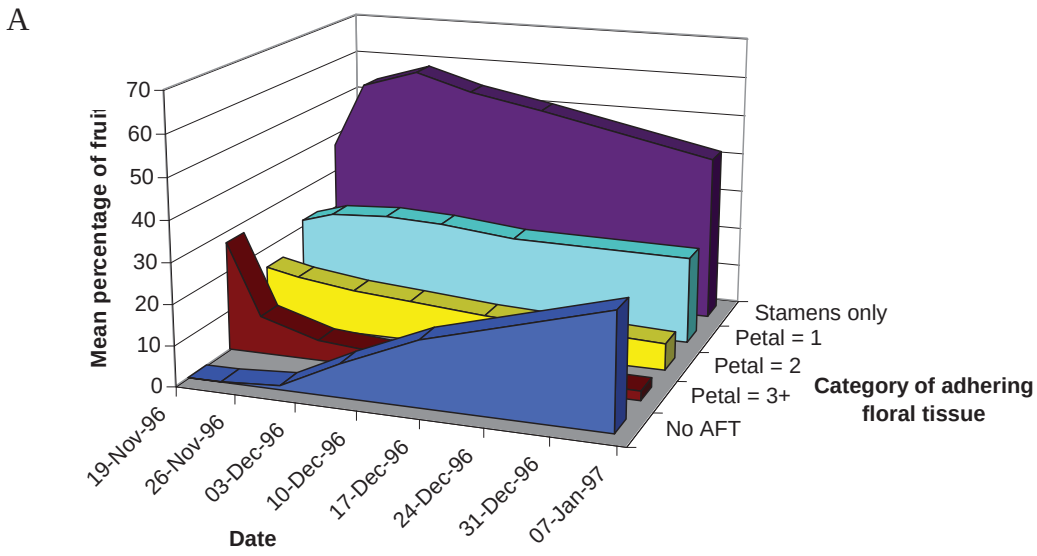


Figure 3.1 Mean percentage of fruit with different categories of adhering floral tissues (AFT) from two orchards during an 8 week period post-flowering (n = 800, max SE ± 3.6%). N.B. Categories 'Petal = 1, 2 and 3^{+}' and 'Stamens only', had 4–30^{+} stamens.

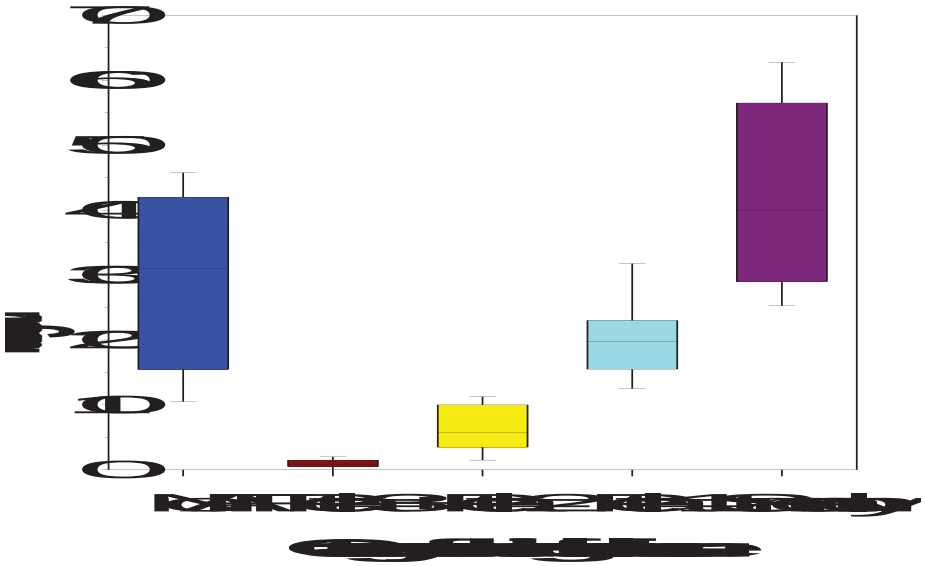


Figure 3.2 Percentage of fruit with different categories of adhering floral tissues (AFT) from 10 orchards sampled once, 7 to 8 weeks after flowering (n = 200), whiskers show 5% and 95% percentiles, horizontal line the median and (•) are outliers. N.B. Categories 'Petal = 1, 2 and 3^{+}' and 'Stamens only', had 4–30^{+} stamens.

3.4 Survey of fruit scarring on fruit with and without adhering floral tissues

3.4.1 Materials and methods

The incidence and severity of sclerotinia scarring on fruit with and without AFT was determined on 7 January 1997 in two Waikato orchards that received no fungicide applications (orchards 22 and 23). Fruit without AFT were designated as having no petals and less than three stamens. Twenty fruit with AFT and 20 fruit without AFT were chosen at random on each of 20 vines from one block in each orchard. The area of scarring on fruit was estimated by measuring the length and width of lesions. Fruit with scarring lesions ≥100 mm^2 were not suitable for export and were termed 'reject fruit'. The category of AFT on fruit was scored as in the previous experiment.

Genstat was used to analyse the mean incidence of fruit with scarring and reject fruit using generalised linear models with logit link and binomial errors. Least squares analysis was used to determine significant differences in the mean area of scarring on fruit with and without AFT.

The category of AFT on fruit with scarring was also recorded on random samples of fruit in 11 orchards during disease assessments as part of field trials described in Chapter 7. Categories of AFT were pooled into seven major categories. These were analysed in Genstat using generalised linear models with log link and errors proportional to Poisson distribution to determine significant differences in the proportion of scarred fruit with different categories of AFT.

3.4.2 Results

The incidence of fruit with scarring symptoms and the percentage of reject fruit was significantly greater ($P<0.001$) on fruit with AFT than on fruit without AFT in both orchards (Table 3.1). The incidence and mean area of scarring was significantly greater ($P<0.01$) on fruit with AFT from orchard 22 compared with similar fruit from orchard 23, and was also the case for fruit without AFT. The mean area of scarring was not significantly different ($P>0.05$) on fruit with and without AFT sampled from within the same orchard (Table 3.1).

Fruit with sclerotinia scarring were observed in 9 of the 11 orchards during disease assessments and there were AFT within 5 mm of most scarring lesions. There were significantly more scarred fruit with AFT that consisted of one petal and 11–30 stamens than two petals and 11–30 stamens (Figure 3.3). These two categories accounted for 64% of AFT on scarred fruit. Eighty four percent of AFT on scarred fruit had stamens clustered beneath the adhering petals. An average of 11% of fruit with scarring had no AFT, i.e. no petals and <4 stamens. Sixteen of the 41 scarred fruit from orchard 22 and 23 that had no AFT, had other diseased tissues in contact or close proximity (<5 mm) to the scarring symptoms, including: diseased fruitlets, AFT on adjacent fruit, adjacent fruit with scarring or field rot, and necrotic lesions on leaves.

Table 3.1 Mean incidence and area of scarring symptoms and percentage reject fruit, from samples of fruit with adhering floral tissues (AFT) and without adhering floral tissues, from two Waikato orchards on 7 January 1997.

Orchard	Fruit sample	Mean % of fruit with scarring	Mean area of scarring[1] (mm^2)	Mean % reject fruit[2]
22	With AFT	42.3 (2.5)[3]	122 (8)	23.3 (2.2)
22	Without AFT	7.5 (1.4)	109 (19)	3.0 (0.8)
23	With AFT	27.8 (2.3)	83 (10)	9.3 (1.5)
23	Without AFT	2.8 (0.7)	62 (31)	0.5 (0.4)

[1] For those fruit with scarring.
[2] Reject fruit are those with scar area ≥ 100 mm^2.
[3] Standard error (n = 400).

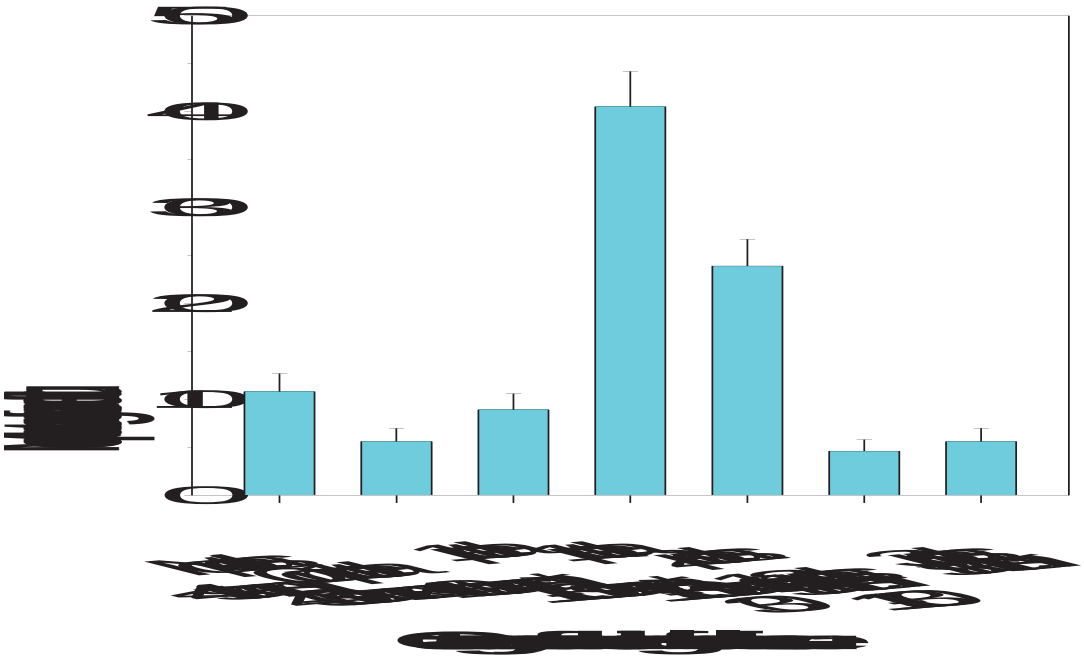


Figure 3.3 Mean percentage of scarred fruit with different categories of adhering petals and stamens from 11 orchards (n = 519 fruit) assessed during the 1996/97 season, (* = stamens clustered beneath adhering petals), bars are standard errors of proportions.

3.5 Incidence of fungi from field collected petals and adhering floral tissues

3.5.1 Materials and methods

During November and December 1996, petals were collected from 3–4 day-old flowers at ca. 50% flowering in each of the 12 orchards used in section 3.3.1. One petal was taken from each of 10 flowers selected at random from each of 10 pistillate vines/orchard. These were surface-sterilised (section 2.2.2) and placed individually on Petri dishes of PDA. During January 1997, AFT were collected from 10 fruit selected at random from each vine, surface-sterilised and placed on Petri dishes of PDA. Fungal colonies growing from tissues were identified to genus level after 5 and 12 days incubation at ambient temperatures on the laboratory bench, as described in section 2.2.3. Although colonies of bacteria were frequently observed these were not recorded because the growth medium and method was not appropriate for quantifying bacteria.

Five diseased fruitlets, five AFT from scarred fruit and five AFT from fruit without scarring, were sampled from each of 10 vines in orchard 22, on 31 January 1997. Each

AFT plus one petal from each diseased fruitlet was surface-sterilised and placed singly on a Petri dish of PDA and incubated on the laboratory bench. The presence of *S. sclerotiorum* and other fungi was recorded after 5 and 10 days.

3.5.2 Results

Isolations from surface-sterilised petals and AFT yielded fungi from 13 and 15 genera respectively (Figure 3.4 A and B). *S. sclerotiorum* was recovered from 19% (SE ± 5, range 0–53%) of petals and 11% (SE ± 5, range 0–43%) of AFT, yet of these only 2% of petals and 3% of AFT yielded pure cultures of *S. sclerotiorum*. *Cladosporium* spp. were most common on petals and *E. purpurescens* was most common on AFT. The percentage recovery of *E. purpurescens* and species of *Fusarium* and *Mucor* was generally higher on AFT than on petals (Figure 3.4 A and B). A further 3–38% of fungal isolates from both tissue types were classed as unknown. Less than 1% of all isolations produced no growth on PDA.

Ninety four percent of the petals taken from diseased fruitlets yielded pure cultures of *S. sclerotiorum*. A further 4% yielded *S. sclerotiorum* plus other fungi, while no growth was recovered from the remaining petal. Forty two percent of the AFT collected from fruit with scarring yielded pure *S. sclerotiorum* cultures and 28% yielded mixed cultures of *S. sclerotiorum* and other fungi. In contrast, only 2% of the AFT collected from fruit without scarring symptoms yielded *S. sclerotiorum*. All remaining AFT from fruit with and without scarring yielded mixed cultures of a range of other fungi.

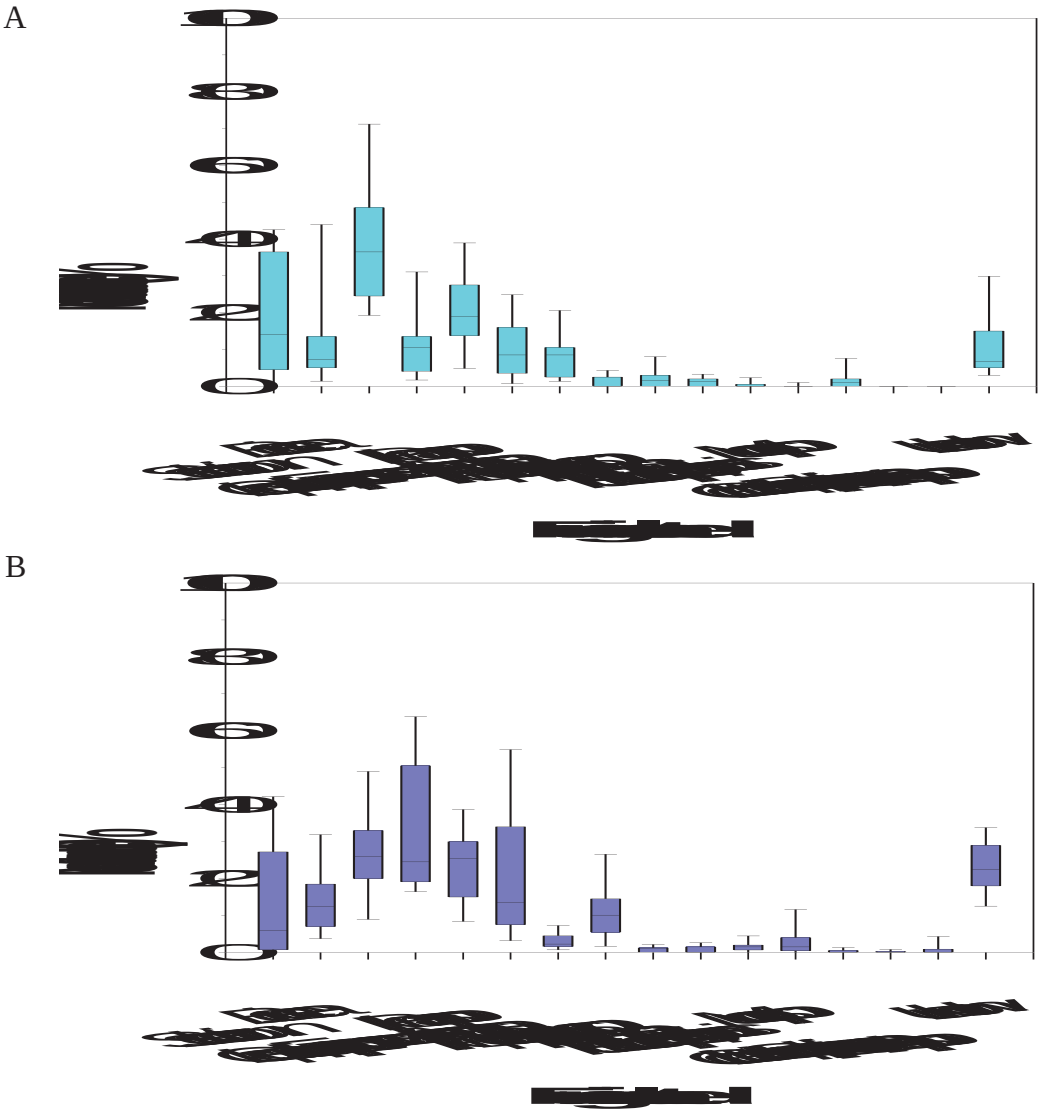


Figure 3.4 A and B. Mean incidence of fungi on PDA isolated from samples of (A) petals during flowering, November–December 1996 and (B) adhering floral tissues on fruit during January 1997, from 12 orchards. Whiskers show 5% and 95% percentiles, horizontal line the median and (•) are outliers.

3.6 Experiment 3-1: Effects of removing adhering floral tissues on disease incidence

3.6.1 Materials and methods

On 7 December 1997, 2 weeks after mid-flowering, 10 fruit with no AFT, 10 fruit with AFT that were immediately removed by hand, and 10 fruit with AFT that were allowed to remain on the fruit, were selected on each of 15 vines at orchard 22. Fruit were selected in groups of three, one from each treatment, not more than 200 mm apart. There were five groups of three fruit on each side of each vine. The fruit in each

treatment had different coloured lengths of wool tied to the pedicel. The vines did not receive any fungicide applications.

On 7 January and 9 February 1998 the incidence of diseased fruitlets and fruit scarring was recorded. The area of each scarring lesion was estimated by measuring their length and width. On 13 January 1998 the incidence of diseased fruit and AFT on fruit was determined on fruit from the same 15 vines at orchard 22, excluding the fruit used above. Fruit were selected by choosing a fruiting shoot at random and assessing all fruit on the selected shoot. Between five and seven shoots were selected on each side of a vine so that at least 40 fruit/vine were assessed. The purpose of this was to establish the background incidence of disease and AFT on fruit for comparison with that obtained from the tagged fruit.

Genstat was used to analyse the proportion of diseased fruitlets and fruit with scarring in each treatment using generalised linear models with logit link and binomial errors. Least squares analysis was used to compare the mean area of scarring on fruit in each treatment.

3.6.2 Results

Small soft white/brown lesions <10 mm^2 in area were observed on several fruit at the point of contact between AFT and the ovary and/or sepals, when AFT were removed. The incidence of diseased fruitlets and fruit with scarring was significantly less ($P<0.001$) on fruit that had AFT removed, compared with fruit with retained AFT (Table 3.2). The fruitlets with no natural AFT only formed one diseased fruitlet (0.7%) and none developed scarring symptoms. The mean area of scarring was not significantly different ($P>0.05$) between fruit with removed and retained AFT or between assessment dates.

On 13 January the incidence of diseased fruitlets was 8.2% (SE ± 0.7) and the incidence of fruit with scarring was 4.2% (SE ± 0.9) on the random sample of fruit (n=655) from the same vines in this block. This level of disease was intermediate between that obtained on fruit with removed and retained AFT (Table 3.2). Thirty five percent of fruit had AFT and 90% of fruit with scarring lesions had AFT in close proximity to the scarring symptoms.

Table 3.2 Mean percentage of diseased fruitlets, fruit with scarring and mean area of scarring from fruit with no natural adhering floral tissues (AFT), fruit with removed adhering floral tissues and fruit with retained adhering floral tissues.

Treatment	Mean % diseased fruitlets	Mean % of fruit with scarring		Mean area of scarring[1] (mm^2)	
	7 January	7 January	9 February	7 January	9 February
No natural AFT	0.7 (0.6)[2]	0 (0)	0 (0)	-	-
Removed AFT	5.3 (1.8)	2.6 (1.3)	2.0 (1.1)	27 (11)	93 (34)
Retained AFT	15.9 (2.6)	17.9 (2.7)	19.2 (2.8)	21 (5)	40 (13)

[1] For those fruit with scarring.
[2] Standard error (n = 150).

3.7 Experiment 3-2: Effects of inoculum type and misting on retention of petals and incidence of diseased fruitlets

3.7.1 Materials and methods

Part A: A culture of *S. sclerotiorum* was isolated on 15 January 1995 from a sclerotium on a kiwifruit fruit with field rot collected from an orchard on Moffat Rd, Bethlehem, near Tauranga[1]. This isolate has been deposited with the culture collection administered by Landcare at Mt Albert Research Centre, New Zealand (ICMP #13844) and for this study is referred to as isolate Sc1. Ascospore inoculum of this isolate was prepared and stored in a desiccator at 4°C on sterile filter membrane discs, as described in Appendix 2, and was used in all experiments requiring ascospore inoculum.

On 25 November 1996, 10 kiwifruit vines were selected from four adjacent rows of eight single planted pistillate vines in a block at orchard 20 (Appendix 1). Vines were separated by at least one buffer vine. The canopy area of each vine was divided into five sectors, three on one side (each 1.6 m wide) and two on the opposite side (each 2.5 m wide). Ten 2 day-old flowers were chosen at random within each sector during mid-flowering and tagged around the pedicel with different coloured wool for each sector. On each tagged flower, a small quantity of bright orange paint (Fast Dry Enamel, Plasti-

[1] Supplied by Dr Kerry Everett

kote Co.) was applied to the sepals directly in line with one petal. This 'marked' petal was used for inoculation and allowed these to be distinguished from un-inoculated petals on the same flower. Each sector within a vine was randomly assigned to one of five treatments that were applied on 26 November:

1. agar plug inoculation, misted with sterile water and bagged
2. ascospore inoculation, misted with sterile water and bagged
3. un-inoculated (control), misted with sterile water and bagged
4. ascospore inoculation, not misted and no bag
5. un-inoculated (control), not misted and no bag

Flowers in treatment 1 were inoculated with a 4 mm diameter agar plug of *S. sclerotiorum* (isolate Sc1) taken from the growing margin of 3 day-old culture on PDA. The agar plug was inverted onto the abaxial surface of the 'marked' petal, ca. 10 mm from the base. Flowers in treatments 2 and 4 were inoculated with dry *S. sclerotiorum* ascospores. A 3 mm wide camel-hair brush was brushed several times over the surface of four grids of membrane filter disc containing ascospores (Appendix 2) and then brushed 4–5 times over both the abaxial and adaxial surfaces of the 'marked' petal. Flowers in treatments 1, 2 and 3 were misted with SDW using a hand held sprayer, ensuring no runoff, and enclosed within waxed paper bags (160 × 140 mm). The top of the bag was held with a twist-tie around the pedicel and one corner of the bag was cut to allow rain water to drain.

The bags were removed after 48 h and the number of petals remaining on each flower, and whether the 'marked' petal remained, was recorded. The number of petals (total and 'marked') remaining on flowers was recorded again after 9 days, together with the incidence of diseased fruitlets.

Part B: Ten vines were selected from five rows of late-flowering pistillate vines in a block at orchard 21 on 26 November 1997 (Appendix 1). Vines were separated by one buffer vine and the canopy of each vine was divided into quarters. Five 2 day-old flowers from within each quarter were randomly selected during petal-fall and tagged around the pedicel with different coloured wool. Sepals were painted as in the previous experiment. Each quarter vine was randomly assigned to one of four treatments:

1. agar plug inoculation, misted with sterile water and bagged

3. agar plug inoculation (control), misted with sterile water and bagged
4. un-inoculated (control), misted with sterile water and bagged

Treatments 1 and 2 were inoculated as in the previous experiment while treatment 3 was inoculated with a sterile agar plug of PDA. The bags were removed after 68 h and the number of 'marked' petals remaining on flowers recorded. Inoculated petals were observed for the presence of lesions. The number of petals remaining on flowers and the incidence of diseased fruitlets and fruit with scarring was recorded 23 days after inoculation. Where only pedicels remained these were surface-sterilised (section 2.2.2) and placed on PDA. If *S. sclerotiorum* was present the pedicels were considered to be from diseased fruitlets.

ANOVA was carried out using Genstat to determine treatment differences in the mean number of petals retained on flowers. Generalised linear models with logit link and binomial errors were used to analyse the proportion of flowers with 'marked' petals attached and proportion of diseased fruitlets in each treatment.

3.7.2 Results

In Part A, flowers inoculated with an agar plug of *S. sclerotiorum* culture had significantly fewer ($P<0.001$) petals/flower remaining after 48 h, compared with un-inoculated controls and flowers that were ascospore-inoculated without misting and bagging (Table 3.3). A similar proportion (51–70%) of 'marked' petals were retained on flowers from each treatment after 48 h (Table 3.3). All of the retained inoculated petals in the *S. sclerotiorum* agar plug treatment had water-soaked lesions typical of sclerotinia colonisation.

After 9 days there were significantly more ($P<0.001$) petals in total and more flowers with 'marked' petals retained on flowers inoculated with an agar plug of *S. sclerotiorum* culture compared with other treatments (Table 3.3). Diseased fruitlets developed from 64% of the flowers inoculated with an agar plug of *S. sclerotiorum* culture. All of these had retained the inoculated petal after 48 h, and all except two had retained the inoculated petal after 9 days. Lesions developed on one retained petal from treatment 2 and on two retained petals from treatment 4. Two fruit from treatments 2 and 4

developed small scarring lesions (25–50 mm^2), however, it was not determined if these corresponded to those that had lesions on petals.

Table 3.3 Retention of petals and incidence of diseased fruitlets from flowers (n=100) inoculated with an agar plug containing *S. sclerotiorum* (misted with SDW and bagged for 48 h) or by dry brushing with *S. sclerotiorum* ascospores, with and without misting and bagging.

Inoculation treatment	Mean number petals retained/flower		% fruit with 'marked' petal retained		% diseased fruitlets
	48 h	9 days	48 h	9 days	9 days
1. Agar[1] + M/Bag[2]	1.9	0.8	68	62	64
2. Ascospore + M/Bag	2.6	0.2	44	2	0
3. Nil + M/Bag	3.1	0.2	51	3	0
4. Ascospore	4.6	0.3	70	2	0
5. Nil	3.7	0.2	64	3	0
	(0.97)[3]	(0.15)	[7.7][4]	[4.5]	[4.8]

[1] 5 mm diameter agar plug of *S. sclerotiorum* culture (isolate Sc1) on PDA.
[2] Flowers hand misted with SDW after inoculation and enclosed within a waxed paper bag for 48 h.
( )[3] Least significant difference ($P<0.05$).
[ ][4] Maximum standard error.

In Part B, significantly fewer petals ($P<0.01$) were retained on the flowers in treatment 1 compared with ascospore-inoculated flowers and un-inoculated flowers, after 62 h (Table 3.4). Forty six percent of the inoculated petals in treatment 1 had extensive lesions covering >90% of the petal area. A further 8% of flowers in treatment 1 had several small lesions on the inoculated petal and 10% had petals with extensive lesions that had abscised and fallen into the bag. On the remaining 36% of flowers the agar plug of *S. sclerotiorum* culture had dried and no lesions had formed. There were no extensive lesions on petals from other treatments, although several lesions ranging from 4–50 mm^2/petal, were present on 32% of petals from treatment 2 after 62 h.

There were no diseased fruitlets after 62 h. All except one of the agar-inoculated flowers that had petals with extensive lesions had developed into diseased fruitlets 23 days after inoculation (Table 3.4). Scarring symptoms developed on only one of the

agar-inoculated flowers that had retained a petal with lesions. Two ascospore-inoculated flowers that had lesions on the petal developed into diseased fruitlets (Table 3.4), but none from this treatment developed scarring symptoms. The diseased fruitlets observed in these two experiments appeared to have smaller ovaries than naturally occurring diseased fruitlets.

Table 3.4 Retention of petals and incidence of diseased fruitlets on flowers (n = 50) inoculated with an agar plug containing *S. sclerotiorum*, a sterile agar plug, and by dry brushing with *S. sclerotiorum* ascospores. All flowers were misted with SDW and bagged for 62 h.

Inoculation treatment	Mean number petals retained/flower		% fruit with 'marked' petal retained		% diseased fruitlets
	62 h	23 days	62 h	23 days	23 days
1. Agar[1]	3.8	1.1	88	53	44
2. Ascospores	5.2	0.5	88	7	4
3. Agar (control)	5.5	0.5	96	6	0
4. Nil (control)	5.0	0.8	84	9	0
	(0.80)[2]	(0.45)	[4.8][3]	[6.8]	[7.1]

[1] 5 mm diameter agar plug of *S. sclerotiorum* culture (isolate Sc1) on PDA
( )[2] Least significant difference (P<0.05).
[ ][3] Maximum standard error.

3.8 Experiment 3-3: Survival of *S. sclerotiorum* within petals

3.8.1 Materials and methods

The survival of *S. sclerotiorum* mycelium within kiwifruit petals was monitored over an 8 week period from 23 December 1996 to 18 February 1997. Three hundred gamma irradiated (4 Mrad.), sterile dried kiwifruit petals[2] were each inoculated by inverting a 4 mm diameter agar plug of *S. sclerotiorum* taken from the growing margin of a 3 day-old culture of isolate Sc1 on PDA. Petals were placed on tissue paper supported on wire mesh in two plastic chambers (380 × 380 × 160 mm) containing 800 ml SDW and were incubated at 20°C. After 4 days, 20 petals were taken at random, surface-sterilised and placed individually on Petri dishes of PDA and kept at ambient temperatures. Growth

[2] Supplied by Dr P.A.G. Elmer

of *S. sclerotiorum* was recorded after 5 and 10 days to confirm colonisation of the petals.

Of the remaining petals, eight were selected at random and attached in a single row on each of 40 rigid plastic Corflute boards (40 × 200 mm) with 10 mm push pins. Five vines were selected in each of four T-bar rows in a block at orchard 20. Two boards were attached to either support wires or canes on each vine, approximately 1.6 m above the ground. One board was positioned near the outer edge of the canopy about 1 m from the main cordon so that it was exposed to direct sunlight for at least part of the day. The other was positioned within 200 mm of the main cordon. These were termed 'exposed' and 'shaded' treatments, respectively. Each week, from 2–8 weeks after the boards were placed in the orchard, one petal was taken at random from each board, surface-sterilised and placed on a Petri dish of PDA. Growth of *S. sclerotiorum* was recorded from each petal after 5 and 10 days.

The percentage recovery of *S. sclerotiorum* within petals was analysed with a statistical package Flexi©, that uses Bayesian smoothers as an extension to non-linear regression (Upsdell, 1994). Although an equation of the fitted curve is not available with this program, Flexi outputs 95% confidence bands. The slope of the fitted curve is significant ($P<0.05$) when the 95% confidence bands associated with a plot of the derivative of the fitted curve (dy/dx, not presented) is non-zero.

3.8.2 Results

Two tropical cyclones passed over the North Island of New Zealand during the first 2 weeks of this experiment, physically damaging some petals, particularly those in the 'exposed' treatment. Damaged petals were sampled, but those less than 100 mm^2 in area were excluded from the results because they were not considered representative.

The average recovery of *S. sclerotiorum* from petals was 86% (SE ± 7) in the 'exposed' treatment and 91% (SE ± 4) in the 'shaded' treatment (Figure 3.5). The lowest incidence of *S. sclerotiorum* occurred on 4 February. Overall, there was no significant difference ($P>0.05$) between 'exposed' and 'shaded' petals and no significant decrease in the percent recovery of *S. sclerotiorum* ($P>0.05$) during the 8 week period.

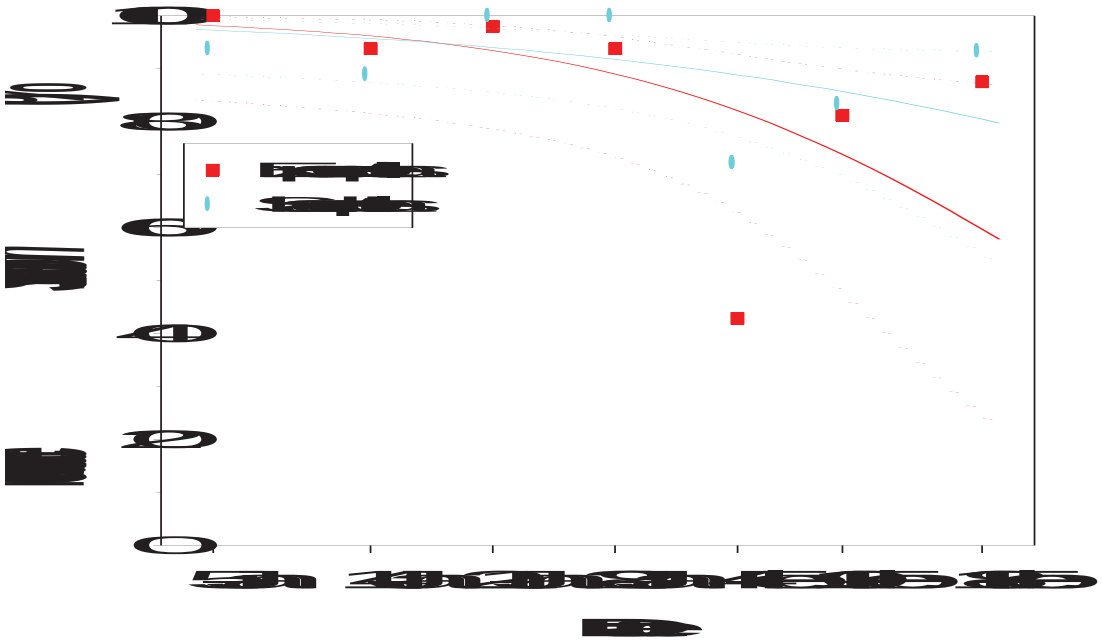


Figure 3.5 Mean incidence of *S. sclerotiorum* recovered from petals colonised by *S. sclerotiorum* ascospores and suspended on plastic boards in 'exposed' and 'shaded' positions within T-bar kiwifruit vines. Solid lines are fitted curves using Flexi, dotted lines are 95% confidence bands.

3.9 Discussion

Petals and stamens have an important role in floral biology because they attract pollinating insects and produce pollen (Free, 1993). However, they do not serve any useful purpose for the plant after fertilisation and are shed from developing fruit in most horticultural crops (Free, 1993; van Doorn & Stead, 1997). The retention of floral tissues on kiwifruit was studied in detail in two Katikati orchards. Petal-fall was well advanced 10 days after the start of anthesis in Hi-cane treated vines. Although petal-fall is considered to be complete soon after anthesis (Brundell, 1975), 30 and 31% of fruit in these two orchards had not undergone complete petal-fall within 6–8 weeks of anthesis. It seems that whether petals remain on fruit is largely determined within 2 weeks of anthesis because there was little change in the proportion of fruit with adhering petals between 2 and 6 weeks after anthesis.

Many fruit had adhering stamens and no adhering petals through December and early January, suggesting that petal-fall occurs more rapidly than stamen-fall. Fruit with adhering petals most often had clusters of stamens adhering near the base of the petal(s).

The one-off survey of the incidence of AFT on fruit from 10 orchards showed there was considerable variation in the percentage of fruit with AFT in early January (52–91%). This suggests there may be site specific factors which influence the retention of AFT on fruit, such as environmental conditions, orchard management or variations in the Hayward cultivar.

The specific reason for petals and stamens failing to abscise after anthesis on some fruitlets is unknown. Rainfall during flowering has been observed to increase the retention of petals and stamens (H. Pak pers. comm.). Petals inoculated with agar plugs containing *S. sclerotiorum* mycelium developed extensive lesions and virtually all of these were retained on fruit, whilst un-inoculated and ascospore-inoculated petals abscised normally. Thus, extensive colonisation by *S. sclerotiorum* may cause floral tissues to remain attached to immature fruit, possibly through the formation of a mycelial 'connection' across the abscission zone. This is supported by the high incidence of *S. sclerotiorum* from adhering petals on diseased fruitlets. Alternatively, this irregularity may be an inherent trait of 'Hayward' kiwifruit grown in New Zealand.

Ascospore-inoculation of individual petals on some flowers resulted in the development of diseased fruitlets or scarring on fruit. This is the first report of sclerotinia disease in kiwifruit resulting from inoculation of intact flowers with ascospores. This supports the hypothesis that ascospores are the primary source of sclerotinia disease in kiwifruit, as in crops such as pea (Huang & Kokko, 1992), bean (Sutton & Deverall, 1983), and canola (Jamaux *et al*., 1995). The low incidence of disease in petals inoculated with ascospores may be related to timing of the applications or to sub-optimal environmental conditions within the waxed paper bags, as evidenced by the drying of several agar plugs in this trial. Furthermore, the development of diseased fruitlets following inoculation of petals with ascospores, and the re-isolation of *S. sclerotiorum* from these diseased tissues, fulfils two key steps in Koch's postulates that confirm the causal organism for a particular disease (Manners, 1993).

Isolations from surface-sterilised petals collected during flowering and from AFT collected from fruit showed that virtually all of these tissues were colonised by numerous fungi and bacteria, and that the incidence of most fungi was similar on petals and AFT. This suggests that there is a high level of microbial competition on petals and

AFT and that the nutrient status of AFT would be considerably depleted compared with fresh petals. AFT on fruit are also often very dry. These factors probably mean that AFT become unfavourable for colonisation by *S. sclerotiorum* ascospores within 1–2 weeks of anthesis. Therefore, it is most likely that the *S. sclerotiorum* mycelium within AFT originates from ascospore colonisation that takes place during flowering and petal-fall rather than later in the season.

The term anthoplane was used by Inglis & Boland (1990) to describe the unique and highly competitive microbial habitat on flowers. *S. sclerotiorum* appears to be successful at colonising this substrate as it was present in 0–53% of kiwifruit petals and 0–43% of AFT on fruit. *S. sclerotiorum* is also prevalent among the microflora within floral tissues of crops such as bean (Boland & Hall, 1987) and canola (Turkington *et al*., 1991). Microbial competition from saprophytes on floral tissues of bean and canola has been shown to reduce infection by ascospores and disease development (Inglis & Boland, 1990; Hutchins & Archer, 1994). Recent experiments have shown that conidial suspensions of *E. purpurescens* sprayed onto kiwifruit flowers inhibited colonisation of petals by *S. sclerotiorum* ascospores and reduced disease on fruit when treated petals were held in contact with the fruit surface (Hoyte *et al*., 1998; Elmer *et al*., 1999a).

The hypothesis that *S. sclerotiorum* mycelium within AFT on kiwifruit is the principal source of secondary spread into permanent floral tissues causing diseased fruitlets and into fruit causing scarring and field rot to develop, is strongly supported by the data:

- Inoculation of flower petals with agar plugs of *S. sclerotiorum* culture caused rapid development of extensive lesions and these petals remained attached to fruitlets; most developing into diseased fruitlets. The smaller size of these diseased fruitlets suggests that the rate of colonisation through the permanent floral tissues was faster or occurred at an earlier stage in development, than in naturally infected flowers.
- The incidence of pure *S. sclerotiorum* cultures from surface-sterilised field-collected petals and AFT was very low, 2% and 3% respectively, but was 94% from petals adhering to diseased fruitlets. It appears likely that if petals become extensively colonised from natural infection then diseased fruitlets will develop, possibly reflecting the high inoculum potential.
- Adhering floral tissues were present on all diseased fruitlets and 93% of fruit with scarring.

- Fruit with AFT in two orchards had 6–10 times higher incidence of sclerotinia scarring symptoms than fruit without AFT (section 3.4).
- Fruit with no naturalAFT rarely develop into diseased fruitlets or fruit with scarring, compared with fruit with AFT (Table 3.2). Furthermore, the removal of AFT within 2 weeks of anthesis significantly reduces the incidence of disease. Similarly, the removal of flower heads on tobacco plants reduced sclerotinia disease caused by fallen corollas landing on leaves (Hartill & Campbell, 1974).
- There was a 70% incidence of *S. sclerotiorum* in AFT sampled from fruit with scarring and a 2% incidence of *S. sclerotiorum* in AFT sampled from fruit without scarring (section 3.5). This suggests that the fruit with AFT are at greatest risk of becoming diseased when the AFT are colonised by *S. sclerotiorum*.
- The survival experiment showed that *S. sclerotiorum* mycelium remained viable within petals for at least 8 weeks under field conditions.

It is also possible that AFT provide a micro-environment which might favour direct infection of fruit surfaces by ascospores, perhaps by trapping moisture or releasing nutrients. Several studies have shown ascospores require a nutrient source such as dead flowers (Abawi & Grogan, 1975; Huang & Kokko, 1992), pollen (Huang & Kokko, 1993; Huang *et al*., 1997), sucrose (Sedun & Brown, 1987), or injury to leaves (Newton & Sequeira, 1972; Abawi & Grogan, 1975), before they can germinate and infect host tissues. Direct infection of healthy host tissues has only been observed on young non-permanent floral tissues of sunflower, bean, soybean and canola (McLean, 1958; Sutton & Deverall, 1983; Lamarque *et al*., 1985; Jamaux *et al*., 1995). It is therefore unlikely that ascospores are capable of infecting directly into kiwifruit fruit, unless an adequate food base such as floral tissues are present. The effects of pollen or nutrient exudates on ascospore infection of fruit warrants investigation.

In conclusion, 'Hayward' kiwifruit retain non-permanent floral tissues on a proportion of fruit. This is the first detailed report of AFT on kiwifruit fruit and of the significance these tissues in the development of sclerotinia disease. The factors responsible for AFT maybe genetic, environmental, micro-biological, or a combination of these. Diseased fruitlets have been shown to develop following extensive colonisation of petals during or soon after flowering. Fruit with AFT, in particular those with petals and clusters of stamens colonised by *S. sclerotiorum*, are the fruit most at risk of sclerotinia infection

and disease i.e. not all fruit are at equal risk of becoming diseased. Contact between fruit and other diseased tissues such as diseased fruitlets, leaves with necrotic lesions and fruit, appears to be the inoculum source for about 10–15% of fruit disease, while the majority of fruit disease results from contact with AFT. In kiwifruit, the role of AFT on fruit in the development of sclerotinia disease highlights the need to understand the interactions and succession of micro-organisms during the colonisation of this substrate while it remains within the vine canopy.

A study of the effects of flower age and environmental conditions on the colonisation of petals by *S. sclerotiorum* ascospores follows in the next two chapters. The environmental conditions that favour infection of permanent floral tissues and fruit, and the infection of fruit surfaces by ascospores with and without the presence of petals, is investigated in Chapter 6.

Chapter 4: Inoculation of petals and quantification of colonisation by *S. sclerotiorum* (*in vitro*)

4.1 Introduction

Experiments in Chapter 3 have shown that adhering petals and stamens colonised by *S. sclerotiorum* ascospores are probably the major source of secondary inoculum in kiwifruit leading to diseased fruitlets and scarring on fruit. Therefore, knowledge of the factors that may influence ascospore colonisation of floral tissues, such as flower age and environmental conditions, will improve our understanding of the epidemiology of sclerotinia disease in kiwifruit.

Achieving this goal requires: choice of a suitable substrate, a reliable and repeatable method for inoculation with ascospores, suitable controlled environmental chambers, and an appropriate means of quantifying the colonisation of host tissues. It was decided that kiwifruit petals would be the most appropriate substrate because they are the dominant non-permanent flower part and the easiest to handle. This chapter describes the inoculation technique, environmental chambers and method for quantifying *S. sclerotiorum* mycelium within kiwifruit petals that are used throughout Chapter 5.

4.1.1 Inoculation techniques

Inoculation techniques used during studies of *S. sclerotiorum* infection often reflect the principal modes of infection (Abawi & Grogan, 1979; Adams & Ayers, 1979). Ascospore suspensions of *S. sclerotiorum* have frequently been used because of the ability to precisely control the inoculum dose, particularly for studies of host resistance on several crops e.g. sunflowers (Tourvielle *et al*., 1978; Vear & Tourvielle, 1984; Pereyra *et al*., 1992), beans (*Phaseolus* spp.) (Abawi *et al*., 1978), carrots (*Daucus carota* L.) (Finlayson *et al*., 1989) and canola (Brun & Renard, 1983). Alternatively, ascospores have been applied directly to plants in a dry state by suspending apothecia close to host tissues to facilitate inoculation on canola (Jamaux *et al*., 1995), pea (Huang & Kokko, 1992) and lettuce (*Lactuca sativa* L.) (Newton & Sequeira, 1972). Dry brush inoculation of *Elsinoe phaseoli* ascospores onto bean provided a high incidence of infection, but did not allow accurate control of the number of spores applied (Phillips,

1994a). This lack of precision in the inoculum dose would also apply when whole apothecia are used to directly inoculate plants.

The degree of infection success following inoculation of tissues can be influenced by the methodology used. Attaching tissues pre-colonised by *S. sclerotiorum* to plant parts has proven to give reliable infection of pea (Kapoor *et al*., 1990), cauliflower (*Brassica oleracea* L.) (Kapoor *et al*., 1985) and bean (Phillips, 1994b). However, inconsistent results were found during comparisons of *S. sclerotiorum* ascospore suspension and mycelium inoculation of sunflower capitula and stems (Vear & Tourvielle, 1984). Also, sclerotinia infection of bean stems was greater with pre-colonised carrot root, compared with either pre-colonised bean petiole or celery stem, pieces of sclerotia or mycelium on agar (Lithourgidis *et al*., 1989). Studies of *E. phaseoli* ascospore infection of bean have shown that either spray or precision application of 10µl droplets of suspensions produced more uniform distribution of lesions and consistent infection, compared with dry brush inoculation (Phillips, 1994a).

Differences between dry and wet inoculation methods have been highlighted in SEM studies of *B. cinerea* infection of rose (*Rosa* × *Hybrida*) and bean (Williamson *et al*., 1995; Cole *et al*., 1996). Dry-inoculated conidia produced very short germ tubes and directly penetrated the host tissue when RH was >94%. By comparison, conidia from wet inoculum produced extensive surface hyphae. Similarly, wet inoculation with *S. sclerotiorum* ascospores has been associated with the formation of appressoria and extensive surface hyphae (Purdy, 1958; Abawi *et al*., 1975; Tariq & Jeffries, 1984). In other studies, dry-inoculated *S. sclerotiorum* ascospores formed short germ tubes with no appressoria or surface hyphal development on canola petals (Jamaux *et al*., 1995) and bean leaves (Sutton & Deverall, 1983). It has been suggested that water soluble components involved in *B. cinerea* spore attachment to host surfaces, and digestive enzymes involved in initial infection processes, may be diluted when spores are placed into suspension during inoculation procedures (Doss *et al*., 1993; Cole *et al*., 1996).

In this study, a dry ascospore inoculation by a sedimentation method was chosen because it avoided the influence of water during suspension preparation and post-inoculation. It was also considered to be more similar to the airborne manner in which ascospores arrive at host surfaces. Dry inoculation with mature apothecia was not

considered because of the difficulty in maintaining a consistent supply of apothecia and the variable nature of ascospore release.

4.1.2 Quantification of tissue colonisation

Colonisation is the important step in pathogenesis when hyphae extend from the point of infection into the surrounding plant tissue (Gaumann, 1950). There have been several approaches to quantifying mycelial colonisation of host tissues. Although lesion formation is frequently used as a measure of infection success, it does not directly quantify colonisation. A more direct method is to microscopically measure hyphal length and identify local areas of colonisation of fluorescent stained material (Bristow *et al*., 1986; McNicol & Williamson, 1988). The volume of *B. cinerea* hyphae within bean and lettuce has been determined with a Laser Scanning Confocal Microscope and image analysis of fluorescing hyphae (Vingnana & Long, 1998). However, considerable time is required for preparation and observation of fluorescent stained material.

Assays that quantify cell-membrane components of hyphae, such as ergosterol, have been positively correlated with fungal biomass in soil (Eash *et al*., 1996; Stahl & Parkin, 1996), and plant tissues (Gretenkort & Helsper, 1993; Schnurer, 1993). Assays for chitin content have also been developed and used to quantify fungal mycelium in contaminated cereal grain (Roberts *et al*., 1991) and wood (Boyle & Kropp, 1992; Nilsson & Bjurman, 1998). No hyphal component assays have been developed specifically for *S. sclerotiorum*.

Considerable advances have been made in the development of immunoassays that have a high degree of specificity for fungi, utilising polyclonal and monoclonal antibodies or DNA probes (Schots *et al*., 1994). The close relatedness of fungi within Sclerotiniaceae, which includes *Sclerotinia* spp. and *Botrytis* spp., inherently show a high level of cross-reactivity, although the adoption of certain selection techniques can provide adequate specificity (Savage & Sall, 1981; Jamaux & Spire, 1994). This would particularly be an issue in kiwifruit because both pathogens are common on flowers and fruit (Pennycook, 1985). Immunological techniques for quantifying *S. sclerotiorum* mycelium within tissue were not pursued for this project because of the considerable development time required and the unavailability of *S. sclerotiorum* specific antibodies from two laboratories that were contacted.

The agar plate count method which yields colony forming units (cfu) is widely used for enumerating micro-organisms, especially bacteria and yeasts within liquid or solid substrates (Postgate, 1969; Atlas & Bartha, 1981). Surface washing of substrates followed by dilution-plating has proved successful to enumerate a wide range of mycoflora from contaminated food (Schnurer, 1993; Abarca *et al*., 1994). The number of cfu of bacteria, yeasts and filamentous fungi recovered from surface washings of bean and canola petals was successfully determined by dilution-plating, although *S. sclerotiorum* was not recovered (Inglis & Boland, 1990). Quantification of mycelium within substrates by maceration and dilution-plating is less frequently reported.

A technique was developed in this study to indirectly quantify *S. sclerotiorum* mycelium in colonised kiwifruit petals by macerating them and incubating aliquots on selective sterile medium to form countable colonies. This technique was chosen because it required little technical equipment, was quick to perform and would not be complicated by identification problems with respect to *B. cinerea.*

4.2 Objectives

To:

- develop an inoculation method to apply ascospores of *S. sclerotiorum* in a dry state onto detached kiwifruit petals.
- design and construct suitable incubation chambers for detached kiwifruit petals.
- develop an assay to quantify the mycelial colonisation of kiwifruit petals by *S. sclerotiorum.*

4.3 General methods

4.3.1 Source of petals

Kiwifruit flowers were collected from two sources. First, whole detached flowers were imported into New Zealand in two separate consignments on 2 and 10 May 1997 from 7 year-old *A. deliciosa* cv. 'Hayward' vines from Kearney Agricultural Centre, University of California[3]. Flowers at the cup stage of opening (section 1.1) were picked in the morning by detaching at the base of the pedicel. They were inverted and the pedicel

[3] Kindly supplied by Dave Morgan

inserted into 3 mm diameter holes in blocks of moist florist's foam set inside berry fruit punnets (T2528, Flight Plastics Ltd.). Twelve punnets, each with 9–16 flowers, were placed into a cardboard berry fruit tray (P.O. 26472, Carter Holt Harvey Packaging Ltd) and five trays were stacked together with a sixth tray on top containing frozen cool packs. On arrival at Los Angles the stack of trays were wrapped with thermal insulation packaging by the exporter (G. Bull, of Rendic International Freight Forwarders, Long Beach, CA.) and air-freighted to New Zealand on the evening of the day they were picked. The flowers reached Ruakura Research Centre between 36–48 h after picking and were kept in a coolstore at 4–6^{o}C for up to 5 days.

Second, samples of *A. deliciosa* cv. 'Hayward' flowers were collected during the period 22 November to 22 December 1997 from pistillate vines at two orchards in the Bay of Plenty (orchard 5 and 6) and two in the Waikato (orchards 20 and 21) (Appendix 1). Up to 15 vines, each separated by at least one vine, were selected from the second, fourth and sixth rows in one block from each orchard. All flowers were collected prior to application of any fungicide by detaching them at the base of the pedicel and placing them into Plix trays within kiwifruit export trays (SLCPE, Carter Holt Harvey Packaging Ltd.). All kiwifruit flowers from New Zealand orchards, except when investigating the effects of flower age, were 3–4 days old. Flower age was determined visually by the presence of partially dehisced anthers (approximately 50%) and change in petal colour from white to off-white/cream (Goodwin, 1986).

4.3.2 Inoculation method

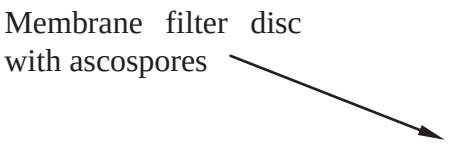
Californian flowers used for inoculation were removed from the coolstore and air-dried in a biohazard cabinet (BH143, Gelman Sciences) for 30–45 minutes. New Zealand flowers were used freshly collected from field sites. Individual petals were removed from flowers with forceps by gently holding the petal base and twisting slightly. Up to 20 petals, depending on the experimental design, were placed onto a 125 mm diameter plastic disc of Corflute (Nylex), and this was termed an 'inoculation batch'.

Petals were inoculated using a Burkard high throughput 'Jet' sampler (Burkard Manufacturing Co. Ltd) originally developed for quantifying viable spores by trapping them onto living susceptible host tissue within a still-air settling chamber (Schwarzbach, 1979). The standard air-intake was replaced with a large funnel positioned vertically

above the settling chamber (Figure 4.1). This device is referred to as the 'Burkard settling chamber'. The Corflute disc with detached petals was placed into the Burkard settling chamber and the motor turned on. The manufactures specified air flow rate during operation is 750 ℓ/minute.

Ascospores of isolate Sc1 were dusted into the funnel space by repeatedly brushing a 3 mm wide camel-hair brush across a strip of membrane filter disc containing ascospores for 10–15 seconds (Appendix 2). Ascospores were sucked into the device and entered the top of the settling chamber through the 'Jet' orifice. The trap motor was switched off and the petals were left within the chamber for 3–4 minutes to allow ascospores to settle. To minimise variation in the number of ascospores applied to petals, a standard area of 16 grids of membrane filter disc (144 mm^2), containing ca. 1.3×10^5 ascospores (Appendix 2), was used for each inoculation batch of petals, except where otherwise stated. A single membrane filter disc was used for each experiment.

Un-inoculated (control) petals were sampled at random from the available flowers after each inoculation batch and there were at least three un-inoculated control petals for each treatment within all assays.



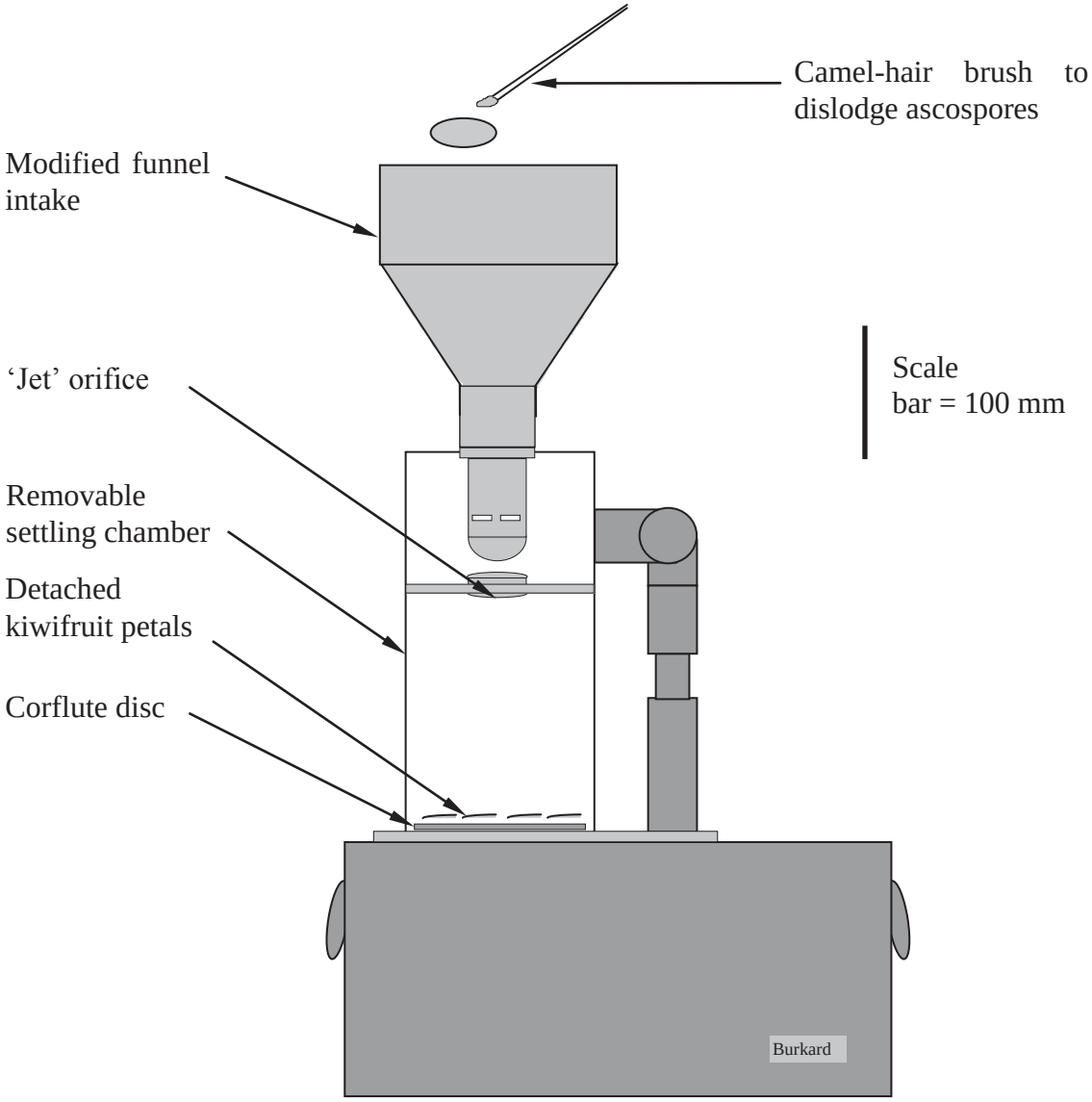


Figure 4.1 Burkard high throughput 'Jet' sampler with modified intake used for inoculation of detached kiwifruit petals with dry *S. sclerotiorum* ascospores, referred to as the 'Burkard settling chamber'.

4.3.3 Petal handling

The Corflute disc holding the petals was removed from the Burkard settling chamber and individual petals transferred with flame-sterilised forceps into a micro-tube holder filled with 1.45 ml SDW (Figure 4.2). The base of each petal was held in place with moist sterile cotton wool. Parafilm was pulled around the sides and over the top of the micro-tube opening. This technique provided an adequate supply of water to the base of the petal to avoid desiccation and reduced the amount of free water exposed to the atmosphere, which might otherwise influence the vapour pressure deficit within controlled environment chambers (section 4.5). Batches of petals were transferred into controlled environment chambers within 15 minutes of inoculation. After each

experiment the parafilm and cotton wool bungs were removed and the bungs and micro-tubes autoclaved (121°C, 15 minutes) to be re-used.

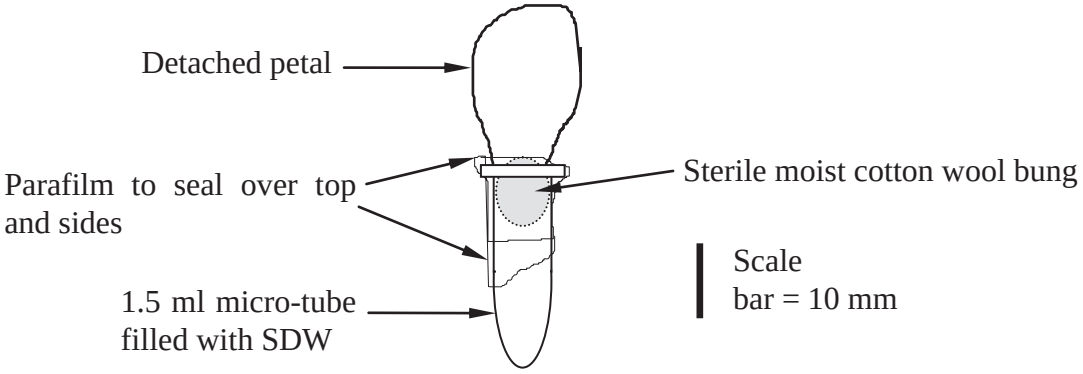


Figure 4.2 Micro-tube holder: kiwifruit petal supported within a micro-tube by means of a cotton wool bung and parafilm.

4.3.4 Determination of colony forming units from petals

On removal from incubation chambers, petals were collected into polystyrene trays. The percentage area of each petal covered with lesions was estimated with reference to disease severity diagrams developed for lesions on pea leaves caused by powdery mildew (*Erysiphe pisi* DC.) (Falloon *et al*., 1995). To determine the number of colony forming units (cfu) petals were removed from the micro-tube holders with forceps and placed on one side of a wooden frame (section 2.2.2). The position of the petals on the frame was ordered so that the identity of each petal could be recognised when they were removed following surface-sterilisation. The petals were surface-sterilised as described in section 2.2.2. The duration and concentration of sodium hypochlorite treatments used for surface-sterilisation were optimised as described in section 4.6.

Petals were removed from the frame with flame-sterilised forceps and placed into a mortar. Petals were ground without addition of any liquid for ca. 10 seconds, after which phosphate buffered saline (PBS) was added to the slurry of macerated tissue. The volume of PBS varied between 1 and 5 ml depending on the extent of soft rot lesions within particular treatments e.g. 0–20% soft rot = 2 ml, >80% soft rot = 5 ml. This provided a greater dilution factor for treatments where more cfu were expected. The petal tissue with PBS was ground for a further 10 seconds and a 200 µl sample was taken with a multi-dispensing micro-pipette. Two 50 µl aliquots were placed ca. 15 mm in from opposite edges of a Petri dish of John Knight's (JK) selective medium (Bourdôt

et al., 2000) (Appendix 3) that had been air-dried for 20–30 minutes in a Biohazard Cabinet. Each droplet was spread evenly across half of the Petri dish surface with a flame-sterilised glass rod, avoiding mixing with the adjacent droplet. The Petri dishes were incubated on the laboratory bench for 2–3 weeks, after which the number of *S. sclerotiorum* colonies growing from each 50 µl droplet was counted (Plate 4.1). Colony counts were re-checked after a further 2–3 weeks.

The mean cfu/petal was calculated as:
colony counts per droplet × 20 × volume PBS used (ml) / number of petals in sample.

Colonies of *B. cinerea* were also counted as this fungus commonly occurred on field collected petals and readily grew on the JK selective medium. Occasionally other colonies, including *Cladosporium* spp. and bacteria, were present but were not counted. Several colony types were sub-cultured on PDA throughout the course of these experiments to provide a consistent identification and distinction between *S. sclerotiorum* and *B. cinerea* colonies (Appendix 3).

To determine the mean dry weight of kiwifruit petals, 20 petals were randomly sampled from the flowers collected from orchard 20 on 26 November 1997 (Experiment 5-9). These were individually weighed with electronic scales (AE260, Mettler) and placed on a plastic tray in an oven for 3 days at 50^{o}C. The mean fresh weight of these petals was 1.12 g (SE ± 0.08) and the mean dry weight was 0.10 g (SE ± 0.01). Therefore, the data presented as cfu/petal in this study can be multiplied by 10 to represent the number of cfu/g dried petal tissue.

A B

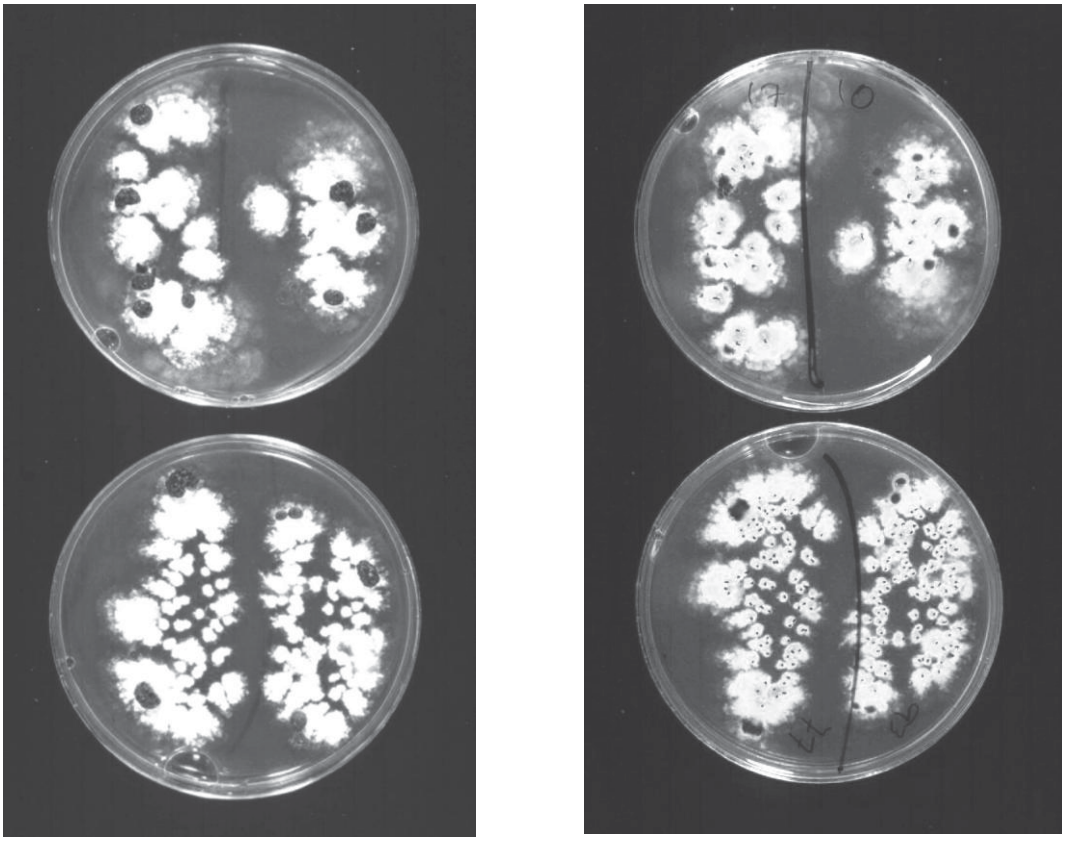


Plate 4.1 A and B. Colonies of *S. sclerotiorum* formed on John Knight's selective medium (Appendix 3) after 2–3 weeks incubation, (A) is top view and (B) is underside view of two JK Petri dishes. Colony counts are 17 and 10 for the upper and 77 and 93 for the lower Petri dish, respectively. N.B. black dots on the underside of Petri dishes are pen marks used for counting.

4.4 Experiment 4-1: Ascospore distribution within the Burkard settling chamber

4.4.1 Objective

- To determine the density and distribution of *S. sclerotiorum* ascospores deposited within the Burkard settling chamber.

4.4.2 Materials and methods

Eleven glass cover slips (22 × 22 mm) were placed concentrically on the Corflute disc used in the Burkard settling chamber. Each cover slip was numbered in the top left-hand corner to identify its position as either 'outer' (1–5), 'middle' (6–10) or 'centre' (11) (Figure 4.3). The Corflute disc was placed into the Burkard settling chamber and *S. sclerotiorum* ascospores from 16 grids of membrane filter disc were brushed into the

intake funnel of the Burkard settling chamber with a 3 mm wide camel-hair brush and allowed to settle for 3 minutes.

The number of ascospore clusters were counted along three 1 mm wide transects on each cover slip with an Axioplan microscope (200×, Carl Zeiss). Each transect was tangential to the Corflute disc and was defined with the aid of an eyepiece graticule. The number of ascospores in each cluster was estimated and recorded in three size categories: 1–3, 4–20 and >20 ascospores/cluster.

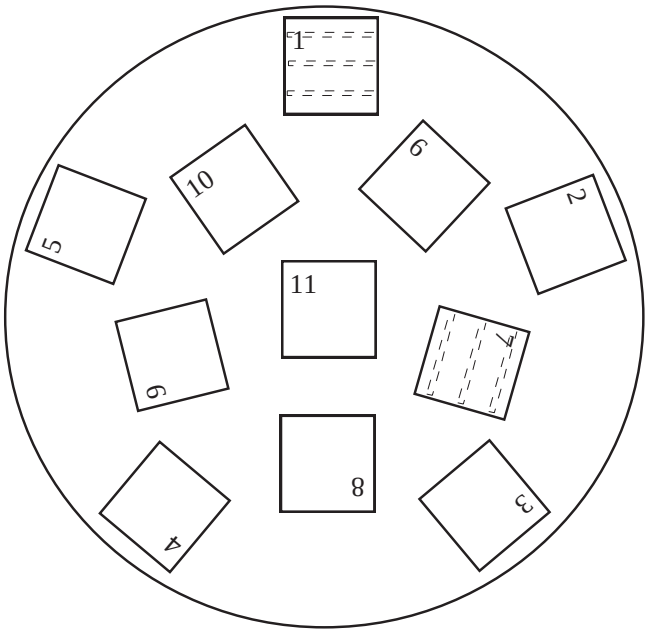


Figure 4.3 Position and numbering of glass cover slips placed on Corflute disc for evaluating ascospore distribution within the Burkard settling chamber. Dashed lines on cover slip 1 and 7 indicate the relative orientation of three transects on each cover slip.

The above procedure was repeated a further two times with the same brush. Following initial inspection of the cover slips considerable clumping of ascospores was noticed. For this reason a second group of three inoculation batches were carried out with a similar brush that had been trimmed to leave only 10 full length hairs.

The number of ascospore clusters was square root transformed prior to analysis by ANOVA using Genstat to test for differences between the first and second group of

inoculation batches, between positions of the cover slips (centre, middle and outer) and for differences in the size of ascospore clusters.

4.4.3 Results

Ascospores were seldom observed to occur singly within the Burkard settling chamber. The mean density of ascospore clusters (all sizes combined) was 26.4 cm^{-2} (SE ± 2.8) using the full brush and 26.5 cm^{-2} (SE ± 2.8) using the trimmed brush. In the first group of inoculation batches there were significantly fewer clusters with 1–3 ascospores ($P<0.01$) compared with clusters with 4–20 and >20 ascospores. The second group of inoculation batches using the trimmed brush had significantly more ($P<0.05$) small clusters of ascospores (1–3) compared with the first group. There were significantly more ascospore clusters ($P<0.05$) with 4–20 and >20 ascospores/clusters on the cover slips positioned in the centre and middle of the settling chamber compared with those in the outer position, for both groups of inoculation batches.

4.4.4 Discussion

Most ascospores were deposited within the Burkard settling chamber as clusters with >4 ascospores. This is consistent with observations that ascospores occur as a multitude of small clusters densely packed on the surface of the membrane filter discs when collected *en mass* from mature apothecia (Appendix 2). The brushing action required to remove ascospores from the membrane filter disc may be the cause for the formation of large clusters. The higher density of large ascospore clusters near the centre of the settling chamber is most likely caused by their greater mass leading to a greater velocity within the chamber and therefore less lateral dispersion during settling (Gregory, 1973).

The trimmed brush was used for all subsequent inoculations because of the more even spread in the size of ascospore clusters. The large number of ascospores/cluster was not considered a serious drawback as clusters of up to 50 ascospores have been observed occurring naturally on canola petals (Lefol & Morrall, 1996). The non-uniform distribution of ascospore clusters may contribute to variation in colonisation between petals within inoculation batches. The effects of this were minimised by ensuring randomisation of petal position with the settling chamber and incubation chambers.

4.5 Design and construction of incubation chambers for detached kiwifruit petals

4.5.1 Introduction

Epidemiological studies of plant pathogens frequently lead to study of disease development under controlled environmental conditions of temperature, RH and/or wetness duration (Zadoks & Schein, 1979; Blakeman & Williamson, 1994). Many different approaches to the incubation of plant material during infection studies are documented, however, detailed explanations of how conditions are maintained and the precision of the environmental variables during experiments is often lacking.

Requirements for controlled temperature facilities are generally easily met by use of laboratory incubators, although refrigeration is necessary for maintaining temperature lower than ambient conditions. Achieving stable RH within standard incubators is less reliable and specially designed systems are often developed for pathological studies. Saturated salt solutions have frequently been used to establish equilibrium RH by passing ambient air over or through the saturated solutions before entry into chambers or by having saturated salt solution within small chambers (O'Brien, 1948; Harrison *et al*., 1994). Different salts have different equilibrium humidities for given temperatures and these have been tabulated (Winston, 1960). Several problems have been encountered with these systems: maintaining saturation, stratification, impurities, and blockages of airlines. Alternatively, sucrose solutions of varying concentrations, glycerol/water mixtures and sulphuric acid/water mixtures can be used (Harrison *et al*., 1994). With these systems the maintenance of a stable concentration of the liquid phase, which affects the equilibrium RH, is essential for reliable RH control.

Ultra fine misting units connected to micro-loggers have also been employed to control RH within Perspex chambers housed in controlled temperature rooms (Hannusch *et al*., 1995). Hannusch *et al.* (1995) reported that RH was maintained to within 0.5% and air temperature was within 0.5^{o}C of set points. Knowledge of psychrometric principles governing RH would suggest that this degree of accuracy in RH is not feasible for the given fluctuations in temperature (Fuchs & Tanner, 1970; Fritschen & Gay, 1979). Careful attention to design would also be required to avoid free moisture.

Precise mixing of saturated air with dry air has been successfully used to control RH to within 1% and is capable of providing programmable step changes in RH or diurnal fluctuations (Butler *et al*., 1995; Gottwald *et al*., 1997). Although these systems provide accurate and dynamic RH and temperature within chambers, the necessary soleniod valves, regulators and electronic meters are expensive. A system has been developed by the Scottish Crop Research Institute that also relies on psychrometric principles but avoids expensive regulation equipment (Harrison & Lowe, 1989). Heat exchangers within waterbaths provide a precise means of maintaining stable air temperatures within submerged tubing and incubation chambers. Relative humidity within incubation chambers is modified by adjusting the temperature differential between saturated air and that of the incubation chambers. The RH of air entering these chambers was within 2% of set points over the range 80–100% RH (Harrison & Lowe, 1989).

There are several difficulties with achieving stable RH conditions where plant:pathogen interactions are studied. Relative humidity is highly sensitive to small changes in temperature, particularly at low temperature and/or high RH (Harrison & Lowe, 1989). Further, transpiration from leaves and air flow interact to produce boundary layers around plant surfaces that create gradients in RH in close proximity to leaves, making it difficult to measure RH at the site of plant:pathogen interactions (Harrison *et al*., 1994).

The overall purpose of this section is to describe the development and operation of a set of controlled environment chambers for detached petal bioassays with a range of set point temperatures (5–34^{o}C) and relative humidities (85–100%), plus a set of chambers where temperature and RH could be independently controlled to create either static or diurnally fluctuating conditions.

4.5.2 Saturated salt solution chambers

Plastic containers (380 × 380 × 160 mm) containing saturated salt solutions or SDW were used to make up a set of incubation chambers. Solubility data were used as a guide to the amount of salt to add (Windholz *et al*., 1976). Saturated salt solutions were prepared by heating 2–3 ℓ distilled water in a 5 ℓ beaker to near boiling, whilst adding enough salt to saturate the solution (Winston, 1960). Four salts were used: KCl, $MgSO_4$, KNO_3, and K_2SO_4, that had nominal relative humidities of 85, 90, 93.5 and 98% at 20^{o}C respectively (Winston, 1960). A small amount (20–30g) of extra salt was added when

they had partially cooled and the solution was stirred occasionally during cooling to break up clumps of salt crystals. Once cooled to room temperature 800 ml of salt solution including salt crystals was added to each of four plastic chambers to give 5–8 mm depth of liquid plus salt. Three 20 mm long sections of PVC pipe were placed in each container to support a square section of wire mesh above the surface of the salt solution. Detached petals in micro-tube holders were held upright by placing them in the holes of the wire mesh.

Each saturated salt solution chamber and SDW chamber was enclosed in a black plastic bag to exclude light. A 20 mm diameter hole cut into one side wall of each chamber provided access for a RH sensor. A rubber bung was used to seal this hole at other times.

Four chambers, one of each salt solution, plus one containing 800 ml SDW were placed into: a coolstore at 10^{o}C, a laboratory incubator (Marford C.W. Martin & Co Ltd.) at 15^{o}C, and two controlled environment rooms (Plant Protection, Ruakura) set at 20 and 25^{o}C. The coolstore and the controlled environment rooms were approximately 3.5 × 2.5 × 2.2 m with forced air circulation. Three additional chambers with 800 ml SDW were placed into the controlled environment room set at 20^{o}C. One chamber with 800 ml SDW was placed into another controlled environment room set at 18^{o}C and into each of five laboratory incubators set at 5, 22.5, 27.5, 30, and 34^{o}C.

4.5.3 Waterbath chambers

4.5.3.1 Design principle

Four controlled environment chambers were constructed within a glasshouse at Ruakura Research Centre, based on the chambers constructed at the Scottish Crop Research Institute, Dundee, Scotland (Harrison & Lowe, 1989). In one waterbath ambient air was brought to a constant temperature (± 0.1^{o}C) using a heat exchanger and then saturated. The air passed into a second waterbath and was brought to a higher temperature to lower the RH. The temperature and RH of air passing through the chambers was therefore controlled by setting the temperature of each waterbath.

4.5.3.2 Design and construction

Compressed air (11–15 Kpa) flowed into waterbath 1 at 40 ℓ/minute, and passed through 22 m of 20 mm diameter copper tubing and a finned condenser unit (Figure 4.4). This acted as a heat exchanger so that the temperature of waterbath 1 determined the air temperature entering the two saturation chambers (800 × 400 × 200 mm) which were in series. Each saturation chamber was partially filled with distilled water and had a header pipe with five laterals, each connected to a 400 mm long aquarium aeration stone. After passing through both saturation chambers the air was at 100% RH and was within 0.5^{o}C of the water temperature in waterbath 1.

The saturated air passed through a water-trap and an insulated pipe into a similar heat exchanger in waterbath 2. When the air exited the condenser unit of waterbath 2 it had re-equilibrated its temperature, thereby determining its RH. A 50 mm diameter header pipe with 20 mm diameter laterals supplied air to each of the four experimental chambers (450 × 300 × 200 mm) situated down the length of waterbath 2 (Figure 4.4). Air entered each chamber through a perforated tube at one end of the chamber (10 ℓ/min) and passed down the length of the chamber and exited via a similar perforated tube at the opposite end (Figure 4.5). The calculated air speed within each chamber was 8 mm sec^{-1}.

The four experimental chambers were constructed of 6 mm Perspex (Waikato Plastics). Each chamber had 10 adjustable non-corrosive draw latches (E8-10-502-20, Southco Inc.) that securely clamped down a 10 mm thick Perspex lid against a rubber seal glued to the rim of each chamber (Figure 4.5). Each latch could be adjusted to ensure an even tension was applied around the perimeter of the lid to form a water tight seal.

A Perspex shelf sealed with silicone grease was supported 70 mm from the top of each chamber and the space beneath this filled with deionised water to counteract the buoyancy of the chambers. Four wet- and dry-bulb psychrometers were constructed from purpose-built temperature sensors (107 thermistor sensors, Campbell Scientific Inc.), 20 × 50 mm glass insect collection vials filled with SDW and cotton wicks, and placed into each chamber. Each psychrometer was located 90–100 mm from the perforated inlet tubing (Figure 4.5). A Humicap® capacitive RH sensor (HMP 35A, Vaisala Sensor Systems) was also positioned within chamber 4 (Figure 4.4).

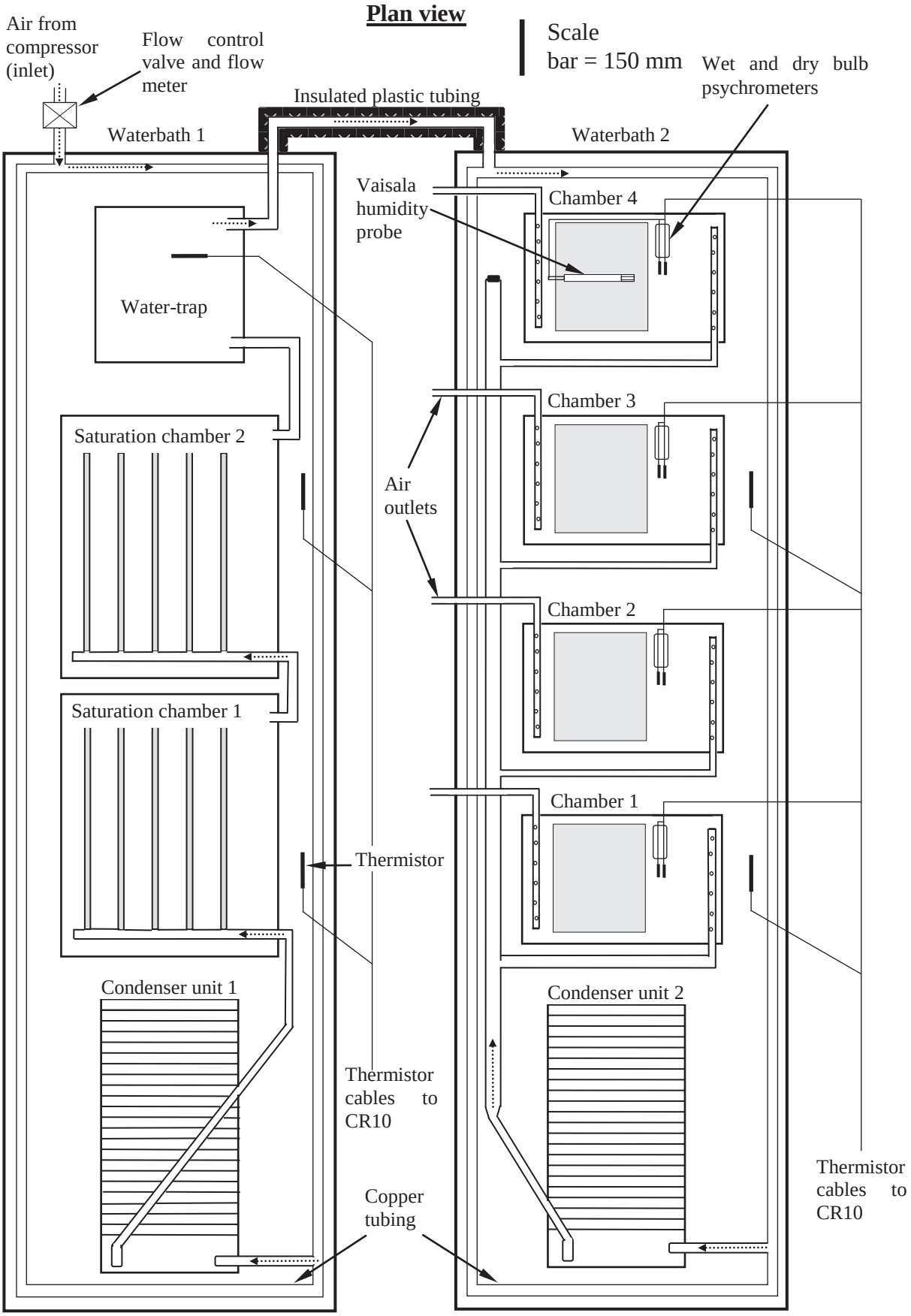


Figure 4.4 Schematic diagram of controlled environment chambers constructed within two large waterbaths, (⋯→ = direction of air flow).

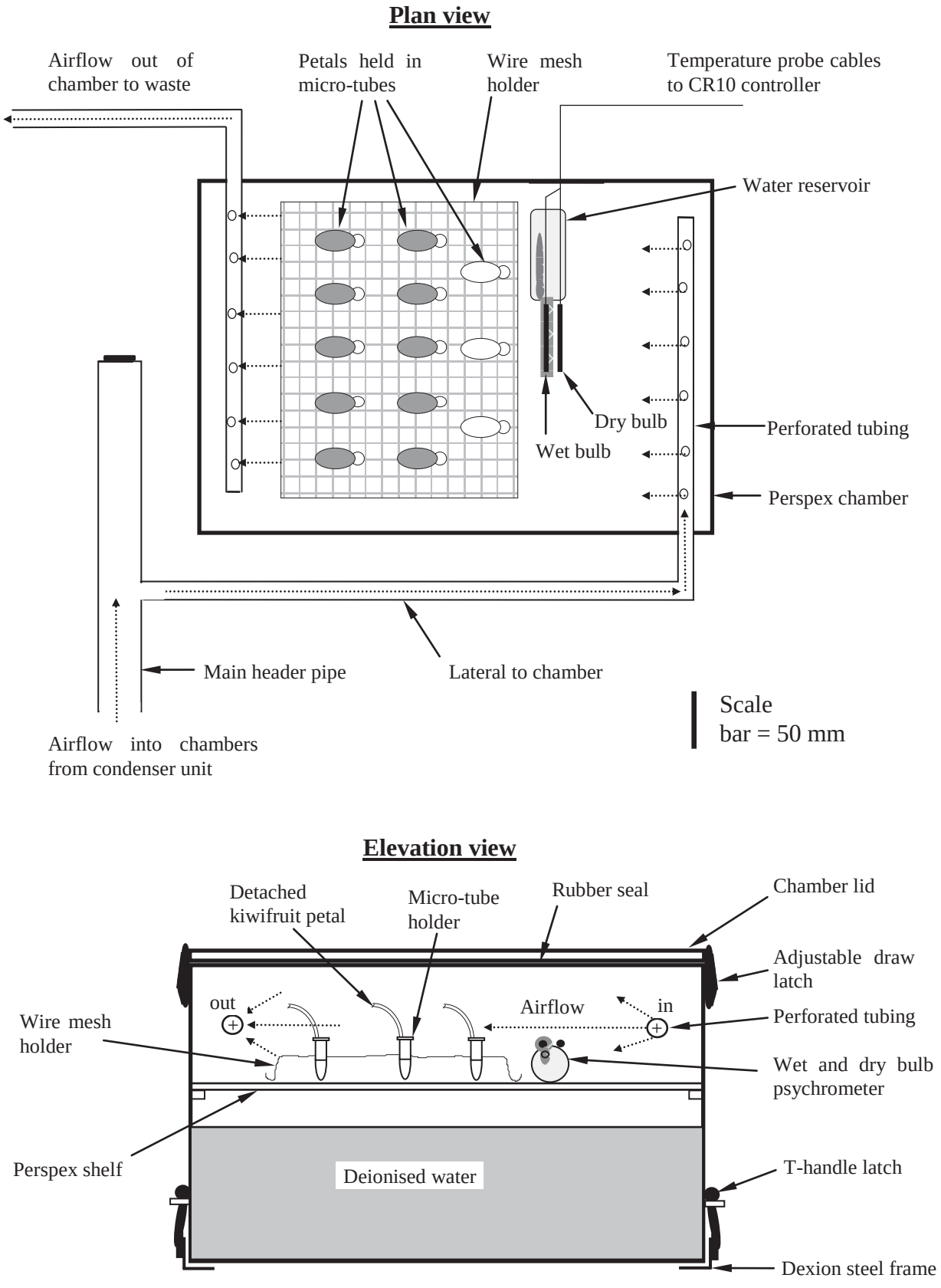


Figure 4.5 Schematic diagram of an individual controlled environment incubation chamber in waterbath 2, showing air flow (⋯▸), position of petals in micro-tubes and wet- and dry-bulb thermistors.

All air lines and thermistor cables had water-tight entry/exit points to the chambers in the form of plastic tank fittings inserted into 20 mm diameter holes in the sides of each chamber. Plastic elbow and straight fittings (Hansen) and 20 mm diameter plastic tubing were used throughout, except for the main header pipe. The condenser units, saturation chambers and experimental chambers were mounted on a Dexion™ steel frame that could be raised and lowered into the waterbaths by a rope and pulley system. Lead weights were attached to the frame to counteract the buoyancy of the saturation and incubation chambers. During normal operation stable temperatures could be achieved by lowering the frame to completely submerge the chambers. The frame in waterbath 2 was partially raised out of the water to allow access as necessary.

The water in each waterbath was circulated independently by two centrifugal water pumps (GAM/1A, Pedrollo) to ensure a uniform temperature. There was a 40 mm diameter PVC intake pipe at one end of the waterbath and a 32 mm diameter outlet pipe that ran the length of each waterbath. At four positions along the outlet pipe there were 200 mm PVC risers (15 mm diameter) each with a high flow rate nozzle (CD FJ 1/2 Floodjet, Delavan). Each waterbath had 4–5 adjoining covers made from 100 mm thick coolstore insulation panels to reduce heat transfer and to exclude light. The glasshouse roof was also covered with a sheet of black polythene to reduce incoming radiation heat flux.

During the detached petal assays that utilised these waterbath chambers (Experiment 5-10 and 5-11), petals in micro-tube holders were placed into the wire mesh holders within each chamber (Figure 4.5, Elevation view). The chamber lids were re-secured, the frames were lowered and the covers were replaced for the duration of the experiment.

4.5.3.3 Control system

The temperature of both waterbaths, and therefore the air temperature and RH in the incubation chambers, were controlled by a datalogger (CR10, Campbell Scientific Inc.). Two thermistor temperature sensors (107B, Campbell Scientific Inc.) were positioned down one side of each waterbath to monitor water temperature (Figure 4.4). The datalogger was programmed (Appendix 7) to record all inputs every 15 seconds via a multiplexer (AM416, Campbell Scientific Inc.). To maintain constant temperature a

comparisons were made between the running average of the waterbath temperature (five most recent readings) and a set of thresholds ranging from ± 0.04°C to ± 0.25°C of the set temperature for each waterbath.

Heating was provided by three 1.2 KW elements equidistant down the length of each waterbath and mounted below the chambers and steel frame. Cooling was provided by a length of copper tubing around the perimeter of each waterbath that circulated coolant. Both the heating and cooling were switched on and off by the datalogger via the control ports and a series of relays connected to the appropriate electrical circuits.

The wet- and dry-bulb air temperature was monitored in each incubation chamber and the datalogger calculated RH with standard algorithms (Sargent, 1980). The datalogger stored waterbath temperatures, water-trap air temperature, wet- and dry-bulb air temperatures in chambers 1–4 and RH in chambers 1–4 every 15 minutes by averaging the 15 seconds readings taken over the previous 15 minutes.

4.5.3.4. Performance testing of environmental conditions

All Campbell 107B thermistor temperature sensors and 10 Type T thermocouples (copper:constantan) were calibrated against a mercury in glass reference thermometer (0.1°C graduations, ± 0.03°C uncertainty of measurement) before installation into the waterbath system. This was carried out with an ice slurry bath (0°C) and a laboratory waterbath set at 25°C, on 26–29 August 1996. Correction values were calculated as the difference between the mean of the sensor readings and the mean of the reference thermometer readings after logging temperatures at 2 minute intervals for at least 30 minutes on three separate occasions at each set point. Correction values ranged from ± 0.07°C for the 107B thermistors and ± 0.24°C for the thermocouples.

Two Vaisala RH sensors were calibrated over saturated salt solution of K_2SO_4 (98% RH) and distilled water (100% RH) in 250 ml conical flasks stored in a controlled environment room at 20°C. The sensors were suspended in the flasks and sealed with parafilm for 30 minutes before making adjustments to the calibration potentiometers. This was repeated at least three times for each solution over a 2 day period. One sensor was positioned in waterbath chamber 4 and the other was used for monitoring temperature and RH in the saturated salt chambers during experiments.

The spatial temperature profile in one chamber chosen at random (Chamber 2) was monitored with 10 thermocouples from 4–7 September 1996 with waterbath 2 operating at a set point of 22°C. The thermocouples were positioned in three rows down the length of the chambers inbetween the inlet and outlet tubing. There were three thermocouples in the two outer rows and four in the central row. During this period the overall mean temperature from the 10 thermocouples was 21.70°C (SE ± 0.015). The mean temperature from each thermocouple was subtracted from the overall mean temperature to estimate the spatial temperature distribution within the chamber. The chamber temperature was marginally cooler (–0.1 to –0.25°C) at the three thermocouples near the inlet end. The average deviation from the overall mean temperature ranged from 0–0.16°C for the remaining seven thermocouples.

The stability of temperature and RH within the waterbath chambers was monitored routinely during the development of this system. Successful operation depended on a multitude of factors, the most important were:

- Maintaining a water tight seal on all components.
- Ensuring air was completely saturated before passing into waterbath 2.
- Minimising the energy influx into and out of the glasshouse and Perspex chambers.
- Ensuring complete submergence of chambers to avoid temperature differential across the chamber lids which could lead to condensation inside the chambers.
- Taking running averages of water temperatures so that smooth cycling of heating and cooling took place.

With these precautions and design features the temperature within each of the four experimental chambers was typically maintained to within 0.3°C of the set temperature and within 3% of the target RH. The mean environmental conditions within the four waterbath chambers operating with set points of 20°C and 98% RH for a 48 h period starting on 12 November 1997 were 20.04°C (SE ± 0.013, minimum 19.71°C and maximum 20.34°C) and 97.5% RH (SE ± 0.5, minimum 93.6% and maximum 98.9 %).

4.5.4 Discussion

The incubation chambers containing saturated salt solutions were chosen for their ease of construction and low requirement for maintenance. Detached petals were positioned as close as practical to the surface of the salt solution to minimise deviation from the

equilibrium RH. Although they provided the necessary range of relative humidities, replication of temperature × RH treatment combinations was not feasible because of limited resource in terms of the number of incubators.

The construction of the waterbath controlled environment chambers provided a system unique in New Zealand where temperature and RH could be controlled independently. The system described here has the same capability of programmable changes in temperature and/or RH as the systems described by Butler *et al* (1995) and Gottwald *et al* (1997). The precise control of the waterbath temperatures led to stable air temperature within the incubation chambers and ensured an acceptable variation in RH. This degree of precision was similar to that achieved by Harrison & Lowe (1989) in similarly designed chambers. The major difference between these chambers and the multiple independent chambers of Harrison & Lowe (1989), was that the four incubation chambers in waterbath 2 must be considered pseudo-replicates. The environmental conditions with the waterbath chambers are illustrated in more detail in Appendix 4 and the psychrometric plots in Figure 5.19 and 5.20.

4.6 Experiment 4-2: Evaluation of surface-sterilisation of kiwifruit petals

4.6.1 Objective

- To optimise surface-sterilisation of petals so that *S. sclerotiorum* ascospores do not contribute to colony forming units during detached petal assays.

4.6.2 Materials and methods

Twelve 3–4 day-old flowers were collected from each of four *A. deliciosa* vines from orchard 5. Petals were inoculated in the Burkard settling chamber with ascospores from 16 grids of membrane filter disc for each of 10 successive batches. Each batch consisted of three petals taken at random from the flowers of each vine. The inoculated petals were laid directly onto the wire mesh, inoculated surface upper-most, within plastic incubation chambers containing SDW for 4 h on the laboratory bench. Two chambers had petals from four inoculation batches in each, and a third chamber had petals from the remaining two batches.

Each of 12 treatments had five replicates of two petals. Each replicate consisted of two petals taken at random from petals originating from two randomly paired batches of inoculation. Petals in treatments 1–3 were held in 1% sodium hypochlorite solution (NaClO) for 1.0, 2.5 and 5.0 minutes, respectively. Petals in treatments 4–6 were held in 1% sodium hypochlorite solution amended with 0.1% acetic acid and 0.1% Tween80, for 1, 2.5 and 5.0 minutes, respectively. Petals in treatments 7–9 were held in drum ethanol for 3, 30 and 60 seconds, respectively, rinsed in tap water for 15 seconds then held in 1% sodium hypochlorite amended with 0.1% acetic acid and 0.1% Tween80 for 2.5 minutes. Petals in treatment 10 were held in SDW amended with 0.1% Tween80 for 2.5 minutes. Petals in treatments 1–10 all had two rinses in tap water for 30 seconds and one rinse in SDW for 15 seconds, immediately afterwards. Treatments 11 and 12 were not surface-sterilised and had no rinsing.

Petals for each treatment replicate were placed into a plastic mesh envelope (150 × 35 mm) and surface-sterilised in 200 mm long plastic containers (section 2.2.2). The order in which treatments were carried out was randomised. The two petals from each replicate were macerated together in 2 ml PBS and one aliquot of 100 µl was spread onto each of two Petri dishes of JK selective medium. Colonies of *S. sclerotiorum* were counted after 2 weeks incubation on the laboratory bench.

The number of cfu/petal (+1) was $\log_{10}$ transformed and analysed by ANOVA using Genstat to determine differences between duration of treatments and the range of solutions used for surface sterilisation. Treatments with a mean of zero were omitted from the analysis because of their influence on sample variance.

4.6.3 Results

There were significantly more ($P<0.001$) colonies of *S. sclerotiorum* (160 cfu/petal) recovered from control treatment 10 (SDW + 0.1% Tween80) than from the other two control treatments that received no rinsing (43 and 55 cfu/petal) (Figure 4.6). Treatments 1–3 (1% sodium hypochlorite for 1–5 minutes) significantly reduced ($P<0.001$), but did not eliminate, the formation of *S. sclerotiorum* colonies (6–13 cfu/petal). Treatments 4–9 resulted in no *S. sclerotiorum* colonies on the JK selective medium (Figure 4.6). When petals were removed from ethanol and placed into water the surface tension normally present on petals appeared to be reduced.

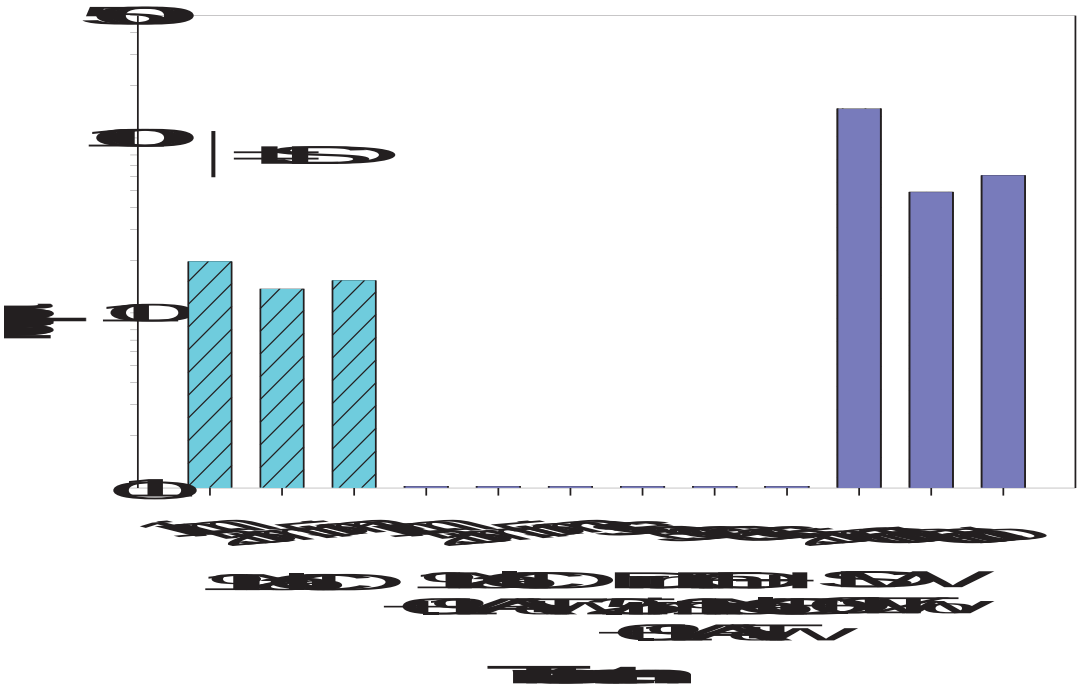


Figure 4.6 Mean colony forming units (cfu)/petal (log scale) following a range of surface-sterilisation treatments, including sodium hypochlorite (NaClO), acetic acid (AA), Tween80 (Tw) and ethanol (EtOH), rinsing in sterile distilled water (SDW) and un-treated controls. Bar = least significant difference.

4.6.4 Discussion

Kiwifruit petals that were inoculated with *S. sclerotiorum* ascospores and surface-sterilised with 1% sodium hypochlorite solution alone for 1, 2.5 or 5 minutes still formed some *S. sclerotiorum* colonies on JK selective medium. This demonstrates that some ascospores survived these treatments. The most effective surface-sterilisation method was treatment with 1% sodium hypochlorite amended with 0.1% acetic acid and 0.1% Tween80, either with or without pre-treatment with drum ethanol, irrespective of the treatment duration. Pre-treatment with drum ethanol was considered appropriate as it reduced surface tension, which may become important when fungal contaminants are present since mycelium and conidia are often hydrophobic. Treatment 8 was chosen as the standard surface-sterilisation method used for all detached petal assays.

4.7 Chapter Discussion

The two shipments of fresh kiwifruit flowers from California were essential for developing the detached bioassay techniques as they were available 6 months before kiwifruit flowering in New Zealand. Kiwifruit flowers were available from the Waikato

and Bay of Plenty over a 6 week period because of the spread of flowering times in different orchards. Because successive experiments described in Chapter 5 used flowers from several orchards, this could lead to variation in the susceptibility to *S. sclerotiorum* infection and in the amount of background inoculum of *S. sclerotiorum* and other micro-organisms. These factors make comparisons between experiments difficult to interpret.

The inoculation and petal handling procedures described above ensured that experiments with up to 12 batches of inoculation could be prepared for placement within the incubation chambers within 3 h from the time of collecting flowers, thus facilitating consistency between experiments. The micro-tube holders were a convenient and effective means of physically supporting detached kiwifruit petals during assays. This technique minimised direct handling of petals, thereby avoiding physical damage, and supplied water to the base of petals which minimised desiccation during incubation. The parafilm that covered the micro-tube and wet cotton wool minimised the exposure of water to the air within incubation chambers, to reduce possible effects on RH.

The construction of incubation chambers using saturated salt solutions and SDW, and the waterbath controlled environment facility provided opportunity to study the effects of temperature and RH on the colonisation of petals by *S. sclerotiorum* ascospores. The formation of discrete colonies of *S. sclerotiorum* on JK selective medium from aliquots of macerated surface-sterilised petals provided a system for indirectly quantifying colonisation of kiwifruit petals. These techniques formed the basis of the detached petal assay methodology that is used throughout Chapter 5 to investigate the effects of host and environmental variables on the infection and colonisation of kiwifruit petals by *S. sclerotiorum* ascospores.

Chapter 5: Factors affecting colonisation of detached petals by ascospores (*in vitro*)

5.1 Introduction

Non-permanent floral tissues such as petals and stamens are probably important sites of primary infection by *S. sclerotiorum* ascospores in kiwifruit (Chapters 2 and 3). The colonisation of permanent flower parts (ovary, sepals and pedicel), shoots, leaves and fruit represent secondary spread and may originate from mycelium within colonised floral tissues.

The concept of inoculum potential represents "the energy of growth of a fungal parasite available for infection of the host at the surface of the host organ to be infected" (Garrett, 1956). The inoculum potential with respect to secondary spread by mycelium from petals and stamens into permanent flower parts, shoots, leaves and fruit might be related to the mycelial biomass of *S. sclerotiorum* within these floral tissues. Thus, the incidence of infection of petals and stamens and the extent of colonisation may be critical factors influencing the development of disease. Therefore, it was considered important to investigate factors affecting the colonisation of kiwifruit petals.

This chapter is divided into four sections: section 5.2 evaluates the appropriateness of the detached petal assay for determining colonisation of petals, section 5.3 investigates the effects of flower age on petal colonisation, section 5.4 investigates the effect of temperature on ascospore germination and mycelial growth *in vitro*, and section 5.5 investigates the effects of temperature and RH on petal colonisation.

5.2 Evaluation of the detached petal assay

5.2.1 Objectives

To:

- establish the optimal incubation period for quantifying colony forming units (cfu) of *S. sclerotiorum* within detached kiwifruit petals.
- confirm that the method for determining colony forming units (section 4.3.4) is sensitive to the degree of colonisation within petals.

- determine whether there is a positive relationship between cfu, inoculum density, number of lesions, and lesion size.

5.2.2 Experiment 5-1: Effect of incubation temperature and time on petal colonisation

5.2.2.1 Materials and methods

Six 3–4 day-old flowers were collected on 16 December 1997 from each of 15 pistillate kiwifruit vines from orchard 5 (Appendix 1). Eight batches of 15 petals, one petal from each vine, were inoculated with ascospores from 16 grids of membrane filter disc in the Burkard settling chamber. The petals were placed into micro-tube holders. From each inoculation batch three groups of five petals were taken at random and placed into each of three SDW incubation chambers held at 15, 20 and 25^{o}C, respectively. Each group of five petals was randomly assigned to one of eight positions within each chamber. Six groups of three un-inoculated petals were interspersed between the eight groups of inoculated petals in each chamber.

One petal from each inoculation batch was sampled randomly after 15, 24, 39, 49, and 72 h from the petals incubated at 20 and 25^{o}C. These were visually assessed for the number of lesions and the percentage area of lesions, surface-sterilised (section 2.2.2) and macerated singly in either 1, 3 or 5 ml PBS, depending on the extent of soft rot. Petals incubated at 15^{o}C were sampled and processed similarly after 24, 39, 49, 72, and 122 h incubation. At each sample interval one group of three un-inoculated petals was taken at random from each chamber, surface-sterilised and macerated together in 3 ml PBS.

The number of lesions/petal, percentage area of lesions and cfu/petal (+1) were square root, angular and $\log_{10}$ transformed, respectively. Data was analysed by ANOVA using Genstat to test for significant effects of temperature and incubation time on the number of lesions/petal, percentage area of lesions and cfu/petal.

5.2.2.2 Results

There was no significant effect of temperature (P=0.85) on the number of lesions and no significant interaction between incubation temperature and time (P=0.54). Combining

the temperature treatments showed the mean number of lesions increased significantly ($P<0.01$) from 17 lesions/petal (SE ± 4.5) after 39 h to 25 lesions/petal (SE ± 3.2) after 49 h. The percentage area of lesions on petals increased at each incubation temperature with increasing incubation time and there was a significant ($P<0.01$) interaction between temperature and time (Figure 5.1). After 49 h there was a significantly higher percentage area of lesions ($P<0.001$) on petals incubated at 20 and 25°C than on petals held at 15°C. There was a significant difference ($P<0.001$) in the percentage area of lesions on petals between each temperature after 72 h incubation (Figure 5.1).

There were <10 cfu/petal recovered between 15 and 49 h at each temperature (Figure 5.2). After 72 h the number of cfu/petal had increased considerably at each temperature and was significantly higher ($P<0.001$) from petals incubated at 20°C (1422 cfu/petal) and 25°C (1506 cfu/petal) compared with those at 15°C. Petals incubated at 15°C sampled after 122 h had reached 1746 cfu/petal which is similar to that recovered from petals incubated at 20 and 25°C for 72 h.

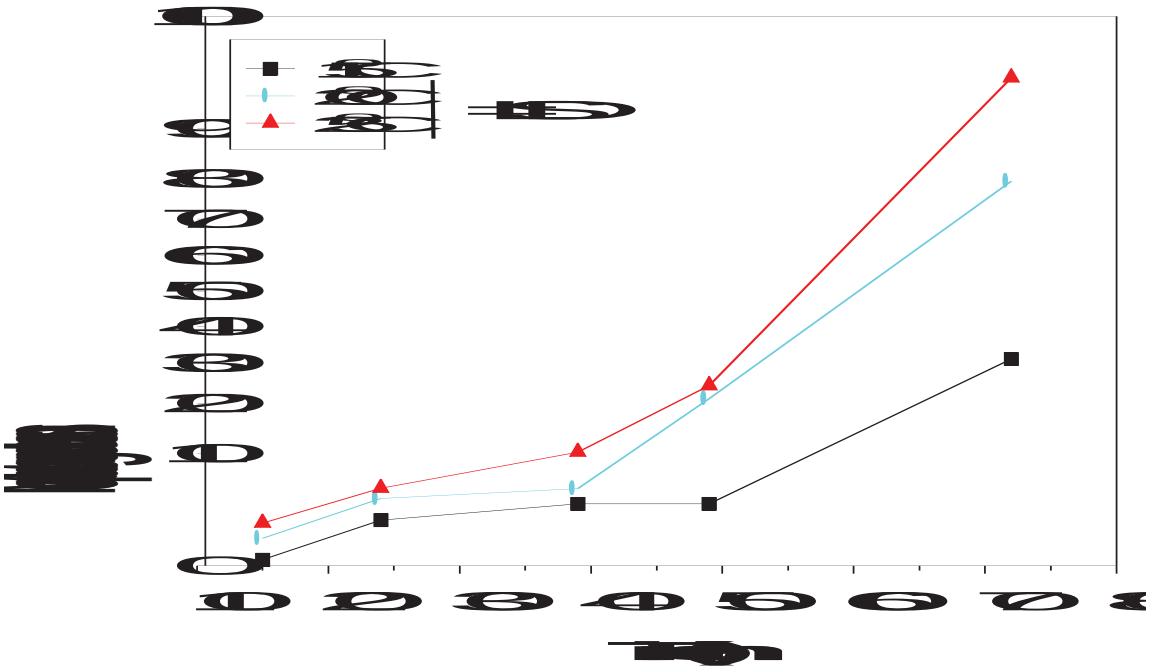


Figure 5.1 Mean percentage area of lesions (angular transformed scale) from petals incubated at 15, 20 and 25°C and sampled 15–72 h after inoculation with *S. sclerotiorum* ascospores from 16 grids of membrane filter disc. Vertical bar = interaction LSD (temperature × time).

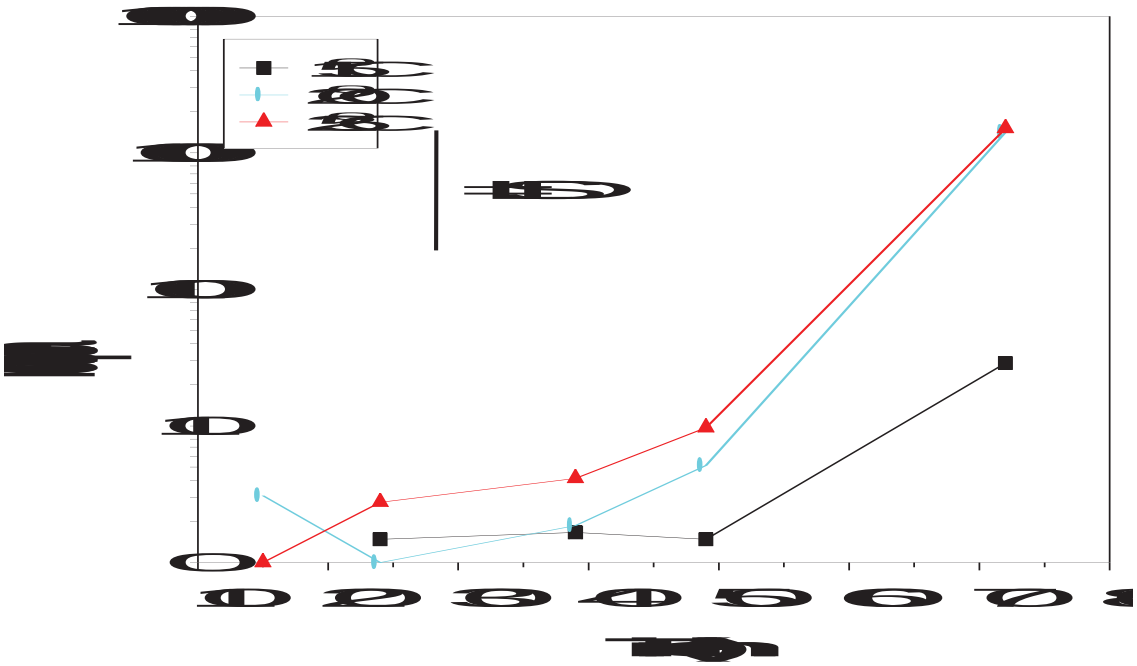


Figure 5.2 Mean colony forming units (cfu)/petal (log scale) from petals incubated at 15, 20 and 25°C and sampled 15–72 h after inoculation with *S. sclerotiorum* ascospores from 16 grids of membrane filter disc using the Burkard settling chamber. Vertical bar = interaction LSD (temperature × time).

5.2.3 Experiment 5-2: Effect of inoculum density on lesion numbers and colony forming units

5.2.3.1 Materials and methods

Five batches of 12 petals from Californian 'Hayward' kiwifruit flowers were inoculated on 16 May 1997 with ascospores from 2, 4, 8, 16 and 32 grids of membrane filter disc in the Burkard settling chamber. Ten un-inoculated control petals were selected and all petals inserted into micro-tube holders. Petals were incubated in a plastic chamber with SDW at 20°C and 100% RH for 70 h. The number of lesions on each petal was counted after 28–30 h and all petals were macerated singly in 2 ml PBS to determine the number of cfu/petal after 72 h as described in section 4.3.4.

The number of lesions/petal and the number of cfu/petal (+1) were square root transformed before analysis. Data was analysed by ANOVA using Genstat to test for the effects of inoculum density on the number of lesions/petal and number of cfu/petal. Curves were also fitted with Flexi to determine significant trends in the data. Colony

forming unit data was also analysed following $\log_{10}$ transformation for graphical presentation, because the conclusions were the same as for square root transformation.

5.2.3.2 Results

There was a significant positive trend ($P<0.05$) in the number of lesions/petal when petals were incoulated with ascospores from increasing area of membrane filter disc, although the slope of the fitted line was non-significant between 16 and 32 grids of membrane filter disc (Figure 5.3). The data was approximately linear between 2 and 8 grids of membrane filter disc and the fitted curve passed close to the origin of both axes (Figure 5.3).

The number of cfu/petal was significantly higher ($P<0.001$) from petals inoculated with 8, 16 and 32 grids of membrane filter disc than from petals inoculated with 2 and 4 grids (Figure 5.4). However, there was no significant trend ($P>0.05$) in the number of cfu/petal in relation to the area of membrane filter disc used for inoculation. The highest level of colonisation from a single petal was 6000 cfu/petal.

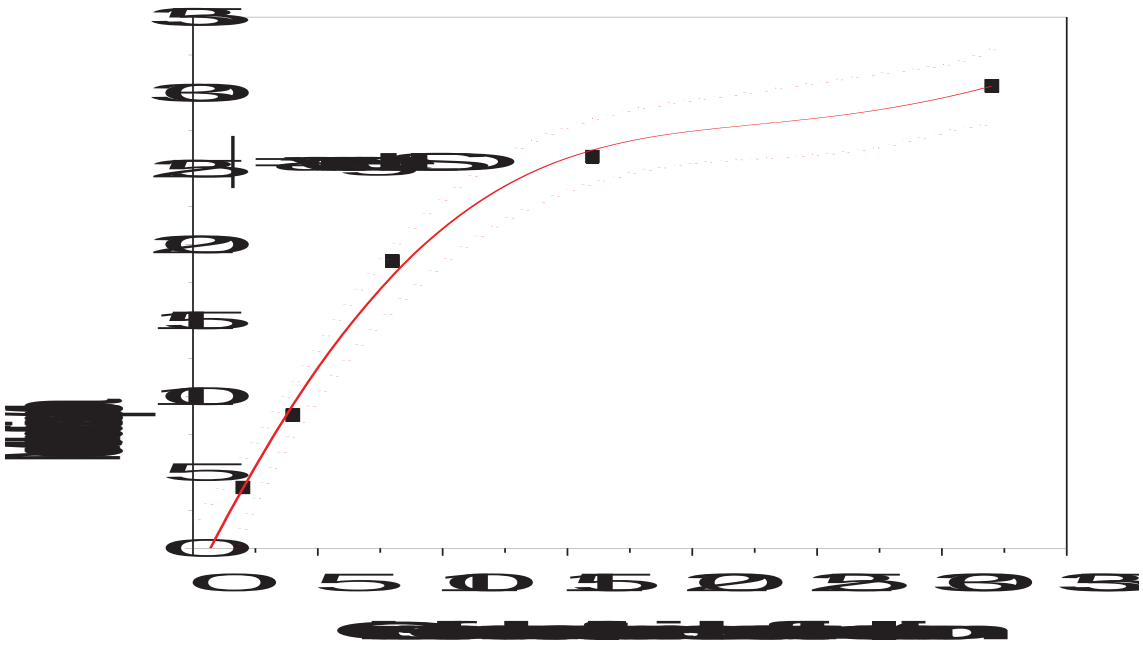


Figure 5.3 Relationship between the mean number of lesions on petals after 30 h and the number of grids of membrane filter disc used for inoculation. Data is back transformed and solid line is a fitted curve using Flexi, dotted lines are 95% confidence bands. LSD = least significant difference.

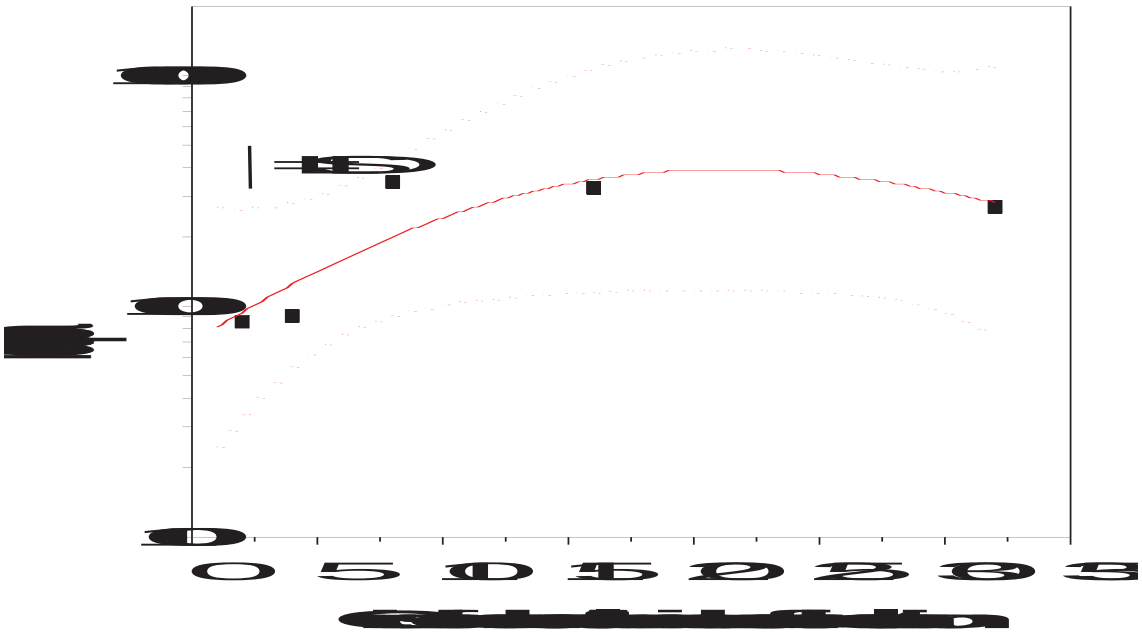


Figure 5.4 Relationship between the mean number of colony forming units (cfu)/petal (log scale) after 72 and the number of grids of membrane filter disc used for inoculation. Solid line is a fitted curve using Flexi, dotted lines are 95% confidence bands. LSD = least significant difference.

5.2.4 Experiment 5-3: Relationship between lesion numbers, lesion area and colony forming units

5.2.4.1 Materials and methods

Twenty petals from Californian 'Hayward' kiwifruit flowers were inoculated on 4 May 1997 with *S. sclerotiorum* ascospores from four grids of membrane filter disc in the Burkard settling chamber. The low ascospore dosage used was to ensure that discrete lesions would form on the petals. Petals were positioned within micro-tube holders and placed into a SDW chamber at 20°C and 100% RH. The number of lesions/petal was counted after 25 h and 48 h, and the percentage area of lesions was measured after 48 h. Petals were surface-sterilised and macerated singly in 2 ml PBS. Two 50 µl drops from each sample were spread onto each of two Petri dishes of JK selective medium and the number of cfu/petal was determined (section 4.3.4). Regression analysis was carried out in SigmaPlot™ (SPSS Inc.) to determine if significant relationships existed between the number of lesions/petal and the percentage area of lesions and between the number of lesions/petal and $\log_{10}$cfu/petal (+1).

5.2.4.2 Results

Distinctive lesions of 0.5–1.0 mm diameter formed on petals within 25 h. There were 16 lesions/petal (SE ± 2.3) after 25 h, increasing to 35 lesions/petal (SE ± 2.8) after 48 h. The percentage area of lesions ranged from 3–80% on individual petals after 48 h. Regression analysis showed a significant positive relationship ($P<0.01$) between the percentage area of lesions after 48 h and the number of lesions after 25 h ($r^2 = 0.82$), but not with the number of lesions after 48 h ($r^2 = 0.21$) (Figure 5.5). The number of cfu/petal ranged from 10–2130 and there was a significant relationship ($P<0.05$) between cfu/petal and the number of lesions on petals after 25 h incubation ($r^2 = 0.72$) (Figure 5.6).

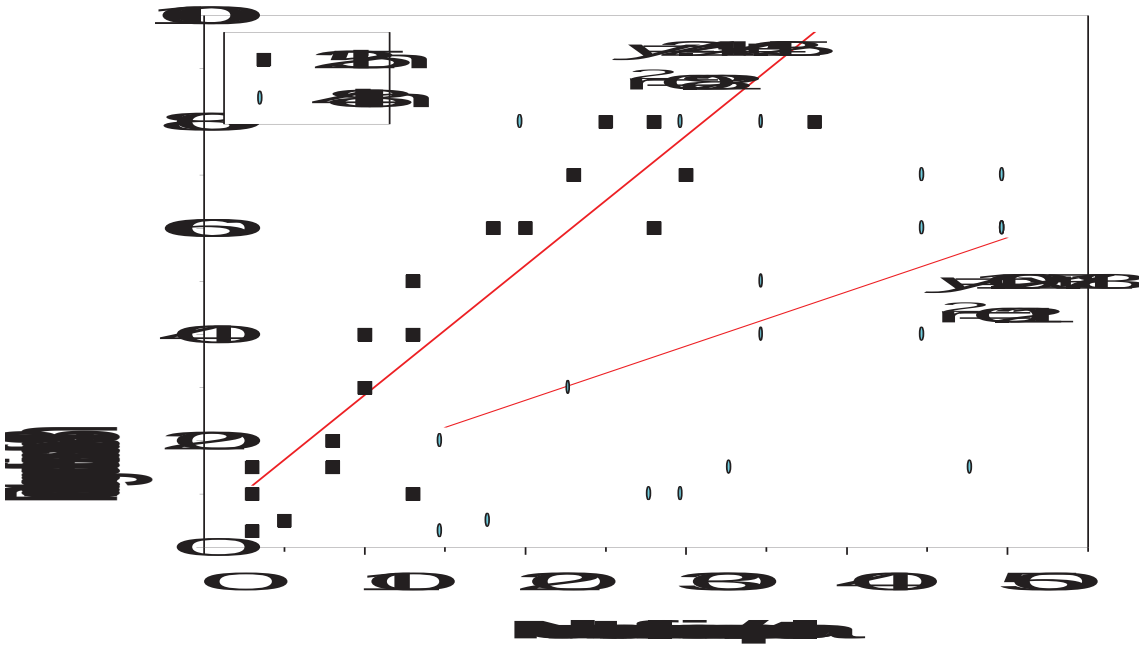


Figure 5.5 Relationship between the number of lesions/petal 25 h and 48 h after inoculation and the percentage area of lesions after 48 h, for 20 petals inoculated in the Burkard settling chamber with *S. sclerotiorum* ascospores from four grids of membrane filter disc.

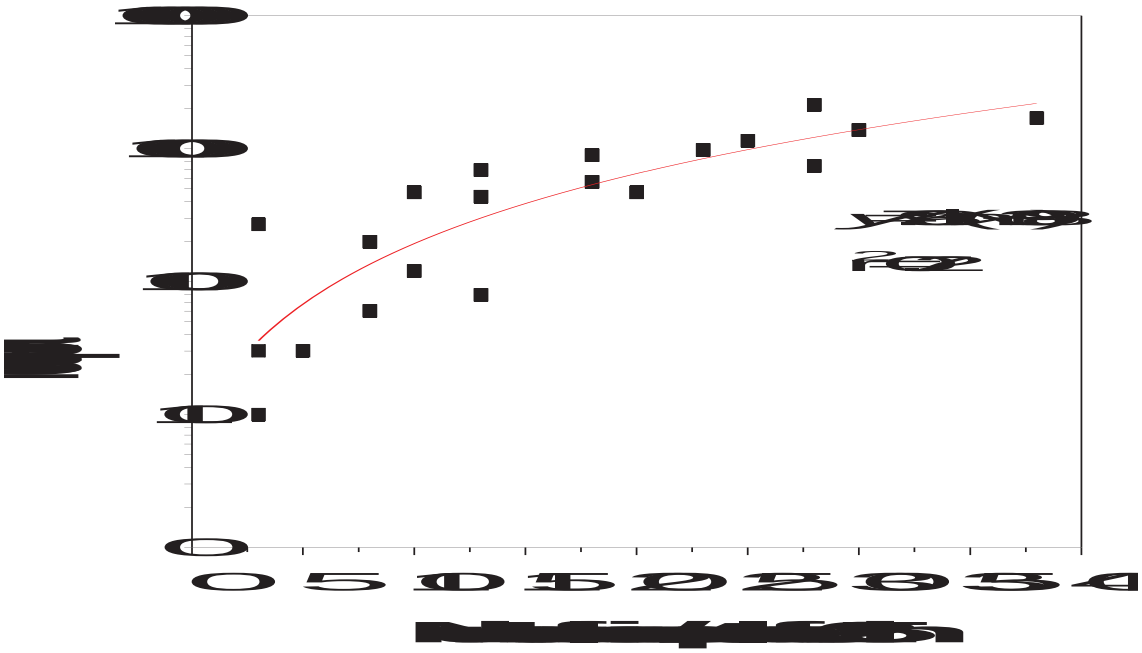


Figure 5.6 Relationship between the number of lesions/petal after 25 h and the mean number of colony forming units (cfu)/petal (log scale) after 48 h for 20 petals inoculated in the Burkard settling chamber with *S. sclerotiorum* ascospores from four grids of membrane filter disc. Solid line is curve fitted using $y = a*\ln(x - x_0)$.

5.2.5 Discussion

Ascospores of *S. sclerotiorum* applied as dry inoculum in the Burkard settling chamber readily infected and colonised detached petals from Californian and New Zealand 'Hayward' kiwifruit flowers. Discrete lesions formed after 25 h incubation at temperatures between 15°C and 25°C in high humidity chambers. These lesions developed into an expanding soft rot during the following 24–72 h, presumably as a result of extensive mycelial proliferation and collapse of the tissue.

The range in the number of lesions between individual petals of a single inoculation batch could be related to variation in ascospore distribution within the settling chamber (section 4.4) or variation in the susceptibility of petals to infection. The absence of a relationship between the number of lesions and percentage area of lesions after 48 h suggests that lesion size was more variable after 48 h incubation than after 25 h. New lesions developed over a 48 h period and at the same time lesions coalesce. Therefore, the number of lesions was not an ideal measure of colonisation and was generally not recorded on subsequent assays.

The percentage area of lesions is a visual estimate of tissue colonisation. Both the mean percentage area of lesions and the number of cfu/petal increased exponentially between 15 and 72 h incubation, suggesting this is a phase of rapid colonisation. The effect of temperature on the number of cfu/petal was similar to reports that lesion expansion on bean leaves, following inoculation with *S. sclerotiorum* ascospores, was significantly greater at 20 and 25^{o}C than at 15^{o}C (Abawi & Grogan, 1975; Phillips, 1994b).

The greatest difference in mean percentage area of lesions and cfu/petal occurred after 72 h incubation. It could be expected that after further incubation the difference in number of cfu/petal between these treatments would be reduced as all infected petals become fully colonised. For this reason an incubation duration of as close as possible to 72 h was used for subsequent experiments.

The presence of lesions on petals may not only be a consequence of colonisation by *S. sclerotiorum* applied during inoculation because contamination of petals by *S. sclerotiorum* and other micro-organisms during exposure in the field before collection is possible. Petals were not surface-sterilised before inoculation because of the risk of altering the surface characteristics of the tissue, causing unknown effects on infection. The percentage area of lesions was recorded during subsequent detached petal assays because it correlated well with the number of cfu/petal. Although lesions were present on some un-inoculated (control) petals, these rots could be distinguished from infection caused by *S. sclerotiorum* by the corresponding absence of *S. sclerotiorum* cfu.

The linear relationship between inoculum density and lesion formation when 2–8 grids were used for inoculation is in agreement with the concept that the number of infections is directly proportional to spore dose at low inoculum densities (Schein, 1964; Zadoks & Schein, 1979; Manners, 1993). The levelling of this relationship between 8 and 32 grids of membrane filter disc might be explained by the increasing incidence of multiple infection (Manners, 1993). The less clear relationship between inoculum density and the number of cfu/petal may be related to a threshold in the density of ascospores, above which increasing ascospore numbers do not substantially increase colonisation when measured 72 h after inoculation. It is possible that an earlier sampling time might have showed a stronger relationship between inoculum dose and cfu/petal.

The method of spreading aliquots of macerated tissue on JK selective medium was effective in producing distinctive, identifiable *S. sclerotiorum* colonies. Up to 2130 cfu were recovered from individual petals sampled at 48 h (Figure 5.6) and up to 6000 cfu/petal at 72 h (Experiment 5-2). It can be assumed that the colonies which formed on JK selective medium originated as mycelial fragments in the macerated tissue because *S. sclerotiorum* does not form conidia (Kohn, 1979b) and surface-sterilisation killed any ascospores remaining from inoculation (section 4.6). Therefore, notwithstanding any unknown differences in the degree of maceration and size of mycelial fragments, greater numbers of cfu/petal can be assumed to correspond to greater mycelial content.

These experiments showed that the number of cfu/petal was sensitive to variation in incubation time and temperature, the percentage area of lesions and inoculum density; factors that are likely to affect the degree of colonisation and mycelial growth within host tissues (Gaumann, 1950; Zadoks & Schein, 1979). The methodology for determining cfu was therefore considered to be suitable for quantifying colonisation by *S. sclerotiorum* within petal tissue. It was decided from these experiments to use ascospores from a standard area of 16 grids of membrane filter disc for inoculation and an incubation time as close as possible to 72 h throughout the remainder of this Chapter.

5.3 Effect of flower age on colonisation of petals

5.3.1 Introduction

Although floral tissues play an important role in many diseases caused by *S. sclerotiorum* (Natti, 1971; Abawi & Grogan, 1975), the effect of flower age on colonisation by ascospores has received little attention. Natti (1971) reported that the average incidence of *S. sclerotiorum* was 0% on freshly opened bean blossoms, 8% on mature attached blossoms, and 58% on blossoms lodged in lower branches. This likely reflects increased time for colonisation, increased spore loading and increased susceptibility of senescing and dead blossoms. Lesion development caused by *B. cinerea* conidial infection of rose (cv. ‘Mercedes’) and geranium (*Pelargonium* × *hortorum* L.H. Bailey) is also favoured by increasing flower age (Shaul *et al*., 1995; Sirjusingh *et al*., 1996). These effects of flower age may be similar for infection of kiwifruit petals by *S. sclerotiorum* ascospores, but has not been investigated.

5.3.2 Objectives

To determine:

- the incidence of *S. sclerotiorum* within petals sampled from flowers at various ages.
- the effect of petal age on the colonisation of detached kiwifruit pet als inoculated with *S. sclerotiorum* ascospores in a dry state.

5.3.3 Experiment 5-4: Incidence of *S. sclerotiorum* in petals from different aged flowers

5.3.3.1 Materials and methods

On 19 November a sample of petals was taken from a block in orchard 23 (Appendix 1) that had been identified as having a moderate apothecial density (2 apothecia m^{-2}). At 50–60% flowering, 10 petals from each of the following categories were collected from each of the 10 tagged pistillate vines:

1. Partially open flower buds with petals but not the internal floral parts exposed.
2. Two day-old flowers with anthers partially brown, petals white and with some resistance to removal.
3. Three to four day-old flowers with anthers largely dehisced, petals slightly yellowing but no petal-fall evident, petals easy to remove.
4. Five day-old flowers, petals still turgid, very easy to remove and flowers were mid-way through petal-fall.
5. Six day-old flowers, petals yellow/orange and with dried edges.
6. Petals with visible lesions.

All collected petals were placed on moist tissue in plastic trays (350 × 300 × 25 mm) and incubated at 20°C. The number of petals in each tray with *S. sclerotiorum* mycelium and/or sclerotia was determined after 6 days. Genstat was used to analyse the incidence of *S. sclerotiorum* from petals from different aged flowers using generalised linear models with logit link and errors proportional to binomial distribution. A curve was fitted to the mean percentage of petals from each category with *S. sclerotiorum* using Flexi.

5.3.3.1 Results

There was a significant (P<0.001) increase in the percentage recovery of *S. sclerotiorum* from petals sampled from flowers with increasing age (Figure 5.7). The incidecne of *S. sclerotiorum* rose progressively from 1% of open buds to 74% of 6 day-old flowers. Petals collected with visible soft rot symptoms had a 95% incidence of *S. sclerotiorum*.

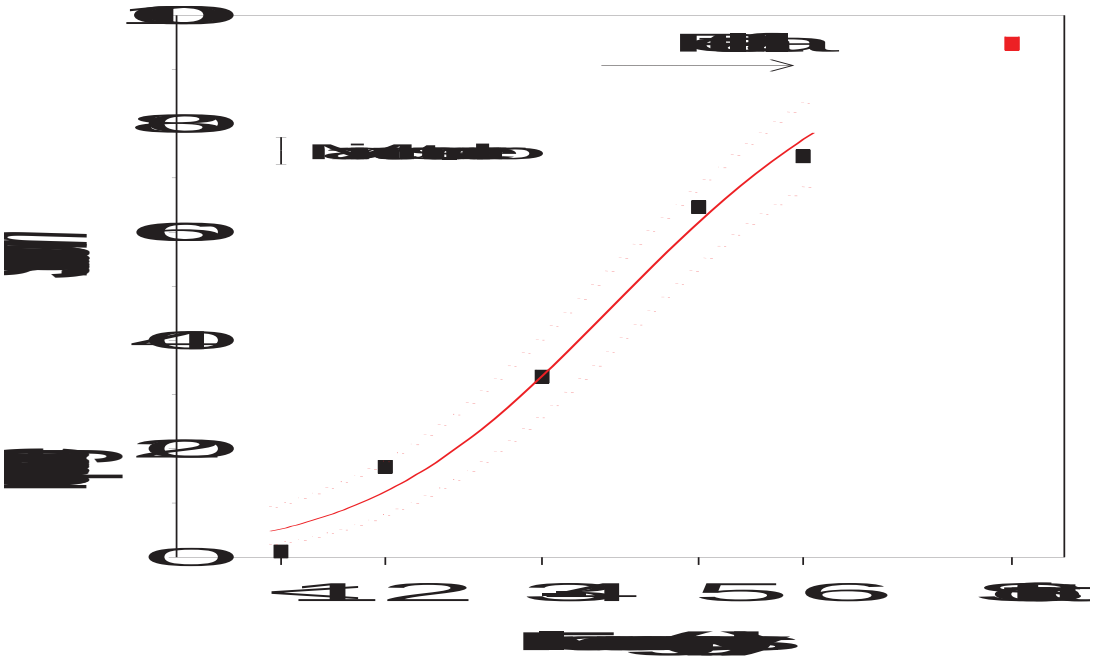


Figure 5.7 Mean percentage recovery of *S. sclerotiorum* from petals sampled from flowers of various ages (days from anthesis) and from petals with visible soft rot (■), collected from orchard 23 on 19 November 1998. Solid line is a fitted curve using Flexi, dotted lines are 95% confidence bands.

5.3.4 Experiment 5-5: Effect of flower age on colonisation of petals by ascospores

5.3.4.1 Materials and methods

On 6 December 1997 four newly opened flowers (termed ‘1 day-old’) were chosen at random on each of 12 pistillate vines at orchard 21 (Appendix 1) and tagged around the pedicel with coloured wool. These were identified by the stamens having no signs of dehiscence. This was repeated on each of the following 5 days using a different coloured length of wool (80–100 mm) for each day. On 11 December all tagged flowers were picked.

The 12 vines were grouped into six pairs and two batches for inoculation were made up from the flowers of each pair of vines. One petal was selected at random from each flower age from one vine, and similar group of six petals was selected from the other vine of each pair. The position of petals in the Burkard settling chamber was randomised within each group of six petals. *S. sclerotiorum* ascospores from 16 grids of membrane filter disc were used to inoculate each batch of petals. Each group of six petals were randomly assigned to a position within one of three incubation chambers with SDW held at 20^{o}C (section 4.5.2), i.e. there were eight groups of six petals in each chamber. Three un-inoculated petals from each flower age were taken at random from among the 12 vines after the third, sixth, and ninth batch of inoculation. Three un-inoculated petals from each flower age were placed into each incubation chamber.

Petals were removed from the chambers after 70 h incubation and assessed for the percentage area of lesions. All petals were surface-sterilised (section 2.2.2). The eight groups of six petals from each chamber were paired randomly and petals of the same age from each pair were macerated together. Two, 3 and 5 ml of PBS was used for petals from flowers that were 1 and 2 days old, 3–4 days old, and 5 and 6 days old, respectively. The three un-inoculated petals from each flower age in each chamber were macerated together in 3 ml PBS. All samples had two 50 µl aliquots spread onto each of two Petri dishes of JK selective medium. The number of cfu/petal of *S. sclerotiorum* and *B. cinerea* were determined (section 4.3.4).

ANOVA was carried out using Genstat to determine the effects of flower age on the percentage area of lesions and cfu/petal. Analysis was carried out before and after angular transformation of the percentage area of lesions and $\log_{10}$ transformation of cfu/petal (+1). Non-transformed data has been presented for the percentage area of lesions because the same conclusions were reached for transformed and non-transformed analyses. Curves were fitted to the data using Flexi.

5.3.4.2 Results

The percentage area of lesions on kiwifruit petals that were 1–6 days old ranged between 8% and 92% on inoculated petals and 2% and 45% on un-inoculated petals (Figure 5.8). There was a significant linear increase ($P<0.001$) in the percentage area of lesions on both inoculated and un-inoculated petals with increasing flower age. The

fitted curves for inoculated and un-inoculated petals were significantly different ($P<0.05$) for flowers that were 3–6 days old (Figure 5.8).

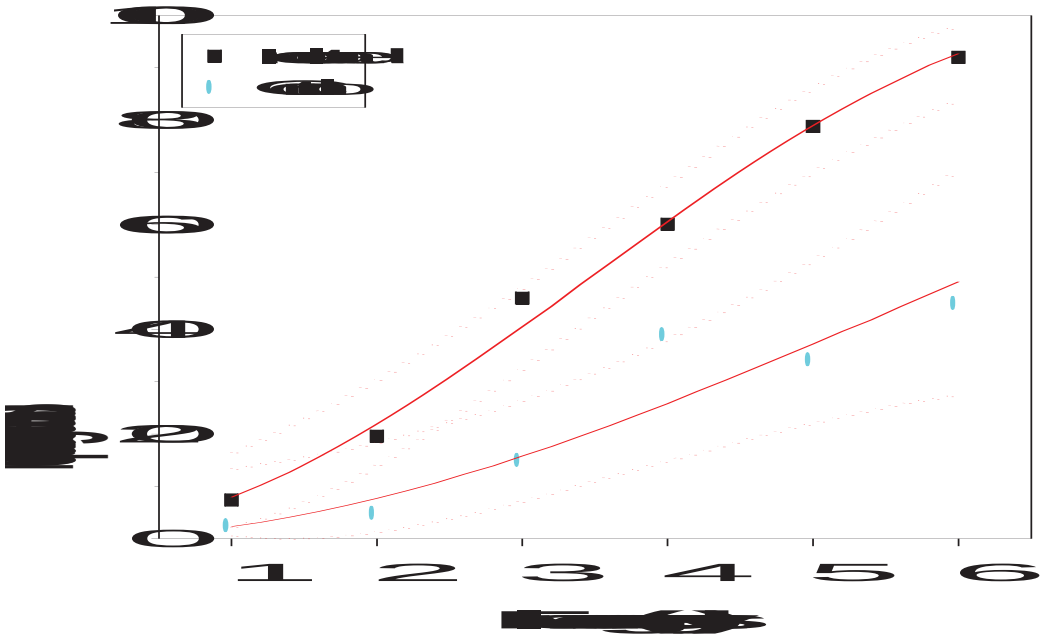


Figure 5.8 Relationship between flower age and mean percentage area of lesions on petals inoculated with *S. sclerotiorum* ascospores and on un-inoculated (control) petals, sampled from 1–6 day-old pistillate flowers. Solid lines are fitted curves using Flexi, dotted lines are 95% confidence bands.

Flower age had a significant effect ($P<0.001$) on the number of *S. sclerotiorum* cfu/petal recovered from inoculated petals. Colony forming units increased from 5 cfu/petal on 1 day-old flower petals to 590 cfu/petal on 4 day-old flower petals. There was no significant difference in the number of cfu/petal between flowers that were 4–6 days old (Figure 5.9). No cfu of *S. sclerotiorum* were recovered from the un-inoculated petals. The number of *B. cinerea* cfu/petal also increased significantly with increasing flower age ($P<0.001$) but was similar on inoculated and un-inoculated petals, except for petals from 4 day-old flowers (Figure 5.9).

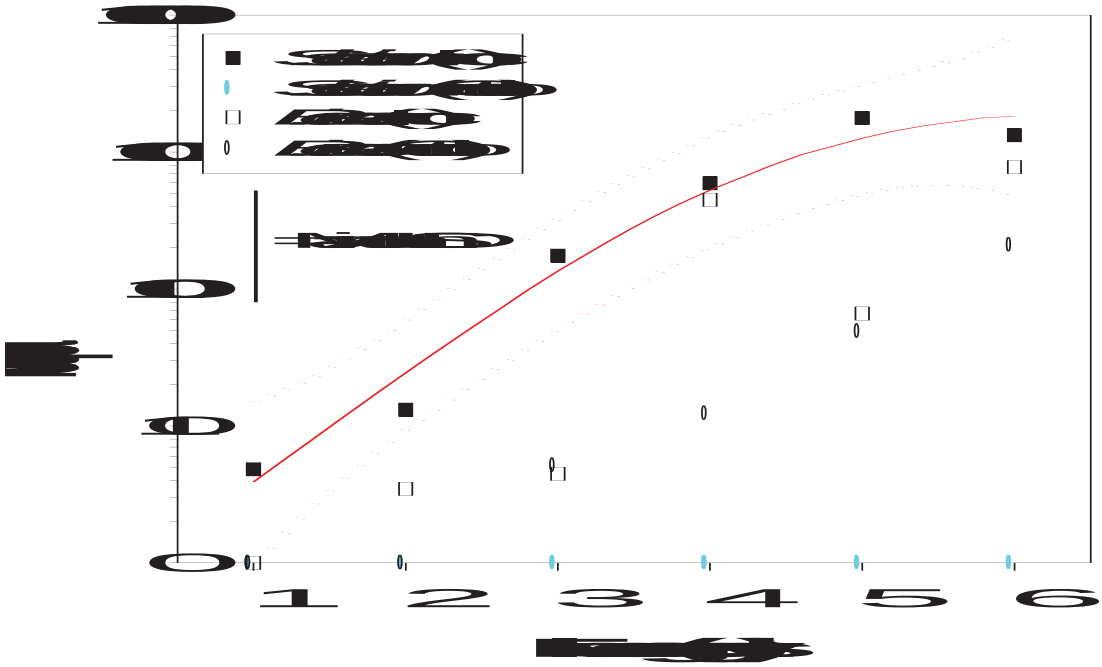


Figure 5.9 Relationship between flower age and mean number of colony forming units (cfu)/petal of *S. sclerotiorum* and *B. cinerea* (log scale) on petals inoculated with ascospores of *S. sclerotiorum* (Inoc) and on un-inoculated (control) petals from 1–6 day-old pistillate flowers. Solid line is a curve fitted to (■) using Flexi, dotted lines are 95% confidence bands. Bar is maximum least significant difference for both *S. sclerotiorum* and *B. cinerea* data.

5.3.5 Discussion

The increase in incidence of *S. sclerotiorum* within petals sampled from flowers of increasing age (Experiment 5-4) may be related to an accumulation of ascospore inoculum. This effect of increasing inoculum on older flowers will be compounded by the increasing susceptibility of petals to colonisation as they become older (Experiment 5-5). This result is comparable to the increase in *S. sclerotiorum* recovered from naturally infected bean flowers of increasing age (Natti, 1971).

It is presumed in this study that this relationship between flower age and the extent of *S. sclerotiorum* colonisation was consistent across a range of inoculum densities, although this was not determined. Studies in canola have shown that the incidence of *S. sclerotiorum* in petals can change significantly during the progression of flowering and between samples collected in the morning and afternoon (Turkington & Morrall, 1993). Further research is required to determine if such differences exist in kiwifruit.

Colonisation of detached kiwifruit petals by *S. sclerotiorum* ascospores significantly ($P<0.01$) increased with increasing flower age, as determined by the percentage area of lesions and the number of cfu recovered after surface-sterilisation. Similarly, infection of a range of geranium floral tissues by *B. cinerea* increased from 0–30% on 1 day-old flowers to 90–100% on 5 day-old flowers (Sirjusingh *et al*., 1996). Also the spore germination and colonisation of petal surfaces by the saprophyte *E. purpurascens* was shown to be significantly greater on snap bean petals just before petal-fall than on unopened or newly opened flowers (Zhou & Reeleder, 1991).

Senescence of petals is one factor that may influence the susceptibility to colonisation. Many different patterns of petal senescence exist in flowering plants, though generally flowers begin to senesce shortly after anthesis, bringing about multiple physiological changes (Sexton & Woolhouse, 1984). Many aspects of petal senescence in kiwifruit have not been studied. Observations of the cessation of petal closure during evenings 1–2 days after anthesis (Goodwin, 1995), noticeable changes in petal colour 2–5 days after anthesis, natural petal abscission 4–5 days after anthesis and the increasing ease of petal removal noted in these experiments, are all indicators that senescence is occurring.

The majority of research into petal senescence relates to commercial cut-flowers and not tree or vine crops. Processes that typically take place include reduced enzyme activity, increased cell membrane microviscosity relating to catabolism of phospholipids and proteins (Borochov & Woodson, 1989; 1990), solute leakage (Celikel & van Doorn, 1995), changes in RNA composition and reduced protein synthesis (Woodson & Handa, 1987; Borochov & Woodson, 1989). Assuming that such physiological changes occur in kiwifruit petals as they age, these factors may contribute to the increased susceptibility of kiwifruit petals to colonisation by *S. sclerotiorum* ascospores. This might be because of stimulation of ascospore germination by solutes, easier penetration of the tissues due to cell wall changes, or reduced host response to the invading hyphae.

Pistillate kiwifruit flowers liberate pollen for 3–5 days after anthesis and bees regularly visit them and transfer staminate pollen (Goodwin & Steven, 1993). It is likely that staminate and pistillate pollen is present on petal surfaces in increasing quantities as flowers age. Tobacco pollen is known to stimulate spore germination of both *S. sclerotiorum* and *B. cinerea* on tobacco leaves (Hartill, 1975). Also, pollen of alfalfa

(*Medicago sativa* L.), pea and bean has been suggested to play a role during sclerotinia infection of these crops (Sutton & Deverall, 1983; Huang & Kokko, 1993; Huang *et al*., 1997). It is possible that kiwifruit pollen may influence ascospore germination and infection of kiwifruit petals by *S. sclerotiorum*. This warrants further investigation.

The degree of soft rot on un-inoculated petals was also related to flower age, however the absence of *S. sclerotiorum* colonies indicates that these symptoms were not caused by *S. sclerotiorum*. *B. cinerea* was also recovered from macerated petals indicating that this fungus was naturally occurring and actively colonised some petals. The increase in *B. cinerea* recovery on inoculated and un-inoculated older petals, may be related to a combination of increasing numbers of conidia with longer exposure to the environment and the effects of petal senescence increasing susceptibility to infection.

Epiphytic fungi, bacteria and yeasts are common on petals and populations have been shown to increase with increasing flower age on canola (Inglis & Boland, 1990; Hutchins & Archer, 1994), sunflower (Kumar & Dwivedi, 1981) and guava (Pandey, 1990). Several fungal saprophytes, e.g. species of *Cladosporium* and *Epicoccum*, are present on necrotic leaf tissues of kiwifruit (Hoyte *et al*., 1994), and these and other fungal saprophytes occur on floral tissues (Figure 2.2). Saprophytes may have contributed to the soft rot on petals in these assays. Furthermore, several saprophytic fungi on bean and canola petals have been shown to be antagonistic to *S. sclerotiorum* in laboratory and field studies (Boland & Inglis, 1989; Inglis & Boland, 1990; Hutchins & Archer, 1994). Competition for nutrients is the likely mechanism of this antagonism (Boland & Hunter, 1988; Kohl & Fokkema, 1994). It is possible that antagonism by naturally occurring micro-organisms may have occurred in these assays.

These results highlight the need to standardise flower age when sampling petals to minimise variation. For this reason 3–4 day-old petals were sampled from vines based on visual examination of the stage of anther dehiscence (Goodwin, 1986). For the detached petal assays this was necessary to ensure consistent susceptibility to infection by ascospores. In Chapter 7, where petals were collected to determine natural incidence of *S. sclerotiorum*, sampling from 3–4 day-old flowers was a necessary compromise between allowing petals time to become colonised but avoiding flowers that had initiated petal-fall.

5.4 Effect of temperature on *S. sclerotiorum* ascospore germination and mycelial growth rate (*in vitro)*

5.4.1 Introduction

Spore germination is the initial step during infection of plant pathogens (Gaumann, 1950; Zadoks & Schein, 1979). Once infection has taken place, necrotrophic fungi like *S. sclerotiorum* spread by mycelial growth invading host tissues and forming discrete lesions. Lesion expansion continues while mycelial growth is extending into the surrounding adjacent healthy tissue (van der Plank, 1963). The rate at which infection and symptom development occurs depends on many factors relating to the host, the pathogen and the environment (Zadoks & Schein, 1979). For a given host tissue and pathogen e.g. *S. sclerotiorum* infection of kiwifruit petals, one of the major determinants of the rate of disease progress is the prevailing environmental conditions.

5.4.2 Objectives

- To determine the effects of temperature on *S. sclerotiorum* ascospore germination on water agar, mycelial growth on PDA and lesion expansion on kiwifruit petals.

5.4.3 Experiment 5-6: Effect of temperature on *S. sclerotiorum* ascospore germination

5.4.3.1 Materials and methods

Ascospores were incubated on water agar at 11 temperatures (3, 7, 12, 15, 18, 20, 23, 25, 27, 30, and 34^{o}C). Ascospores were prepared by rinsing one membrane filter disc containing ascospores (isolate Sc1) with 2 ml SDW (0.05% Tween80) for 1 minute using a sterile plastic spatula. The concentration of ascospores was determined with a haemacytometer (Hawksley & Sons Ltd) and adjusted to 1×10^4 ascospores ml^{-1}. Two 100 µl aliquots of ascospores were spread on each of three replicate Petri dishes of water agar for each temperature treatment. Petri dishes were placed inside a black 4 ℓ plastic container with a lid to exclude light and placed in a coolstore, incubator or controlled environment room set at the appropriate temperatures (p. 73). A min/max thermometer (Diplex) was placed in each container and the temperature was recorded at each sampling interval and all were within 1.0^{o}C of the set temperature.

The percentage germination of ascospores was determined after 3, 9 and 24 h by examining 100 ascospores in each droplet with a compound microscope (Axioplan, Carl Zeiss) (100 ×). Germination was considered to have taken place if germ tubes were at least equal to the length of the ascospore (10–14 μm). The order in which treatments were assessed was randomised at each sample interval. The mean percentage germination was calculated for each sampling time and curves were fitted using Flexi to describe the response of ascospore germination to changes in temperature and time.

5.4.3.2 Results

The percentage germination of *S. sclerotiorum* ascospores peaked between 20 and 27°C after 3 h and was significantly lower at temperatures outside this range (Figure 5.10). The fitted curve showed the maximum germination of 32% occurred at 24.5°C after 3 h. After 9 and 24 h incubation >69% germination had occurred over a wide temperature range (12–30°C) and the highest germination recorded was 98% at temperatures in the range 20–30°C. The percentage germination after 24 h incubation was significantly higher than after 9 h incubation at 7 and 12°C (Figure 5.10).

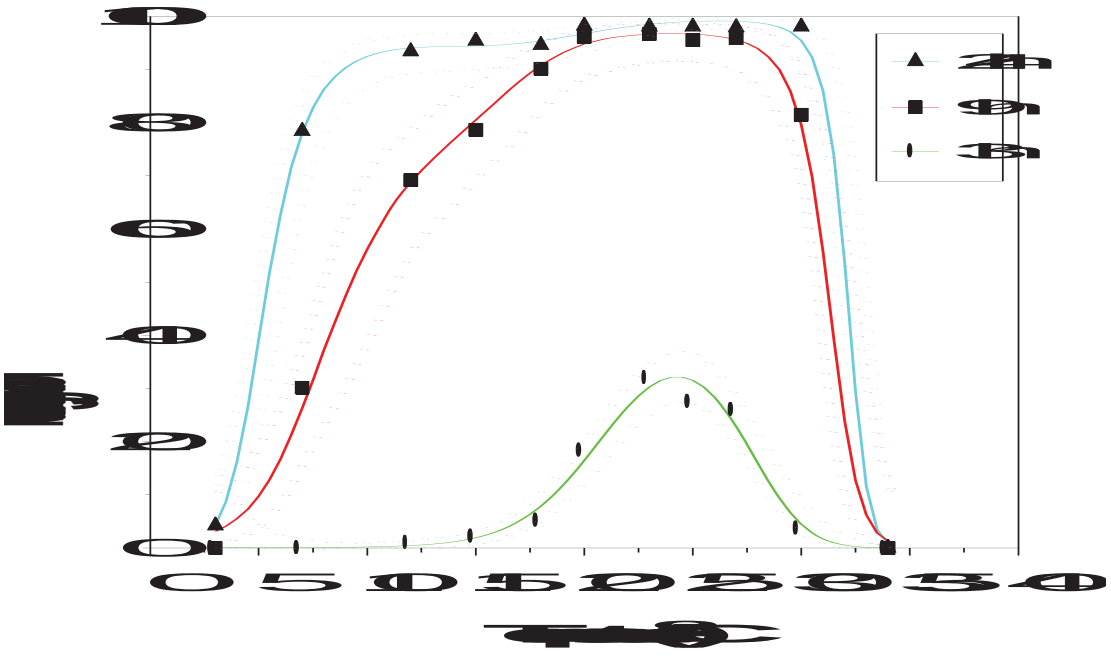


Figure 5.10 Mean percentage germination of *S. sclerotiorum* ascospores on water agar at temperatures between 3 and 34°C after 3, 9 and 24 h incubation in the dark. Solid lines are curves fitted using Flexi, dotted lines are 95% confidence bands.

5.4.4 Experiment 5-7: Effect of temperature on mycelial growth rate of *S. sclerotiorum* on PDA and lesion expansion on petals

5.4.4.1 Materials and methods

Part A: One isolate of *S. sclerotiorum* was cultured from a diseased fruitlet collected from each of 12 orchards (section 2.2.1) and maintained on PDA. On 17 August 1998, each isolate, plus isolate Sc1, were sub-cultured onto fresh Petri dishes of PDA. After 2 days growth nine 4 mm diameter agar plugs were taken from the growing margin of each isolate. Each agar plug was inverted onto the centre of a Petri dish of PDA, on the underside of which two lines at right angles were marked, centred on the agar plug. Three Petri dishes from each isolate were placed into separate black 4 ℓ containers with a lid and each was randomly assigned to one of three temperature treatments (15, 18 and 20^{o}C). A min/max thermometer was placed into each container and temperatures were recorded daily. After 21, 30, 45 and 53 h the colony margin on each radial line was marked on the underside of each Petri dish. The order in which isolates were assessed was randomised at each sampling interval. The mycelial growth rate (mm h^{-1}) was calculated at the end of the experiment by dividing the distance between each mark by the respective time intervals.

Part B: On 20 August 1998, isolate Sc1 was cultured onto a single Petri dish of PDA. After 2 days growth, 30 agar plugs were cut from the growing margin and each was inverted onto the centre of a Petri dish of PDA, as described above. Three Petri dishes were placed into each of 10 black 4 ℓ containers with a lid and each was randomly assigned to a temperature treatment (5, 10, 15, 18, 20, 23, 25, 27, 30, and 34^{o}C), as for Experiment 5-6. A min/max thermometer was placed into each container and temperatures were recorded daily. After 17, 27, 40 and 53 h colony growth was determined as described above. All temperature measurements taken during Part A and B were within 1.0^{o}C of the set temperature.

Part C. In a separate experiment, one petal from each of eight flowers, sampled from orchard 20 (Appendix 1), was inoculated in the centre with a 4 mm diameter agar plug taken from the growing margin of a 3 day-old culture of isolate Sc1 on PDA. The petals were placed into micro-tube holders and incubated in a SDW chamber at 20^{o}C. The length of lesions, along a line from the base of the petal towards the tip, was determined

after 24 and 38 h and the rate of lesion expansion (mm h^{-1}) was calculated between these time intervals.

ANOVA was carried out using Genstat to test for significant differences in mycelial growth rate between isolates (Part A) and sampling intervals (Part B). A non-linear thermodynamic model based on absolute reaction-rate theory was used to describe the relationship between temperature and mycelial growth rate (Schoolfield *et al*., 1981; Scherm & van Bruggen, 1994):

$$r(T) = \frac{p_{25}\frac{T}{298}\exp\left[\frac{\Delta H_A}{R}\left(\frac{1}{298}-\frac{1}{T}\right)\right]}{1+\exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{1/2L}}-\frac{1}{T}\right)\right]+\exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{1/2H}}-\frac{1}{T}\right)\right]} \qquad \text{equation 1}$$

where $r(T)$ = development rate (h^{-1}), T = temperature in degrees Kelvin (298K = 25°C), R = universal gas constant (1,987 cal deg^{-1} mol^{-1}), p_{25} is an estimate of the development rate at 25°C assuming no enzyme inactivation, ΔH_A = enthalpy of activation of enzyme reaction (cal mol^{-1}), ΔH_L = change in enthalpy associated with low temperature enzyme inactivation (cal mol^{-1}), ΔH_H = change in enthalpy associated with high temperature enzyme inactivation (cal mol^{-1}), $T_{1/2L}$ and $T_{1/2H}$ = temperatures (K) at which enzyme is ½ low temperature inactive and ½ high temperature inactive, respectively. Biological interpretations of these parameters have been proposed by Schoolfield *et al.* (1981). Non-linear curve fitting of this model was performed in Origin™ (Microcal Software, Inc.). For graphical presentation the formula has been simplified to the form:

$$r(T) = \frac{p_{25}\frac{T}{298}\exp(a)}{1+\exp(b)+\exp(c)} \qquad \text{equation 2}$$

where parameters a, b and c are the respective components from equation 1.

5.4.4.2 Results

The mycelial growth rate of all isolates on PDA during the first 21 h (Part A) and the first 17 h (Part B) was significantly less ($P<0.05$) than between this first measurement and the final measurement after 53 h. Because of this lag phase in growth the mean mycelial growth rate was calculated by omitting the first growth measurement. In Part A, the mean mycelial growth rates of the 13 *S. sclerotiorum* isolates were 0.54 mm h^{-1}

(SE ± 0.019), 0.86 mm h^{-1} (SE ± 0.034), and 0.87 mm h^{-1} (SE ± 0.035), at 15, 18 and 20°C, respectively, and are shown as the box plot in Figure 5.11. Isolate Sc1 was not significantly different (P>0.05) from these overall means. One isolate had significantly different (lower) mycelial growth rates (P<0.001) compared with other isolates.

In Part B, there was a significant relationship (P<0.001) between temperature and the mean mycelial growth rate of isolate Sc1 growing on PDA over the range 5–34°C (Figure 5.11). The fitted curve was asymmetrical about the maximum of 0.94 mm h^{-1} at 24.2°C. There was no growth at 34°C and the low temperature minimum was <5°C. The fitted parameters of the thermodynamic model (equation 1) are presented in Appendix 5. The mean rate of lesion expansion on kiwifruit petals inoculated with *S. sclerotiorum* culture agar plugs and incubated at 20°C was 0.37 mm h^{-1} (SE ± 0.03), approximately half the mycelial growth rate on PDA (Figure 5.11).

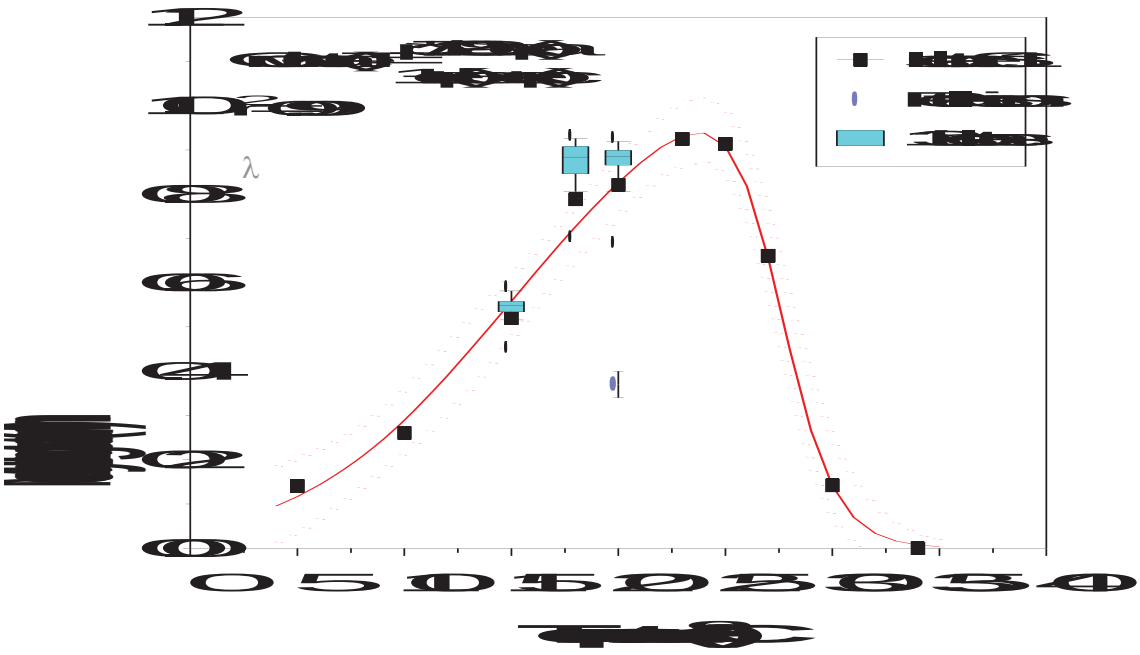


Figure 5.11 Mean mycelial growth rate of isolate Sc1 at a range of temperatures (5–34°C) and 13 isolates of *S. sclerotiorum* (box plot) at three temperatures (15, 18 and 20°C), 17–53 h after inoculation on PDA. Single point (●) is mean rate of expansion of petals lesions (bar = standard error). Solid line is a curve fitted to (■) using a thermodynamic model (equation 1), dotted lines are 95% confidence bands. For box plots, whiskers show 5% and 95% percentiles, horizontal line is the median and (○) are outliers.

5.4.5 Discussion

In these experiments germination was highest at 23°C after 3 h. Percentage germination after 9 and 24 h was similar between 20–27°C with a maximum of 98%. Similarly, Abawi & Grogan (1975) found that *S. sclerotiorum* ascospores had a maximum germination of 98% at 25°C after 3 h on PDA and that a wide temperature range (10–30°C) was equally favourable for germination after 6 h. Singh & Srivastava (1993) determined that after 24 h in SDW ascospore germination was <90%, but was stimulated to 97% by addition of 100µg/ml sucrose. It is likely that the differences in percentage germination between these experiments can be explained by the relative nutrient status of the medium used, i.e. PDA > sucrose > water agar > SDW. It is apparent from these experiments that germination of *S. sclerotiorum* ascospores did not exceed 98%. The few ascospores that failed to germinate may represent a proportion of the population that is inherently non-viable or has an extremely slow development rate.

The fitted curve describing the temperature response of *S. sclerotiorum* culture growing on PDA was bell-shaped and skewed to the right, with minimum, optimum and maximum temperatures (<5, 23, and 34°C) that are similar to cardinal temperatures of most temperate fungal pathogens (Gaumann, 1950). In comparable experiments a similar temperature response of *S. sclerotiorum* cultures on PDA was found (Spotts & Cervantes, 1996); however, Abawi & Grogan (1975) reported no growth at 30°C after 48 h. The maximum mycelial growth rate of 0.94 mm h^{-1} in Experiment 5-7 was slightly greater than growth rates recorded from nine *S. sclerotiorum* isolates (0.77–0.92 mm h^{-1}) after 48 h on PDA at 25°C (Tores, 1990). The lag phase recorded in colony growth during the first 17 h is also in agreement with other reports (Tariq *et al*., 1985; Tores, 1990).

Part A of Experiment 5-7 showed that isolate Sc1 had similar mycelial growth rates on PDA when compared with 11 of the 12 other isolates. Although other aspects of isolate variability were not investigated in this study, isolates of *S. sclerotiorum* from different host plant species and/or geographic locality have been shown to differ in growth rate, sclerotial size, ascospore dimensions, and degree of pathogenicity towards a range of host species (Price & Colhoun, 1975a; Price & Colhoun, 1975b). Isolate Sc1 from New Zealand kiwifruit was used throughout this study and exhibited similar ascospore germination rates and mycelial growth rates to *S. sclerotiorum* isolates studied overseas.

5.5 Effect of temperature and relative humidity on colonisation of petals

5.5.1 Introduction

Colonisation of host tissues and disease development is often strongly influenced by environmental conditions (Gaumann, 1950; Manners, 1993). Many studies have quantified the effects of temperature and leaf wetness on pathogen sporulation and infection of susceptible hosts and used this information for developing disease prediction models. For example, temperature optima and minimum periods of surface leaf wetness have been experimentally determined for *B. cinerea* on grape bunches (*Vitis vinifera* L.) (Broome *et al*., 1995) and geranium flowers and leaves (Sirjusingh & Sutton, 1996), *Botrytis squamosa* on onion leaves (*Alium cepa* L.) (Alderman & Lacy, 1983), and *Ascochyta rabiei* infection of chickpea (*Cicer arietinum* L.) (Trapero-Casas & Kaiser, 1992). In their studies, increasing wetness duration in the range of 4–30 h increased sporulation and infection, and optimum temperatures for infection were generally in the range of 20–25^{o}C.

The majority of research on the effects of temperature on colonisation by *S. sclerotiorum* has been carried out on bean in chambers where RH was maintained close to 100%. Lesions appeared within 48 h on bean leaves inoculated with agar plugs of culture, and lesion growth was optimal at 20^{o}C (Abawi & Grogan, 1975). Similarly, inoculation of bean leaves with mycelial fragments of *S. sclerotiorum* resulted in lesion formation after 40 h and 60 h at 25^{o}C and 20^{o}C respectively, and lesion growth was greatest at 25^{o}C (Weiss *et al*., 1980b). Boland & Hall (1987) reported that disease on bean developed between 15 and 25^{o}C, most rapidly at 20^{o}C, and required at least 54 h continuous plant surface wetness in controlled environments and at least 39 h plant surface wetness in field studies. Phillips (1994b) showed that initiation of infection of bean leaves by *S. sclerotiorum* does not require a continuous period of plant surface wetness and that the minimum required period of plant surface wetness is temperature dependent. In the above reports, symptoms of infection by *S. sclerotiorum* did not develop in beans at 5^{o}C or at 30^{o}C.

Field studies of white rot of peas have also shown a positive relationship between disease incidence and severity, and temperature, RH, and rainfall (Singh, 1991).

Temperatures in the range of 10–20^{o}C were suitable for disease development with an optimum of 14^{o}C, considerably lower than for beans as described in the laboratory studies above.

Atmospheric humidity has a large effect on the development and growth of pathogenic fungi and associated diseases (Manners, 1993; Harrison *et al*., 1994). Infection and lesion development is favoured by high RH (90–100%) for pathogens such as *Cercospora carotae* (Pass.) on carrots (Carisse & Kushalappa, 1992), *B. cinerea* on tomato flowers and stems (Eden *et al*., 1996) and *Monilinia fructicola* (Wint.) Honey on sour cherry (*Prunus cerasus* L.) (Koball *et al*., 1997). The initiation and expansion of lesions caused by *S. sclerotiorum* on bean leaves is favoured by 95–100% RH and 20–28^{o}C (Hannusch & Boland, 1996).

The above studies all seek to gain an understanding of the influence of temperature and water availability on infection. Relative humidity is the measure of water available for infection by plant pathogens that has most often been used in epidemiological studies. However, most of these studies have been carried out under constant temperature and RH. Studies have shown that fungal development responds non-linearly to temperature (Hau *et al*., 1985; Scherm & van Bruggen, 1994) and that there can be differences between development rates under constant and fluctuating temperatures (Phillips, 1994b; Scherm & van Bruggen, 1994; Gottwald *et al*., 1997). The magnitude of this difference is dependent on the degree of non-linearity of the temperature growth rate function and the extent of temperature fluctuation (Xu, 1996). These differences have prompted studies that utilise experimentation under precise controllable environmental conditions to allow close matching to actual conditions of the natural environments (Harrison & Lowe, 1989; Butler *et al*., 1995; Gottwald *et al*., 1997).

Although all of the studies mentioned above deal with psychrometric principles they do not make direct reference to psychrometrics. Psychrometrics describes the water status of air and its energy balance in terms of the inter-relationships between the variables of: dry bulb temperature (^{o}C), wet bulb temperature (^{o}C), absolute humidity (g water/kg air), relative humidity (ratio of air vapour pressure and saturation vapour pressure × 100%), dew point (temperature at which saturation occurs if moist air is cooled at constant pressure), and enthalpy (kJ kg^{-1} dry air) (Fritschen & Gay, 1979). These

variables can be represented graphically on psychrometric charts. In this study, psychrometric charts are used to visualise the temperature and RH during experiments in relation to naturally occurring diurnal fluctuations in temperature and RH.

The effects of environmental conditions on colonisation of kiwifruit petals by *S. sclerotiorum* ascospores have not been studied previously. Knowledge of these effects under experimental conditions could be related to the actual environmental conditions experienced in kiwifruit. This would lead to increased understanding of the weather conditions that favour sclerotinia disease progress and assist the development of a disease prediction model.

5.5.2 Objectives

To:

- determine the effects of temperature and relative humidity on the colonisation of kiwifruit petals by *S. sclerotiorum* ascospores.
- use psychrometric principles to relate the response of *S. sclerotiorum* infection *in vitro* to the environmental conditions in a kiwifruit orchard.

5.5.3 Experiment 5-8: Effect of temperature on colonisation of petals

5.5.3.1 Materials and methods

Ten 3–4 day-old flowers were collected from each of 10 pistillate vines in orchard 21 on 6 December 1997. One petal was taken at random from each vine for each of 10 batches of inoculation. *S. sclerotiorum* ascospores from 16 grids of membrane filter disc were used to inoculate each batch. One petal from each inoculation batch was randomly assigned to one of 10 incubation chambers containing SDW held at different temperatures (5, 10, 15, 18, 20, 22.5, 25, 27.5, 30, and 34^{o}C). Inoculated petals were arranged in two rows of five in order of inoculation batch (Figure 5.12). Three un-inoculated control petals were taken at random from the flowers of successive vines between completing each inoculation batch. These were randomly assigned to one of the incubation chambers. The temperature within each incubation chamber was measured twice daily with the thermistor in the Vaisala RH sensor. All these readings were within 1.0^{o}C of the specified set temperature.

All petals were removed from the chambers after 80 h and assessed for percentage area of lesions. Petals from each chamber were grouped into five pairs by combining inoculated petals from the two rows and the three un-inoculated control petals separately (Figure 5.12). Petals were surface-sterilised and macerated in 2 ml (5, 10, 15 and 34^{o}C), 4 ml (18^{o}C) and 5 ml PBS (20, 22.5, 25, 27.5, and 30^{o}C), respectively. The three control petals from each treatment were macerated together in 3 ml PBS. Two aliquots of 50 µl from each sample were spread on a Petri dish of JK selective medium and cfu were determined (section 4.3.4).

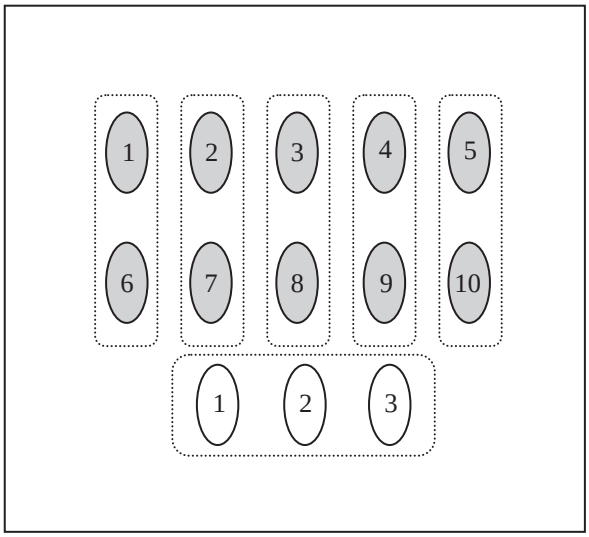


Figure 5.12 Relative position of 10 inoculated petals (shaded and numbered according to inoculation batch) and three un-inoculated petals within incubation chambers for Experiment 5-8, and groupings (dotted lines) of petals used for determining colony forming units.

The relationships between temperature and the percentage area of lesions on petals and the number of cfu/petal was described using non-linear curve fitting in Origin using the thermodynamic model of equation (1). Absolute humidity was calculated from the set temperature and RH (100%) for each treatment with a psychrometric calculator (PsyCalc©, Linric Company) and was used to present the environmental conditions of this experiment on a simplified psychrometric chart (section 5.5.7, Figure 5.19).

5.5.3.2 Results

The percentage area of lesions on kiwifruit petals inoculated with *S. sclerotiorum* ascospores and incubated for 80 h was zero at 5^{o}C and increased steadily up to 27% at 15^{o}C (Figure 5.13). There was a significant increase ($P<0.01$) in the percentage area of

lesions between 15 and 18°C, however, temperature did not significantly (P>0.05) affect the percentage area of lesions between 18 and 27.5°C (84–100%). There was a significant (P<0.01) decline in the percentage area of lesions between 27.5°C and 34°C. The percentage area of lesions on un-inoculated petals was significantly higher (P<0.05) at 22.5, 25 and 27.5°C than at temperatures outside this range, and was significantly less (P<0.01) than inoculated petals at 18, 20 and 30°C (Figure 5.13). Un-inoculated petals without soft rot had remained turgid at all temperatures, except at 34°C, and had changed to a pale orange colour, similar to naturally ageing petals on vines.

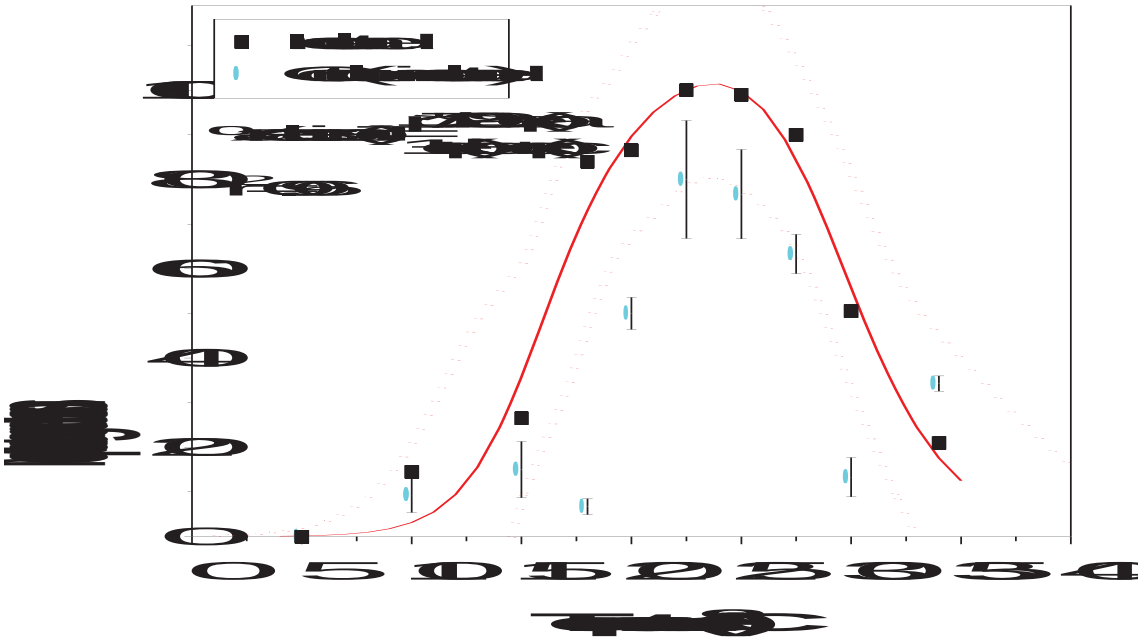


Figure 5.13 Relationship between temperature and mean percentage area of lesions on petals inoculated with *S. sclerotiorum* ascospores (Inoculated) and on un-inoculated petals (Control), incubated for 80 h. Solid line is a curve fitted to (■) using a thermodynamic model (equation 1), dotted lines are 95% confidence bands. Bars indicate standard error of the mean for the control.

The number of cfu recovered from inoculated petals showed a similar temperature response curve to the mean percentage area of lesions. There were very low numbers of *S. sclerotiorum* cfu on inoculated petals incubated at 5–15°C (<100 cfu/petal) and cfu were not recovered from petals incubated at 34°C (Figure 5.14). There was a significant increase (P<0.001) in the mean cfu/petal between 15°C and 18°C. The mean cfu/petal remained high over the temperature range 18–27.5°C (1972–2255 cfu/petal). The fitted curve had a maximum at 24.2°C (Appendix 5). There were no *S. sclerotiorum* cfu recovered from un-inoculated petals. Colony forming units of *B. cinerea* were

recovered from petals inoculated with *S. sclerotiorum* ascospores and from un-inoculated petals over the temperature range 15–27.5°C (Figure 5.14).

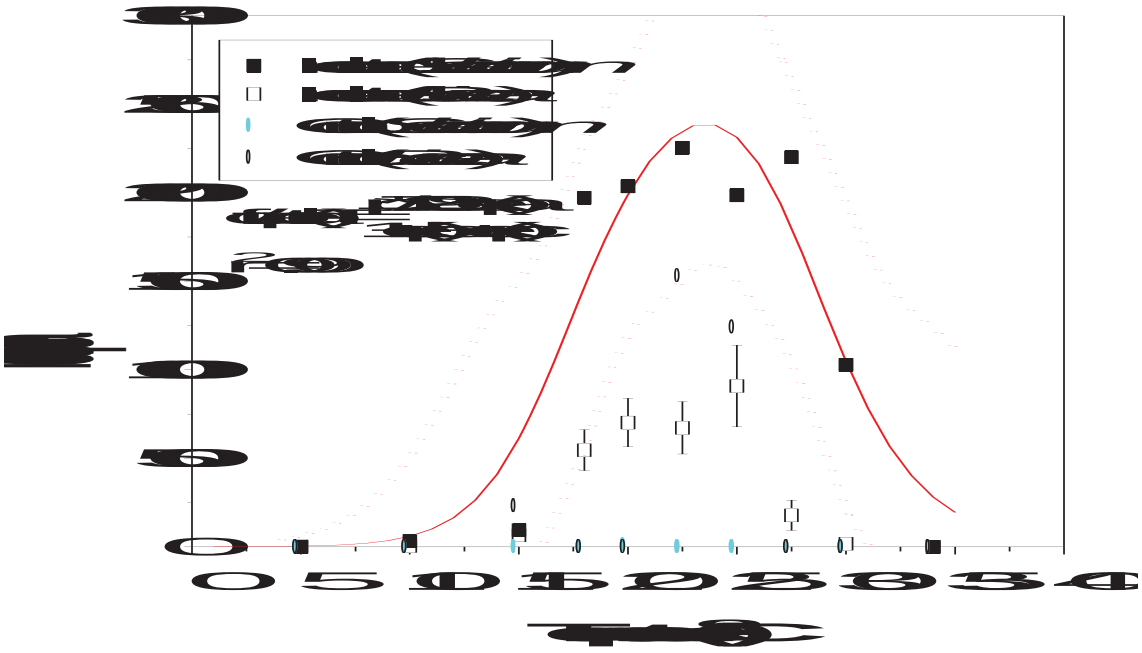


Figure 5.14 Relationship between temperature and the mean number of colony forming units (cfu/petal) of *S. sclerotiorum* and *B. cinerea* recovered from petals inoculated with *S. sclerotiorum* ascospores (Inoculated) and from un-inoculated petals (Control), incubated for 80 h. Solid line is a curve fitted to (■) using a thermodynamic model (equation 1), dotted lines are 95% confidence bands. Bars indicate standard error of the mean for *B. cinerea* (Inoculated).

5.5.4 Experiment 5-9: Effect of temperature and relative humidity on colonisation of petals

5.5.4.1 Materials and methods

Ten 3–4 day-old flowers were collected from each of 10 pistillate vines from orchard 20 on 26 November 1997. There were 20 treatments comprising different combinations of temperature (set points of 10, 15, 20 and 25°C) and RH (set points of 85, 90, 93, 98 and 100%). Each of 20 batches of inoculation were prepared by taking one petal at random from the flowers of each vine i.e. 10 petals per batch. *S. sclerotiorum* ascospores from 16 grids of membrane filter disc were used to inoculate each batch. Petals from the first 10 batches of inoculation were randomly assigned to one of 10 treatments. A second set of 10 batches of inoculation were similarly assigned to the remaining 10 treatments. Three un-inoculated control petals were taken at random from flowers after each

inoculation batch, placed directly into micro-tube holders, and randomly assigned to one of the treatments. Petals were positioned within chambers as shown in Figure 5.12.

All petals were removed from the first set of 10 treatments after 70 h and assessed for percentage area of lesions. Petals were surface-sterilised and macerated in either 2 or 4 ml PBS depending the degree of soft rot. The control petals from each chamber were combined and macerated together in 3 ml PBS. Two 50 µl aliquots from each sample were spread on a Petri dish of JK selective medium. Petals from the second set of 10 treatments were then removed from the chambers and processed similarly. Colony forming units were determined as described in section 4.3.4.

The temperature and RH within the distilled water and saturated salt chambers was determined on four occasions within each chamber with a Vaisala humidity sensor. The mean temperature readings in the incubation chambers were within 0.8°C of the set temperatures with standard errors of ≤0.2°C (Table 5.1).

Table 5.1 Set temperature, mean temperature and mean relative humidity, within sterile distilled water and saturated salt chambers during Experiment 5-9.

Set temperature (°C)	Mean temperature (°C)	Distilled water [100] [1]	Saturated salt chamber			
			K_2SO_4 [98]	KNO_3 [93.5]	$MgSO_4$ [90]	KCl [85]
10	10.4 (0.2)[2]	99.9%	98.1%	96.8%	93.4%	88.7%
15	14.8 (0.1)	99.8%	97.2%	95.7%	92.4%	86.5%
20	19.2 (0.1)	99.8%	97.5%	95.1%	92.6%	87.2%
25	24.8 (0.1)	100%	97.2%	93.9%	92.6%	86.4%
		{0.3}[3]	{0.7}	{0.4}	{0.4}	{0.7}

[ ][1] = nominal relative humidity (%) at 20°C.
( )[2] = standard error for temperature, across all relative humidities, n = 20.
{ }[3] = maximum standard error (relative humidity), n = 4.

The mean percentage area of lesions and cfu/petal for each temperature and RH treatment combination are presented as three dimensional bar plots. Absolute humidity was calculated from the mean temperature and RH of each treatment (Table 5.1) using

PsyCalc© and was used to present the environmental conditions during this experiment on a simplified psychrometric chart (section 5.5.7, Figure 5.19).

5.5.4.2 Results

Although there was variation in the actual temperature and RH during detached petal assays, e.g. Table 5.1 and 5.2, for simplicity, relative humidities are referred to as the nominal value at 20°C (Table 5.2). Un-inoculated petals incubated at 90–100% RH were turgid at the completion of this experiment, while those incubated at 85% RH had become partially flaccid, indicating that some desiccation had occurred.

The highest percentage area of lesions (47–96%) occurred on inoculated petals incubated at 20–25°C and 98–100% RH (Figure 5.15 A). At all other temperature and RH treatment combinations the percentage area of lesions was <40%. In particular, percentage area of lesions was <7% where temperatures of 10–20°C were combined with relative humidities of 85–93%. The percentage area of lesions at 15°C and 98% RH and 100% RH was lower than might be expected from the general trend of increasing lesion area at higher humidities. The percentage area of lesions on un-inoculated petals followed a similar pattern, but was <25% at 93–100% RH and <2% at 85 and 90% RH (Figure 5.15 B).

The number of *S. sclerotiorum* cfu/petal was highest in the treatment combinations 25°C × 90–100% RH (258–2530 cfu/petal) and 20°C × 98–100% RH (520–960 cfu/petal) (Figure 5.16 A). There was <30 cfu/petal recovered from petals incubated at 10 and 15°C regardless of the RH conditions. The number of *S. sclerotiorum* cfu/petal recovered from un-inoculated petals was <20 for all treatments (Figure 5.16 B).

A B

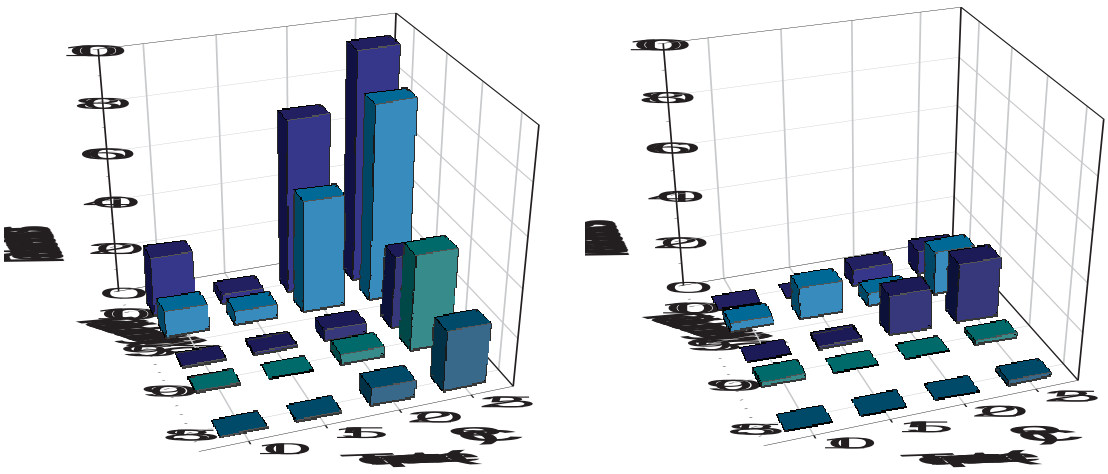


Figure 5.15 A and B. Effect of temperature and relative humidity on percentage area of lesions on petals (A) inoculated with *S. sclerotiorum* ascospores and (B) un-inoculated petals (control), incubated for 72 h.

A B

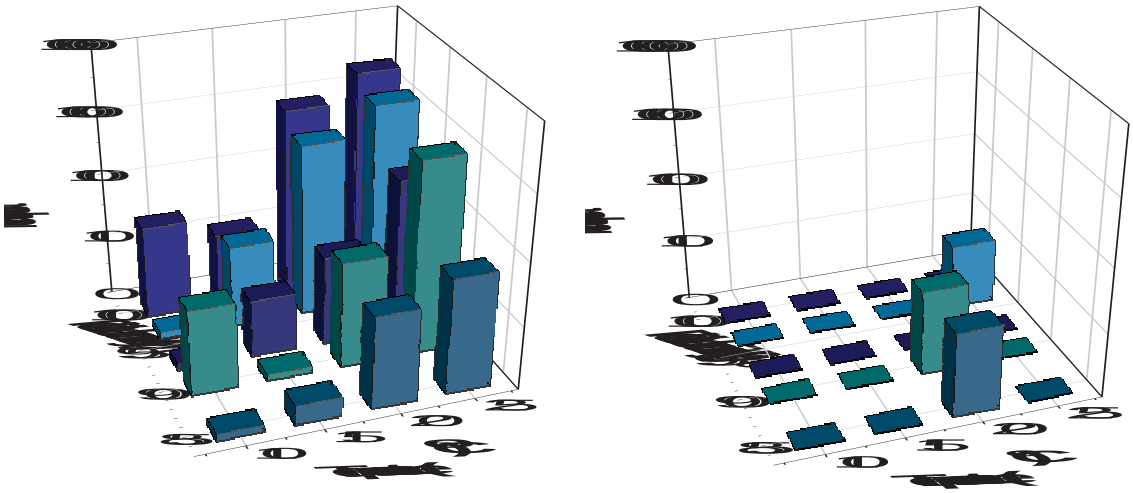


Figure 5.16 A and B. Effect of temperature and relative humidity on the number of colony forming units (cfu)/petal (log scale) from (A) petals inoculated with *S. sclerotiorum* ascospores and (B) from un-inoculated petals (control), incubated for 72 h.

5.5.5 Experiment 5-10: Effect of fluctuating temperature and relative humidity on colonisation of petals

5.5.5.1 Materials and methods

For this experiment four treatments (3–6) were carried out in the four waterbath chambers during four consecutive runs over a period of 16 days from 22 November. Petal from treatments 1 and 2 were incubated in 98 and 85% RH saturated salt chambers at 20^{o}C, respectively, and were repeated during each waterbath treatment. Each treatment had set points and actual conditions (measured during the first run of each treatment) as shown in Table 5.2.

Pistillate flowers were collected from six vines in orchard 20 on 22 and 25 November 1997 and from six vines in orchard 21 on 30 November and 5 December 1997 (Appendix 1). The whole experiment was repeated once with flowers sampled from six vines in orchard 6 on 9, 15, 19 and 22 December. Six flowers were collected from each vine on each sampling date. Two petals were taken at random from the flowers of each vine for each of five batches of inoculation. *S. sclerotiorum* ascospores from 16 grids of membrane filter disc were used to inoculate each batch. The 12 petals from each inoculation batch were placed into micro-tube holders and two were randomly assigned to one of six incubation chambers i.e. the two saturated salt chambers (treatments 1 and 2) and the four chambers within the waterbath. Petals were positioned within chambers as shown in Figures 5.12 and 4.5.

Three un-inoculated control petals were taken at random from the flowers immediately after each of the five batches of inoculation were completed. A sixth set of three control petals was taken after the fifth inoculation batch. These were placed directly into micro-tube holders and randomly assigned to one of the six chambers. All petals were removed from the chambers after 70–72 h incubation and assessed for percentage area of lesions before surface-sterilisation. Inoculated petals from each treatment were combined into pairs (Figure 5.12) and macerated together in either 2, 3 or 4 ml PBS depending on the extent of soft rot within each treatment. Un-inoculated control petals from within each chamber were macerated together in 3 ml PBS. Two 50 µl aliquots from each sample were spread onto a Petri dish of JK selective medium and cfu were determined (section 4.3.4).

Table 5.2 Set points, mean, standard deviation and range of temperature and relative humidity conditions for treatments 1–6 during the first run of Experiment 5-10.

Treat-ment	Temperature		Relative humidity	
	Set point (°C)	Actual conditions (°C)	Set point (%)	Actual conditions (%)
1	20	19.3 ± 0.4 [1] (18.7–19.8)[2]	98	98 ± 0.5 [1] (97–99)[2]
2	20	19.6 ± 0.4 (18.9–19.8)	85	87 ± 0.5 (85–89)
3	20	20.0 ± 0.09 (19.9–20.3)	97	95 ± 2.1 (91–99)
4	20	19.8 ± 0.09 (19.6–19.9)	85	83 ± 1.6 (81–87)
5	20	19.7 ± 0.14 (19.4–20.1)	70–100	83 ± 7.4 (69–96)
6 [3]	10–20	14.8 ± 2.7 (10.2–19.2)	70–100	88.5 ± 8.8 (73–100)

[1] Standard deviation.
[2] Range in parentheses.
[3] See Appendix 4 for graphs of temperature and RH.
NB. for treatment 1 and 2, n = 8 and for treatments 3–6, n = 864 (5 minute readings).

The mean temperature and RH for the period October–December 1996 at the Te Puke Research Orchard weather station was calculated for each hour of the day. This diurnal pattern of temperature and RH was used to model the conditions within the waterbath chambers during treatments 5 and 6 (Appendix 4), by programming changes to the set points of waterbath 1 and 2 (Appendix 7).

Subsequently, the diurnal pattern of temperature and RH for the period 1 November to 31 January 1996-99 was calculated from the available hourly data at the Te Puke Research Orchard weather station. The data was separated into a November–December period, coinciding with kiwifruit flowering (n = 130 days) and a January–February period, coinciding with early fruit development (n = 137 days). Each period of data was split into days with ≥5 mm rainfall day^{-1} (22 days for November–December and 18 days for January–February) and days with <5 mm rainfall day^{-1}. The threshold of 5 mm was arbitrary, but approximates the rainfall required to thoroughly wet a vine canopy.

Absolute humidity was calculated for each hour of the day for each of the four data sets using Psychrometric Function software (PKSFX© ver. 2.5 demo, PKWARE Inc.). The dry bulb temperature and absolute humidity for each of the above data sets was then

used to plot treatment and field environmental conditions on a simplified psychrometric chart (section 5.5.7, Figure 5.20).

Following angular transformation the percentage area of lesions and $\log_{10}$ transformation of cfu/petal (+1), data was analysed by REML (Residual maximum likelihood) (Patterson & Thompson, 1971) using Genstat. Treatment differences were assessed by likelihood ratio tests (Welham & Thompson, 1992).

5.5.5.2 Results

The mean percentage area of lesions was significantly greater ($P<0.01$) on petals in treatment 1 (98% RH 'static') than in treatment 3 (97% RH waterbath) (Figure 5.17). The mean percentage area of lesions on petals in treatments 2 and 4 (85% RH) and from treatments with diurnally fluctuating conditions was <5% and significantly less ($P<0.001$) than on petals in treatments 1 and 3. The number of *S. sclerotiorum* cfu/petal recovered from petals in treatment 1 (390 cfu/petal) was also significantly greater ($P<0.05$) than from petals in treatment 3 (80 cfu/petal) (Figure 5.18). All other treatments had <5 of cfu/petal *S. sclerotiorum* and there were no cfu from un-inoculated (control) petals.

There was no significant difference ($P>0.05$) in the number of *B. cinerea* cfu/petal recovered from inoculated and un-inoculated petals. There were significantly higher ($P<0.05$) numbers of *B. cinerea* cfu/petal in treatment 1 and 3 (97% RH) than the other treatments.

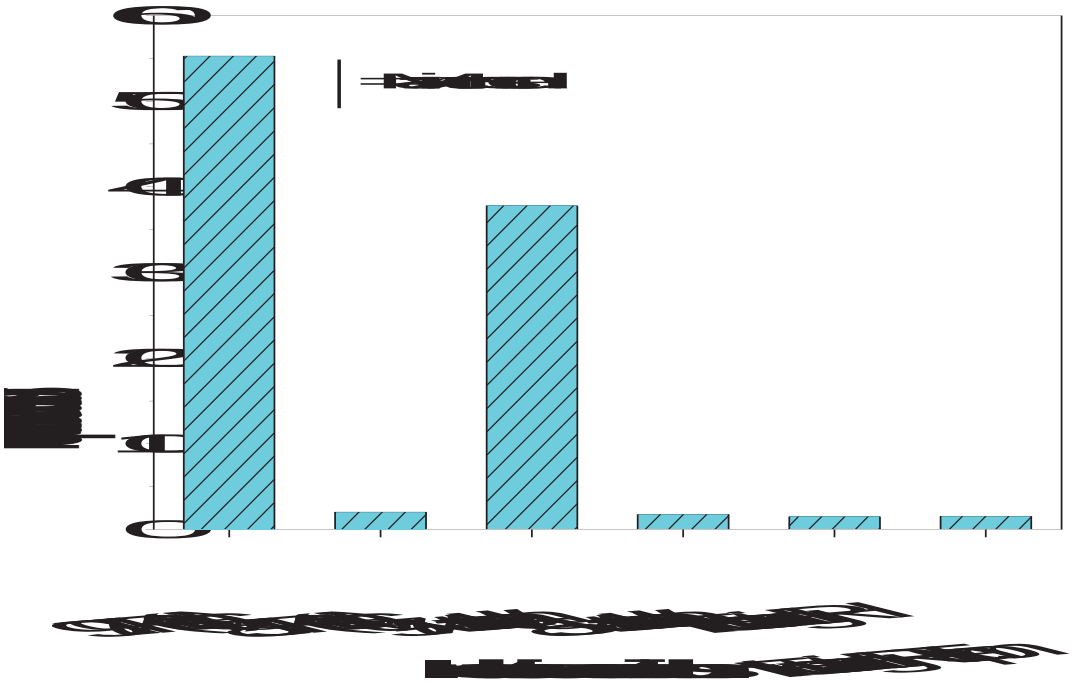


Figure 5.17 Mean percentage area of lesions on petals inoculated with *S. sclerotiorum* ascospores and incubated at 20°C for 72 h at 98% RH and 85% RH in saturated salt chambers (static), 97% RH, 85% RH, fluctuating RH (70–100%), and fluctuating RH (70–100%) and temperature (10–20°C) in waterbath chambers. Data is back transformed after angular transformation.

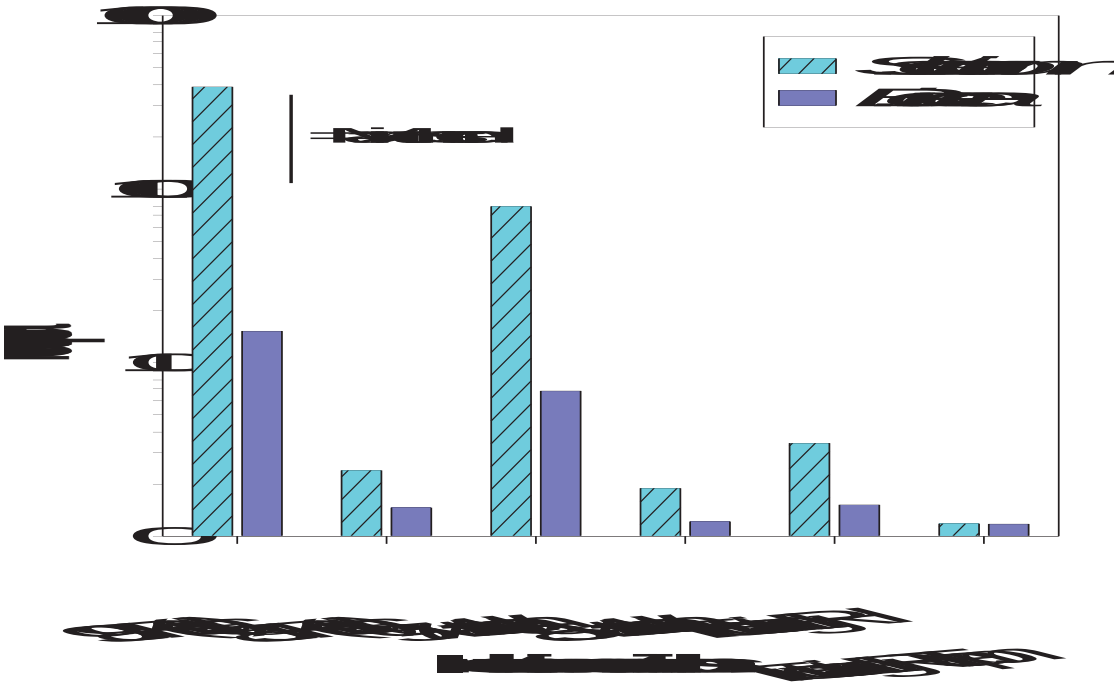


Figure 5.18 Mean cfu/petal of *S. sclerotiorum* and *B. cinerea* (log scale) on petals inoculated with *S. sclerotiorum* ascospores and incubated at 20°C for 72 h at 98% RH and 85% RH in salt chambers (static), 97% RH, 85% RH, fluctuating RH (70–100%), and fluctuating RH (70–100%) and temperature (10–20°C) in waterbath chambers.

5.5.6 Experiment 5-11: Effect of duration of fluctuating temperature and relative humidity on colonisation of petals

5.5.6.1 Materials and methods

For this experiment petals were incubated for different duration's (0–72 h) in a SDW incubation chamber at 20°C and in the waterbath chambers operating with diurnally fluctuating temperature and RH, i.e. as for treatment 6 in Experiment 5-10 (Table 5.2 and Appendix 4). Petals in treatment 1 were incubated for 72 h in the waterbath chambers. Petals in treatments 2 and 3 were initially incubated in the SDW chamber for 30 h and 48 h respectively and then transferred to the waterbath chambers. Petals in treatment 4 were incubated for 72 in a SDW incubation chamber at 20°C.

Five 3–4 day-old flowers were collected from each of eight pistillate vines from orchard 5 on 16 December 1997. Two petals were taken at random from the flowers of each vine for each of four batches of inoculation, each using ascospores from 16 grids of membrane filter disc. Four petals from each batch were randomly assigned to each of four treatments. Three un-inoculated petals were taken at random from all the flowers after each inoculation batch and randomly assigned to a treatment.

After inoculation each group of four petals (excluding treatment 1) and the groups of three un-inoculated petals, were randomly assigned to a position within a 4 × 4 grid layout in a plastic incubation chamber with SDW at 20°C. As petals from treatments 2 and 3 were transferred to the waterbath chambers one petal from each group of four petals was randomly assigned to each of the four waterbath chambers. Within each waterbath chamber, individual petals (including treatment 1) were randomly assigned to a position within a 4 × 4 grid layout on the wire mesh holder. The un-inoculated petals associated with treatments 1–3 were placed one into each of three out of the four chambers. The temperature and RH within the SDW chamber was 19.7°C (SE ± 0.5, n = 8) and 99.8% (SE ± 0.2).

All petals were removed after 72 h incubation and assessed for the percentage area of lesions before surface-sterilisation. Inoculated petals from each treatment were paired according to the original inoculation batch (2 pairs per batch) and macerated in 2 ml (treatment 1 and 2), 3 ml (treatment 3) and 4 ml (treatment 4) PBS. The three control

petals from each group were combined and macerated together in 3 ml PBS. Each sample had two 50 µl aliquots spread on a Petri dish of JK selective medium and cfu were determined after incubation for 2–3 weeks.

The percentage area of lesions was angular transformed and cfu/petal (+20) was $\log_{10}$ transformed. ANOVA was carried out using Genstat to determine the effects of the delay in transferring petals into the waterbath chambers. This experiment was repeated once with a second sample of flowers collected on the 22 December 1997. Data was combined from both experiments when analysis showed there were no significant differences between them.

5.5.6.2 Results

Petals incubated for 72 h in the SDW incubation chamber at 20°C and 100% RH had the highest percentage area of lesions and number of cfu/petal (Table 5.3). Treatments placed directly into the waterbath chambers with fluctuating temperature and RH or transferred to them after 30 h, had <5% area of lesions and <35 cfu/petal. Petals that were transferred to the waterbath chambers after 48 h had significantly higher ($P<0.05$) percentage area of lesions and cfu/petal than petals in treatment 1 and 2 (Table 5.4). The percentage area of lesions on un-inoculated (control) petals was <3% for treatments 1–3 and 10% for treatment 4. No cfu/petal recovered from un-inoculated petals.

Table 5.3 Effect of incubation time within waterbath chambers under fluctuating temperature and RH conditions on the percentage area of lesions and log(cfu/petal + 20) for petals inoculated with *S. sclerotiorum* ascospores and incubated for 72 h.

Treatment	Duration of 20°C and 100% RH	Duration of fluctuating temperature and RH	% area lesions	log(cfu/petal + 20)
1	0 h	72 h	3.3	1.59
2	30 h	42 h	4.4	1.41
3	48 h	24 h	20.9	2.07
4	72 h	0 h	38.6	2.58
			(2.4)[1]	[0.22][2]

( )[1] Maximum standard error of the difference.
[ ][2] Least significant difference.

5.5.7 Psychrometric summary

The environmental conditions during the detached petal assays in Experiments 5-8, 5-9 and 5-10 are shown on a simplified psychrometric chart that displays the inter-relationships between dry bulb temperature (^{o}C), absolute humidity (g water/kg air) and relative humidity (%) (Figure 5.19). Each treatment is colour coded to represent five degrees of colonisation taken as a percentage of the maximum $\log_{10}$(cfu/petal) in each experiment respectively.

Points along the saturation line (100% RH), between 18 and 30^{o}C inclusive (Experiment 5-8), are all within 20% of the maximum colonisation (Figure 5.19). The conditions required for successful colonisation at 10, 15 and 20^{o}C appeared to be more dependent on higher RH values than at 25^{o}C (Experiment 5-9). Petals from treatment 3 (Experiment 5-10) has a similar degree of colonisation as petals incubated at 98% RH under static conditions. Petals from treatments 4 and 5 (Experiment 5-10) had a relatively lower degree of colonisation than the lower range of RH treatments under static conditions (Experiment 5.9). The region of the psychrometric chart occupied by treatment 6 (Experiment 5-10), fluctuating temperature and RH, overlaps the 10^{o}C and 15^{o}C treatments from Experiment 5-9 and extends to RH values lower than those tested at 20^{o}C (Figure 5.19).

The mean 24-hourly psychrometric plot for treatment 6 (Experiment 5-10) closely matched the data on which these diurnally fluctuating conditions were modelled (October–December 1996), except for a 3 h period when RH was higher mid-morning and when RH was lower around mid-afternoon (Figure 5.20). The mean 24-hourly psychrometric plot for days with <5 mm rain during November–December 1996–99 was similar to October–December 1996, except that the minimum and maximum temperatures were about 1.5^{o}C higher and RH was below 70% when temperature was at a maximum. In contrast, the mean 24-hourly psychrometric plot for days with ≥5 mm rain during November–December 1996–99 had a temperature range of 15.5–19^{o}C and RH >85% (Figure 5.20). The mean 24-hourly psychrometric plots for January–February 1997–2000 had higher minimum and maximum temperatures than during November–December and had a similar separation between days with ≥5 mm rain and days with <5 mm rain.

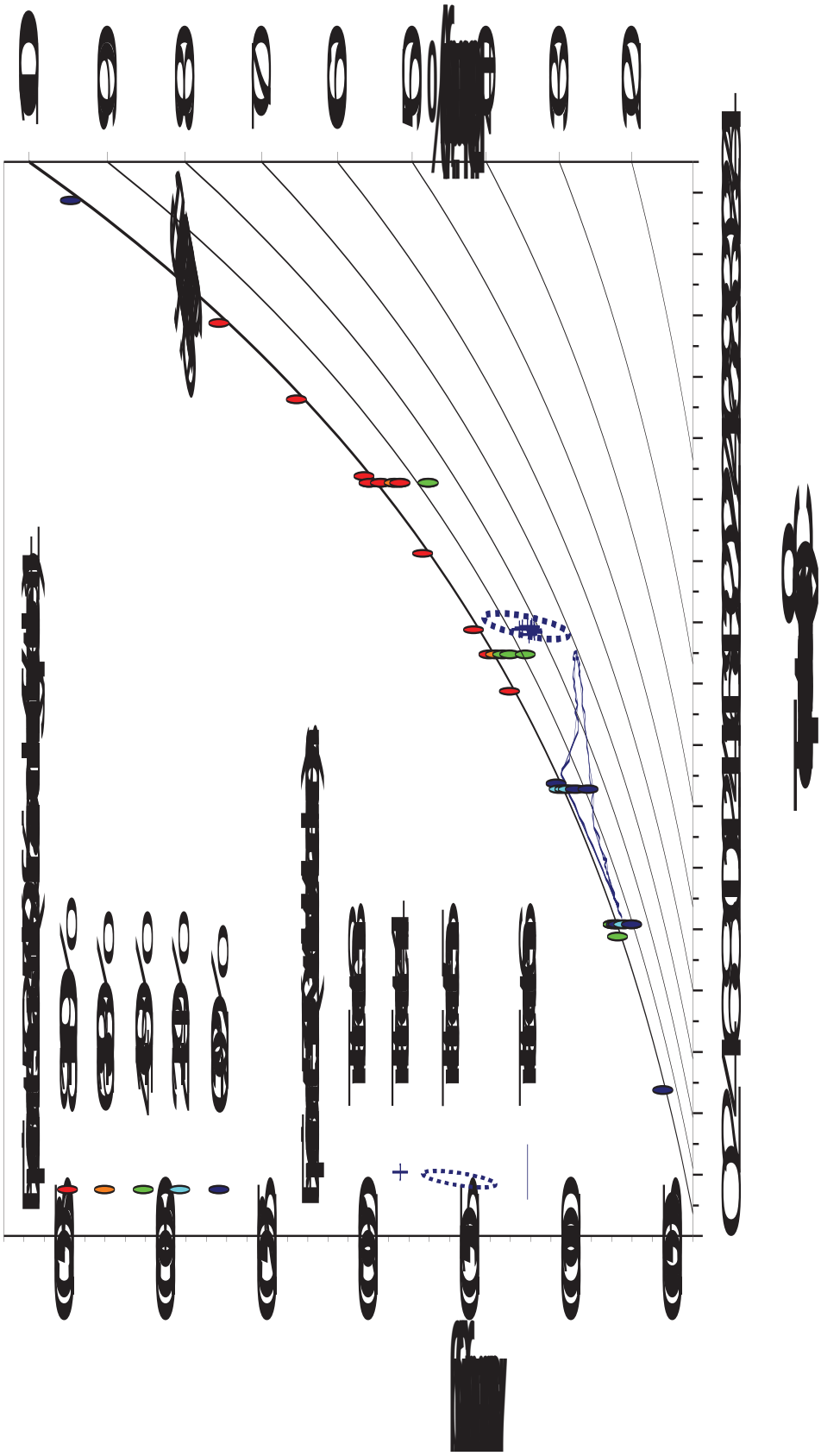


Figure 5.19 Psychrometric points for treatments from Experiments 5-8 and 5-9 and mean 24-hourly psychrometric plot for waterbath chambers during treatments 3–6 in Experiment 5-10, colour coded as a percentage of the maximum log(cfu/petal) for each experiment.

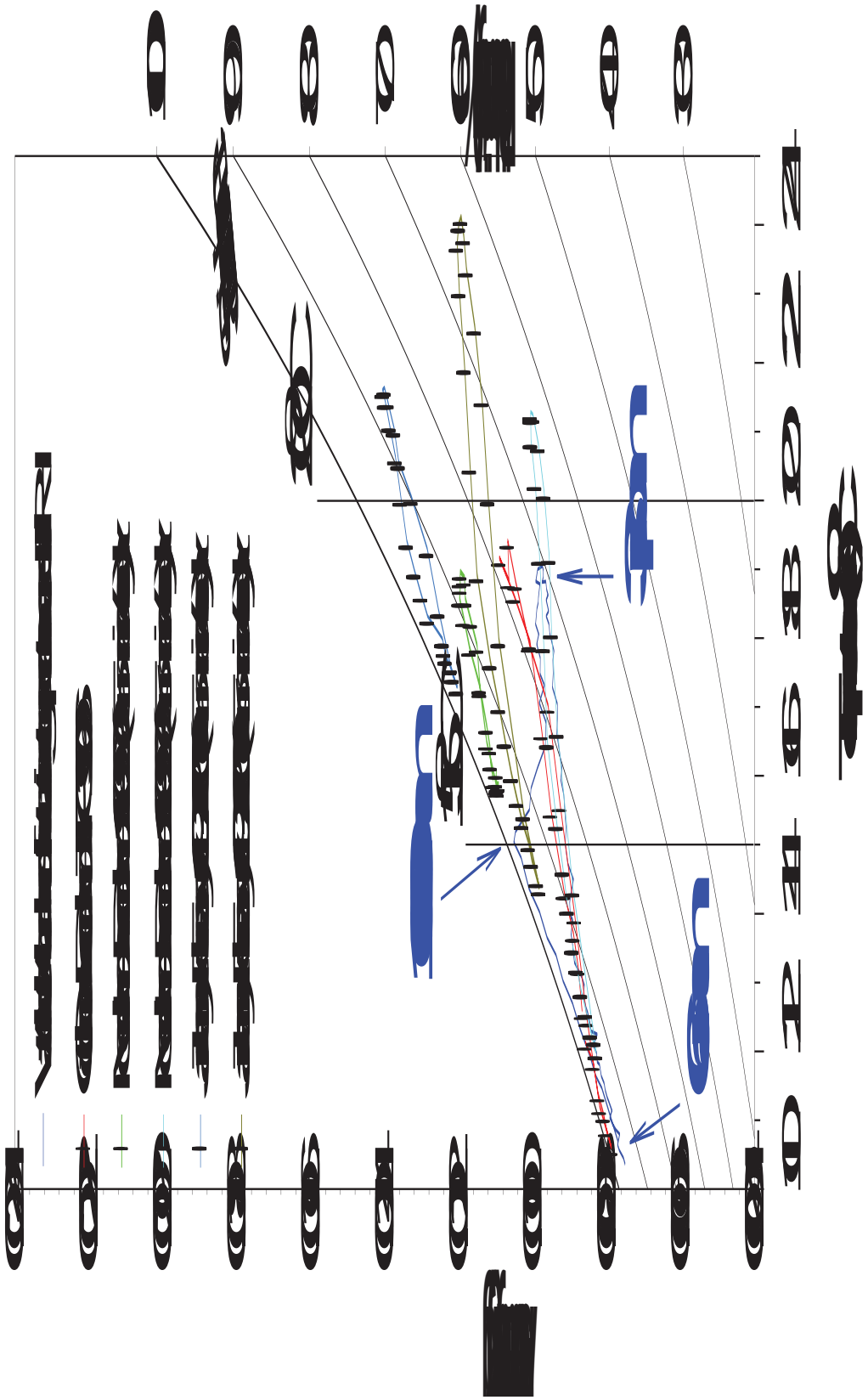


Figure 5.20 Mean 24-hourly psychrometric plots within the waterbath chambers under fluctuating temperature and relative humidity including three reference times, and for data from the Te Puke Research Orchard weather station during October–December 1996 and during November–December 1996–1999 and January–February 1997–2000, each split into days with ≥5 mm rainfall/day and days with <5 mm rainfall/day.

5.5.8 Discussion

The relationship between incubation temperature, mean percentage area of lesions and the number of *S. sclerotiorum* cfu/petal shows that the cardinal temperatures for ascospore colonisation of detached kiwifruit petals are similar to those for ascospore germination and mycelial growth rate *in vitro* (section 5.4). This feature is typical of many plant pathogens (Gaumann, 1950; Manners, 1993). The optimum rate of petal infection occurred at 23°C, however, the data suggests there is little response to temperature between 20–27°C at 100% RH. Previously reported optimum temperatures for *S. sclerotiorum* infection of bean leaves are: 20°C (Abawi & Grogan, 1975; Boland & Hall, 1987), 23°C (Phillips, 1994b) and 25°C (Weiss *et al*., 1980b). Infection of kiwifruit petals by *S. sclerotiorum* took place at 30°C in this study, in contrast to the above reports where no infection of bean leaves by *S. sclerotiorum* took place at 30°C. However, comparison between such experiments is confounded by isolate variation, the coarseness of temperature increments used, type of host tissue used, inoculation techniques, and the method of determining successful infection.

Detached kiwifruit petals became infected at 20 and 25°C over the full range of RH tested (85–100%). The only previous study of the effects of RH on infection by *S. sclerotiorum* was carried out at three temperatures (20, 24 and 28°C) and three relative humidities (90, 95 and 100%) (Hannusch & Boland, 1996). In their study, lesions developed on leaves after 96 h in their treatment combinations, although they were significantly smaller at 90% RH for each temperature, and at 28°C and 100% RH, when compared with other treatments. Experiment 5-9, and those carried out by Hannusch & Boland (1996), show that infection of petals by *S. sclerotiorum* ascospores readily takes place over the temperature range of 20–25°C and 90–100% RH. Ascospore infection of kiwifruit petals may therefore not require the presence of surface wetness.

The colonisation of petals in the waterbath chambers was significantly greater at 97% RH than 85% RH and therefore the same trend as found in Experiment 5-9. The reduced number of cfu/petal from petals incubated in the waterbath chambers at 97% RH, compared with petals at 98% RH in the saturated salt chamber (Experiment 5-10), might be explained by the air flow within the waterbath chambers. This air flow is lkely to have reduced the RH at the petal surface by reducing the thickness of the boundary air layer (Harrison *et al*., 1994).

Incubating petals within the waterbath chambers under constant temperature and diurnally fluctuating RH (treatment 5) or diurnally fluctuating temperature and RH (treatment 6) reduced lesion growth and number of cfu/petal to a level similar to petals incubated at 20°C and 85% RH. The temperature and RH regimes during treatments 5 and 6 were therefore not conducive to ascospore colonisation of detached kiwifruit petals. This is not surprising, given that the mean 24-hourly psychrometric plot for treatment 6 overlapped the 10 and 15°C treatments from Experiment 5-9, which also had very little colonisation (<30 cfu/petal).

When inoculated petals were given 30–48 h in conditions of 20°C and 98% RH and then placed into the less favourable diurnally fluctuating temperature and RH conditions of the waterbath chambers (Experiment 5-11), there was less colonisation of petals than when petals were held at 20°C and 98% RH throughout. The environment in the waterbath chambers during this experiment therefore appears to have influenced the colonisation of the petals even after ascospore infection had been initiated. This experiment highlights the need to study dynamic environmental conditions during epidemiological studies.

There was high degree of overlap between the mean 24-hourly psychrometric plots derived for October–December 1996, treatment 6 (Experiment 5-10) and November–December 1996–1999 for days with <5mm rainfall day^{-1} (Figure 5.20). Because the latter of these coincides with the timing of kiwifruit vines flowering, weather conditions that produce 24-hourly psychrometric plots similar to this would not be favourable for ascospore infection of petals.

Further experimentation under diurnally fluctuating conditions that closely match the mean 24-hourly psychrometric plots shown in Figure 5.20 or others based on different criteria for separating micro-climate data, would provide new knowledge of the response of *S. sclerotiorum* infection under realistic field conditions. This information would be useful for developing disease prediction models that utilise a measure of ascospore infection risk during flowering.

This is the first reported study of the effects of a range of environmental conditions and host factors on *S. sclerotiorum* ascospore infection and colonisation of kiwifruit.

Because this method indirectly quantifies mycelial content within petals, it measures treatment effects on ascospore germination and penetration of petals and subsequent colonisation of the petal through the spread of mycelium. Variation in the number of cfu therefore represents effects on one or several of these stages of disease progress.

Flowers had to be supplied from several different orchards for these experiments because they were carried out over a 5 week period. Direct comparison between experiments must be done with caution because variation between orchards was not investigated. The data on colonisation of petals by *S. sclerotiorum* ascospores may not relate directly to the infection of other tissue, such as the ovary and fruit, because these most likely result from mycelial infection. Furthermore, variables such as light and moisture, that were excluded during these controlled experiments, may affect disease progress by affecting ascospore survival (Caesar & Pearson, 1983; Hong *et al*., 1997) or their ability to infect tissues.

The occurrence of *B. cinerea* colonies in several experiments indicates this fungus is present on kiwifruit flowers. This is in agreement with findings that senescent petals attached to fruit were the main source of *B. cinerea* inoculum at petal-fall (Elmer *et al*., 1994). *S. sclerotiorum* and *B. cinerea* have been observed to co-exist on petals and within necrotic lesions on leaves in kiwifruit orchards (M. Manning and P. Elmer, pers. comm. and Chapter 2 and 3). It is possible that colonisation of petals by *B. cinerea* in these assays could reduce colonisation by *S. sclerotiorum* because of competition for space and nutrients. The few *S. sclerotiorum* colonies from un-inoculated petals in these experiments may have arisen from contamination in the field or from cross contamination during inoculation procedures and petal handling in the laboratory.

5.6 Chapter Discussion

Work described in this chapter determined that inoculum density, flower age, temperature and RH have a significant effect on the infection and colonisation of detached petals. Un-inoculated control petals were still turgid after 3 days incubation, except at 34^{o}C or 85% RH. It is assumed therefore, that petals were not adversely affected when detached from flowers, inoculated and placed in micro-tube holders, especially considering they were at least 6 days old by the completion of assays and

under field conditions would have undergone petal-fall. However, it remains to be determined whether similar effects occur on intact petals.

Colonisation of kiwifruit petals and stamens during flowering will be favoured by increasing exposure to ascospore deposition with increasing flower age (Experiment 5-2) and due to ageing of petals (Experiment 5-4 and 5-5). Opposing this increasing likelihood of infection is the process of petal-fall. If petal-fall occurs before establishment of infection within a petal or before spread of infection into ovary or sepal tissues, then fruit disease can not develop from that primary infection unit.

Kiwifruit petals contact the ovary at their point of attachment, i.e. the base of the petal, and may contact sepals near the base of the petal. It could be assumed that colonisation within petals must reach the base of the petal before mycelium can spread into the adjacent ovary or sepal tissue. It is conceivable that lesions initiated mid-way down the length of a petal would reach the petal base within 1–2 days under favourable conditions because the rate of expansion of lesions on petals was 8.9 mm day^{-1} at 20^{o}C and 100% RH. Therefore, petals that don't remain attached to fruit have only a small window of time before petal-fall when infection can pass from a petal into the ovary or sepals.

The environmental conditions most favourable for colonisation of petals by *S. sclerotiorum* ascospores are temperatures between 18–27^{o}C and relative humidities >90%. The response of colonisation of kiwifruit petals held at saturation was very steep between 15 and 20^{o}C (Figure 5.2, 5.14 and 5.16 A). Because of the lack of replication of individual treatments (incubators) it is difficult to accurately determine the true response to increasing temperature in this region from this data. However, there are other reports of substantial changes in the rate of lesion growth over this temperature range. Phillips (1994b) reported a 70% increase in lesion size 180 h after inoculation of bean leaves at 18^{o}C compared with 13^{o}C. The wetness duration necessary for appearance of symptoms on bean leaves was greater at 15^{o}C than 20^{o}C (Boland & Hall, 1987). Weiss *et al.* (1980b) reported a 50% increase in lesion area on leaves incubated at 20^{o}C compared with 15^{o}C.

This steep temperature response is of considerable importance since the mean daily temperatures during spring/early summer in New Zealand kiwifruit orchards are

typically 12–18^{o}C (Morley-Bunker & Salinger, 1987; Seager, 1993; McPherson *et al*., 1994). Therefore, small changes in conditions could have large effects on the rate of colonisation and disease progress.

The mean 24-hourly psychrometric plots of diurnally fluctuating temperature and RH from Te Puke Research Orchard (Figure 5.20) demonstrate that microclimate conditions can be separated into categories that reflect different psychrometric states. Further research is necessary to evaluate the colonisation of kiwifruit petals by *S. sclerotiorum* under a wider range of diurnal fluctuating conditions than those used in this study. This would determine more precisely the natural environmental conditions that lead to primary colonisation and disease development.

Chapter 6: Effect of misting duration on flower and fruit disease (*in vivo*)

6.1 Introduction

Results described in Chapter 3 showed that kiwifruit petals are a potential source of inoculum and that petals with high inoculum potential may result in the development of diseased fruitlets. Results described in Chapter 5 showed that the colonisation of detached kiwifruit petals is strongly influenced by the age of flowers, temperature and RH, and that free moisture is not essential. Adhering floral tissues colonised by *S. sclerotiorum* act as an inoculum source for infection of fruit and lead to the development of scarring symptoms (Chapter 3). The inoculum potential of these tissues may be related to the extent of colonisation by *S. sclerotiorum* mycelium, environmental conditions and proximity to the fruit surface.

Previous reports state that field rot can develop on fruit from December to February when favourable conditions of wet weather continue for at least 72 h, and that if dry weather intervenes once infection has established, shallow lesions dry out leaving fruit badly scarred (Pennycook, 1982; Pennycook, 1985; Manning, 1991). However, there is no data to support these comments or define what is meant by 'favourable conditions'.

It is possible that all scarring symptoms are preceded by the formation of field rot symptoms and the severity of scarring symptoms may be related to the area and depth of these lesions. Fruit that develop field rot symptoms which spread throughout the fruit readily fall to the ground, while most fruit with scarring remain attached to vines until harvest, unless removed during fruit thinning (Pennycook, 1985). The environment, host and pathogen factors that determine whether field rot or scarring symptoms develop are not understood.

6.2 Objectives

To test four hypotheses:

1. that the development of diseased fruitlets, fruit scarring and field rot symptoms requires free moisture.
2. that the duration of wetness conditions determine whether scarring or field rot symptoms develop and affects the severity of these symptoms.
3. that at least 72 h wetness duration is required for the development of fruit disease following inoculation.
4. that the inoculum potential of the source of secondary spread affects the type and severity of symptoms that develop on fruit.

6.3 Experiment 6-1: Effects of inoculum type, flower age and misting duration on the incidence of diseased fruitlets

6.3.1 Materials and methods

A misting system was set up in 12 pistillate vines (2.5 m vine spacing) in block 10 at orchard 20 (Appendix 1). A 15 mm irrigation line was supported 300 mm beneath the main cordons for 15–20 m either side of a 50 mm main water line that passed across the block, half way down the length of two rows that were separated by a buffer row. At each vine a single misting nozzle was positioned 200–300 mm to one side of the trunk. Bore water was supplied to each plot and regulated by a series of in-line taps.

The vines with misting were grouped into 6 pairs (plots 1–6) such that vines within each pair were separated by one buffer vine (Figure 6.1), and pairs of vines were separated by at least three buffer vines or the buffer row. Plot 6 was not used in Experiment 6-1. There were two additional plots in a third row that did not have a misting system (plots 7 and 8) and a further plot of two adjacent vines that was enclosed within a 4 m high plastic whole vine canopy, supported by a galvanised pipe frame (plot 9) (Buwalda *et al*., 1992).

Each side of the main cordon on each of the two vines was considered a sub-plot and was labelled West, North, East or South (Figure 6.1). Each of the sub-plots with misting nozzles (plots 1–5) were randomly assigned to one of five ‘misting duration’

treatments: (i) 9 h, (ii) 22 h, (iii) 32 h, (iv) 44 h and (v) 77 h. The eight sub-plots from plots 7 and 8 were randomly assigned to one of two treatments: (vi) hand-misted with SDW and enclosed within waxed paper bags for 48 h ('mist and bag'), and (vii) no misting or bagging ('ambient' - control). Treatment (viii) consisted of the four sub-plots from plot 9 and these received no misting or bagging ('tent' - no rainfall control).

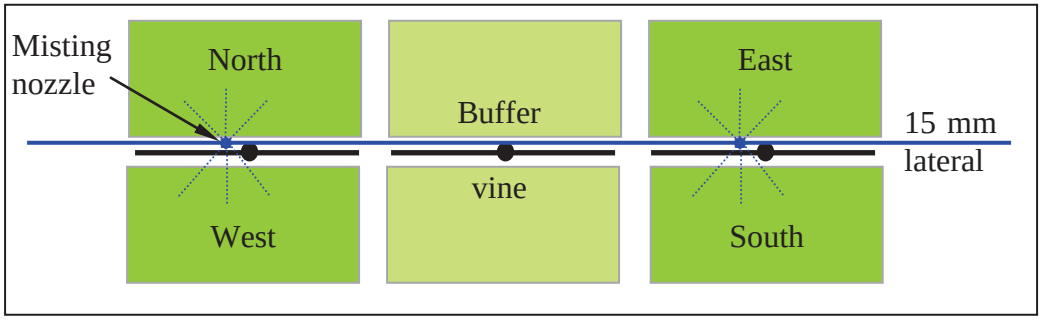


Figure 6.1 Diagram of a two-vine plot with four half vine sub-plots (North, East, West and South), one buffer vine and the misting system, used for experiments in Chapter 6.

Pistillate flowers that were 1, 2 and 3 days old were inoculated on 22 November 1997. Flowers were identified on the previous 3 days by tagging freshly opened flowers in each sub-plot with coloured wool. Three different colours were used each day to identify the inoculation treatment designated for each flower. As flowers were tagged they were hand pollinated by gently brushing the anthers of freshly picked staminate flowers across the stigmatic surfaces, as the block had no staminate vines. On plots 1–5, only flowers that were exposed directly to the misting were tagged, generally 1.0–2.0 m down the length of the canes. On the remaining plots, flowers from a similar region of the canopy were tagged. Individual flowers received one of three treatments:

1. inoculation by inverting a 4 mm diameter agar plug, taken from the growing margin of a 4 day-old *S. sclerotiorum* culture on PDA, onto the abaxial surface of two petals.
2. inoculation by brushing dry *S. sclerotiorum* ascospores from sections of filter membrane disc onto the abaxial and adaxial surfaces of three petals with a camel-hair brush.
3. un-inoculated (control).

The number of flowers within each inoculation treatment on each sub-plot varied depending on the number of flowers available. Within each sub-plot there were 1–6 flowers in treatment 1, 1–4 flowers in treatment 2 and 1–3 flowers in treatment 3. In total there were 327, 165 and 164 for treatments 1, 2 and 3 respectively.

The eight West sub-plots were blocked together, as were the North, East and South sub-plots. The West sub-plots were inoculated first and the misting was started in these sub-pots 1 h after completing the inoculation. The other blocks were then inoculated in turn and similarly the misting was initiated 1 h after completing the inoculation in each respective block. The misting was intermittent (turned on for 1 minute every 10–15 minutes) and was regulated by a solenoid valve controlled by an electronic timer (Flash Compact 12DC 24h time cycle, Brodersen), a relay (RH2B-U DC12V, Idec) and a 12V battery power supply. This provided continuous surface wetness without significant runoff. Plastic sheets were hung between sub-plots of the same vine to restrict the misting when only one of the two sub-plots had been inoculated. Misting was continued for the duration specified above and excluded from sub-plots where necessary by hanging plastic sheets and/or blocking misting nozzles completely with plastic cling film.

On 3 December 1997, 11 days after inoculation each fruit was examined and the incidence of diseased fruitlets, pedicels from which fruit had aborted, and lesions on sepals, was recorded. All suspected diseased tissues were taken to the laboratory, surface-sterilised (section 2.2.2) and placed on PDA to determine if they were colonised by *S. sclerotiorum*. A similar disease assessment and series of isolations was carried out on 18 December 1997, 26 days after inoculation. Pedicels colonised by *S. sclerotiorum* were classified as diseased fruitlets and disease incidence from both assessments was pooled before analysis.

Daily rainfall and minimum and maximum air temperatures throughout the duration of each experiment in this chapter were obtained from the meteorological station located 500 m from the trial block. Mean daily air temperature was calculated as (max – min)/2.

6.3.2 Statistical analysis

The percentage incidence of diseased fruitlets, scarring and field rot in the 'misting duration' treatments in all experiments of this chapter were analysed using Genstat with generalised linear models with logit link and errors proportional to binomial distribution. REML analysis was carried out on $\log_{10}(\text{area} + x)$ transformation of the area of scarring and field rot lesions on fruit, where x was the minimum area of scarring in each experiment. Statistical comparisons between 'misting duration' treatments and 'ambient' and 'tent' treatments was not carried out as the trial design did not allow for valid comparisons because of the isolation of the control treatments.

6.3.3 Results

Misting duration's ranging from 9–77 h did not affect the incidence of diseased fruitlets ($P>0.05$) and there was no significant interaction between duration of misting and either the inoculation method ($P=0.12$) or flower age ($P=0.43$), hence the data from the five misting treatments were combined. Within these misting treatments, agar plug inoculation with *S. sclerotiorum* culture resulted in a significantly higher incidence of diseased fruitlets than dry ascospore inoculation (Table 6.1). The incidence of diseased fruitlets decreased ($P<0.001$) with increasing flower age for flowers inoculated with agar plugs. Flowers inoculated with dry ascospores were not significantly affected by flower age. No diseased fruitlets occurred on the un-inoculated flowers in any treatment.

No diseased fruitlets developed in the 'tent' no rainfall control treatment, from dry ascospores in the 'mist and bag' treatment or from agar plugs of *S. sclerotiorum* culture in the 'ambient' treatment (Table 6.1). When paper bags were removed there was no free moisture present in the bag or on the fruit. The only rainfall during the experiement was 62 mm over the 5–12 day period following inoculation and a further 24 mm between the first and second assessment. The mean daily temperature between inoculation and the second assessment was 15^{o}C (SE ± 0.3, range 11.9–17.4^{o}C).

Table 6.1 Effects of flower age and misting on the mean percentage incidence of diseased fruitlets 26 days after flower petals were inoculated with agar plugs of *S. sclerotiorum* culture or with dry ascospores.

		Inoculation type			
Treatment	Flower age	Agar plug	n [1]	Dry ascospore	n
Misting duration (9–77 h)	1	96 (3.8)[2]	15	22 (5.8)	50
	2	59 (7.9)	39	13 (3.8)	78
	3	31 (8.1)	33	16 (4.7)	63
Mist and Bag	1–3	46 (9.6)	21	0	42
Ambient	1–3	0	24	4 (2.8)	48
Tent	1–3	0	23	0	46

[1] Number of flowers inoculated in each treatment.
[2] Standard error.

6.4 Experiment 6-2: Effects of inoculum source and misting on severity of fruit scarring

6.4.1 Materials and methods

Two types of pistillate kiwifruit petals were used to inoculate 7–10 day-old fruitlets. First, 100 gamma irradiated (4 Mrad.) sterile dried kiwifruit petals[4] were re-hydrated overnight by placing on sterile moist paper in a plastic tray enclosed in a plastic bag. They were inoculated with *S. sclerotiorum* ascospores by repeatedly brushing a camel-hair brush over four grids of membrane filter disc containing ascospores and then gently over the surface of four petals. A fresh section of membrane disc was used for each set of four petals. Inoculated petals were incubated at 20°C for 48 h to allow colonisation to take place and are referred to as 'colonised' petals. A sub-sample of 50 of these petals were surface-sterilised (section 2.2.2) and placed individually on PDA in Petri dishes to confirm colonisation by *S. sclerotiorum*.

Second, 300 'field' petals were collected from 3–4 day-old flowers at orchard 22 (Appendix 1), a block with an apothecial density of 7.3 apothecia m^{-2} (section 7.4.1). A

[4] Supplied by Dr P.A.G. Elmer

random sample of 50 petals was surface-sterilised and placed individually on PDA. The incidence of *S. sclerotiorum* growth was recorded after 10 days.

On 22 November 1996, seven replicate single vine plots were labelled in block 12B at orchard 20, 7–10 days after 50% flowering. On each vine four sets of 10 fruitlets and four sets of five fruitlets were tagged around the pedicel with lengths of different coloured wool. Fruitlets were inoculated by attaching petals directly below the petal scar region with a 10 × 60 mm strip of elastic material tied around the fruit and petal to secure the petal in place. Twenty fruit on each vine were inoculated with 'field' petals and 10 fruit per vine were inoculated with 'colonised' petals. Ten fruit had an elastic tie attached without a petal ('tie control') and twenty fruit had no tie and no petal ('control'). Half of the fruit from each treatment were hand-misted with SDW and enclosed within waxed paper bags for 48 h ('mist and bag') and the remainder were left un-misted ('ambient').

The elastic ties were removed from fruit 12 days after inoculation on 4 December. Disease assessments were carried out 5 and 10 weeks after inoculation on 28 December 1997 and 28 January 1997. The incidence of fruit with small brown lesions (brown speckling) and the incidence of typical sclerotinia scarring lesions was recorded. The area of scarring symptoms was estimated by measuring the length and width of lesions.

6.4.2 Results

S. sclerotiorum was recovered from all 'colonised' petals sub-sampled from those used for inoculation of fruit. Forty three percent of 'field' collected petals had *S. sclerotiorum*. There was 4.7 mm rainfall on the day of inoculation and a further 68 mm between 27 November and 3 December. Fruit with 'colonised' petals attached had a significantly higher incidence ($P<0.001$) of brown speckling ('ambient' only) and scarring, compared with fruit that had 'field' collected petals attached (Table 6.2). On 28 January 1997, the incidence of brown speckling had decreased to <10% on all treatments. The incidence of scarring on fruit had increased to 5 and 14% on fruit with 'field' collected petals and was over 60% on fruit with 'colonised' petals attached (Table 6.2). There had been a further 7 days between the first and second assessment which had >2 mm rainfall day^{-1}. The mean air temperature was 16.4^{o}C (SE ± 0.26,

range 11.9–21.7°C) between inoculation and the second assessment. There were no disease symptoms on the un-inoculated controls.

The mean area of scarring was significantly greater ($P<0.001$) on fruit in the 'mist and bag' treatment with 'colonised' petals attached, compared with the 'ambient' treatment, on both assessment dates (Table 6.3). Although the mean area of scarring on fruit on 28 January was greater than on 28 December for each treatment, this was not a significant increase ($P>0.05$).

Table 6.2 Mean percent incidence of brown speckling and scarring symptoms on fruit 5 and 10 weeks after 'field' collected and 'colonised' petals were attached to fruit 1–2 weeks after anthesis.

Date	Misting treatment	'Field' petal		'Colonised' petal	
		Brown Sp.[1]	Scarring	Brown Sp.	Scarring
28 Dec 1996	Mist and bag	28 (5.5)[2]	3 (2.0)	37 (8.4)	33 (7.9)
	Ambient	14 (4.4)	2 (1.6)	55 (9.6)	20 (8.0)
28 Jan 1997	Mist and bag	9 (3.6)	14 (4.1)	4 (4.1)	63 (9.1)
	Ambient	3 (2.3)	5 (2.9)	8 (5.2)	61 (9.3)

[1] Brown speckling on fruit surface (i.e. mild scarring symptoms).
[2] Standard errors for comparisons within a symptom type and assessment date.

Table 6.3 Mean area of scarring per fruit, of those with scarring, 5 and 10 weeks after 'field' collected petals and 'colonised' petals were attached to fruit 1–2 weeks after anthesis.

Date	Misting treatment	'Field' petal (mm^2/fruit)[1]	'Colonised' petal (mm^2/fruit)
28 Dec 1996	Mist and bag	35	89
(sed = 23)[2]	Ambient	40	38
28 Jan 1997	Mist and bag	56	114
(sed = 42)	Ambient	71	71

[1] Combined area from brown speckling and scarring symptoms.
[2] Maximum standard error of difference for comparisons within an assessment date.

6.5 Experiment 6-3: Effect of misting duration on fruit disease in December

6.5.1 Materials and methods

This experiment was carried out on plots 1–6 and plot 9 used in Experiment 6-1 at orchard 20, and used the inoculation method described for Experiment 6-2. One month after flowering, four sets of five fruit in each sub-plot were tagged with coloured wool to designate the inoculation treatment they would receive. Two hundred and fifty gamma irradiated (4 Mrad.) sterile dried kiwifruit petals were re-hydrated and colonised by *S. sclerotiorum* ascospores as described for Experiment 6-2, and a sub-sample of 50 were surface-sterilised (section 2.2.2) and placed on PDA. One hundred and fifty petals were re-hydrated but not inoculated. On 19 December 1997, 5 weeks after flowering, 140 of the 'colonised' petals were used to inoculate five fruit on each sub-plot by securing them to the side of the fruit with elastic ties. Similarly, five fruit per sub-plot had an un-inoculated petal attached to the fruit with an elastic tie, five fruit had a tie but no petal ('tie control') and five fruit had no tie or petal ('control').

The order in which sub-plots were inoculated was blocked and randomised as in Experiment 6-1. The misting was initiated after each set of seven sub-plots had been inoculated and was continued within plots 1–6 to provide six different 'misting duration' treatments: (i) 9 h, (ii) 24 h, (iii) 50 h, (iv) 72 h, (v) 96 h and (vi) 122 h. The seventh treatment was the 'tent' (no rainfall control) in plot 9 protected by the plastic canopy.

All elastic ties were carefully removed 17 days after inoculation on 5 January 1998 and the incidence and area (length and width) of scarring symptoms on fruit recorded. The incidence and area of scarring lesions was also recorded on 5 February 1998, together with the incidence of fruit that had dropped to the ground.

6.5.2 Results

S. sclerotiorum was recovered from all 'colonised' petals sub-sampled from those used to inoculate fruit. There was no significant effect of misting duration ($P>0.10$) on the incidence or area of scarring on fruit with 'colonised' petals, 17 or 48 days after

inoculation. There was 14 mm rainfall within 36 h of inoculation, intermittent rainfall during the following week (0.1–5.8 mm day^{-1}) and no rainfall between 29 December and the first assessment on 5 January 1998. On 5 January the incidence of scarring in the six 'misting duration' treatments ranged from 90–100% on fruit with 'colonised' petals attached, compared with 0–17% on fruit with un-inoculated petals attached. The incidence of scarring decreased slightly to 85–95% and 0–9% respectively, after 48 days.

The mean area of scarring on fruit with 'colonised' petals attached in the six 'misting duration' treatments was 85 mm^2 (SE ± 8) 17 days after inoculation and 135 mm^2 (SE ± 13) after 48 days. This was not a significant increase ($P>0.05$). Fruit with un-inoculated petals attached had a mean area of scarring of 11 mm^2 (SE ± 8). Six percent of the 120 fruit inoculated with 'colonised' petals in the 'misting duration' treatments became completely diseased and were shrivelled and dry on the vines or rotten on the ground. There were only 4 days between the first and second assessment that had >2 mm rainfall day^{-1}.

Seventeen days after inoculation there was a 5% incidence of scarring on fruit with 'colonised' petals attached in the 'tent' treatment and the area of these lesions were all <7 mm^2/fruit. There was no scarring on these fruit or on any control fruit 48 days after inoculation.

6.6 Experiment 6-4: Effect of misting duration on fruit disease in January

6.6.1 Materials and methods

This experiment was a repeat of Experiment 6-3, except that it included four sub-plots each separated from one of four misted plots by a single buffer vine. These additional sub-plots were treated as 'Ambient controls'. The misting system was shifted down the rows by 10 m to avoid using the same vines as those used for Experiment 6-3. On 13 January 1998, four sets of five fruit were tagged with coloured wool to designate the treatment they would receive. On 15 January five fruit per sub-plot were inoculated with one of the four treatments described in Experiment 6-3. Similarly, a sub-sample of

'colonised' petals were surface-sterilised and placed on PDA. The 'misting duration' treatments were (i) 9 h, (ii) 24 h, (iii) 48 h, (iv) 72 h, (v) 96 h and (vi) 120 h and were blocked and randomised among the sub-plots of plots 1–6 in a similar way to those of Experiment 6-1 and 6-3.

On 20 January the incidence and area of both scarring and field rot lesions was recorded for all tagged fruit. On 29 January the elastic ties were removed, and the incidence and area of scarring and field rot lesions was re-assessed, together with the incidence of field rot on the ground. On 5 February, the incidence of additional field rot on the ground was recorded.

6.6.2 Results

S. sclerotiorum was recovered from all 'colonised' petals sub-sampled from those used to inoculate fruit. There was no significant effect of misting duration on the incidence or severity of disease symptoms on fruit with 'colonised' petals attached and no symptoms developed on fruit with non-inoculated petals attached. Therefore, the data from the six 'misting duration' treatments were combined. Five days after fruit were inoculated with colonised petals, field rot lesions had developed on 85% of the fruit in the 'misting duration' treatments, a further 11% had dry scarring lesions and all were still attached to the vines (Figure 6.2 A). In comparison, fruit in the 'ambient' treatment had 20% incidence of field rot and 5% incidence of scarring. Fruit in the 'tent' treatment had a 35% incidence of scarring and no field rot (Figure 6.2 A). The mean area of field rot on fruit in the 'misting duration' treatments was 450 mm^2 (SE ± 39) and the mean area of scarring and field rot lesions on other fruit was <50 mm^2 (Figure 6.2 B). There was a small amount of rainfall (4.5 mm) over a 3 day period 40–100 h after inoculation, coinciding with the misting treatments.

Two weeks after inoculation 40% of the fruit in the 'misting duration' treatments had fallen from the vines and were completely rotten, and a further 57% had large sunken scarred lesions but remained attached to the vines (Figure 6.3 A). The mean area of scarring on the latter was 450 mm^2 fruit (Figure 6.3 B). Field rot and scarring lesions developed on 25% percent and 35% of fruit respectively in the 'ambient' treatment. Fruit in the 'tent' treatment had no field rot and a 40% incidence of scarring lesions.

The mean area of scarring on ‘ambient’ and ‘tent’ fruit was significantly less than on those in the ‘misting duration’ treatments (Figure 6.3 B). The incidence of field rot that had fallen to the ground had increased to 61, 30 and 10% in the ‘misting duration’, ‘ambient’ and ‘tent’ treatments, respectively, 3 weeks after inoculation. There was no rainfall between the first and second assessment and 17 mm rainfall on the 2 days before the final assessment on 5 February.

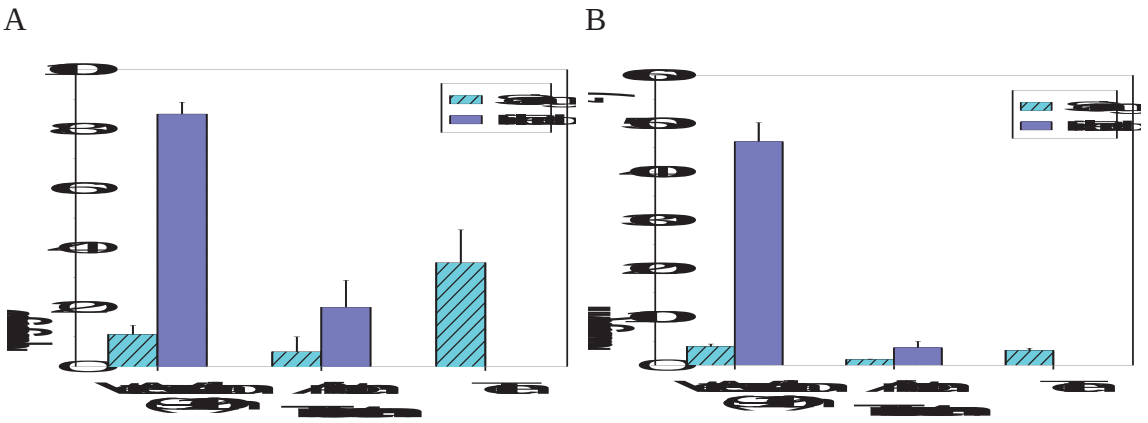


Figure 6.2 A and B. The effect of misting for 9–120 h on (A) mean incidence of scarring and field rot symptoms and (B) mean area of scarring and field rot on fruit, compared with fruit under Ambient conditions and within a plastic Tent, 5 days after colonised petals were attached to the side of fruit with elastic ties. Bars are standard errors.

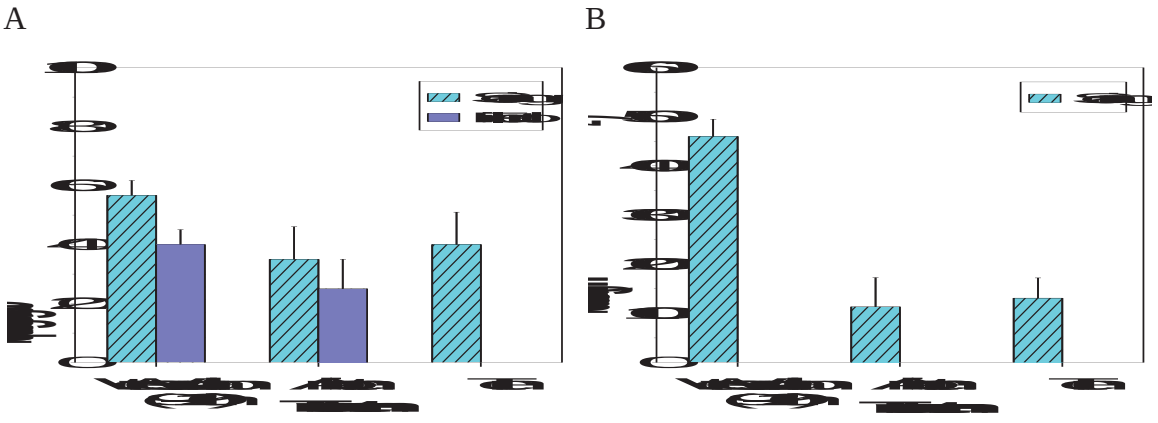


Figure 6.3 A and B. The effect of misting for 9–120 h on (A) mean incidence of scarring symptoms and field rot on the ground and (B) mean area of scarring on fruit, compared with fruit under Ambient conditions and within a plastic Tent, 14 days after colonised petals were attached to the side of fruit with elastic ties. Bars are standard errors.

6.7 Experiments 6-5 and 6-6: Effects of type and size of inoculum on fruit disease in January and February

6.7.1 Materials and methods

For Experiment 6-5, 14 fruit on each sub-plot from plots 1–6 and plot 9 at orchard 20 were each tagged on 28 January 1998 with coloured wool to identify the designated inoculation treatment. One hundred gamma irradiated (4 Mrad.) sterile dried kiwifruit petals were colonised with *S. sclerotiorum* ascospores as described in Experiment 6-2. Twenty of these were bisected with flame-sterilised scissors and tweezers. Thirty petals were cut into 40 pieces 0.5 cm^2 and 40 pieces 0.25 cm^2.

Stamens were removed from 15 intact 3–4 day-old pistillate flowers that had been collected during flowering and stored at –18^oC in a plastic bag. Clusters of approximately 30 stamens were inoculated and colonised by *S. sclerotiorum* ascospores as described for petals. Sub-samples of 10 pieces of each type and size (see below) of inoculum were surface-sterilised and placed on PDA Petri dishes to confirm colonisation.

On 31 January, tagged fruit within each sub-plot were inoculated with ascospores (treatments 1–3) or by tying petals or stamens to the side of fruit (treatments 4–12):

1. Dry ascospores applied with a camel-hair brush to the region where two adjacent fruit were touching. The two fruit were held against each other in this position with a 120 mm elastic tie.
2. Dry ascospores applied to opposite sides of one fruit.
3. Dry ascospores applied to the side of one fruit and a re-hydrated non-inoculated sterile petal tied onto the side of the fruit covering the area where the ascospores had been applied.
4. A whole sterile petal colonised by *S. sclerotiorum*.
5. Half a sterile petal (approximately 1 cm^2) colonised by *S. sclerotiorum*.
6. A 0.5 cm^2 section of sterile petal colonised by *S. sclerotiorum*.
7. A 0.25 cm^2 section of sterile petal colonised by *S. sclerotiorum*.
8. A cluster of ca. 30 stamens colonised by *S. sclerotiorum*.
9. A cluster of 5 stamens colonised by *S. sclerotiorum*.

10. A single stamen colonised by *S. sclerotiorum*.
11. A non-inoculated sterile petal (petal control).
12. A cluster of 30^{+} non-inoculated stamens (stamen control).
13. A tie only attached to the fruit (tie control).
14. No tie or petal attached to the fruit (untreated control).

The misting started within an hour of completing the respective sets of sub-plots, and was continued for 96 h in plots 1–6. On 10 February the ties were removed and all fruit were assessed for the incidence of scarring or field rot symptoms. On 18 February fruit were again assessed for the incidence of scarring and field rot symptoms.

Experiment 6-5 was repeated on 20 February (Experiment 6-6) because there had been no symptom development on the inoculated fruit in Experiment 6-5. The misting system was kept in the same position and the same combination of inoculation treatments was used, but the misting was continued for 120 h in an attempt to encourage infection. Elastic ties were removed after 10 days and all fruit were assessed for the presence of scarring or field rot symptoms. On 10 March fruit were again assessed for scarring or field rot symptoms.

6.7.2 Results

S. sclerotiorum was recovered from all 'colonised' petals and stamens sub-sampled from those used to inoculate fruit. No scarring or field rot symptoms were observed on any fruit from Experiment 6-5 or 6-6 within 3 weeks of inoculation with either dry ascospores or the range of colonised petals and stamen tissues used as inoculum. However, white mycelium was observed on the surface of many petals and spreading outwards 3–10 mm from the margin of the attached petal/stamens on the surface of several fruit.

Throughout Experiment 6-5 (31 January–18 February) there was only 1.2 mm rainfall, on 11 February. The mean daily temperature was 21.6°C (SE ± 0.4) and the mean daily maximum temperature was 28.3°C (SE ± 0.6) with a maximum of 31.1°C on 4 February. During Experiment 6-6 (20 February–10 March), there was 42 mm rainfall within 4 days of inoculation, coinciding with the misting, and 6 consecutive days of

rainfall (15.2 mm) from 5–10 March. The mean daily temperature was 19.1°C (SE ± 0.6) and the mean daily maximum temperature was 25.4°C (SE ± 0.6) with a maximum of 29.3°C.

6.8 Discussion

The 'misting duration' treatments in Experiments 6-1–6-4 all provided favourable conditions for disease symptom development, compared with fruit in 'ambient' conditions and those in the 'tent' treatments that were protected from rainfall under the plastic canopy. This strongly supports the first hypothesis of this chapter that development of sclerotinia disease on kiwifruit fruit requires free moisture. The second hypothesis was not supported because misting duration's ranging from 9–122 h did not have a significant effect on the incidence of diseased fruitlets, scarring and field rot, nor on the area of scarring and field rot.

The actual wetness period within each treatment can only be considered as approximate because wetness duration was not monitored within treatment plots. Also, the ambient conditions and the time of day when different misting treatments ceased could have affected the actual duration of wetness. For example, the 9 h misting treatment ceased during the evening of the day that inoculum was attached to fruit and may therefore have had a wetness duration period that extended throughout the night, in which case the actual wetness duration may have been as long as 20 h. The minimum period of wetness duration was not accurately established from these experiments, but it could be between 9 and 20 h, and therefore suggests that the third hypothesis, which stated 72 h of wetness are required for disease development, is unlikely to be correct.

Diseased fruitlets developed when flower petals were inoculated with ascospores and agar plugs of *S. sclerotiorum* culture. Scarring symptoms developed on 10 day-old fruit and 4–5 week-old fruit inoculated by attaching 'colonised' and 'field' collected petals. Field rot developed on 9–10 week-old fruit also inoculated by attaching colonised petals and these readily fell from vines. Therefore, these experiments have shown that different symptoms develop depending on the growth stage at which inoculum is applied. These results also support the observations from Chapter 2 that fruit with scarring tend to remain on vines while fruit with field rot tend to fall from vines.

6.8.1 Diseased fruitlets

Inoculation of petals on 1, 2 or 3 day-old flowers with an agar plug of *S. sclerotiorum* culture or by dry brushing with ascospores resulted in the development of diseased fruitlets within 12 days, but only when misting was provided (Table 6.1). This is the first report that provides direct evidence that diseased fruitlets can result from ascospore infection of petals, and is in agreement with research on other crops that has shown ascospores to be an important source of primary inoculum (Abawi & Grogan, 1975; Huang & Kokko, 1992; Jamaux *et al*., 1995).

The higher incidence of diseased fruitlets resulting from agar plug-inoculation of petals, compared with ascospore-inoculation of petals, possibly reflects that agar plugs have a higher inoculum potential and faster rate of colonisation. The decreased incidence of diseased fruitlets with increasing flower age on flowers inoculated with agar plugs in the 'misting duration' treatments, may have been related to the reduced time between inoculation and petal-fall. Although inoculated petals may have become colonised, petal-fall might have prevented colonisation of other floral tissues. This may also apply to flowers inoculated with dry ascospores, although petals on 1 and 2 day-old flowers would be less susceptible to infection by ascospores than 3 day-old petals (Figure 5.9). These opposing factors may explain why on ascospore-inoculated flowers there was no significant effect of flower age on the incidence of diseased fruitlets.

Misting and bagging of agar plug inoculated flowers provided conditions suitable for the development of diseased fruitlets, as reported earlier (Table 3.3 and 3.4). For the experiments in this Chapter there is no explanation why disease incidence was not affected by flower age in the ‘mist and bag’ treatment compared with the 'misting duration' treatments. The absence of diseased fruitlets in the ‘mist and bag’ treatment inoculated with dry ascospores suggests that conditions within the paper bags were not as favourable for disease progress as in the ‘misting duration’ treatments, where misting was continued for 9–77 h. Possibly the flowers enclosed in bags may have dropped their petals earlier. Also, since the bags and flowers were dry after 48 h, it was not known for how long flowers remained wet. This lack of symptom development is similar to earlier attempts to infect flowers with ascospores (section 3.7).

The absence of symptom development from the ‘ambient’ and ‘tent’ controls was expected, as agar plugs most probably dried out before petals could be colonised. The substantially higher incidence of diseased fruitlets from ascospore-inoculated flowers in the 'misting duration' treatments compared with the ‘ambient’ treatment, suggests disease development was favoured by the free moisture provided immediately post-inoculation, supporting the hypothesis that free moisture is required for the development of diseased fruitlets. Rainfall 5–12 days after inoculation did not result in diseased fruitlets in the ‘ambient’ controls, suggesting moisture was required within 5 days of ascospore inoculation.

6.8.2 Scarring and field rot

Scarring symptoms observed in Experiment 6-2 and 6-3 were typical of natural disease symptoms. The incidence of scarring on 10 day-old fruitlets inoculated with ‘colonised’ petals increased between 28 December and 28 January proportional to the decrease in brown speckling. This suggests that brown speckling may be an early stage of scarring symptoms. The 4.7 mm of rainfall on the day of inoculation and the considerable rainfall during the 12 days following inoculation, may have contributed to the similar incidence of scarring on fruit with ‘colonised’ petals attached in the ‘mist and bag’ and ‘ambient’ treatment. However, misting and bagging increased the mean area of scarring symptoms on these fruit, suggesting that moisture immediately after inoculation may affect symptom development. The evidence of these experiments does not support the fourth hypothesis of this Chapter that wetness duration affects symptom severity.

The ratio of disease incidence (brown speckling and scarring combined) to *S. sclerotiorum* incidence within petals was only slightly less for ‘field’ collected petals (23/43) compared with ‘colonised’ petals (67/100). Thus, ‘field’ collected petals had only a slightly reduced incidence of infection relative the incidence of *S. sclerotiorum* within the petals. This is perhaps surprising, because ‘colonised’ petals could be considered to have a considerably higher inoculum potential.

There was a very high incidence of scarring on 5 week-old fruit in the ‘misting duration’ treatments, although there was no significant effect of misting duration on symptom incidence or severity. A small percentage of fruit became fully diseased and

this is consistent with observations of shrivelled immature fruit on vines (Chapter 2, p. 27). The increase in mean area of scarring in Experiment 6-2 and 6-3, although not statistically significant, could be influenced by the active growth of lesions and/or expansion of lesion area as a result of fruit growth. The latter could fully account for the increase in lesion area, since two-thirds of the increase in fruit size occurs during the first 10 weeks after anthesis (Beever & Hopkirk, 1990). If this were the case, then active lesion expansion may have ceased within 2 ½ to 5 weeks of inoculation.

Fruit inoculated in mid-January (Experiment 6-4) developed a high incidence of field rot, compared with earlier inoculations which generally only resulted in fruit scarring. The duration of misting in the range of 9–120 h did not influence whether scarring or field rot developed. However, the absence of field rot on fruit in the 'tent' treatment and the very small area of scarring in the 'ambient' and 'tent' treatments, suggests that the free moisture provided by the misting system did create conditions favourable for the development of scarring and field rot symptoms. The area of scarring on fruit remaining in the 'misting duration' treatments was similar to the area of field rot on fruit 5 days after inoculation. This indicates that the lesions on these fruit had not expanded significantly between 5 and 14 days after inoculation.

The absence of symptom development in Experiment 6-5 and 6-6 was unexpected and cannot be fully explained. The air temperatures following inoculation of fruit in Experiment 6-5 were at times higher than the optimum for *S. sclerotiorum* mycelial growth (Figure 5.11). The air temperature within the kiwifruit block may have been higher than these recorded values because of the effects of shelter. This may have adversely affected the ability of the *S. sclerotiorum* mycelium to grow and infect into the fruit tissue. Despite the rainfall during Experiment 6-6, it is also possible that the dry warm conditions may have caused the fruit surfaces to dry out during the misting period and these interrupted wet periods may have limited infection (Abawi & Grogan, 1975; Phillips, 1994b). It is unlikely that the susceptibility of fruit to infection decreases significantly in late-January because field rot is often observed during January and February (Chapter 2 and (Pennycook, 1985; Manning, 1991)). The effects of type and size of inoculum on the infection of fruit and symptom severity, and whether ascospores are capable of infecting fruit surfaces, remain unknown and leaves the fourth hypothesis un-answered.

Chapter 7: Relationships between inoculum and disease incidence

7.1 Introduction

Plant epidemics occur when the amount of disease changes in a population of host plants over a period of time (Campbell & Madden, 1990). The conditions required for an epidemic include susceptible host individuals, a pathogen with high infective capacity, and optimal environmental conditions (Gaumann, 1950). Study of the inter-relationships between these components is necessary to improve disease management and reduce crop losses. Epidemics caused by *S. sclerotiorum* have been studied in annual field crops e.g. bean (Boland & Hall, 1987; Nelson *et al*., 1989), but not in kiwifruit.

A key factor involved in the development of plant disease epidemics, and one which is often limiting during the early stages, is the number of pathogen spores landing on susceptible host tissues (Manners, 1993). Ascospores are considered to be the principal source of primary inoculum for sclerotinia disease in annual field crops such as bean (Abawi & Grogan, 1975; Boland & Hall, 1987), lettuce (Newton & Sequeira, 1972), canola (Gugel & Morrall, 1986; Turkington & Morrall, 1993), tomato (Purdy & Bardin, 1953) and pea (Huang & Kokko, 1992). Chapters 2, 3, 5 and 6 provide evidence that ascospores are the most likely source of primary inoculum in New Zealand kiwifruit, as has been proposed by Pennycook (1985) and Sale (1981). Although airborne *S. sclerotiorum* ascospores have been trapped on Kerssies selective medium (Kerssies, 1990) in two Bay of Plenty kiwifruit orchards (Hoyte, 1996), there are no published reports of the role of ascospores as primary inoculum for sclerotinia disease in kiwifruit.

The quantity of ascospore inoculum produced by *S. sclerotiorum* is largely determined by two factors. First, the density and distribution of sclerotia within the soil, which is influenced by the production of sclerotia from host tissues during previous growing seasons, cultural practices and sclerotial survival (Williams & Western, 1965; Abawi & Grogan, 1979). Numerous reports on sclerotial survival show it is affected by factors such as burial in soil, soil moisture, and micro-organisms (Coley-Smith & Cooke, 1971; Willetts, 1971; Merriman, 1976; Abawi & Grogan, 1979). Second, the production of

apothecia from sclerotia, which is largely determined by soil moisture and temperature (Abawi & Grogan, 1975; Adams & Ayers, 1979; Grogan, 1979). Further, the quantity of airborne inoculum reaching susceptible host surfaces is determined by factors affecting spore release, dispersal and settlement (Gregory, 1973).

There is limited information on the density or distribution of sclerotia and apothecia in kiwifruit orchards. Sclerotia were recovered from soil in two Te Puna orchards at densities ranging from 0–0.8 kg^{-1} soil, but apothecia were not counted *in-situ* (Goh & Lyons, 1992b). More recently, apothecial density ranging from 0–10.3 m^{-2} were determined by sampling 160 0.25 m^2 quadrats in kiwifruit blocks from 18 orchards (Pak *et al*., 1997).

There are several reports of a relationship between three separate measures of primary inoculum of *S. sclerotiorum* (sclerotial density, ascospore density and apothecial density) and disease incidence. The rate of disease progress of sclerotinia wilt of sunflower in eight fields was positively correlated with the number of sclerotia extracted from soil samples (Holley & Nelson, 1986; Nelson *et al*., 1989). Yet, Schwartz & Steadman (1978) found no correlation between sclerotial populations and white mold incidence in bean. In lettuce field plots fumigated to kill sclerotia, disease incidence was positively correlated with the mean number of ascospores trapped on selective medium in Petri dishes (Ben-Yephet & Siti, 1987). These ascospores were assumed to originate from outside the field. The density of apothecia has been shown to be positively correlated with disease incidence in bean and soybean (Boland & Hall, 1988a; Boland & Hall, 1988b), canola (Gugel & Morrall, 1986) and kiwifruit (Pak *et al*., 1997). For the latter, the percentage of quadrats with at least one apothecium was a better predictor of the incidence of *S. sclerotiorum* within petals than was apothecial density (Pak *et al*., 1997).

Adhering floral tissues colonised by *S. sclerotiorum* play a key role in disease progress and are considered to be the most likely source of secondary spread leading to flower and fruit disease (Chapter 2, 3 and 6). This link between floral tissues and disease progress is further supported by findings that the incidence of scarring on fruit was positively correlated with the incidence of petal colonisation (Pak *et al*., 1997). The incidence of stem infection disease in canola has also been positively correlated with the

incidence of *S. sclerotiorum* within petals and the latter was considered to be a better predictor of disease than apothecial density (Gugel & Morrall, 1986; Turkington & Morrall, 1993).

7.2 Objectives

To:

- quantify parts of the disease cycle relating to primary inoculum (production of sclerotia and apothecia).
- determine the relationship between apothecial density (primary inoculum) and incidence of *S. sclerotiorum* within surface-sterilised petals (secondary inoculum).
- determine if there is a positive relationship between secondary inoculum and the incidence of diseased fruitlets and fruit with scarring.
- consider whether inoculum-disease relationships could be used for disease forecasting or prediction of disease risk.

7.3 Factors affecting inoculum production

7.3.1 Production of sclerotia from diseased fruitlets and fruit

7.3.1.1 Materials and methods

On 3 February 1997, three two-vine plots (5×2.5 m^2) were selected in 'nashi' block at orchard 22 (Appendix 1). All diseased fruitlets <15 mm in length still attached to vines, plus those that had fallen to the ground, were collected separately from each plot. Fruit with field rot ≥15 mm in length were collected from the vines and off the ground and pooled together for each plot. The length of diseased fruitlets and diseased fruit was recorded. Sclerotia were removed with the aid of forceps from the external surfaces and internal cavities of each diseased fruitlet and fruit. Sclerotia were counted and the number of sclerotia with damage marks was recorded.

After 3 weeks air drying sclerotia from all diseased fruitlets were pooled and passed through a series of metal sieves (5.0, 3.5, 2.0, and 1.0 mm). The mean sclerotial weight was derived by dividing the weight of sclerotia in each sieve by the number of sclerotia. This was repeated for sclerotia extracted from fruit with field rot. The mean sclerotial

size was calculated for each vine by multiplying the number of sclerotia in each sieve by the mid-point of the sieve sizes and dividing by the total number of sclerotia. Paired t-test was used to determine any significant difference in the mean sclerotial weight and mean sclerotial size between diseased fruitlets and fruit with field rot, using the three vines as replicates.

7.3.1.2 Results

There were 104 diseased fruitlets attached to the vines, 352 diseased fruitlets on the ground, and 230 fruit with field rot. The majority of sclerotia were extracted from fruit with field rot and most sclerotia from diseased fruitlets were extracted from within internal cavities (Table 7.1). Damage marks were present on 25% and 13% of sclerotia extracted from the external surfaces of fruit with field rot and diseased fruitlets collected from on the ground, respectively. Often the sclerotial rind had reformed over the damaged portion of the sclerotia. No damage marks were observed on sclerotia extracted from diseased fruitlets attached to the vines or from sclerotia produced internally within diseased fruitlets or fruit with field rot (Table 7.1).

Table 7.1 Total number of sclerotia extracted from diseased fruitlets and fruit with field rot, collected from three kiwifruit vines at orchard 22, and mean number of sclerotia, percentage of sclerotia extracted from external surfaces, and percentage of external sclerotia with damage marks.

Plant part	Total sclerotia	Mean sclerotia per unit[1]	External sclerotia (%)	External sclerotia with damage marks (%)
Diseased fruitlets on vine	248	2.7 (0.1) [2]	39 (6.8)	0
Diseased fruitlets on ground	821	2.7 (0.2)	16 (5.3)	13 (9.7)
Field rot	3316	18.8 (1.3)	64 (4.8)	25 (5.1)

[1] Excludes diseased fruitlets and fruit that had no sclerotia.
[2] Standard error.

Fifteen percent of diseased fruitlets had no sclerotia, 79% had 1–4 sclerotia and there was a maximum of 11 sclerotia/diseased fruitlet (Figure 7.1). The mean length of diseased fruitlets was 9.5 mm (SE ± 0.08). Fruit with field rot had a mean length of 33

mm (SE ± 0.6, range 15–52 mm) and 15% of these also had no sclerotia. All fruit with field rot <20 mm in length had 0–10 sclerotia, while fruit >20 mm in length were highly variable in the number of sclerotia they produced and ranged from 0–101 sclerotia/fruit.

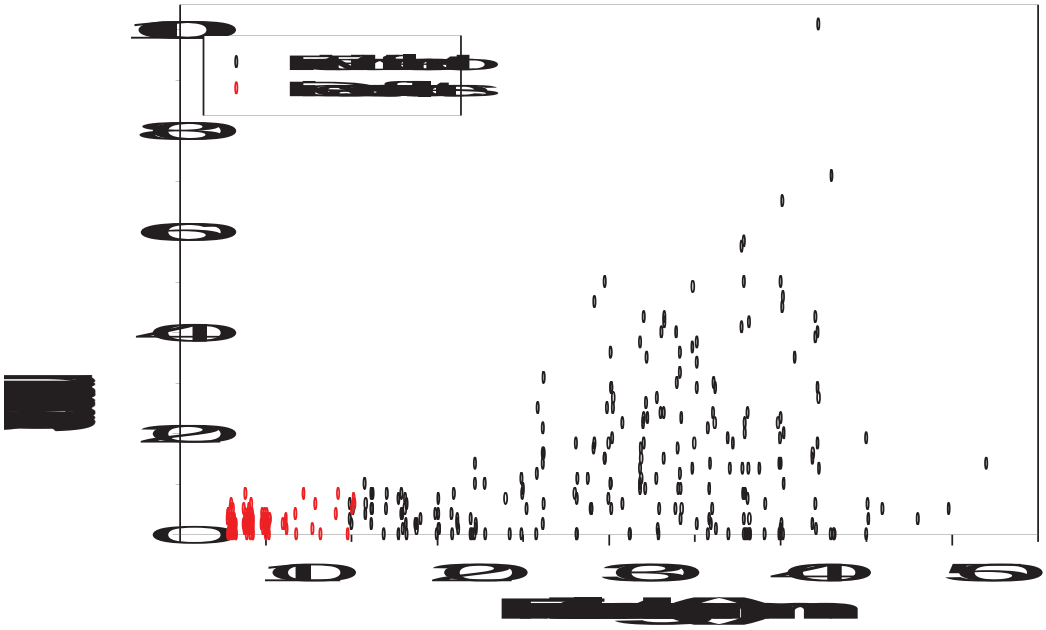


Figure 7.1 Relationship between the number of sclerotia extracted and length of diseased fruitlets (random sub-set of 100) and fruit with field rot (n = 230) collected from three vines at orchard 22. To distinguish overlapping points a normally distributed increment between ⁻0.8 and 0.8 was added to fruit length ('jitter' function).

Figure 7.2 shows the distribution of sclerotial sizes from diseased fruitlets and fruit with field rot. Ninety five percent of sclerotia from diseased fruitlets were in the 1.0–3.5 mm size range and 73% of sclerotia from fruit with field rot were in the 2.0–5.0 mm size range. The overall mean size of sclerotia from diseased fruitlets (2.1 mm, SE ± 0.03) was significantly less than for sclerotia from fruit with field rot (2.8 mm, SE ± 0.07). The mean sclerotial weight was similar for diseased fruitlets and fruit within each respective size grade (Figure 7.2).

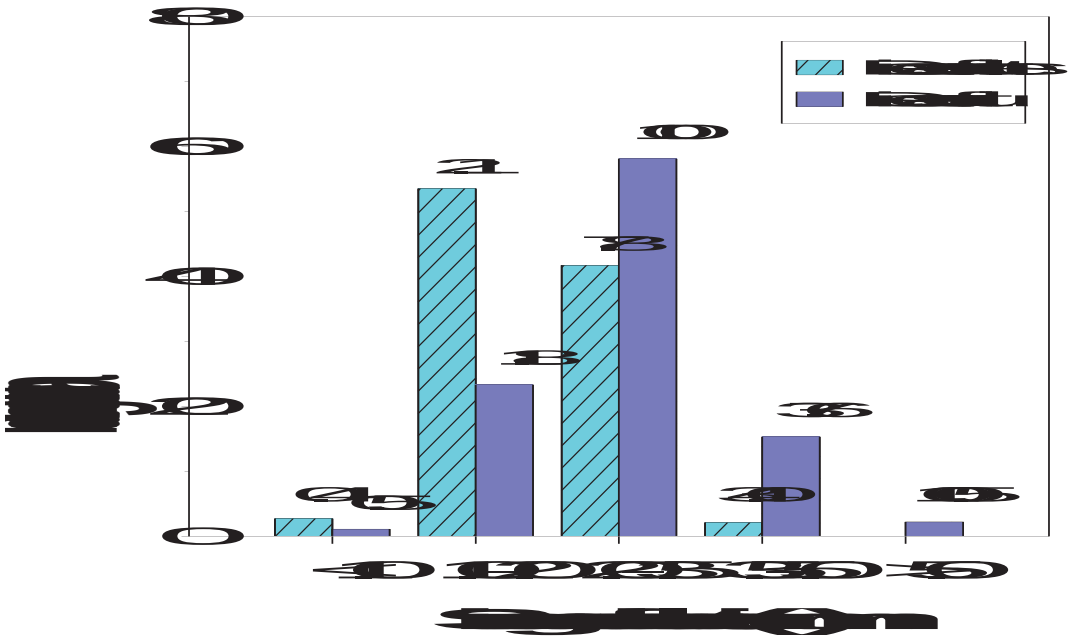


Figure 7.2 Mean percentage of sclerotia within various size categories determined by passing sclerotia through 1.0, 2.0, 3.5, and 5.0 mm sieves, collected from diseased fruitlets and fruit with field rot from three vines at orchard 22. Values above each column are the mean sclerotial weight (mg) for each category.

7.3.2 Effects of size and source of sclerotia on apothecial production

7.3.2.1 Materials and methods

A batch of sclerotia was produced as described in Appendix 2. These were air dried for 3 months and graded according to size with a series of metal sieves (5.0, 3.5, 2.0, and 1.0 mm). Naturally produced sclerotia were extracted by hand from diseased fruitlets and fruit with field rot collected from vines at orchard 7 (Appendix 1) during March 1992 (Hoyte *et al.*, 1992). These sclerotia were surface-sterilised (section 2.2.2), air dried for 3 months and graded according to size with the same metal sieves as above. The categories of sclerotia used are shown in Table 7.2.

On 13 June 1992, 96 sclerotia from each category (Table 7.2) were buried in 12 of 16 replicate 0.25 m^2 plots beneath kiwifruit vines in a block at orchard 7. Plots were located in four rows of vines and spaced 5 × 5 m. Vegetation was removed by hand from each plot and the soil was lightly scraped to remove debris and to provide a uniform surface. Rubber bands were stretched between nails in a wooden frame (240 × 320 mm) to form a grid pattern of 6 × 8 squares. The frame was positioned within each

plot by aligning one corner next to a wooden peg. Eight sclerotia from each of the six categories were placed into 10–15 mm deep holes at the centre of each square of the grid and buried by filling the holes with soil and lightly compacting. The location of each of the six sclerotial categories was randomised separately within each row of the grid pattern for each plot. There were four control plots which had no buried sclerotia.

Table 7.2 Source and size categories of sclerotia used for burial trial at orchard 7.

Source of sclerotia	Size of sclerotia (mm)
Diseased fruitlets	2.0–3.5
Diseased fruitlets	1.0–2.0
Field rot	3.5–5.0
Field rot	2.0–3.5
Autoclaved wheat	3.5–5.0
Autoclaved wheat	2.0–3.5

By re-positioning the wooden frame in each plot the number of mature apothecia emerging from each sclerotium was recorded on six occasions (1 to 3 weeks apart) from 20 October 1992 to 6 January 1993. The diameter of each apothecium was estimated visually and recorded. Apothecia that were not in line with the expected position of buried sclerotia were considered to be naturally occurring. Daily rainfall, 10 cm soil temperature and air temperature data was collected from the Te Puke Research Orchard (15 km to the East) for the period 1 October–6 January.

The percentage germination of sclerotia was angular transformed before ANOVA using Genstat. The number of apothecia per sclerotium was $\log_e$ transformed and analysed for differences between sclerotial types with REML. The mean apothecial size was analysed with REML.

7.3.2.2 Results

The percentage germination of sclerotia was significantly ($P<0.05$) lower for sclerotia collected from fruit with field rot than for other sclerotia (Table 7.3). Sclerotia grown

on autoclaved wheat had a significantly ($P<0.05$) lower percentage germination for the smaller size grade (2.0–3.5 mm) compared with the larger grade. The larger grade of sclerotia from wheat produced significantly more apothecia/sclerotia ($P<0.05$) than all other categories (Table 7.3). For each source of sclerotia, the larger of each pair of sclerotial size categories had significantly more apothecia/sclerotia and significantly larger apothecia ($P<0.05$). The number and diameter of apothecia produced by the three 2.0–3.5 mm size grades of sclerotia was similar ($P>0.05$). The mean diameter of naturally occurring apothecia was 2.9 mm (n = 33).

Table 7.3 Mean percent germination, number of apothecia/sclerotium (of those that germinated) and mean apothecial diameter, from sclerotia of various sizes from diseased fruitlets, fruit with field rot and autoclaved (A/c) wheat, buried at orchard 7 on 13 June 1992 and monitored from 20 October to 6 January 1993.

Source of sclerotia	Sclerotial size	Germination (%)	Apothecia per sclerotium [1]	Mean apothecial diameter (mm) [2]
Diseased fruitlets	2.0–3.5	89.6	1.7	5.3
Diseased fruitlets	1.0–2.0	82.3	1.2	3.7
Field rot	3.5–5.0	35.4	2.7	5.9
Field rot	2.0–3.5	48.9	1.7	5.2
A/c wheat	3.5–5.0	88.5	3.9	6.1
A/c wheat	2.0–3.5	67.7	1.4	4.7
		SED[3] = 6.1	LSR[4] = 1.23	SED = 0.33

[1] Back transformed.
[2] Calculated from mature apothecia on 27 October, 18 November and 8 December.
[3] Standard error of difference ($P<0.05$).
[4] Least significant ratio ($P<0.05$), i.e. values within column are significantly different if their ratio is >LSR.

The number of apothecia from 96 sclerotia in each category is shown in Figure 7.3. Daily rainfall and soil temperature at 10 cm depth from Te Puke Research Orchard during the period when apothecia were monitored is shown in Figure 7.4. Most of the apothecia observed from culture-grown sclerotia were present on 20 and 27 October 1992, during a period when rainfall was frequent and the 10 cm soil temperature was

10–15°C. In contrast, on the 27 October very few apothecia had been produced by sclerotia collected from diseased fruitlets and fruit with field rot.

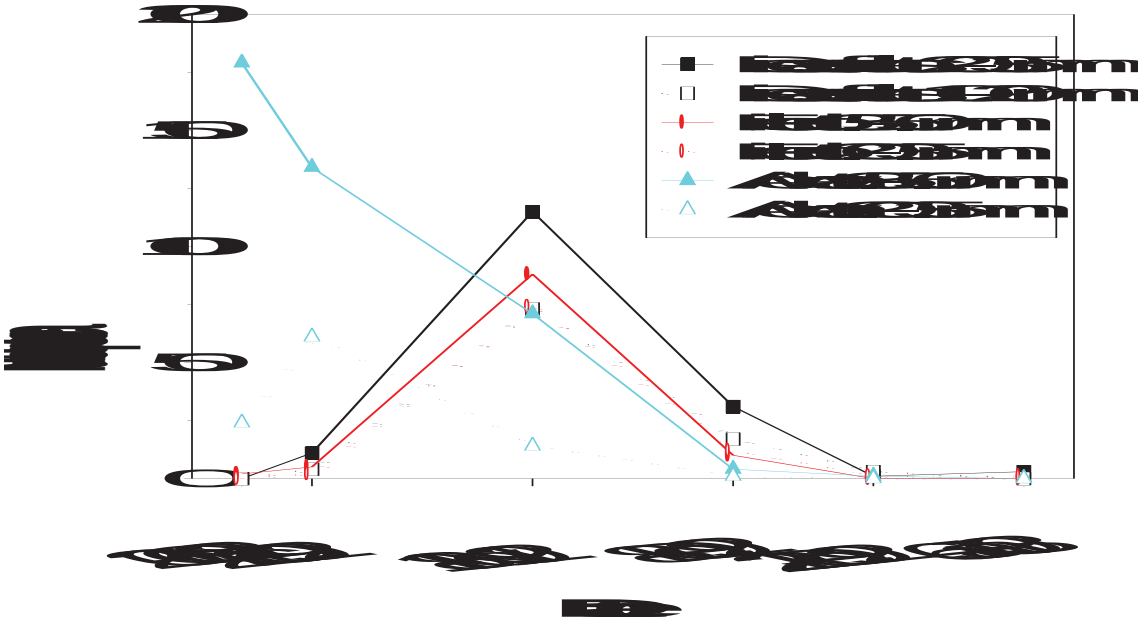


Figure 7.3 Number of mature apothecia present within 12 plots of sclerotia collected from diseased fruitlets, fruit with field rot and grown on autoclaved (A/c) wheat (96 sclerotia/category), and graded into size categories with 5, 3.5, 2 and 1 mm sieves and buried on 13 June 1992.

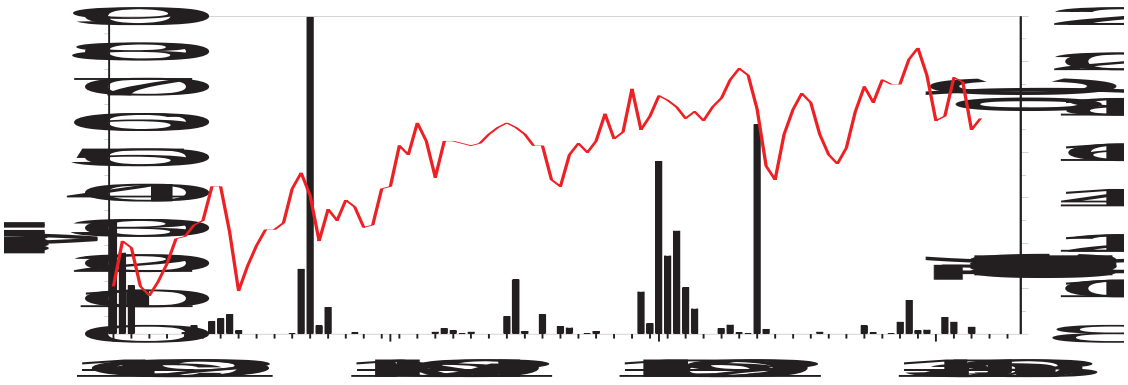


Figure 7.4 Daily rainfall and soil temperature at 10 cm depth (9 a.m. readings) at Te Puke Research Orchard from 1 October 1992–6 January 1993.

By 18 November, the number of apothecia from culture-grown sclerotia had declined, while those from kiwifruit had peaked (Figure 7.3). This was 4 days after a 19 day period without substantial rainfall (<5 mm) that had been broken by 2 wet days (20 mm); soil temperature had been 15–18°C for the previous week (Figure 7.4). All

classes of sclerotia had few or no apothecia on 22 December and 6 January, despite considerable rainfall in early December (Figure 7.4). There were 0, 5, 19, 9, 0, and 0 naturally occurring apothecia present on the six sampling dates, respectively.

7.3.3 Discussion

These experiments showed that sclerotia of *S. sclerotiorum* are produced within internal cavities and on external surfaces of both diseased fruitlets and fruit with field rot, as reported previously for kiwifruit (Hoyte *et al*., 1992) and several field crops (Rainbow, 1970; Hoes & Huang, 1975; Merriman *et al*., 1979). The source of naturally produced sclerotia influenced the number and size distribution of sclerotia produced and the percentage germination following burial in orchard soil.

The number of sclerotia produced on fruit with field rot increased with increasing fruit size. During the first 5 weeks after anthesis fruit have <2% soluble carbohydrate and the fresh weight is <20 g, however fruit weight and carbohydrate content increase by 4–5-fold between 5 and 25 weeks after anthesis (Pratt & Reid, 1974; Okuse & Ryugo, 1981; Reid *et al*., 1982). The type and concentration of utilisable nutrients e.g. forms of sugars and inorganic nitrogen, have been shown to influence sclerotial formation in laboratory studies (Wang & Tourvielle, 1971; Bakr & Grewal, 1987; Budge & Whipps, 1991a). The changes in weight and composition of kiwifruit fruit are the likely explanation of the limited number of sclerotia produced from diseased fruitlets and small fruit and the increased number and size of sclerotia produced by larger fruit.

The range of sclerotial sizes in this study (0.8–5.0^{+} mm) is similar to that reported from sunflowers (Hoes & Huang, 1975) and lettuce (Ben-Yephet *et al*., 1993), and is typical of isolates studied in culture (Tariq *et al*., 1985; Tores, 1990). There was a significant effect of sclerotial size on the number of apothecia produced, but this was not influenced by the source of sclerotia. A similar relationship was reported for culture-grown sclerotia under laboratory conditions (Dillard *et al*., 1995), and increasing sclerotial weight was related to increased number of apothecia for sclerotia collected from lettuce crops (Ben-Yephet *et al*., 1993). In this study, the size of sclerotia was also positively related to apothecial size, and is in agreement with the findings of Rajender *et al*. (1996). This information in combination with the yield of sclerotia from diseased

fruitlets and fruit with field rot can be used to estimate the potential number and size of apothecia from a given level of disease in pistillate kiwifruit vines.

Although the processes of sclerotial decay were not studied, the differences in percentage germination between sclerotia from different sources were presumably caused by variation in the loss of viability during burial. These differences in viability can be attributed to the source of sclerotia because all sclerotia were exposed to similar conditions of soil temperature, moisture, and depth of burial, factors known to affect sclerotial survival and apothecial formation (Coley-Smith & Cooke, 1971; Abawi & Grogan, 1979).

Cultured sclerotia germinated earlier than naturally formed sclerotia from kiwifruit. Also, sclerotia from fruit with field rot had a lower percentage germination compared with cultured sclerotia and sclerotia from diseased fruitlets. This is in agreement with similar studies where cultured sclerotia formed apothecia several weeks earlier and had greater percentage recovery and viability than sclerotia from canola (Mitchell & Wheeler, 1990) and lettuce (Merriman, 1976). In their studies these differences were attributed to naturally produced sclerotia having more perforated rind cells and a higher incidence of micro-organisms. Other studies have shown that sclerotia produced internally within bean stems were recovered in greater numbers than externally produced sclerotia following burial (Merriman *et al*., 1979). Merriman *et al.* (1979) concluded that greater longevity of internally produced sclerotia was related to a significantly lower incidence of degrading micro-organisms, as a result of reduced microbial competition during sclerotial formation. This may explain the different germination rates between sclerotia from fruit with field rot compared with those from diseased fruitlets, the latter being largely formed internally.

Diseased fruitlets in kiwifruit are not in contact with soil during sclerotial formation because they initially remain attached to the vine (section 2.3.2.1). This minimises contact with soil borne micro-organisms. Like the culture-grown sclerotia observed by Merriman (1976), these sclerotia may have better survival characteristics as a result of reduced microbial competition during sclerotial formation and may have well formed rind cells that can better resist colonisation by micro-organisms when they do eventually contact the soil. In contrast, fruit with field rot readily fall to the ground (Chapter 6, p.

140) and sclerotia formed on these are in close contact with the soil and its associated micro-flora, which may have reduced their survival in this study.

Previously, in a similar burial experiment at the same site, 99% of culture-grown sclerotia produced apothecia after burial for 5–6 months, indicating a very high survival rate (Hoyte *et al*., 1992). The only other study of sclerotial survival in kiwifruit, tested by mycelial germination, showed a 30% and 48% decline in viability of buried culture-grown sclerotia after 111 and 208 days respectively (Goh & Lyons, 1992b). There is conflicting data on the effects of sclerotial size on survival. Ben-Yephet *et al*. (1993) showed that in four fields small sclerotia increased in proportion compared with large sclerotia over time, partially due to the formation of secondary sclerotia. Harvey *et al*. (1995) showed that smaller, culture-grown sclerotia had a higher rate of degradation than larger sclerotia buried in pasture. In this study there was no consistent relationship between sclerotial size and percentage germination.

Sclerotial production from staminate vines was not determined in this study, although sclerotia were seen on diseased flower clusters, leaves and canes on staminate vines. Infection of weed plants in contact with diseased vine prunings was also evident. It remains unknown whether sclerotia formed from staminate vines and weeds have similar survival and germination characteristics as those from pistillate vines used in this study. This warrants investigation to more fully understand the factors affecting inoculum production in kiwifruit orchards.

Damage marks were observed only on externally produced sclerotia formed on diseased fruitlets collected from the ground and fruit with field rot. These damage marks were consistent with feeding of gastropod and arthropod soil fauna, such as slugs (G. Barker pers. comm.). Williams & Western (1965) reported similar damage on sclerotia extracted from soil and suggested this contributed to sclerotial decay. Jones (1970) reported that sclerotial rinds readily regenerated when cut. Regeneration of the sclerotial rind occurred on some but not all damaged sclerotia in this study. This form of damage to sclerotia may have contributed to the lower germination rate for sclerotia from field rot compared with diseased fruitlets.

Apothecial production from sclerotia in the burial trial occurred from late October to early December when soil temperatures ranged from 10–18^{o}C. This is within the generally accepted optimum temperature range of 10–20^{o}C (Willetts & Wong, 1980) and is similar to laboratory observations that apothecia are produced most abundantly between 11 and 15^{o}C (Abawi & Grogan, 1975; Saito, 1977). Soil moisture content near saturation is required for apothecial formation (Coley-Smith & Cooke, 1971; Abawi & Grogan, 1979; Willetts & Wong, 1980). A delay in stipe development and apothecial emergence was attributed to dry soil conditions in the previous year (Hoyte *et al*., 1992) and interruption of irrigation has been shown to delay sclerotial germination in canola (Twengstrom *et al*., 1998). It is possible that the peak in apothecial numbers on 18 November may have been delayed related to dry soil conditions during the first half of the month, when rainfall was minimal (<5 mm from 26 October–13 November 1992).

Numbers of apothecia declined during early December, despite frequent rainfall. Several studies have suggested an increased susceptibility to soil micro-organisms following carpogenic germination (Williams & Western, 1965; Mitchell & Wheeler, 1990). It is possible that degradation of the buried sclerotia in this trial may have occurred following germination.

This is the first report of the yield of sclerotia produced from individual pistillate kiwifruit vines. The two-vine plots at orchard 22 produced equivalent to 150 sclerotia m^{-2}, each presumably capable of producing 1.2–2.7 apothecia on average (Table 7.2). The average density of apothecia measured within this same block during November–December 1996/97 and 1998/99 ranged from 6.0–7.2 apothecia m^{-2}. This is high relative to the apothecial densities recorded from 18 orchards by Pak *et al*. (1997) (mean 0.91, maximum 8.0). Even though data from orchard 22 is not available for consecutive seasons, there is a large disparity between the measured sclerotial production and the apothecial density. This suggests a large proportion of naturally produced sclerotia may not produce apothecia in subsequent seasons, possibly due to loss of viability or non-favourable conditions for germination. Similarly, Grogan (1979) noted that "the numbers of sclerotia at the time of planting seems to be considerably less than expected in view of the large numbers that usually are produced on infected tissues during an epidemic. Thus, the percent of survival, even in the short term, seems to be low".

7.4 Relationships between inoculum and disease incidence

The relationships between inoculum and disease incidence was studied during 1996/97 and the results were used as the basis for designing larger scale field trials during 1998/99 to allow more effective statistical analysis.

7.4.1 1996/97 season

7.4.1.1 Materials and methods

S. sclerotiorum inoculum was measured in the nine Bay of Plenty kiwifruit orchards used in section 3.3.1 and two Waikato orchards (orchards 22 and 23), by counting apothecia in quadrats and trapping *S. sclerotiorum* ascospores on JK selective medium. Ten pistillate vines and three staminate vines spaced evenly across one block in each orchard were selected and labelled 1 month before flowering. On two occasions during November and December 1996 apothecia were counted beneath the staminate vines and six pistillate vines randomly selected from the labelled vines in each orchard. The assessments were done 5–10 days before the expected date of flowering and at 50–80% flowering. Beneath each vine, one quadrat (0.5 × 1 m) was placed on each side of the main cordon, with the short side directly beneath the cordon and 0.5–2.0 m away from the vine trunk. For each quadrat, the distance from the vine trunk was arbitrarily determined by tossing the quadrat whilst standing next to the trunk. The total number of apothecia within each quadrat was counted during approximately 2 minutes of searching.

Petri dishes with JK selective medium were used to passively trap airborne *S. sclerotiorum* ascospores in a similar manner to previous studies (Williams & Stelfox, 1979; Ben-Yephet & Siti, 1987; Kerssies, 1990), except that Petri dishes were suspended with the agar surface facing downwards to prevent rain water collecting. These were held 150–200 mm below the vine canopy by attaching each dish to a three pronged wire hanger secured to flowering shoots. One Petri dish was suspended in each of the six pistillate vines used for apothecial assessments. Orchardists exchanged the Petri dishes for fresh ones each day between 8–9 am, starting from ca. 10% flowering and continuing for 12–14 days. Petri dishes were incubated in ambient conditions for up to three weeks and the number of *S. sclerotiorum* colonies counted. These were

identified by their white colour, small domed shape and flat spreading margin that developed as they aged (Appendix 3). Several colonies of *S. sclerotiorum* from each orchard were sub-cultured on PDA to confirm their identity.

The incidence of *S. sclerotiorum* within pistillate flower petals and within AFT on fruit was determined as a measure of disease. Ten petals were collected from 3–4 day-old flowers, one petal/flower, selected at random from each labelled pistillate vine in each orchard at 60–95% flowering. Petals were surface-sterilised (section 2.2.2) and two petals placed on opposite sides of Petri dishes of PDA with flame-sterilised forceps. Characteristic white colony growth of *S. sclerotiorum* was recorded after 5 days incubation on the laboratory bench (15–20^{o}C) and confirmed 2 weeks later when sclerotia had formed. Adhering floral tissues were collected from 10 fruit from each labelled pistillate vine in each orchard, 6–8 weeks after flowering. They were surface-sterilised and placed singly on PDA and growth of *S. sclerotiorum* recorded as described above. Five of the 11 orchards were treated with Rovral fungicide during petal-fall, after petals had been sampled.

At least 40 flowers/pistillate vine were identified during flowering by marking 8–10 flowering shoots with a 200 mm length of coloured tape. The number of diseased fruitlets were assessed on the marked shoots 3 weeks after flowering. When only the pedicel remained attached to the vine it was removed, surface-sterilised and placed on PDA. When *S. sclerotiorum* growth was recorded from pedicels it was assumed that they originated from diseased fruitlets. Six to eight weeks after flowering the number of fruit with scarring symptoms was assessed on the same tagged shoots. The length and width of scarring symptoms, and the presence of AFT on fruit with scarring symptoms was also recorded.

Inoculum and disease data from 1996/97 and 1998/99 (section 7.4.2) were analysed using Genstat with generalised linear models with logit link and errors proportional to binomial distribution. Where apothecial densities were plotted against each other log link with errors proportional to Poisson was used. Tests of significance relate to the slope of the fitted line. Apothecial density was square root transformed. The incidence of JK Petri dishes with *S. sclerotiorum* colonies, the incidence of *S. sclerotiorum* within petals and the incidence of disease were angular transformed.

7.4.1.2 Results

S. sclerotiorum colonies were present on 10% (SE ± 2.6, range 1–23%) of JK Petri dishes from nine orchards, but were absent from the remaining two orchards. Petri dishes with *S. sclerotiorum* colonies had 4.6 colonies per dish (SE ± 1.6, maximum 96). *Cladosporium* spp. were the most common among the fungal colonies on JK selective medium, typically ranging from 10–20 colonies per Petri dish. *Penicillium* spp. and bacterial colonies were also present. Fungal and bacterial contaminants precluded accurate determination of *S. sclerotiorum* colonies on 5% of JK Petri dishes which were not included in the analysis.

Apothecia were observed in seven of the nine orchards from which *S. sclerotiorum* colonies were recorded on the JK Petri dishes and one orchard where no *S. sclerotiorum* colonies were recorded. Orchard 12 and 22 had apothecia present within 69% and 89% of quadrats and a mean apothecial density of 4.7 apothecia m^{-2} (SE ± 0.8) and 7.3 apothecia m^{-2} (SE ± 1.5), respectively. All other orchards had <1.9 apothecia m^{-2}. Across all orchards there was a mean of 3.0 apothecia per quadrat (SE ± 0.3, range 1–22) for those quadrats with apothecia present. There was a significant positive regression ($P<0.05$) between apothecial density and the percentage of JK Petri dishes with *S. sclerotiorum* colonies (Figure 7.5).

Four orchards had <8% incidence of *S. sclerotiorum* within petals, and each of these had a low apothecial density (<0.12 apothecia m^{-2}) and low incidence of JK Petri dishes with colonies (<7%). The incidence of *S. sclerotiorum* within petals from the other seven orchards ranged from 12–53%. Overall there was a significant positive regression between the incidence of *S. sclerotiorum* within petals and both apothecial density ($P<0.005$) and the percentage of JK Petri dishes with *S. sclerotiorum* colonies ($P<0.05$) (Figure 7.6 A and B).

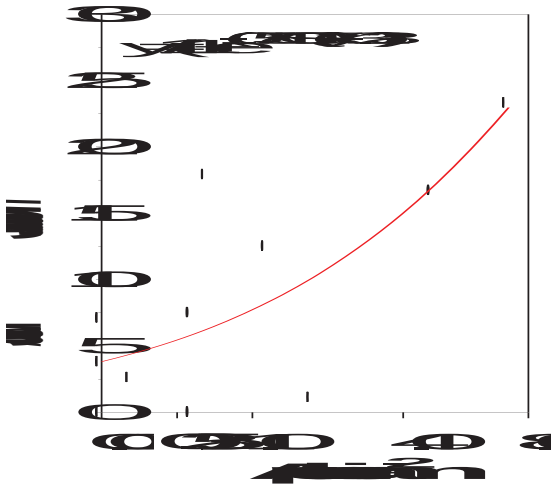


Figure 7.5 Relationship between the mean percentage of JK Petri dishes with *S. sclerotiorum* colonies and mean apothecial density (square root transformed scale), during flowering in 11 orchards during 1996. SQRT = square root transformation.

A B

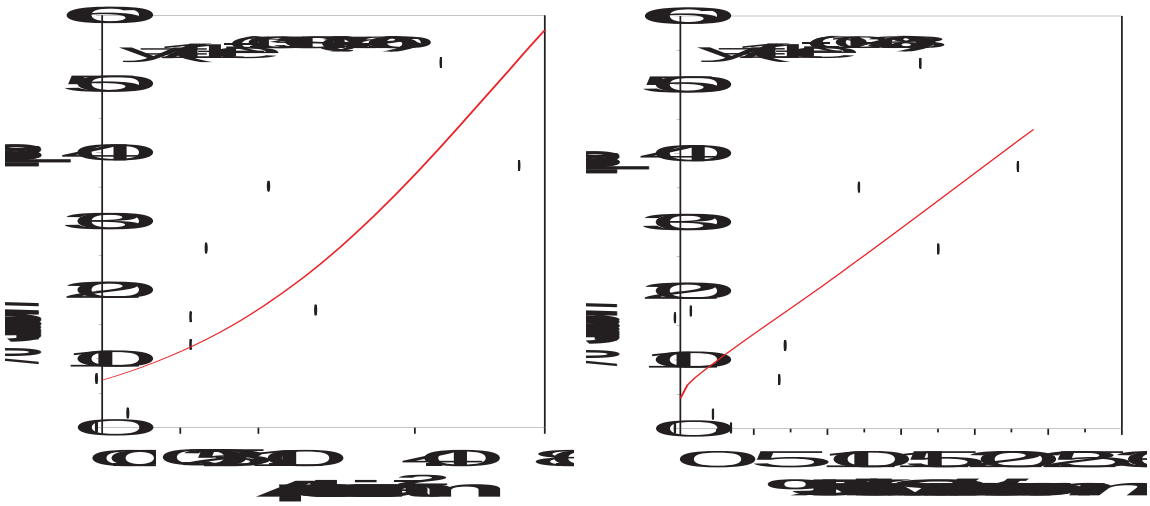


Figure 7.6 A and B. Relationships between the mean incidence of *S. sclerotiorum* within petals and (A) mean apothecial density (square root transformed scale) and (B) the mean percentage of JK Petri dishes with *S. sclerotiorum* colonies during flowering, in 11 orchards during 1996. SQRT = square root transformation.

There was a significant positive regression ($P<0.01$) between the incidence of *S. sclerotiorum* within petals and the incidence of *S. sclerotiorum* in AFT (Figure 7.7). One orchard had a 35% incidence of *S. sclerotiorum* in petals but 0% incidence in the AFT and was considered an outlier. Forty one percent of fruit had AFT (SE ± 3.3,

range 24–58%) and there was no positive relationship between this and the incidence of *S. sclerotiorum* within petals (P=0.87).

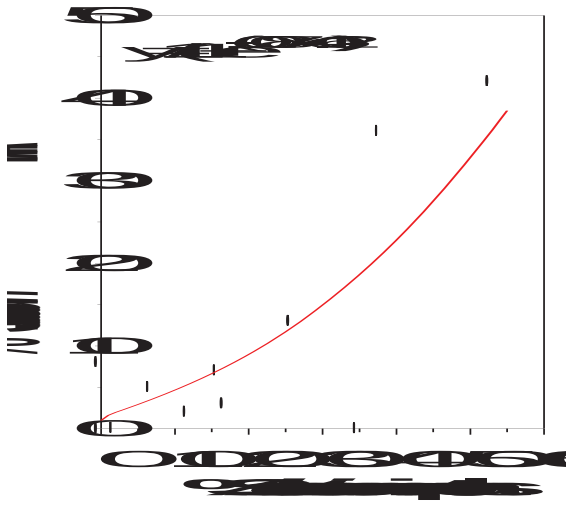


Figure 7.7 Relationship between the mean incidence of *S. sclerotiorum* within adhering floral tissues (AFT) and within petals in 11 orchards during 1996.

The incidence of diseased fruitlets ranged from 0–12% and was positively correlated (P<0.005) with the incidence of *S. sclerotiorum* within petals (Figure 7.8 A). Similarly, the incidence of scarring on fruit ranged from 0–18% and was positively correlated (P<0.001) with the incidence of *S. sclerotiorum* in AFT (Figure 7.8 B). The combined incidence of diseased fruitlets and fruit with scarring was not significantly different (P=0.18) between orchards with and without fungicide applications.

Because orchards 22 and 23 had particularly high inoculum levels combined with a high incidence of *S. sclerotiorum* within floral tissues and disease incidence compared with the other orchards, these data points tended to have a large influence on these regression analyses. Therefore, the level of significance of these linear regressions needs to be interpreted with caution.

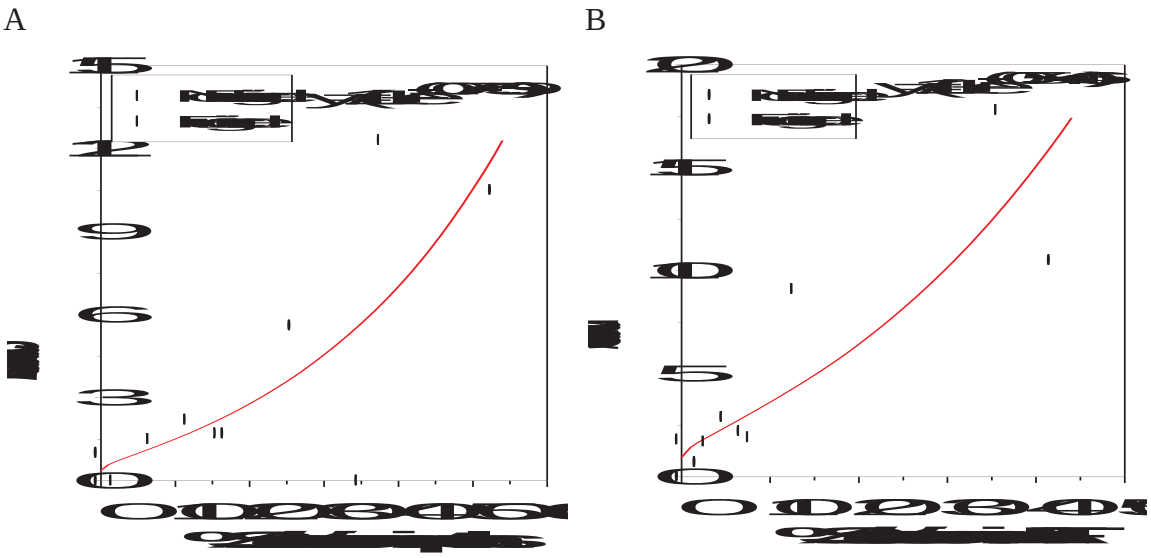


Figure 7.8 A and B. Relationships between (A) the mean incidence of diseased fruitlets and the mean incidence of *S. sclerotiorum* within petals and (B) the mean incidence of scarring on fruit and the incidence of *S. sclerotiorum* within adhering floral tissues (AFT) on fruit, in 11 orchards with and without fungicide applications during 1996/97.

7.4.2 1998/99 season

7.4.2.1 Materials and methods

Inoculum levels and disease incidence was determined in 13 orchards from the Bay of Plenty (orchards 1, 2, 3, 6, 7, 9, 10, 12, 15, 16, 17, 18, and 19, Appendix 1), three orchards from the Waikato (orchards 22, 23, and 24) and two orchards from South-west Auckland (orchards 13 and 14). Five staminate vines and 10 pistillate vines, distributed evenly across each of two blocks per orchard, were marked and numbered before flowering. Apothecial density beneath marked vines was determined in each orchard within 5 days of the onset of anthesis on pistillate vines (6–26 November) and again 2 to 3 weeks later. Two 0.25 m^2 quadrats were randomly positioned on each side of the main cordon, and the number of apothecia in each quadrat was counted during at least 1 minute of searching in each quadrat. A weekly survey of apothecial density was carried out in four orchards, one from each region (orchard 2, Katikati; orchard 12, Bay of Plenty; orchard 13, South Auckland; and orchard 22, Waikato). Between 13 October and 14 December 1998, apothecial density was measured as described above, except that two quadrats were sampled per vine.

Fifty petals were collected from 3–4 day-old flowers on each marked vine. Staminate and pistillate vines were sampled when they reached about 50–80% flowering. Petals from each vine were placed on moist tissue paper in a plastic tray (350 × 300 × 25 mm). Four plastic caps (from 15 ml vials) were positioned in the corners of each tray so that they could be stacked. Stacks of 10 trays were placed into a plastic bag and the open end folded over to minimise water loss. Trays were then placed in a controlled environment room at 20°C. The number of petals in each tray with *S. sclerotiorum* mycelium and/or sclerotia was determined after 6 or 7 days incubation. Fifteen of the 36 orchard blocks were treated with Rovral fungicide during petal-fall, after petals had been sampled.

Disease incidence was determined on the pistillate vines 6–8 weeks after flowering. Fruiting shoots were sampled at random on each side to the main cordon. Diseased fruitlets, dry brown pedicels and fruit with scarring were counted on each shoot. Further shoots were sampled so that at least 50 fruit and diseased fruitlets were assessed on each side of the vine. All pedicels were collected, surface-sterilised and placed individually on PDA. If *S. sclerotiorum* was isolated then these pedicels were considered to be from diseased fruitlets that had fallen from the vines.

7.4.2.2 Results

The apothecial density in the four orchards where weekly assessments were carried out and the daily rainfall at a meteorological site within 15 km of each orchard is shown in Figure 7.9. Apothecia were observed in five of the orchard blocks in mid-October and numbers had increased in seven of the eight blocks by 5–10 November. No apothecia were observed in the 'pond' block at orchard 22 throughout the sampling period. Apothecial density decreased in all blocks between 8–17 November, declining to zero in four blocks (Figure 7.9). This coincided with a 16-day period when no rainfall was recorded at any of the meteorological sites and the soil surface became very dry at each site except at orchard 12. There was a second increase in apothecial density during late-November to early-December, at a time when rainfall was frequent. In the 'kaituna' block (Orchard 12) and 'nashi' block (Orchard 22) the apothecial density reached levels that were higher than before the dry period in mid-November.

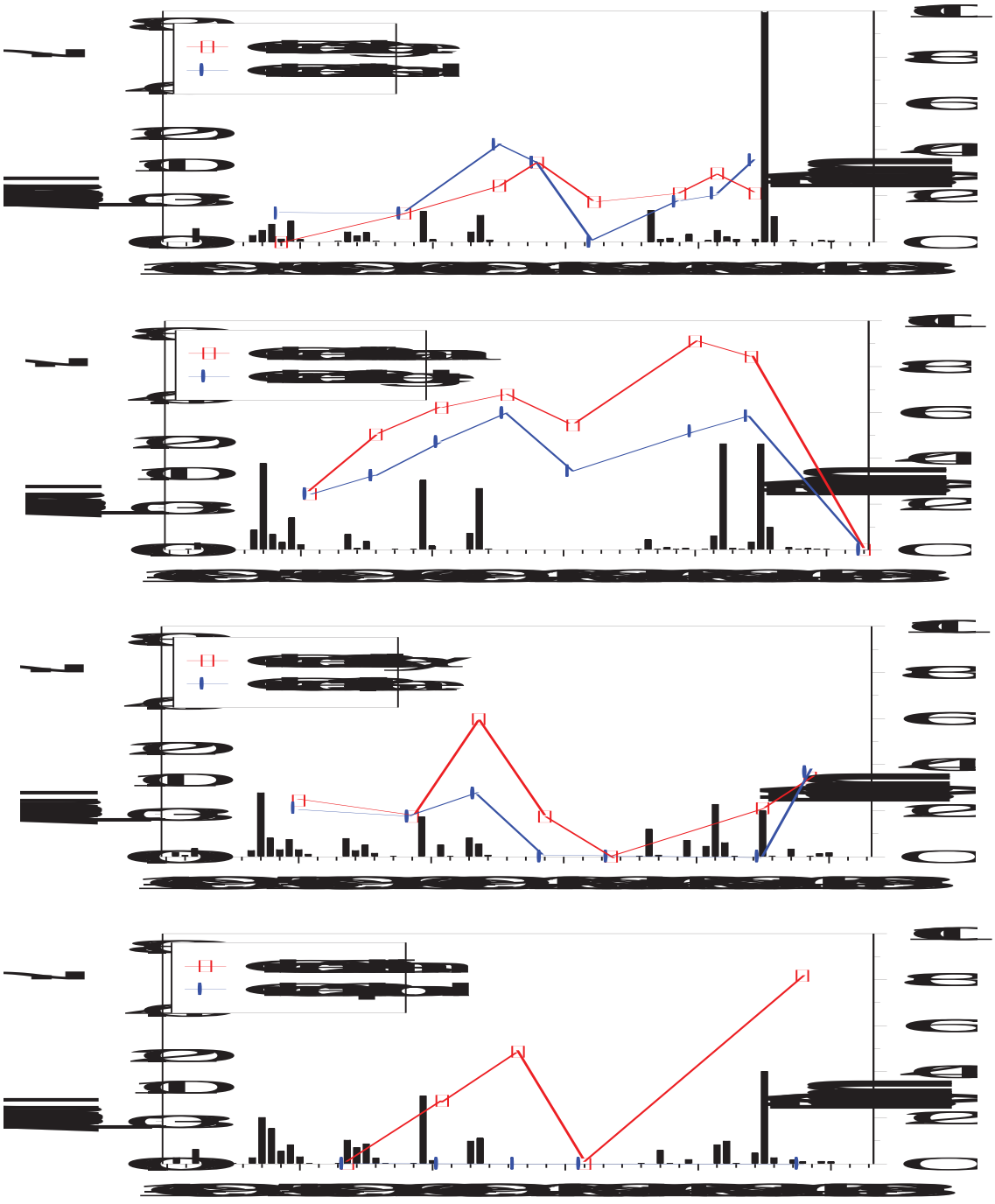


Figure 7.9 Mean apothecial density (square root scale) determined in two blocks from orchard 2, 12, 13, and 22 by sampling two 0.25m^2 quadrats beneath 10 pistillate and 5 staminate vines, and daily rainfall ( ) from 1 October to 15 December 1998 from meteorological site within 15 km.

In the main survey blocks, apothecia were observed in 20 of the 36 blocks during the first assessment and 29 blocks during the second. This included seven blocks (first assessment) and 14 blocks (second assessment) that had apothecia beneath only pistillate or staminate vines. Six blocks had no apothecia in both assessments and two

of these were from one orchard. There was no rainfall recorded at any of the four meteorological sites between 4 and 21 November, when most of the first assessments were carried out (Figure 7.9). There was frequent rainfall at each site during the week before commencing the second assessment and during the second assessments.

The mean apothecial density was similar beneath pistillate and staminate vines and between the first and second assessments (Table 7.4). There was a significant positive regression ($P<0.001$) between the apothecial density (mean of two assessments) beneath pistillate and staminate vines (Figure 7.10). However, the significance of this relationship was strongly influenced by the cluster of 23 blocks which had <0.5 apothecia m^{-2} beneath both vine types, while the remaining 13 blocks were highly variable. For the latter, the ratio of apothecial density beneath these vines types ranged from 0.25 to 4.0 in eight blocks and was outside this range for the other five blocks.

Table 7.4 Apothecial density (per m^{-2}) from two assessments beneath 10 pistillate vines (40 quadrats) and 5 staminate vines (20 quadrats) in each of two blocks from 18 orchards, during 1998.

Vine type	Assessment 1 (9–26 November)		Assessment 2 (2–9 December)	
	Mean	Range	Mean	Range
Pistillate	0.92 (0.27)[1]	0–4.7	1.09 (0.33)	0–9.4
Staminate	1.13 (0.37)	0–9.6	0.93 (0.33)	0–7.0

[1] Standard error.

There was a significantly higher incidence of *S. sclerotiorum* ($P<0.05$) within petals sampled from pistillate vines (15%, SE ± 3.4) than staminate vines (9%, SED ± 2.9) and a positive correlation between them (Figure 7.11 A). The incidence of *S. sclerotiorum* within pistillate and staminate petals was low (1%, SE ± 0.3) from blocks with no apothecia, moderate (7%, SE ± 2.3) from blocks with 0–0.25 apothecia m^{-2} and high (20%, SE ± 4.6) from blocks with >0.25 apothecia m^{-2}. Overall the incidence of *S. sclerotiorum* within petals was positively correlated with the apothecial density from both the first ($P<0.001$) and second ($P<0.005$) assessments. Regression analysis was repeated using the mean of the two apothecial assessments (Figure 7.11 B) because

several blocks had no apothecia recorded in one or both assessments, and because of the considerable changes in apothecial density during November–December (Figure 7.9).

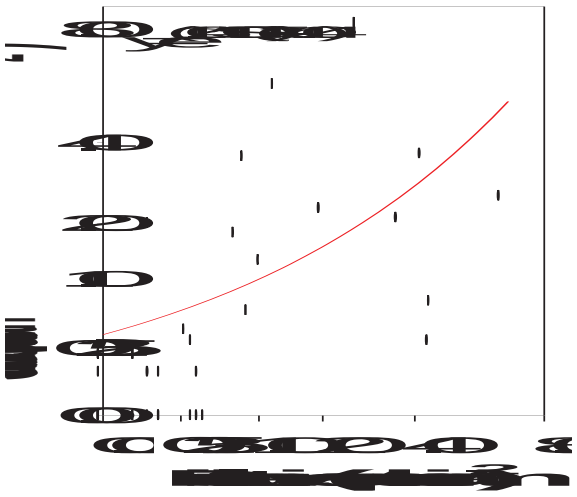


Figure 7.10 Relationship between the mean apothecial density (square root transformed scales) beneath staminate vines (40 quadrats) and pistillate vines (80 quadrats) within 36 orchard blocks , mean of two assessments, during 1998. SQRT = square root transformation.

A B

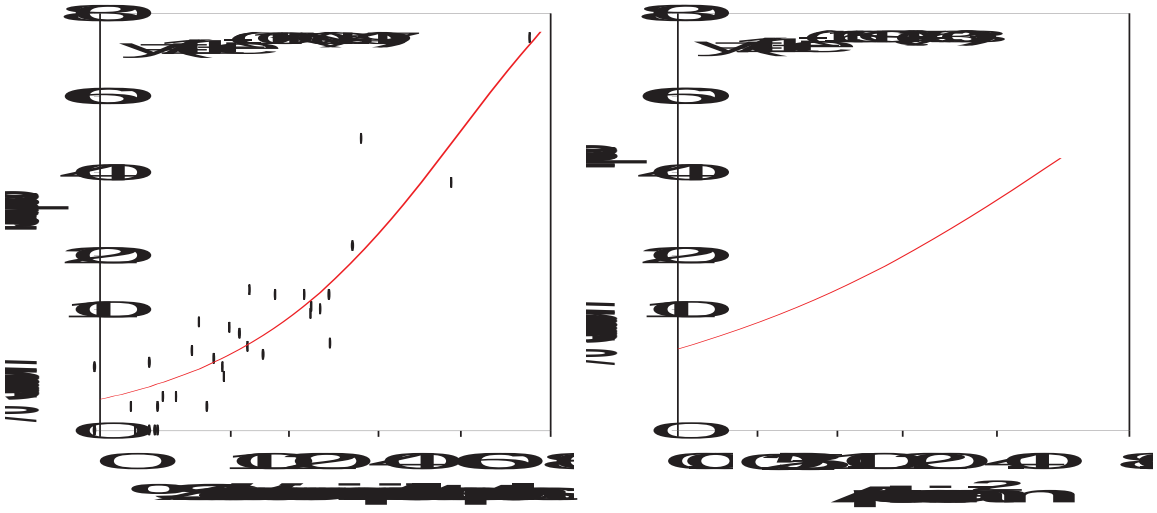


Figure 7.11 A and B. Relationship between (A) the mean incidence of *S. sclerotiorum* within petals sampled from staminate and pistillate vines and (B) the mean incidence of *S. sclerotiorum* within petals, pistillate and staminate vines combined, and mean apothecial density (square root transformed scale), during 1998. ANG = angular transformation and SQRT = square root transformation.

Regression analysis of the data from the orchard blocks with and without fungicide applications showed no significant difference between them, therefore the data was combined. The mean incidence of diseased fruitlets was 1.4% (SE ± 0.3) and the mean incidence of fruit with scarring was 1.0% (SE ± 0.3). Two of the four blocks that had no diseased fruitlets or fruit with scarring, had zero apothecia and two had <0.3 apothecia m^{-2}. Each of these four blocks had <5.2% incidence of *S. sclerotiorum* within petals. The other four blocks with zero apothecia had 0–2.3% incidence of *S. sclerotiorum* within petals and 0.6–1.3% incidence of disease. The incidence of diseased fruitlets and fruit with scarring was positively correlated (P<0.001) with the mean incidence of *S. sclerotiorum* within petals (Figure 7.12).

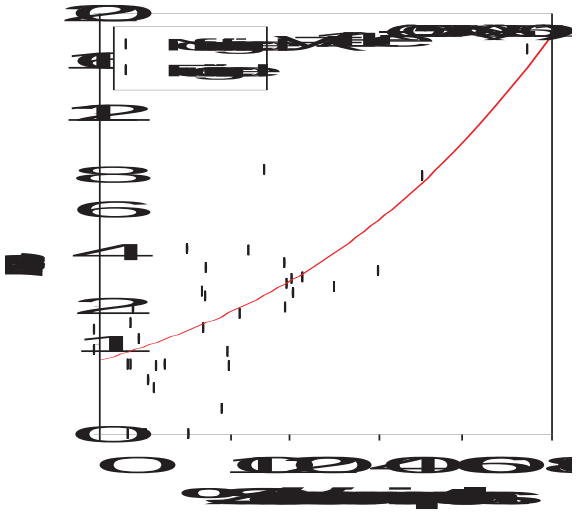


Figure 7.12 Relationship between the mean incidence of disease (diseased fruitlets and scarred fruit combined) and the mean incidence of *S. sclerotiorum* within petals (pistillate and staminate vines combined) in orchard blocks with and without fungicide applications, during 1998/99. Angular transformed scales. ANG = angular transformation.

7.4.3 Discussion

A major objective of this study was to examine the role of primary and secondary inoculum in disease progress and to determine whether positive relationships exist between *S. sclerotiorum* inoculum and disease incidence in kiwifruit. Data from 11 orchards during 1996/97 suggested positive relationships between: (i) the two measures of primary inoculum, apothecial density and incidence of JK Petri dishes with *S. sclerotiorum* colonies; (ii) primary inoculum and secondary inoculum, incidence of *S.*

sclerotiorum within petals; (iii) secondary inoculum and the incidence of diseased fruitlets and fruit with scarring. Orchard blocks with low apothecial density had a low incidence of *S. sclerotiorum* within petals and low disease incidence. Conversely, two orchards with >4 apothecia m^{-2} had >30% incidence of *S. sclerotiorum* in petals and >20% incidence of disease.

There were similar positive relationships between primary inoculum, petal infection and disease incidence during the 1998/99 season. Therefore, despite the limitations of the number of sites and sample size used during 1996/97, the data appears to be representative. Very low primary inoculum levels (0–0.25 apothecia m^{-2}) were associated with low levels of petal infection (0–25%). Very low petal infection (0–5%) was associated with a low incidence of diseased fruitlets and fruit scarring (0–1.5%). A similar relationship has been shown during the 1999/00 season when inoculum-disease relationships were again studied in these same orchards (H. Pak, pers. comm.).

These significant positive relationships are similar to those reported for sclerotinia disease of bean (Boland & Hall, 1988a), canola (Gugel & Morrall, 1986; Turkington & Morrall, 1993), soybean (Boland & Hall, 1988b) and sunflower (Holley & Nelson, 1986), and support the hypothesis that ascospores produced by apothecia are the primary inoculum source in kiwifruit. The data also suggest that the ability of ascospore inoculum to cause disease is readily expressed in floral tissues from orchards with a range of management practices and environmental conditions. This may be explained by the inherent susceptibility of petals to colonisation by ascospores over a wide range of temperature (10–27^{o}C) and RH (90–100%) (Chapter 5, p.114).

Turkington *et al.* (1991) also showed significant positive relationships between stem rot of canola and the incidence of *S. sclerotiorum* within petals, but noted there was considerable variation between locations and seasons. These relationships were most accurate when inoculum levels were low, and less so when moderate or high, suggesting that additional factors influenced disease incidence (Turkington *et al*., 1991). This also appears to be the case in kiwifruit, as the variability of petal infection and incidence of diseased fruitlets and scarring was greater at higher inoculum levels than at low inoculum levels. Similar variation in the relationship between the number of apothecia and disease incidence have also been reported in bean (Schwartz & Steadman, 1978;

Boland & Hall, 1988a), canola (Morral & Dueck, 1982) and kiwifruit (Pak & Manning, 1998).

The upper boundary of data points in Figure 7.11 B can be seen to represent increasing potential for disease as the available inoculum increases in the range of 0–8 apothecia m^{-2}. Various modifying factors may be operating within individual orchard blocks to reduce actual disease levels below the potential disease level for a given density of apothecia. These modifying factors include: dense ground cover, that may reduce ascospore dispersal (Aylor, 1998); bare soil allowing a faster decline in water potential during dry weather, leading to decline in apothecial numbers; periods of RH less than 90%, which will reduce colonisation of floral tissues (Chapter 5, p. 115); poor synchrony between flowering and inoculum production, which could reduce the inoculum loading on floral tissues; fungicide applications, which are likely to reduce primary infection and/or secondary spread depending on the timing of application.

Although fungicide applications are said to be effective at controlling sclerotinia in kiwifruit (Pennycook, 1985), there is no published data to support this. In this study, fungicide treated blocks had similar disease levels to un-treated blocks, but this was not studied as part of the trial design and therefore conclusions can not be made on the efficacy of these applications. If fungicide applications in the blocks used for this study did reduce disease levels then the slope of the regressions in Figure 7.8 and 7.12 may have been affected by these treatments.

With respect to ascospore escape from ground cover, spore trajectory simulation modelling has shown that the likelihood of *V. inequalis* ascospores escaping from a grass/weed sward is highly sensitive to wind speed and turbulence (Aylor, 1998). It is unknown what proportion of *S. sclerotiorum* ascospores are trapped in ground cover and whether disease incidence would change if sward density were increased or the interval between mowing was changed.

The variation in disease incidence may also be attributed to sampling error in the measurement of inoculum, aggregation of apothecia and disturbance of plots during quadrat assessments, or in the measurement of disease. Also, ascospores from neighbouring fields have been suggested to contribute to the inoculum within crops with

low natural inoculum levels (Williams & Stelfox, 1979; Ben-Yephet & Siti, 1987; Boland & Hall, 1987). This is also a possibility in kiwifruit because orchards are often adjacent to each other.

The absence of a relationship between the incidence of *S. sclerotiorum* in petals and the proportion of fruit that have AFT suggests that for fruit remaining on the vines, colonisation of petals by *S. sclerotiorum* does not appear to strongly influence the retention of floral tissues following petal-fall.

Although there was a similar mean and range in apothecial density beneath pistillate and staminate vines, a linear relationship did not adequately describe this data. Many blocks had low apothecial density (<0.5 m^{-2}) beneath both vine types, while others varied considerably between pistillate and staminate vines. This variability could be a direct consequence of different levels of sclerotial production from these respective vine types during the previous seasons. Differences in disease levels between staminate and pistillate vines may contribute to this through possible differences in susceptibility, inoculum availability and the timing of flowering which can impact on the environmental conditions during flowering.

Different environmental conditions beneath the two vine types may also account for these observed differences in apothecial density. It was noted that canopies of staminate vines were denser than pistillate vines, therefore the ground beneath them was more heavily shaded. In some circumstances apothecial formation and survival under staminate vines may have been greater than under pistillate vines because of greater retention of soil moisture, creating more favourable conditions (Adams & Ayers, 1979; Grogan, 1979). This possible effect of vine canopy may be similar to the relationship in bean crops between canopy density, soil moisture retention, wetness duration and temperature (Blad *et al*., 1978; Schwartz & Steadman, 1978; Boland & Hall, 1987).

Although there was not a strong relationship between apothecial density beneath pistillate and staminate vines, there was a strong positive relationship between the incidence of *S. sclerotiorum* within petals sampled from pistillate and staminate vines. This could be explained by ascospore inoculum being more dispersed than the source of the original inoculum (Gregory, 1973). The reason for a significantly higher incidence

of *S. sclerotiorum* on pistillate petals than on staminate petals is unknown, but may relate to differences in the timing of flowering with respect to inoculum levels, susceptibility to colonisation or effects of different canopy structure on environmental conditions or ascospore dispersal.

Observations of apothecia in kiwifruit during this study suggest that apothecial production occurs during November and December, therefore the availability of primary inoculum coincides with kiwifruit flowering. High soil moisture ($>^{-}7.5$ bars) is required for apothecial development and survival (Abawi & Grogan, 1975; Morral, 1977). The likely decrease in soil moisture caused by the lack of rainfall from 4–21 November 1998 could explain the decline in apothecial numbers during mid-November in the monitor blocks and perhaps the absence of apothecia from several blocks in the main survey. Absence of apothecia could also be related to non-detection because of sampling limitations and/or aggregation of low numbers of apothecia. This could also explain the situation in the four blocks that had no apothecia but 0.6–1.3% disease incidence. Pak & Manning (1998) also reported disease in blocks without apothecia and proposed that monthly sampling intervals limited the accuracy of their results. In addition, they only determined apothecial density beneath staminate vines, which may have increased the variation in their inoculum-disease relationships.

Three orchards (7, 10, and 12) had between 3.2–6.2 apothecia m^{-2} in mid-November 1998, despite the absence of significant rainfall. Each of these blocks did not use herbicides for weed control beneath vines and therefore had a well established dense ground cover. This resulted in a noticeable retention of soil moisture compared with the other sites which all had herbicide weed control and may explain the presence of apothecia in such large numbers during dry weather.

Because dry soil conditions do not favour apothecial development, the time required for apothecia to appear following dry conditions is important to consider when scheduling apothecial assessments. It is also possible that irrigation of kiwifruit vines during November and December may influence apothecial formation and survival (Blad *et al*., 1978; Twengstrom *et al*., 1998). In kiwifruit, the mean development time from the first appearance of stipes until maturation of apothecia was reported to be 8.5 days (Hoyte *et al*., 1992). Other reports suggest that apothecia usually appear within 3–7 days of heavy

rainfall following a dry period (Hartill, 1980; Gugel & Morrall, 1986; Boland & Hall, 1987). At orchards 2 and 12, apothecial density increased 4–5 days after rainfall was recorded on 21 November 1998, suggesting a similar delay period in this study.

The selective nature of JK medium successfully minimised the growth of fungal and bacterial contaminants and in all but a few cases permitted effective quantification of *S. sclerotiorum* colonies during flowering in 9 of 11 orchards. In the absence of any reports of mycelial fragments of *S. sclerotiorum* becoming airborne, it is probable that these colonies on JK selective medium originate from ascospores. Colonies of *S. sclerotiorum* were also recorded on Kerssies selective medium from late-November until early-April in 1992/93 when used in Burkard high throughput 'Jet' spore-traps in two Te Puna kiwifruit orchards (Hoyte, 1996).

Data from Petri dish spore traps, as used in this study, cannot be translated to concentration (spores m^{-3}) as they merely measure surface deposition. The trapping efficiency of exposed Petri dishes is generally poor compared with forced-air impaction traps such as the 'Hirst' (Hirst, 1952) and Burkard 'Jet' (Schwarzbach, 1979) because of the effects of variable particle size, wind speed, and edge effects. In this study, inverting Petri dishes to avoid rainfall collecting on them may have considerably reduced the trapping efficiency, particularly under low wind speeds. This may have resulted in under-estimation of colony counts and inoculum levels and possibly have lead to a correlation with wind speed. This technique was not pursued during 1998/99 because of the unknown trapping efficiency.

Although disease symptoms first appear on flowers during November–December, apothecia were observed from mid-October in this study, and have been observed in orchards from late-September (Manning, 1991). Therefore, ascospore inoculum is often present in orchards several weeks before flowering and the onset of disease development. This is similar to reports in other crops, where initial disease has been linked to the onset of flowering in sunflower and bean, even though apothecia are generally observed several weeks earlier (Natti, 1971; Cook *et al*., 1975; Nelson *et al*., 1989). The absence of disease appearing earlier is probably because ascospores require a food base such as senescent flower parts for germination and infection of healthy host tissue (Abawi & Grogan, 1975; Sutton & Deverall, 1983; Gugel & Morrall, 1986).

7.5 Chapter Discussion

This chapter provides quantitative information on several stages of sclerotinia disease that can be utilised in the development of a sclerotinia disease model for kiwifruit:

- The number and size distribution of sclerotia produced from diseased fruitlets and fruit with field rot, in combination with the seasonal nature of symptom development, allows the production of sclerotia from pistillate vines to be estimated from disease incidence data.
- The size and source of sclerotia affects survival and the number and size of apothecia produced. Sclerotia within diseased fruitlets probably develop and mature with little or no contact with soil-borne micro-organisms. This suggests that different 'ecological populations' of sclerotia may exist in kiwifruit.
- The importance of apothecia within orchards, the role of ascospores as primary inoculum and the importance of colonisation of floral tissues in disease progress (discussed in Chapter 3) has been reinforced.
- The typical range of apothecial density and relationships between pistillate and staminate vines in terms of apothecial density and petal infection has been shown.
- Consistent relationships were shown between primary inoculum and petal infection and between petals infection and the incidence of diseased fruitlets and fruit.

It is feasible that the relative potential for disease in orchards could be estimated from these inoculum-disease relationships. The relationship between apothecial density and petal infection suggests that the incidence of *S. sclerotiorum* within petals may also be an appropriate indicator of disease risk. Because a measurement of petal infection takes into account factors relating to inoculum loading and environmental conditions it may be a more suitable measure of disease risk than apothecial density.

There is potential to establish inoculum thresholds of apothecial density or levels of petal infection and use these to categorise disease risk within orchard blocks. Before a disease risk prediction system could be used, additional information is required to determine: the minimum number of quadrats required to accurately determine low inoculum densities, the effects of soil moisture and rainfall on apothecial emergence, the effects of the weather on the relationships between apothecial density and disease, and possible changes in *S. sclerotiorum* incidence within petals during flowering.

Chapter 8: General Discussion

8.1 Introduction

Many aspects of the epidemiology of *S. sclerotiorum* in New Zealand kiwifruit were investigated for the first time in this study. This includes the role of adhering floral tissues (AFT) in disease progress, the effects of environmental conditions on primary infection and secondary spread, factors affecting inoculum production, and the relationships between inoculum and disease.

The first objective of this thesis was to identify disease pathways. Apothecia were observed in orchards and there was a the higher incidence of *S. sclerotiorum* in petals and stamens than other floral tissues. Rapid colonisation of flower petals by ascospores was demonstrated. This suggests the most probable primary infection pathway of sclerotinia disease in kiwifruit is through ascospore infection of petals and stamens. In addition, it is likely that the majority of primary infections occurs during flowering and petal-fall. This is because the extensive colonisation of these susceptible tissues by *S. sclerotiorum* and other micro-organisms would leave little opportunity for colonisation by *S. sclerotiorum* later in the season.

Observations of disease symptoms in orchards confirmed that AFT on developing fruit were frequently a site for secondary spread of this disease, as proposed by Pennycook (1985) and Manning (1991). Adhering floral tissues were common on 'Hayward' kiwifruit and were shown to be a component of sclerotinia epidemiology as a source of mycelial inoculum. Secondary spread of this disease led to the formation of diseased fruitlets, scarring and field rot (Chapter 3 and 6). This highlights similarities with sclerotinia disease in crops such as bean (Abawi & Grogan, 1979), pea (Huang & Kokko, 1992) and brassica spp. (McLean, 1958; Gugel & Morrall, 1986).

The reason for petals and stamens failing to abscise from pistillate flowers at petal-fall was not specifically established in this study. In some instances the extensive colonisation of petals and stamens by *S. sclerotiorum*, as occurred in those from diseased fruitlets and fruit with scarring symptoms, may contribute to the incidence of AFT. There may also be a genetic basis for petals and stamens failing to abscise. Fruit of the cultivar 'Hort16A' (Zespri Gold™) have a very low incidence of AFT (Henry

Pak, pers. com.), but this has yet to be quantified. If the tendency for these floral tissues to adhere has a genetic basis, then cultivars that have a lower incidence of AFT may be less prone to sclerotinia disease. Further research is required to determine the incidence of AFT on the existing and new commercial *Actinidia* cultivars.

The presence of petals on flowers and AFT on fruit that are colonised by *S. sclerotiorum* represent a pool of secondary inoculum from which disease develops when moist conditions are favourable (Chapter 6). This has four important epidemiological consequences: (i) floral tissues are the site of deposition of disease-causing primary inoculum, (ii) reduction in the incidence and severity of *S. sclerotiorum* colonisation of floral tissues should reduce subsequent disease risk, (iii) disease progress is incremental and sclerotinia in kiwifruit can therefore be considered a simple interest epidemic, (iv) disease risk may be predicted by determining primary inoculum levels or the quantity of *S. sclerotiorum* within petals. The latter is a similar technique to that used for sclerotinia disease risk prediction in Canadian canola crops (Turkington *et al*., 1991; Turkington & Morrall, 1993).

The detached petal assay was developed (Chapter 4) to study the effects of host and environmental conditions on the colonisation of kiwifruit petals by ascospores (Chapter 5). The positive relationship between the percentage area of lesions on petals and with increasing temperature in the range of 15–20^{o}C, suggested this assay was adequately sensitive to the degree of colonisation by *S. sclerotiorum*. The maximum number of cfu/petal achieved in these experiments ranged from 2400–6000, equivalent to 24 000–60 000 cfu/g of dried petal tissue. The minimum detectable limit ranged from 40–100 cfu/petal.

Changes in petal physiology during senescence, such as reduced enzyme activity and solute leakage (Borochov & Woodson, 1989; Celikel & van Doorn, 1995) could explain the large increase in susceptibility of kiwifruit petals to colonisation by *S. sclerotiorum* ascospores. The steep response of mycelial growth rate and petal colonisation to temperature between 15 and 20^{o}C suggests that prevailing temperatures during flowering may strongly influence the rate of infection and extent of colonisation of petals by ascospores.

S. sclerotiorum ascospores colonised detached kiwifruit petals without the addition of free water (Chapter 5) and the positive relationship between apothecial density and petal colonisation across several orchards during two seasons (Chapter 7, p. 164 and 170) suggests ascospores can colonise petals under a range of field conditions. However, petal colonisation was significantly reduced at relative humidities below 90% and under fluctuating temperature and/or RH. Under field conditions ascospore infection probably takes place during periods when RH remains above 90% for at least 48 h, including conditions under which dew forms on susceptible plant surfaces. Rainfall is probably also conducive to infection, but the effects of free water on infection of petals were not examined in these studies.

Diurnally fluctuating environmental conditions within kiwifruit orchards and during controlled environment experiments were represented on psychrometric charts (Figure 5.19 and 5.20). The very low number of cfu/petal under conditions that closely matched the mean 24-hourly psychrometric plots for low rainfall days during November and December 1996–99, suggests that weather patterns with similar plots will not be favourable for primary infection. Further experimentation is required to determine the rate of colonisation of petals under other diurnally fluctuating conditions also representative of those occurring during kiwifruit flowering and petal-fall.

Free moisture supplied by a misting system for 9–120 h within kiwifruit vines, as opposed to ambient conditions or those within a protective plastic tent, provided favourable conditions for the development of diseased fruitlets (ascospore inoculation), scarring, and field rot (Chapter 6). This is the first report that sclerotinia disease symptoms in kiwifruit have been artificially induced and supports the modes of primary infection and secondary spread proposed above.

The ability of ascospores to incite infections on fruit during the growing season requires further investigation, because Experiments 6-5 and 6-6 were inconclusive. The minimum period of wetness duration required for infection of fruit from colonised petals was not determined in this study because disease symptoms developed with the shortest duration of misting tested (9 h). Further work is necessary to better understand the interaction between actual wetness duration and temperature under a range of field conditions.

This is the first report on kiwifruit that the substrate on which sclerotia develop (diseased fruitlets vs. field rot) affects germination rate, sclerotial size and the number and size of apothecia produced. These effects are possibly mediated through the degree of exposure to soil micro-organisms (Merriman *et al*., 1979; Mitchell & Wheeler, 1990) and the limitation of reserves within small sclerotia. Because sclerotia occupy distinct ecological niches, it is proposed that there are ecologically distinct populations of sclerotia in kiwifruit. Further study of the effects of pruning staminate vines and removal of these prunings on sclerotial production is recommended.

The potentially large numbers of sclerotia produced from individual fruit with field rot (up to 100/fruit) and from infection of weeds beneath staminate vines after pruning, can lead to intensely aggregated production of sclerotia and therefore aggregation of apothecia. Aggregation of apothecia will lead to a variable ascospore concentration in the air and ascospore density on plant surfaces (Gregory, 1973) possibly contributing to variation of disease incidence (Boland & Hall, 1988a; Boland & Hall, 1988b). Ascospore density on kiwifruit floral tissues could be modelled using known principles of spore liberation, dispersion and sedimentation (Gregory, 1973; Aylor, 1998) together with modelling of air flow dynamics in kiwifruit blocks (Judd *et al*., 1993) and the spore trapping efficiency of flowers.

Apothecia were produced by *S. sclerotiorum* during the period of flowering of staminate and pistillate kiwifruit vines in South Auckland, Waikato and Bay of Plenty. The level of primary inoculum during flowering and petal-fall when susceptible tissues are present is likely to be variable between locations and growing seasons due to different sclerotial densities and because carpogenic germination is dependent on a high soil moisture content for several days or weeks (Abawi & Grogan, 1979; Purdy, 1979; Imolehin *et al*., 1980). Therefore the influence of rainfall, wind, crop canopy and ground cover on soil moisture, apothecial production and longevity, and ascospore release and net deposition on floral tissues warrant further investigation.

Several distinct disease symptoms were caused by *S. sclerotiorum* over a 2–3 month period, starting at flowering (Chapter 2, p. 23–30). This raises the question as to why all occurrences of *S. sclerotiorum* within floral tissues do not lead to disease symptoms immediately after anthesis? This could be because some secondary spread of disease is

dependent on new ascospore infection of fruit. However, this infection pathway remains unproven.

Another explanation might be that varying inoculum potential of *S. sclerotiorum* within petals at flowering and within AFT on fruit affects the ability of *S. sclerotiorum* mycelium to spread into adjacent healthy tissues, i.e. to cause secondary spread. For example, a fully colonised petal, such as those incubated at 22.5 and 25^{o}C in Experiment 5-8 and used as inoculum in Experiments 6-2, 6-3 and 6-4, can be expected to have a higher inoculum potential compared with a partially colonised petal or AFT. A flower with a petal that has a high inoculum potential may have a higher probability of developing into a diseased fruitlet. Adhering floral tissues on fruit will tend to have variable degrees of colonisation because of the other fungi (Figure 3.4), bacteria and yeast's present. If this equates to variable inoculum potential in AFT, then the optimal conditions for secondary spread from individual AFT may also vary. A diseased fruitlet or fruit with field rot will likely have high inoculum potential if it is in contact with adjacent fruit/leaves (Plate 2.6 and 2.9 B). The consequence of this variability in the level of inoculum potential within floral tissues and between different sources of infection, may be that secondary spread occurs sporadically depending on the micro-climate conditions.

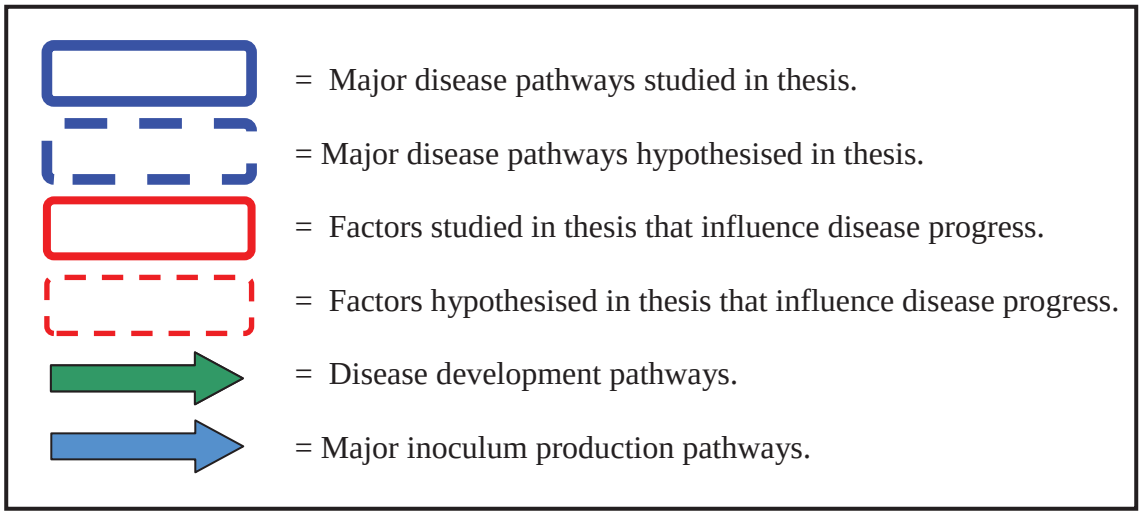
It is proposed that there are several key components of sclerotinia disease in kiwifruit:

- The presence of apothecia as a source of primary ascospore inoculum.
- Flowering of kiwifruit provides a source of susceptible host tissue.
- Primary infection of petals, and probably stamens, by *S. sclerotiorum* ascospores.
- Secondary spread into the permanent floral tissues leading to diseased fruitlets.
- Developing fruit which have AFT colonised by *S. sclerotiorum*.
- The presence of surface moisture to facilitate secondary spread of disease by mycelial infection between diseased, especially AFT, and healthy tissues.
- The production and over-wintering of sclerotia from disease tissues.

8.2 Conceptual model of sclerotinia disease in kiwifruit

Interpretation of the experimental work and observations carried out in this study and of the literature on *S. sclerotiorum* in other crops has led to the proposal of a conceptual model of sclerotinia disease in kiwifruit. Figure 8.1 gives a diagrammatic representation of inoculum production and disease development of sclerotinia within New Zealand kiwifruit and illustrates the linkages between the different stages in the life cycle and the various factors that influence disease progress. This conceptual model also distinguishes between information that has been directly studied in this thesis and hypothetical relationships.

Figure 8.1 KEY:



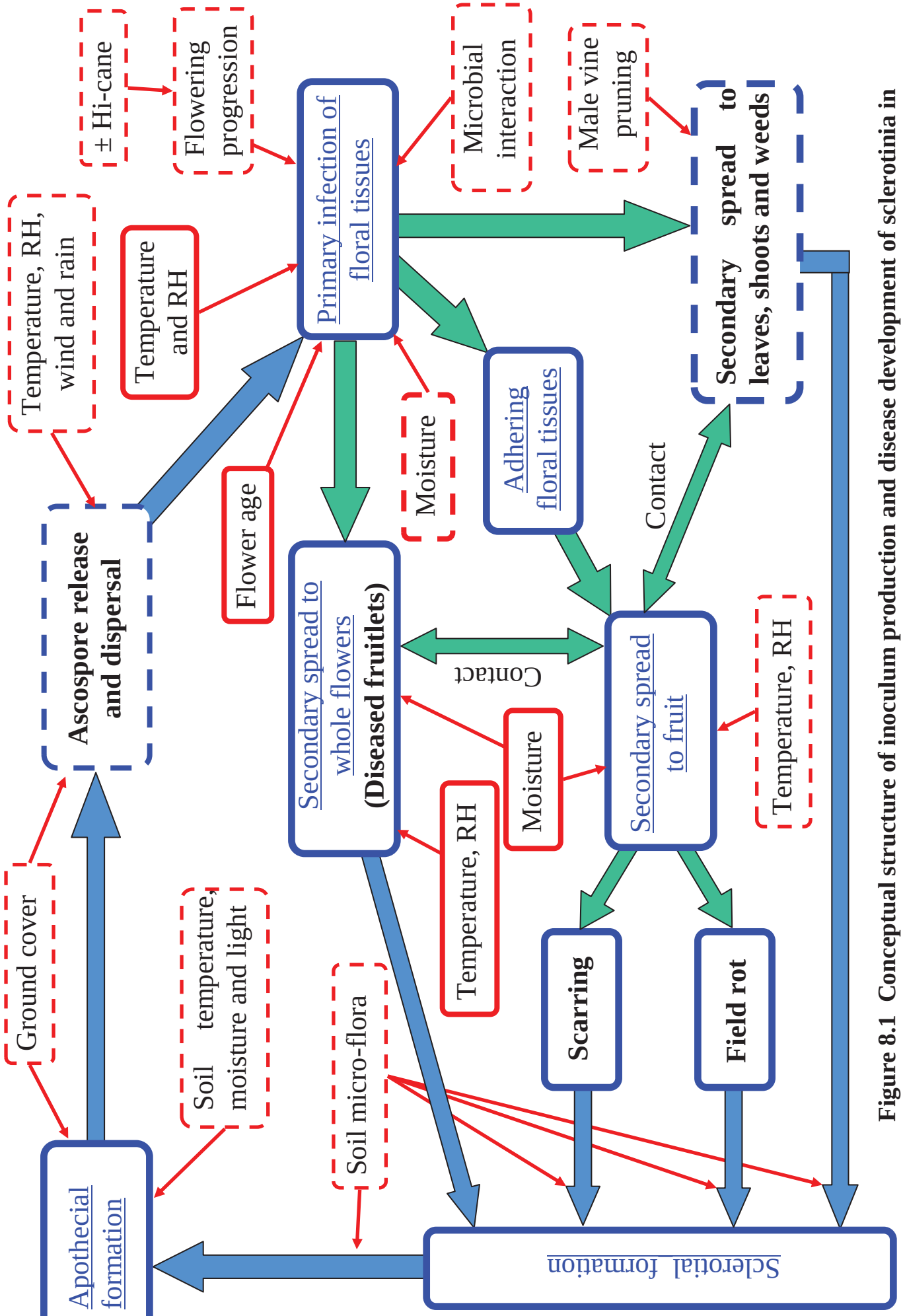
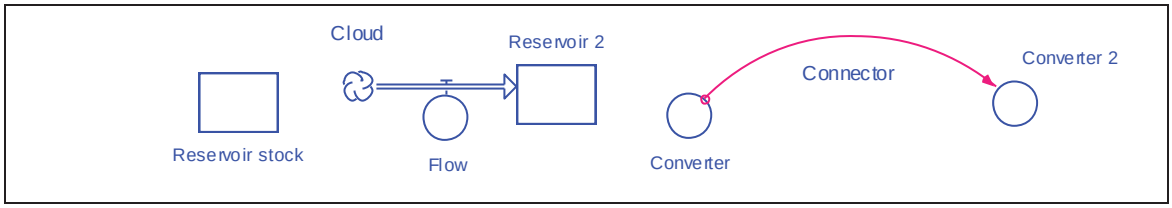


Figure 8.1 Conceptual structure of inoculum production and disease development of sclerotinia in

8.3 Disease prediction

The understanding of the disease cycle and the epidemiological knowledge gained from these studies gives insights into factors that affect disease progress of sclerotinia in kiwifruit. When this knowledge is applied to kiwifruit crop management and industry practices it can be used to help plan disease management procedures to minimise the impact of sclerotinia. The extent to which this knowledge can be taken further to predict disease losses using inputs which reflect weather, inoculum and host susceptibility, warrants further research. Accurate prediction of crop loss may be difficult given the variability shown in these studies. An alternative approach could be prediction of disease risk potential using relevant components of disease progress. Disease risk can be defined as the upper limit for expression of actual disease for a given inoculum level when all other factors contributing to disease progress are optimal.

A proposed structure for models that describe disease risk prediction (Figure 8.2) and disease incidence prediction (Figure 8.3) has been developed for sclerotinia in kiwifruit using a systems analysis modelling approach (Jeffers, 1978) and the modelling software Ithink® (High Performance Systems Inc.). The mathematical relationships required to make the model quantitative have not yet been developed. The symbols used in these structural models are defined below:



Reservoir stocks (rectangular box) are accumulators i.e. they collect whatever flows into and out of them.

Flows serve the purpose of filling and emptying reservoir stocks, according to relevant relationships.

Clouds are an unlimited source of units that can be added to (or taken from) reservoir stocks via flows.

Converters (circle) hold values for constants, define external inputs to the model, calculate algebraic relationships, and serve as the repository for graphical functions.

Connectors (arrows) connect elements within the model.

The proposed inputs for determining 'Current Risk' (Figure 8.2) are:

- 'Environmental Conditions' influence disease risk by affecting 'Soil Moisture', 'Ascospore Concentration' and the 'Primary Colonisation' of floral tissues.
- 'Soil moisture' under kiwifruit vines will strongly influence apothecial production and survival and is a key variable currently used in a sclerotinia risk prediction system in Canadian canola crops (Canola Connection – Weather and crop forecasts http://www.canola-council.org/).
- 'Ground Cover' beneath kiwifruit vines is likely to influence changes in soil moisture, particularly during periods with no rainfall, and possibly affects ascospore concentration through interaction with dispersal mechanisms.
- 'Apothecial density' or the incidence of quadrats with apothecia as suggested by Pak *et al.* (1997), affects disease risk because apothecia are the source of primary inoculum and is related to disease through the relationship shown in Figure 7.11 B.
- 'Ascospore Concentration' in the air is largely determined by 'Apothecial density', 'Environmental Conditions' and the effects of 'Ground Cover'.
- The incidence and extent of colonisation of floral tissues by *S. sclerotiorum* is represented by 'Primary Colonisation' and equates to inoculum potential for disease. This is determined by the interaction between 'Ascospore Concentration', the prevailing 'Environmental Conditions', 'Microbial Interaction' and the timing and progression of 'Flowering'.
- 'Primary Colonisation' in this study has been quantified by 'Petal Incubation' on sterile agar medium and in humid chambers, but could also be determined by 'Rapid Detection' utilising DNA-based or immunological-based technologies.
- 'Previous Disease Risk', based on a historical 'Database' of measured disease incidence, disease risk predictions or by quantification of 'Sclerotial Population' within orchard soil.
- 'Current Risk' could be refined 2–3 weeks after flowering by taking into account the 'Incidence of AFT' on fruit and the 'AFT Colonisation' (i.e. incidence of AFT with *S. sclerotiorum*), which are measures of the main inoculum source for fruit scarring and field rot.

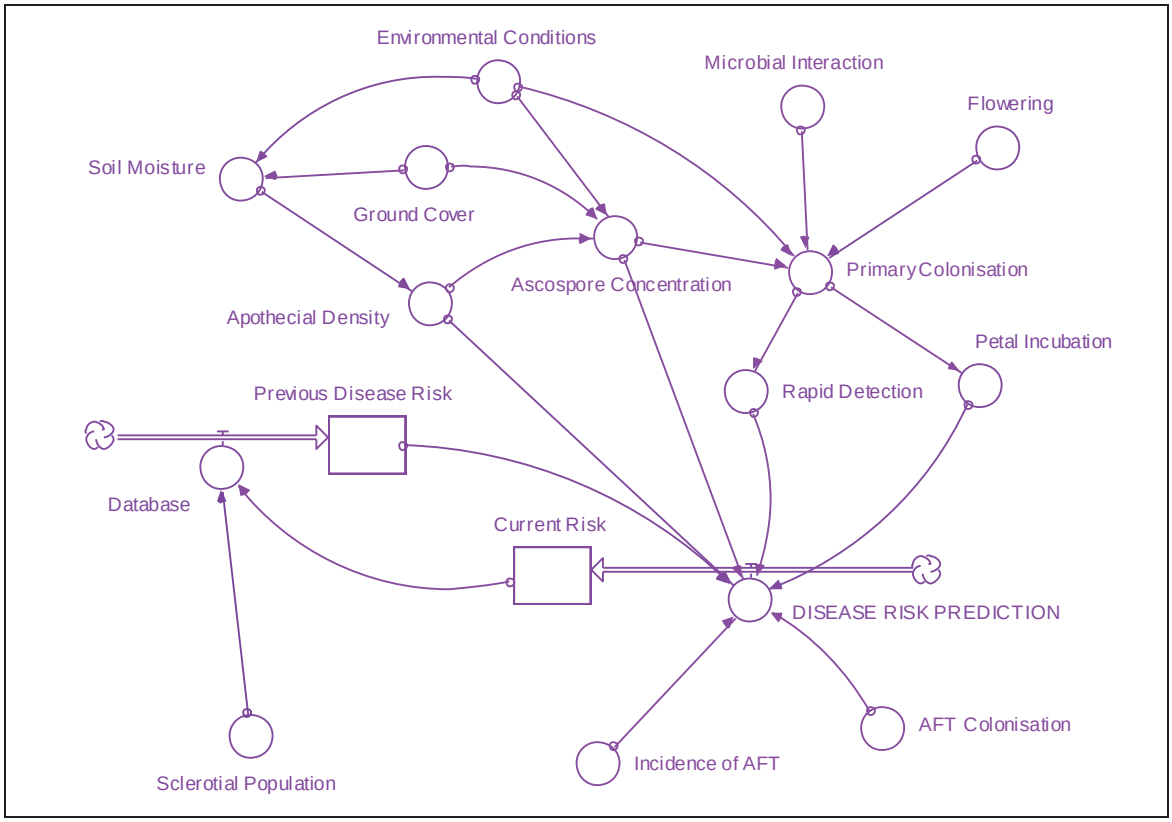


Figure 8.2 Structure of a systems analysis model of disease risk of sclerotinia in kiwifruit constructed using Ithink modelling software.

'Ascospore Concentration' could be estimated by use of agar plate spore trapping, however, selective media such as Kerrsies and John Knight's require ca. 2 weeks incubation and therefore give results too late for making decisions on fungicide use during flowering and petal-fall. Efficient sampling strategies need to be developed for determining the density of apothecia within orchards. Knowledge of soil moisture on a regional basis could provide useful information for scheduling apothecial assessments.

Methods used to measure *S. sclerotiorum* incidence in petals in this study included incubation of non-surface-sterilised petals and plating of petals on PDA after surface sterilisation. The former takes 5–7 days for a result, whilst *S. sclerotiorum* growth on PDA can be determined after ca. 4–5 days. Use of a semi-selective PDA-based medium (Steadman *et al*., 1994) can distinguish *S. sclerotiorum* growth in 3 days (unpublished data). A method for rapidly quantifying petal infection is currently not available to the industry, but could be developed with existing products, such as the monoclonal antibody plate trapped antigen Enzyme Linked Immunosorbant Assay (ELISA) Identikit Q™ specific to *S. sclerotiorum* and which produces results within 20 h (Agden, 2000).

The effects of micro-climate on colonisation of petals by *S. sclerotiorum* ascospores could be modelled using results from infection assays carried out under diurnally fluctuating conditions that match a range of micro-climate conditions in kiwifruit orchards during November and December (Chapter 5, p. 124).

The proposed inputs for determining 'Disease Incidence' (Figure 8.3) are:

- An estimate of the maximum disease can be derived from 'Current Risk' (Figure 8.2) and used to set the upper limit of 'Disease Incidence'.
- 'Environmental Conditions', particularly surface wetness, will affect the mycelial infection of fruit (Chapter 6).
- 'Disease Assessment' data derived from actual sampling will increase prediction accuracy and could be used to modify prediction of 'Disease Incidence'.
- The 'Incidence of AFT' on fruit and 'AFT Colonisation' used for determining 'Current Risk' are also relevant to the prediction of 'Disease Incidence'.
- The 'Growth Stage' determines the symptom types that develop and impacts on the production of sclerotia (Figure 7.1).
- 'Fruit Set' represents the initial crop load and predicted disease incidence will be a proportion of this value.
- The effects of disease control options carried out within orchards e.g. fungicide application, is represented by 'Managed Disease Reduction' and depends on reliable efficacy data under a range of disease risk levels and seasonal conditions.
- 'Sclerotial Production' can be estimated from the predicted 'Disease Incidence' and production of sclerotia from diseased fruitlets and fruit with field rot (Chapter 7, p. 151).

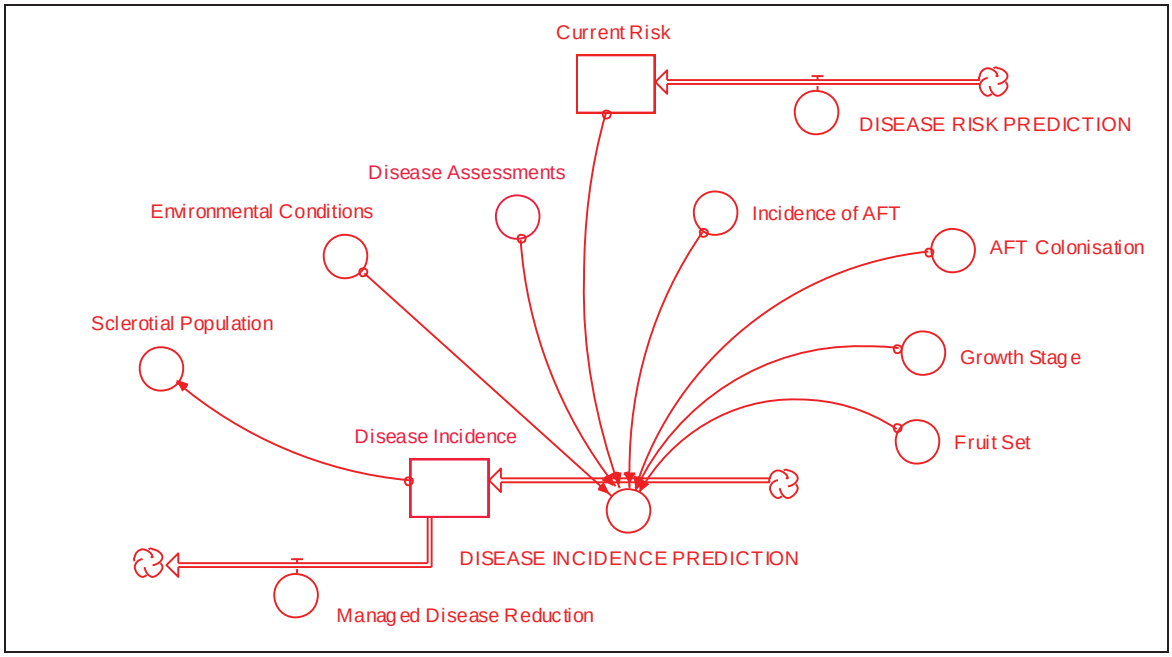


Figure 8.3 Structure of a systems analysis model of disease incidence of sclerotinia in kiwifruit constructed using Ithink modelling software.

8.4 Management of sclerotinia

There are several implications of this research for the management of sclerotinia disease in kiwifruit, many of which are apparent from the conceptual model of sclerotinia (Figure 8.1) and the two systems analysis models of disease risk and disease incidence (Figure 8.2 and 8.3). Many of the orchardists spoken to during orchard visits were not fully aware of the range of sclerotinia symptoms and either did not recognise sclerotinia diseased fruitlets or confused these symptoms with bacterial blossom blight. Clearly, the education of orchardists, managers and consultants with respect to disease symptoms and the basic aspects of disease pathways is an important first step to improved disease management. This has been achieved, in part, during the course of this study through presentations at two grower field days, two seminars (Hoyte, 1997; Hoyte *et al.*, 1998), and two research conferences (Hoyte *et al.*, 1999; Hoyte, 2000). Other industry-based publications include a popular article (Hoyte, 1996) and an update of the KiwiGreen© Manual (Appendix 6 and 7).

Effective disease management for sclerotinia needs to be based on the understanding that disease risk is largely determined by the extent of petal colonisation during flowering and petal-fall. Therefore, orchard management that reduces primary infection of floral tissues will potentially reduce the risk of disease progress throughout the

remainder of the season. Pennycook (1985) highlights the importance of fungicide treatment during flowering. The results from this study confirm that fungicides should be applied during flowering. Emphasis should be made to ensure maximum coverage of open flowers because floral tissues are the site of primary infection and are the source of secondary spread into fruit. Possible effects of fungicide applications on kiwifruit pollination requires further investigation.

The optimal timing of fungicide applications warrants investigation in view of the effects of flower age on susceptibility to colonisation (Chapter 5, p. 94–98) and the influence of Hi-cane on the timing and duration of flowering (Henzell, 1986; Walton & Fowke, 1993). The ability of Benlate™ to protect un-opened flowers through its partial systemic activity has not been directly studied in kiwifruit and this also warrants investigation.

Current recommendations are for a second fungicide application to be made during high disease risk periods from December to February (Pennycook, 1985; Sale, 1993; Walton & Sommerville, 1998). The suggestion from Pennycook (1985) that fungicides be timed to coincide with 'ascospore showers' may not be appropriate during the fruit growth stage because this study showed that most fruit disease arises from AFT colonised by *S. sclerotiorum* before symptom development. Focusing disease control to ensure protection of flowers during flowering and petal-fall may be more effective than a single application later in the season or splitting two fungicide applications between flowering and later in the season.

The application of fungicides to the orchard soil to disrupt or destroy apothecia has been suggested (Pennycook, 1982), although the presence of grass and weed ground cover may limit the penetration of fungicides. This is very unlikely to become a feasible disease control option because such treatment would not meet the standards of the environmental management system currently being developed in the industry (Linda Harley, pers. comm.).

The cultural control strategies of early pruning of staminate vines, removal of vine prunings and use of air-blast sprayers to dislodge senescing petals and stamens, suggested by Manning (1991) and Pennycook (1985), are sound recommendations

based on the research in this study, although neither has been validated. Spraying fungicide beneath staminate vines before pruning may also reduce production of sclerotia by reducing secondary spread onto weeds. Removal of any diseased fruitlets and fruit with field rot from kiwifruit blocks during fruit thinning could also decrease the level of sclerotial populations in orchard soils. Pruning management of shelter belts may assist with increasing air-flow to reduce wetness periods, since well sheltered orchard blocks tend to have low wind velocities (1–3 m s^{-1}) beneath vines (Judd *et al*., 1993).

The secondary spread of disease onto kiwifruit fruit is reduced by removal of AFT within 1–2 weeks of the petal-fall (Chapter 3, p. 48). The technique of passing air-blast sprayers through orchard blocks is unlikely to be effective unless AFT colonised by *S. sclerotiorum* are removed from most fruit. If this technique was shown to be effective, then the efficacy of fungicide applications made with air-blast sprayers would therefore also include a non-fungicidal component of disease control. Removal of AFT from fruit by hand is un-likely to be economic. Each of the above potentially useful cultural control techniques need to be evaluated within moderate–high disease risk orchards to determine the cost of these measures and the extent to which they can reduce crop loss and inoculum production.

Biological control strategies may reduce sclerotinia disease and inoculum build-up within kiwifruit blocks. Biological control of sclerotinia disease of bean and canola using competitive and/or anti-fungal microbes to reduce the colonisation of floral tissues by *S. sclerotiorum* has been studied with some success (Boland & Inglis, 1989; Zhou & Reeleder, 1991; Hutchins & Archer, 1994). In kiwifruit, Elmer *et al.* (1999a) have shown that the application of an *E. purpurescens* spore suspension reduced petal infection as effectively as the standard fungicide, Rovral, suggesting biological control of sclerotinia at flowering may be feasible. The fungal myco-parasite *Coniothyrium minitans* has been researched for its ability to degrade sclerotia of *S. sclerotiorum* and other fungi (Trutmann *et al*., 1982; Budge & Whipps, 1991b; Gerlagh *et al*., 1995), but has only recently been evaluated in kiwifruit (A. Stewart, pers. comm.).

Quantitative prediction of sclerotinia would provide the New Zealand kiwifruit industry with a useful tool for the prediction of crop loss and efficient intervention with

fungicides or other control actions. Targeting of disease management to areas (several blocks or whole orchards) where disease risk is high may offer scope for a reduction in fungicide applications. The adoption of a disease risk prediction system requires a considerable shift in management philosophy away from an 'insurance' approach to fungicide application. Yet, because of recent good profits for kiwifruit orchardists (Anonymous, 1999a), the cost of spraying fungicides on kiwifruit can be justified even if the level of potential fruit loss is very small (<1% of crop load). Thus, the economics of chemical control and the apprehension associated with the withholding of sprays in low disease risk sites can be expected to hinder the uptake of a management philosophy based on disease risk.

A proposed structure of a sclerotinia management system for kiwifruit has also been developed using a systems analysis modelling approach (Figure 8.4) and the proposed inputs are:

- 'Current Risk' from Figure 8.2 and 'Disease Incidence' from Figure 8.3.
- 'Botrytis Risk' can have an impact on the orchardists choice of fungicide for sclerotinia disease control (Manning *et al*., 1995).
- Specifications for 'Export Requirements' relating to fungicide-free fruit can also impact on the choice of fungicide for sclerotinia disease control. There may also be other factors that orchardists could take into account and these are represented by 'User Defined 1'.
- A range of 'Control Options' are available to orchardists, including 'Synthetic Fungicides', 'Cultural Techniques' and possibly in the future 'Biological Control Agents'. For each of these there will be a range of considerations relating to timing and costs.
- The decisions made within the 'Sclerotinia Management System' may have a series of possible 'Outcomes to Reduce Sclerotinia' including: 'Apply Fungicide', use an 'Air Blast Sprayer' to dislodge adhering petals, adjust the 'Pruning Schedule' and 'Removing Prunings' from staminate vines, 'Fruit Thinning' to remove diseased fruitlets or fruit with field rot, and possibly in the future 'Apply BCA' (biological control agents). Any other control techniques are represented by 'User Defined 2'.
- The 'Efficacy Data' specific to each 'Control Option' will determine the impact of any implemented disease control on the level of 'Managed Disease Reduction', and this remains to be determined for current and future 'Control Options'.

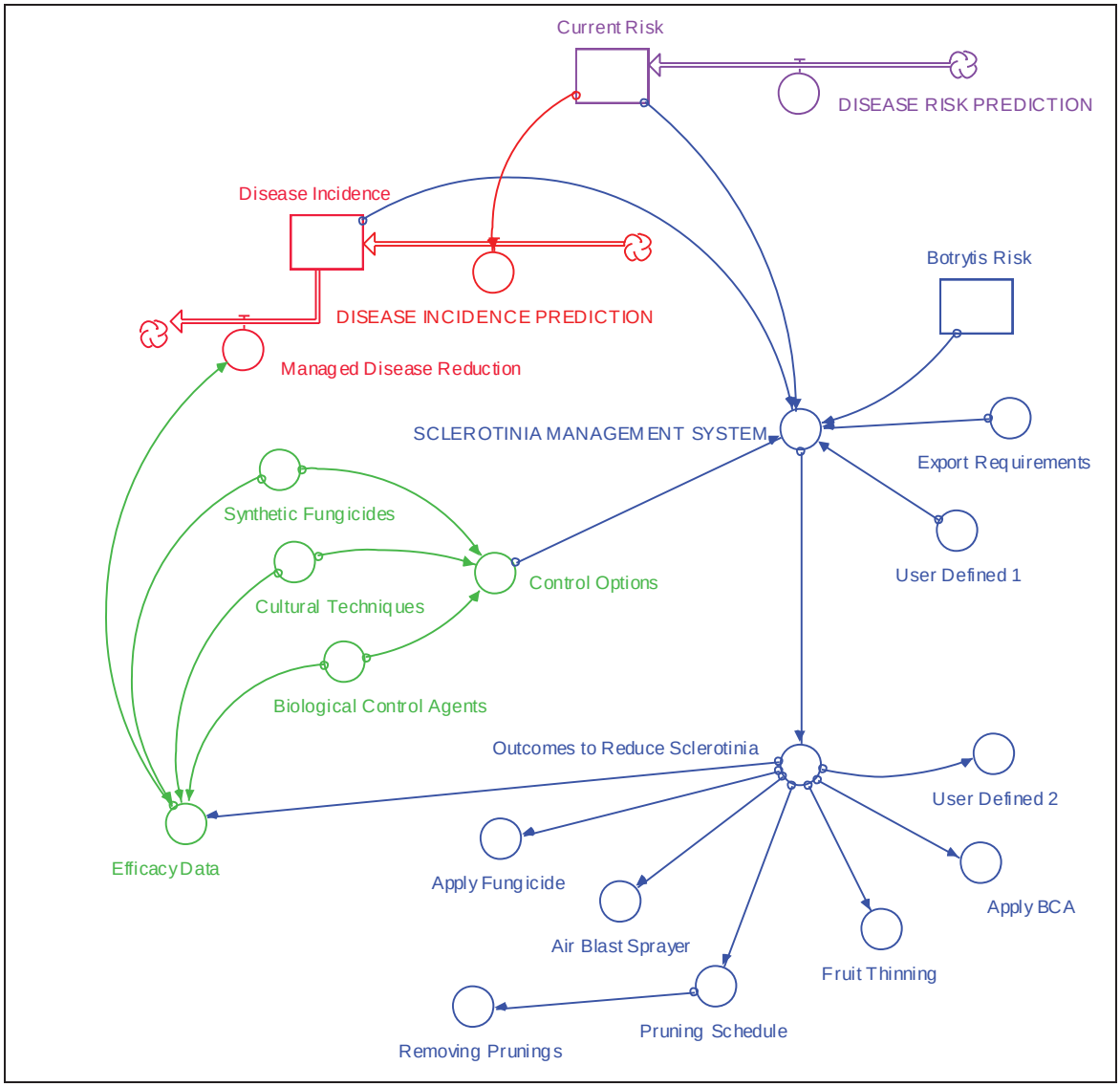


Figure 8.4 Structure of a systems analysis model of a sclerotinia management system for kiwifruit constructed using Ithink modelling software.

8.5 Conclusions

This study has advanced the understanding of many aspects of *S. sclerotiorum* epidemiology in kiwifruit. Disease progress has been more clearly defined through field observations and investigation of the role of AFT, relationships between apothecial density and petal infection, the effects of environmental conditions on ascospore colonisation of petals, and the effects of environmental conditions on mycelial infection of fruit.

Aspects of this research could be utilised to formulate many of the under-lying processes represented in the proposed models of disease risk and disease incidence.

Completion of these models will also depend on a sound understanding of relevant biological and physical relationships, mathematical principles, information from the literature, personal knowledge and intelligent guesses (Analytis, 1980). The validation of the performance and operation of such models needs to form the basis of further research and may offer many opportunities for improvements in disease management (Zadoks & Schein, 1979).

When these proposed models are combined within a sclerotinia management system, a unit will be created that can be further integrated with other models that have been generated for kiwifruit e.g. timing of bud-burst and flowering (Morley-Bunker & Salinger, 1987; McPherson *et al*., 1994), fruit growth (Gandar *et al*., 1996), environmental variables (Atkins *et al*., 1992; Judd *et al*., 1993), and economics of management changes (Doyle *et al*., 1989; Lescourret & Habib, 1996).

To date there has been little published information on the epidemiology of *S. sclerotiorum* in kiwifruit and on the short to medium term effects of any cultural or biological disease management practices. Validation of the relationships between existing and new control options and disease progress is a necessary step to provide a sound basis to implementing an effective sclerotinia disease management system in the kiwifruit industry.

References

Abarca, M.L., Bragulat, M.R., Castella, G. & Cabanes, F.J. (1994). Mycoflora and aflatoxin-producing strains in animal mixed feeds. *Journal of Food Protection* **57,** 256-258.

Abawi, G.S. & Grogan, R.G. (1974). Ascospores of *Whetzelinia sclerotiorum* as primary inoculum causing white mold of beans in New York. *Phytopathology* **64,** 578.

Abawi, G.S. & Grogan, R.G. (1975). Source of primary inoculum and effects of temperature and moisture on infection of beans by *Whetzelinia sclerotiorum*. *Phytopathology* **65,** 300-309.

Abawi, G.S. & Grogan, R.G. (1979). Epidemiology of diseases caused by *Sclerotinia* species. *Phytopathology* **69,** 899-904.

Abawi, G.S., Polach, F.J. & Molin, W.T. (1975). Infection of bean by ascospores of *Whetzelinia sclerotiorum*. *Phytopathology* **65,** 673-678.

Abawi, G.S., Provvidenti, R., Crosier, D.C. & Hunter, J.E. (1978). Inheritance of resistance to white mold disease in *Phaseolus coccineus*. *Journal of Heredity* **69,** 200-202.

Adams, P.B. & Ayers, W.A. (1979). Ecology of *Sclerotinia* species. *Phytopathology* **69,** 896-899.

Agden. (2000). Plant Disease Diagnostics Catalogue 2000. Agden Agrifoods Diagnostics. Ayr. pp. 35.

Agostini, D. & Habib, R. (1996). A stochastic model of flowering for kiwifruit. *Acta Horticulturae* **416,** 65-72.

Alderman, S.C. & Lacy, M.L. (1983). Influence of dew period and temperature on infection of onion leaves by dry conidia of *Botrytis squamosa*. *Phytopathology* **73,** 1020-1023.

Alexander, B.J.R. & Stewart, A. (1994). Survival of sclerotia of *Sclerotinia* and *Sclerotium* spp. in New Zealand horticultural soil. *Soil Biology and Biochemistry* **26,** 1323-1329.

Alexandra, G.P. (1986). Kiwifruit - male vines selection, distribution and husbandry techniques. In: Proceedings of the Ministry of Agriculture and Fisheries, Kiwifruit Pollination Seminar, Tauranga. **October,** 1-8.

Analytis, S. (1980). Obtaining of sub-models for modelling the entire life cycle of a pathogen. *Journal of Plant Diseases and Protection* **87,** 371-382.

Anderson, J.B., Kohn, L.M. & Leslie, J.F. (1992). Genetic mechanisms in fungal adaption. In: *The Fungal Community: its organisation and role in the ecosystem*, vol. 9. (ed. G. C. Carroll and D. T. Wicklow) pp. 73-98. Marcel Dekker, Inc., New York.

Anonymous. (1998). Annual Report '98. Kiwifruit New Zealand Ltd. pp. 62.

Anonymous. (1999a). Annual Report '99. Kiwifruit New Zealand Ltd. pp. 62.

Anonymous. (1999b). KiwiGreen Manual. Kiwifruit New Zealand Ltd. Tauranga. pp. 135.

Anonymous. (1999c). KNZ Quality Manual. New Zealand Kiwifruit Marketing Board. pp. 290.

Atkins, T.A., Laurenson, M.R., Mills, T.M. & Ogilvie, D.K. (1992). Orchard 2000: Towards a decision support system for New Zealand's orchard industries. *Acta Horticulturae* **313,** 173-182.

Atlas, R.M. & Bartha, R. (1981). *Microbial Ecology: fundamentals and applications*. Addison-Wesley Publishing Co., Reading. 560 p.

Aylor, D.E. (1998). The aerobiology of apple scab. *Plant Disease* **82,** 838-849.

Bakr, M.A. & Grewal, J.S. (1987). Effect of various nitrogen source on growth and sclerotia formation of *Sclerotinia sclerotiorum*. *Indian Journal Mycology and Plant Pathology* **17,** 150-153.

Beever, D.J. & Hopkirk, G. (1990). Fruit development and fruit physiology. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 97-126. Ray Richards, Auckland.

Ben-Yephet, Y. & Bitton, S. (1985). Use of a selective medium to study the dispersal of ascospores of *Sclerotinia sclerotiorum*. *Phytoparasitica* **13,** 33-40.

Ben-Yephet, Y., Genizi, A. & Siti, E. (1993). Sclerotial survival and apothecial production by *Sclerotinia sclerotiorum* following outbreaks of lettuce drop. *Phytopathology* **83,** 509-513.

Ben-Yephet, Y. & Siti, E. (1987). Lettuce drop caused by inoculum of *Sclerotinia sclerotiorum* blown-in from a distance. *Phytoparasitica* **15,** 69-71.

Beresford, R.M. & Spink, M. (1992). A national disease forecasting system for apple black spot (*Venturia inaequalis*) in New Zealand. *Acta Horticulturae.* **313,** 285-296.

Blad, B.L., Steadman, J.R. & Weiss, A. (1978). Canopy structure and irrigation influence white mold disease and microclimate of dry edible beans. *Phytopathology* **68,** 1431-1437.

Blakeman, J.P. & Williamson, B. (1994). *Ecology of plant pathogens*. CAB International, Wallingford. 362 p.

Boland, G.J. & Hall, R. (1987). Epidemiology of white mold of bean in Ontario. *Canadian Journal of Plant Pathology* **9,** 218-224.

Boland, G.J. & Hall, R. (1988a). Numbers and distribution of apothecia of *Sclerotinia sclerotiorum* in relation to white mold of white bean (*Phaseolus vulgaris*). *Canadian Journal of Botany* **66,** 247-252.

Boland, G.J. & Hall, R. (1988b). Relationships between the spatial pattern and number of apothecia of *Sclerotinia sclerotiorum* and stem rot of soybean. *Plant Pathology* **37,** 329-336.

Boland, G.J. & Hall, R. (1994). Index of plant hosts of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* **16,** 93-108.

Boland, G.J. & Hunter, J.E. (1988). Influence of *Alternaria alternata* and *Cladosporium cladosporioides* on white mold of bean caused by *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* **10,** 172-177.

Boland, G.J. & Inglis, G.D. (1989). Antagonism of white mold (*Sclerotinia sclerotiorum*) of bean by fungi from bean and rapeseed flowers. *Canadian Journal of Botany* **67,** 1775-1781.

Borochov, A., Drori, A., Tirosh, T., Borochov, N.H. & Mayak, S. (1990). Quantitative and qualitative changes in membrane proteins during petal senescence. *Journal of Plant Physiology* **136,** 203-207.

Borochov, A. & Woodson, W.R. (1989). Physiology and biochemistry of flower petal senescence. *Horticultural Reviews* **11,** 15-43.

Bourdôt, G.W., Saville, G.A., Hurrell, G.A., Harvey, I.C. & de Jong, M.D. (2000). Risk analysis of *Sclerotinia sclerotiorum* for biological control of *Cirsium arvense* in pasture: sclerotium survival. *Biocontrol Science & Technology* **10,** 411-425.

Boyle, C.D. & Kropp, B.R. (1992). Development and comparison of methods for measuring growth of filamentous fungi on wood. *Canadian Journal of Microbiology* **38,** 1053-1060.

Bristow, P.R., McNicol, R.J. & Williamson, B. (1986). Infection of strawberry flowers by *Botrytis cinerea* and its relevance to grey mould development. *Annals of Applied Biology* **109,** 545-554.

Brook, P.J. (1990a). Diseases in kiwifruit. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 420-428. Ray Richards, Auckland.

Brook, P.J. (1990b). Review: *Botrytis cinerea* and fruit rot of kiwifruit, *Actinidia deliciosa*. Report to New Zealand Kiwifruit Marketing Board. pp. 92.

Broome, J.C., English, J.T., Marois, J.J., Latorre, B.A. & Aviles, J.C. (1995). Development of an infection model for *Botrytis* bunch rot of grape based on wetness duration and temperature. *Phytopathology* **85,** 97-102.

Brun, H. & Renard, M. (1983). *Sclerotinia sclerotiorum* on rape: inoculation techniques. *Agronomie* **3,** 93.

Brundell, D.J. (1975). Flower development of the Chinese gooseberry (*Actinidia chinensis* Planch.) I. Development of the flowering shoot. *New Zealand Journal of Botany* **13,** 473-483.

Budge, S.P. & Whipps, J.M. (1991a). Effect of sucrose concentration on sclerotia production and subsequent apothecial formation by *Sclerotinia sclerotiorum*. *Mycological Research* **95,** 195-198.

Budge, S.P. & Whipps, J.M. (1991b). Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* spp. for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. *Plant Pathology* **40,** 59-66.

Butler, D.R., Reddy, R.K. & Wadia, K.D.R. (1995). Single-plant chambers to control humidity for pathological studies. *Plant Pathology* **44,** 1-9.

Buwalda, J.G., Green, T.G.A., Meekings, J.S. & Coneybear, D.J. (1992). Measurement of canopy gas exchange of kiwifruit vines using a suite of whole-canopy cuvettes. *Environmental Experimental Botany* **32,** 425-438.

Caesar, A.J. & Pearson, R.C. (1983). Environmental factors affecting survival of ascospores of *Sclerotinia sclerotiorum*. *Phytopathology* **73,** 1024-1030.

Campbell, C.L. & Madden, L.V. (1990). *Introduction to Plant Disease Epidemiology*. John Wiley & Sons, New York. 532 p.

Carisse, O. & Kushalappa, A.C. (1992). Influence of interrupted wet periods, relative humidity, and temperature on infection of carrots by *Cercospora carotae*. *Phytopathology* **82,** 602-606.

Carpenter, M.A., Frampton, C. & Stewart, A. (1999). Genetic variation in New Zealand populations of the plant pathogen *Sclerotinia sclerotiorum*. *New Zealand Journal of Crop and Horticultural Science* **27,** 13-21.

Celikel, F.G. & van Doorn, W.G. (1995). Solute leakage, lipid peroxidation and protein degradation during the senescence of Iris tepals. *Physiologia Plantarum* **94,** 515-521.

Cole, L., Dewey, F.M. & Hawes, C.R. (1996). Infection mechanisms of *Botrytis* species: pre-penetration and pre-infection processes of dry and wet conidia. *Mycological Research* **100,** 277-286.

Coley-Smith, J.R. & Cooke, R.C. (1971). Survival and germination of fungal sclerotia. *Annual Review Phytopatholgy* **9,** 65-92.

Coley-Smith, J.R., Verhoeff, K. & Jarvis, W.R. (ed.). (1980). *The biology of Botrytis*. Academic Press Inc., London. 318 p.

Cook, G.E., Steadman, J.R. & Boosalis, M.G. (1975). Survival of *Whetzelinia sclerotiorum* and initial infection of dry edible beans in western Nebraska. *Phytopathology* **65,** 250-255.

Cooper, K. & Marshall, R. (1990). Losses in fruit numbers from flowering to harvest. In: Proceedings of the National Kiwifruit Research Conference, Rotorua. **Special publication No. 3,** 5-8.

Davidson, R.M. (1973). Pollination and fruit set in Chinese Gooseberries. *Horticultural Newsletter* **February,** 22-24.

Davidson, R.M. (1990). The physiology of the kiwifruit vine. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 127-155. Ray Richards, Auckland.

Dillard, H.R., Ludwig, J.W. & Hunter, J.E. (1995). Conditioning sclerotia of *Sclerotinia sclerotiorum* for carpogenic germination. *Plant Disease Reporter* **79,** 411-415.

Dingley, J.M. (1969). Records of plant disease in New Zealand. *Bulletin* 192, Department of Scientific and Industrial Research. Wellington. pp. 298.

Doss, R.P., Potter, S.W., Chastagner, G.A. & Christian, J.K. (1993). Adhesion of non-germinated *Botrytis cinerea* conidia to several substrata. *Applied Environmental Microbiology* **59,** 1786-1791.

Doyle, C.J., Moore, W.B. & Henzell, R.F. (1989). Modelling the economic consequences of potential management changes in a mature kiwifruit orchard in New Zealand. *Agricultural Systems* **31,** 321-347.

Dutton, M.V. & Evans, C.S. (1996). Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. *Canadian Journal of Microbiology* **42,** 881-895.

Eash, N.S., Stahl, P.D., Parkin, T.B. & Karlen, D.L. (1996). A simplified method for extraction of ergosterol from soil. *Soil Science Society of America Journal* **60,** 468-471.

Eden, M.A., Hill, R.A., Beresford, R.M. & Stewart, A. (1996). The influence of inoculum concentration, relative humidity, and temperature on infection of greenhouse tomatoes by *Botrytis cinerea*. *Plant Pathology* **45,** 795-806.

Elmer, P.A.G., Boyd-Wilson, K., Whelan, H., Pyke, N.B., Gaunt, R.E. & Frampton, C.M. (1994). Sources of *Botrytis* in vines and spores on the fruit surface at harvest. In: Proceedings of the National Kiwifruit Research Conference, Rotorua. **Special publication No. 5,** 19-21.

Elmer, P.A.G., Hoyte, S.M. & Reglinski, T. (1999a). Biological suppression of *Sclerotinia sclerotiorum* in kiwifruit. In: Proceedings of the 12th Biennial Australasian Plant Pathology Society Conference, Canberra. p. 63.

Falloon, R.E., Viljanen Rollinson, S.L.H., Coles, G.D. & Poff, J.D. (1995). Disease severity keys for powdery and downy mildews of pea, and powdery scab of potato. *New Zealand Journal of Crop and Horticultural Science* **23,** 31-37.

Ferguson, A.M. (1980). Major causes of imperfections in the Bay of Plenty kiwifruit crop. In: Proceedings of the thirty third New Zealand Weed and Pest Control Conference, Tauranga. **33,** 92-95.

Ferguson, A.R. (1984). Kiwifruit: a botanical review. *Horticultural Reviews* **6,** 1-64.

Ferguson, A.R. (1990a). Botanical Nomenclature: *Actinidia chinensis, Actinidia deliciosa*, and *Actinidia setosa*. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 36-57. Ray Richards, Auckland.

Ferguson, A.R. (1990b). The genus *Actinidia*. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 15-35. Ray Richards, Auckland.

Ferguson, A.R. (1990c). Stem, branches, leaves and roots of the kiwifruit vine. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 58-70. Ray Richards, Auckland.

Ferguson, A.R. & Bollard, E.G. (1990). Domestication of the kiwifruit. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 165-246. Ray Richards, Auckland.

Finlayson, J.E., Rimmer, S.R. & Pritchard, M.K. (1989). Infection of carrots by *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* **11,** 242-246.

Ford, I. (1971). Chinese gooseberry pest and disease control. *New Zealand Journal of Agriculture* **122,** 86-89.

Free, J.B. (1993). *Insect pollination of crops*, 2nd edition. Academic Press Ltd., San Diego. 684 p.

Fritschen, L.J. & Gay, L.W. (1979). *Environmental Instrumentation*. Springer-Verlag, New York. 216 p.

Fry, W.E. & Fohner, G.R. (1985). Construction of predictive models: I. Forecasting disease development. In: *Mathematical modelling of crop disease*, vol. 3. *Advances in plant pathology* (ed. C. A. Gilligan) pp. 161-178. Academic Press Inc., London.

Fuchs, M. & Tanner, C.B. (1970). Error analysis of Bowen Ratio measured by differential psychrometry. *Agricultural Meteorology* **7,** 329-334.

Gandar, P.W., Hall, A.J. & de Silva, H.N. (1996). Deterministic models for fruit growth. *Acta Horticulturae* **416,** 103-112.

Garrett, S.D. (1956). *Biology of root-infecting fungi*. Cambridge University Press, Cambridge. 293 p.

Gaumann, E. (1950). *Principles of plant infection*. Crosby Lockwood & Son, Ltd., London. 543 p.

Gerlagh, M. (1986). The role of flower infection by *Sclerotinia sclerotiorum* (White mold). *International Organisation for Biological and Integrated Control WPRS Bulletin* **4,** 21-24.

Gerlagh, M., Goossen-van de Geijn, H.M., Verdam, B. & Fokkema, N.J. (1995). Biological control of White mould (*Sclerotinia sclerotiorum*) in various crops by application of *Coniothyrium minitans*. In: Proceedings of the workshop: Biological control of sclerotium-forming pathogens, Wellesbourne. **18,** 13-17.

Goh, H.H. & Lyons, S.N. (1992a). *Sclerotinia* infection in two kiwifruit orchards in the Bay of Plenty. In: Proceedings of the forty fifth New Zealand Plant Protection Conference, Wellington. **45,** 180-183.

Goh, H.H. & Lyons, S.N. (1992b). Survival and viability of sclerotia of *Sclerotinia sclerotiorum* in two kiwifruit orchards in the Bay of Plenty. In: Proceedings of the forty fifth New Zealand Plant Protection Conference, Wellington. **45,** 184-187.

Goodwin, R.M. (1986). Kiwifruit flowers: anther dehiscence and daily collection of pollen by honey bees. *New Zealand Journal of Experimental Agriculture* **14,** 449-452.

Goodwin, R.M. (1995). Afternoon decline in kiwifruit pollen collection. *New Zealand Journal of Crop and Horticultural Science* **23,** 163-171.

Goodwin, R.M. & Steven, D. (1993). Behaviour of honey bees visiting kiwifruit flowers. *New Zealand Journal of Crop and Horticultural Science* **21,** 17-24.

Goodwin, R.M., Ten Houten, A. & Perry, J.H. (1999). Effect of staminate kiwifruit vine distribution and flower number on kiwifruit pollination. *New Zealand Journal of Crop and Horticultural Science* **27,** 63-67.

Gottwald, T.R., Trocine, T.M. & Timmer, L.W. (1997). A computer-controlled environment chamber for the study of aerial fungal spore release. *Phytopathology* **87,** 1078-1084.

Gregory, P.H. (ed.). (1973). *The microbiology of the atmosphere*, 2nd edition. Leonard Hill., Aylesbury. 377 p.

Gretenkort, M.A. & Helsper, J. (1993). Disease assessment of pea lines with resistance to root rot pathogens: protocols for *in vitro* selection. *Plant Pathology* **42,** 676-685.

Grogan, R.G. (1979). *Sclerotinia* species: Summary and comments on needed research. *Phytopathology* **69,** 908-910.

Gugel, R.K. & Morrall, R.A.A. (1986). Inoculum-disease relationships in *Sclerotinia* stem rot of rapeseed in Saskatchewan. *Canadian Journal of Plant Pathology* **8,** 89-96.

Hancock, J.C. (1967). Hemicellulase degradation in sunflower hypocotols infected with *Sclerotinia sclerotiorum*. *Phytopathology* **57,** 203-206.

Hannusch, D.J. & Boland, G.J. (1996). Influence of air temperature and relative humidity on biological control of White mold of bean (*Sclerotinia sclerotiorum*). *Phytopathology* **86,** 156-162.

Hannusch, D.J., James, T.D.W., Gillespie, T.J. & Boland, G.J. (1995). A simple and inexpensive control of relative humidity in a flow-through environmental chamber. *Environmental Experimental Botany* **35,** 411-415.

Harrison, J.G. (1988). The biology of *Botrytis* spp. on *Vicia* beans and chocolate spot disease - a review. *Plant Pathology* **37,** 168-201.

Harrison, J.G. & Lowe, R. (1989). Effects of humidity and air speed on sporulation of *Phytophthora infestans* on potato leaves. *Plant Pathology* **38,** 585-591.

Harrison, J.G., Lowe, R. & Williams, N.A. (1994). Humidity and fungal diseases of plants - problems. In: *Ecology of plant pathogens*. (ed. J. P. Blackman and B. Williamson) pp. 79-97. CAB International, Wallingford.

Hartill, W.F.T. (1975). Germination of *Botrytis* and *Sclerotinia* spores in the presence of pollen on tobacco leaves. *New Zealand Journal of Agricultural Research* **18,** 405-407.

Hartill, W.F.T. (1980). Aerobiology of *Sclerotinia sclerotiorum* and *Botrytis cinerea* spores in New Zealand tobacco crops. *New Zealand Journal of Agricultural Research* **23,** 259-262.

Hartill, W.F.T. & Campbell, J.M. (1974). Effects of flower removal on the development of the *Sclerotinia*/*Botrytis* complex of tobacco. *New Zealand Journal of Agricultural Research* **17,** 147-152.

Hartill, W.F.T. & Underhill, A.P. (1976). "Puffing in *Sclerotinia sclerotiorum* and *Sclerotinia minor*". *New Zealand Journal of Botany* **14,** 355-358.

Harvey, I.C., Foley, L.M. & Saville, D.J. (1995). Survival and germination of shallow-buried sclerotia of *Sclerotinia sclerotiorum* in pastures in Canterbury. *New Zealand Journal of Agricultural Research* **38,** 279-284.

Hau, B., Eisensmith, S.P. & Kranz, J. (1985). Construction of temporal models: II Simulation of aerial epidemics. In: *Advances in Plant Pathology: Mathematical modelling of crop disease*, vol. 3. (ed. C. A. Gilligan) pp. 31-65. Academic Press, London.

Hawthorne, B.T. (1974). *Sclerotinia minor* on lettuce: effect of plant growth on susceptibility to infection. *New Zealand Journal of Agricultural Research* **17,** 387-392.

Henzell, R.F. (1986). Hydrogen cyanamide and its effect on kiwifruit. Ministry of Agriculture and Fisheries, Kiwifruit Seminar. Havelock North. pp. 33-39.

Hirst, J.M. (1952). An automatic volumetric spore trap. *Annals of Applied Biology* **39,** 257-265.

Hoes, J.A. & Huang, H.C. (1975). *Sclerotinia sclerotiorum*: Viability and separation of sclerotia from soil. *Phytopathology* **65,** 1431-1432.

Holley, R.C. & Nelson, B.D. (1986). Effects of plant population and inoculum density on incidence of Sclerotinia wilt of sunflower. *Phytopathology* **76,** 71-74.

Holliday, P. (1992). *A dictionary of plant pathology*. Cambridge University Press, Cambridge. 369 p.

Hong, T.D., Ellis, R.H. & Moore, D. (1997). Development of a model to predict the effect of temperature and moisture on fungal spore longevity. *Annals of Botany* **79,** 121-128.

Hopping, M.E. (1976). Structure and development of fruit and seeds in Chinese gooseberry (*Actinidia chinensis* Planch.). *New Zealand Journal of Botany* **14,** 63-68.

Hopping, M.E. (1981). Kiwifruit pollination: influence of male clones. Ministry of Agriculture and Fisheries, Kiwifruit seminar. Tauranga. pp. 21-25.

Hopping, M.E. (1990). Floral biology, pollination and fruit set. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 71-96. Ray Richards, Auckland.

Hopping, M.E. & Jerram, E.M. (1979). Pollination of kiwifruit (*Actinidia chinensis* Planch.): stigma-style structure and pollen tube growth. *New Zealand Journal of Botany* **17,** 233-240.

Hoyte, S.M. (1994). *Sclerotinia* and its control. In: Proceedings of the National Kiwifruit Research Conference, Rotorua. **Special publication No. 5,** 18-19.

Hoyte, S.M. (1996). Does Sclerotinia infect your floral tissue? *New Zealand Kiwifruit* **November,** 11.

Hoyte, S.M. (1997). Sclerotinia - the pathogen and its management. HortResearch Internal Report 97/53. pp. 5-10.

Hoyte, S.M. (2000). The role of adhering floral tissues in sclerotinia disease of kiwifruit. *New Zealand Plant Protection* **53,** 138-142.

Hoyte, S.M., Bedford, R.J. & Gouk, S.C. (1992). Field observations of *Sclerotinia sclerotiorum*: Apothecial development and sclerotial production. In: Proceedings of the forty fifth New Zealand Plant Protection Conference, Wellington. **45,** 188-192.

Hoyte, S.M., Elmer, P.A.G., Reglinski, T. & Perry, J. (1998). Biological Suppression of Sclerotinia: A future option for Disease Control. HortResearch Internal Report 98/48. pp. 39-44.

Hoyte, S.M., Pak, H.A. & Maning, M.A. (1999). Development of a disease risk prediction system for *Sclerotinia*. Kiwifruit New Zealand Research Ltd. "Creating Wealth Through Research" Conference. Rotorua. pp. 52-57.

Hoyte, S.M., Perry-Meyer, L.J. & Hill, R.A. (1994). Incidence of *Botrytis cinerea* and colonisation of necrotic leaf tissue in kiwifruit canopies. In: Proceedings of the forty seventh New Zealand Plant Protection Conference, Waitangi. **47,** 356-358.

Huang, H.C. & Kokko, E.G. (1992). Pod rot of dry peas due to infection by ascospores of *Sclerotinia sclerotiorum*. *Plant Disease Reporter* **76,** 597-600.

Huang, H.C. & Kokko, E.G. (1993). Infection of pea pollen by *Sclerotinia sclerotiorum*. *Trends Agricultural Science - Plant Pathology* **1,** 13-17.

Huang, H.C., Kokko, E.G. & Erickson, R.S. (1997). Infection of alfalfa pollen by *Sclerotinia sclerotiorum*. *Phytoparasitica* **25,** 17-24.

Huang, H.C. & Kozub, G.C. (1993). Survival of mycelia of *Sclerotinia sclerotiorum* in infected stems of dry bean, sunflower, and canola. *Phytopathology* **83,** 937-940.

Hunter, J.E., Steadman, J.R. & Cigna, J.A. (1982). Preservation of ascospores of *Sclerotinia sclerotiorum* on membrane filters. *Phytopathology* **72,** 650-652.

Hutchins, J.D. & Archer, S.A. (1994). Biocontrol of *Sclerotinia sclerotiorum* by manipulation of petal microflora. In: Proceedings of the Brighton Crop Protection Conference, Pests and Diseases, Farnham. **2,** 935-936.

Imolehin, E.D., Grogan, R.G. & Duniway, J.M. (1980). Effect of temperature and moisture tension on growth, sclerotial production, germination, and infection by *Sclerotinia minor*. *Phytopathology* **70,** 1153-1157.

Inglis, G.D. & Boland, G.J. (1990). The microflora of bean and rapeseed petals and the influence of the microflora of bean petals on white mold. *Canadian Journal of Plant Pathology* **12,** 129-134.

Ingold, C.T. (1971). *Fungal spores: their liberation and dispersal*. Claredon, Oxford. 302 p.

Jamaux, I., Gelie, B. & Lamarque, C. (1995). Early stages of infection of rapeseed petals and leaves by *Sclerotinia sclerotiorum* revealed by scanning electron microscopy. *Plant Pathology* **44,** 22-33.

Jamaux, I. & Spire, D. (1994). Development of a polyclonal antibody-based immunoassay for the early detection of *Sclerotinia sclerotiorum* in rapeseed petals. *Plant Pathology* **43,** 847-862.

Jeffers, J.N.R. (1978). *Introduction to systems analysis: with ecological applications*. Edward Arnold, London. 198 p.

Jones, D. (1970). Ultrastructural and composition of the cell walls of *Sclerotinia sclerotiorum*. *Transactions of the Bristish Mycological Society* **54,** 351-360.

Judd, M.J., Prendergast, P.T. & McAneney, K.J. (1993). Carbon dioxide and latent heat flux measurements in a windbreak-sheltered orchard. *Agricultural and Forest Meteorology* **66,** 193-210.

Kapoor, A.S., Singh, D. & Singh, B.M. (1990). A technique for artificial inoculation of pea seedling with *Sclerotinia sclerotiorum*. *Indian Phytopathology* **43,** 469-470.

Kapoor, K.S., Gill, H.S. & Sharma, S.R. (1985). A technique for artificial inoculation of cauliflower seedlings with *Sclerotinia sclerotiorum* (Lib.) de Bary. *Phytopathologische Zeitschrift* **112,** 191-192.

Kapoor, K.S., Gill, H.S. & Sharma, S.R. (1987). Survival and carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. *Indian Pathology* **40,** 500-502.

Kerssies, A. (1990). A selective medium for *Botrytis cinerea* to be used in a spore-trap. *Netherlands Journal of Plant Pathology* **96,** 247-250.

Khare, K.B. & Bompeix, G. (1976). Proteolytic activities of *Sclerotinia sclerotiorum* and *S. minor*: possible role during pathogenesis. *Revue-de-Mycologie* **40,** 65-84.

Koball, D.C., Wilcox, W.F. & Seem, R.C. (1997). Influence of incubation-period humidity on the development of brown rot blossom blight of Sour Cherry. *Phytopathology* **87,** 42-49.

Kohl, J. & Fokkema, N.J. (1994). Fungal interactions on living and necrotic leaves. In: *Ecology of plant pathogens*. (ed. J. P. Blackman and B. Williamson) pp. 321-334. CAB International, Wallingford.

Kohli, Y., Morall, R.A.A., Anderson, J.B. & Kohn, L.M. (1992). Local and trans-Canadian clonal distribution of *Sclerotinia sclerotiorum* of canola. *Phytopathology* **82,** 875-880.

Kohn, L.M. (1979a). Delimitation of the economically important plant pathogenic *Sclerotinia* species. *Phytopathology* **69,** 881-886.

Kohn, L.M. (1979b). A monographic revision of the genus *Sclerotinia*. *Mycotaxon* **9,** 365-444.

Kohn, L.M., Stasovski, E., Carbone, J.R. & Anderson, J.B. (1991). Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum*. *Phytopathology* **81,** 480-485.

Kondo, N., Kodama, F., Ozaki, M. & Akai, J. (1988). Occurrence and control of Sclerotinia head rot of sunflower in Hokkaido. *Annals of the Phytopathological Society of Japan* **54,** 198-203.

Kranz, J. (1990). Epidemics, their mathematical analysis and modelling: an introduction. In: *Epidemics of plant diseases: mathematical analysis and modelling*, vol. 13. *Ecological studies* pp. 1-11. Springer-Verlag, New York.

Kumar, V. & Dwivedi, R.S. (1981). Mycoflora associated with floral parts of sunflower. *Indian Phytopathology* **34,** 314-317.

Lamarque, C., Leconte, M., Berrier, J. & Jaunet, A.M. (1985). Recherche des sites de contaminations du capitule de tournesol par les ascospores de *Sclerotinia sclerotiorum* (Lib.) de Bary. *CETIOM, Informations Techniques* **92,** 27-35.

Lawes, G.S. (1990). Propagation of kiwifruit. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 297-321. Ray Richards, Auckland.

Lefol, C. & Morrall, R.A.A. (1996). Immunofluorescent staining of *Sclerotinia* ascospores on canola petals. *Canadian Journal of Plant Pathology* **18,** 237-241.

Lescourret, F. & Habib, R. (1996). An object-oriented analysis method for modelling orchard management. *Acta Horticulturae* **416,** 235-242.

Liang, C.F. & Ferguson, A.R. (1984). Emendation of the Latin name of *Actinidia chinensis* Pl. var. *hispida* C.F. Liang. *Guihaia* **4,** 181-182.

Lightner, G.W. & Steiner, P.W. (1990). Computerization of blossom blight prediction model. *Acta Horticulturae* **273,** 159-162.

Lithourgidis, A.S., Tzavella-Klonari, K. & Roupakias, D.G. (1989). Methods of inoculation of Faba bean plants with *Sclerotinia sclerotiorum*. *Journal of Phytopathology* **127,** 123-128.

Lowe, R.G., Wang, Z.Y., Patterson, K.J. & Gould, K.S. (1992). Rootstocks - enhancing the vine's potential. In: Proceedings of the National Kiwifruit Research Conference, Rotorua. **Special publication No. 4,** 33-36.

Lumsden, R.D. (1969). *Sclerotinia sclerotiorum* infection of bean and the production of cellulase. *Phytopathology* **59,** 653-657.

Lumsden, R.D. (1976). Pectolytic enzymes of *Sclerotinia sclerotiorum* and their localisation in infected bean. *Canadian Journal of Botany* **54,** 2630-2641.

Lumsden, R.D. & Dow, R.L. (1973). Histopathology of *Sclerotinia sclerotiorum* infection of bean. *Phytopathology* **63,** 708-715.

Lyford, P. (1981). Review of the 1980/81 kiwifruit season. Ministry of Agriculture and Fisheries, Kiwifruit Seminar. Tauranga. pp. 1-5.

Manandhar, D.N. & Lawes, G.S. (1980). Blossom sprays and their effect on fruit set. *Orchardist of New Zealand* **53,** 269-272.

Manners, J.G. (1993). *Principles of plant pathology*, 2nd edition. Cambridge University Press, Cambridge. 343 p.

Manning, M. (1991). *Sclerotinia* - a summer disease. *New Zealand Kiwifruit* **November,** 16.

Manning, M.A., Pak, H.A. & Pennycook, S.R. (1995). Population dynamics of fungicide resistant strains of *Botrytis cinerea* in New Zealand kiwifruit orchards. In: Proceedings of the 10th Biennial Australasian Plant Pathology Society Conference, Lincoln University. p. 38.

Maxwell, P.D. & Lumsden, R.D. (1970). Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. *Phytopathology* **60,** 1395-1398.

McGregor, S.E. (1976). Chinese gooseberry ('kiwi'). In: *Insect pollination of cultivated crop plants*. Agriculture Handbook 496. pp. 146-148. United States Department of Agriculture, Washington D.C.

McLean, D.M. (1958). Role of dead flower parts in infection of certain crucifers by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Plant Disease Reporter* **42,** 663-666.

McNicol, R.J. & Williamson, B. (1988). Systemic infection of black currant flowers by *Botrytis cinerea* and its possible involvement in premature abscission of fruits. *Annals Applied Biology* **114,** 243-254.

McPherson, H.G., Hall, A.J. & Stanely, C.J. (1994). Seasonal and regional variation in budbreak and flowering of kiwifruit vines in New Zealand. *New Zealand Journal of Crop and Horticultural Science* **22,** 263-273.

Merriman, P.R. (1976). Survival of sclerotia of *Sclerotinia sclerotiorum* in soil. *Soil Biology and Biochemistry* **8,** 385-389.

Merriman, P.R., Pywell, M., Harrison, G. & Nancarrow, J. (1979). Survival of sclerotia of *Sclerotinia sclerotiorum* and effects of cultivation practices on disease. *Soil Biology and Biochemistry* **11,** 567-570.

Mitchell, S.J. & Wheeler, B.E.J. (1990). Factors affecting the production of apothecia and longevity of sclerotia of *Sclerotinia sclerotiorum*. *Plant Pathology* **39,** 70-76.

Mordue, J.E.M. & Holliday, P. (1976). *Sclerotinia sclerotiorum*. Commonwealth Mycological Institute descriptions of pathogenic fungi and bacteria, No. 513. pp. 2.

Morley-Bunker, M.J. & Salinger, M.J. (1987). Kiwifruit development: the effect of temperature on budburst and flowering. *Weather and climate* **7,** 26-30.

Morral, R.A.A. (1972). Variations and correlations within and between morphology, pathogenicity, and pectolytic enzyme activity in Sclerotinia from Saskatchewan. *Canadian Journal of Botany* **55,** 8-11.

Morral, R.A.A. (1977). A preliminary study of the influence of water potential on sclerotium germination of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* **55,** 8-11.

Morral, R.A.A. & Dueck, J. (1982). Epidemiology of sclerotinia stem rot of rapeseed in Saskatchewan. *Canadian Journal of Plant Pathology* **4,** 161-168.

Natti, J.J. (1971). Epidemiology and control of bean white mold. *Phytopathology* **61,** 669-674.

Nelson, B.D., Hertsgaard, D.M. & Holley, R.C. (1989). Disease progress of Sclerotinia wilt of sunflower at varying plant populations, inoculum densities and environments. *Phytopathology* **79,** 1358-1363.

Newton, H.C. & Sequeira, L. (1972). Ascospores as the primary infective propagule of *Sclerotinia sclerotiorum* in Wisconsin. *Plant Disease Reporter* **56,** 798-802.

Nilsson, K. & Bjurman, J. (1998). Chitin as an indicator of the biomass of two wood-decay fungi in relation to temperature, incubation time, and media composition. *Canadian Journal of Microbiology* **44,** 575-581.

Noyes, R.D. & Hancock, J.G. (1981). Role of oxalic acid in the *Sclerotinia* wilt of sunflower. *Physiological Plant Pathology* **18,** 123-132.

O'Brien, F. (1948). The control of humidity by saturated salt solutions. *Journal of Scientific Instruments* **25,** 73-76.

Okuse, I. & Ryugo, K. (1981). Compositional changes in the developing 'Hayward' kiwifruit in California. *Journal of the American Society for Horticultural Science* **106,** 73-76.

Pak, H.A. & Manning, M.A. (1998). *Sclerotinia* Prediction System 1998 Results. *Report to Kiwifruit New Zealand* 99/42, HortResearch client report No. 1999/42. pp. 25.

Pak, H.A., Manning, M.A. & Hoyte, S.M. (1997). Apothecial density and disease incidence in *Sclerotinia* on kiwifruit. In: Proceedings of the 11th Biennial Conference of the Australasian Plant Pathology Society Conference, Perth. p. 96.

Pak, H.A., Manning, M.A. & Pennycook, S.R. (1995). Effect of early season fungicides on resistance dynamics of *Botrytis cinerea* populations in New Zealand kiwifruit orchards. In: Proceedings of the 10th Biennial Australasian Plant Pathology Society Conference, Lincoln University. p. 55.

Palmer-Jones, T. & Clinch, P.G. (1974). Observations on pollination of Chinese gooseberry variety 'Hayward'. *New Zealand Journal of Experimental Agriculture* **2,** 455-458.

Pandey, R.R. (1990). Mycoflora associated with floral parts of guava (*Psidium guajava* L.). *Acta Botanica Indica* **18,** 59-63.

Patterson, H.D. & Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika* **58,** 545-554.

Pennycook, S.R. (1982). *Sclerotinia* rot of Kiwifruit (*Actinidia chinensis*). *Orchardist of New Zealand* **55,** 407-408.

Pennycook, S.R. (1985). Fungal fruit rots of *Actinidia deliciosa* (kiwifruit). *New Zealand Journal of Experimental Agriculture* **13,** 289-299.

Pereyra, V.R., Sala, C.A. & Bazzalo, M.E. (1992). A comparison between Argentine and French sunflower hybrid varieties for their resistance to head rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. In: Proceedings of the 13th International Sunflower Conference, Pisa. **2,** 1199-1204.

Phillips, A.J.L. (1987). Carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*: a review. *Phytophylactica* **19,** 279-283.

Phillips, A.J.L. (1994a). A comparison of methods for inoculating bean plants with *Elsinoe phaseoli* and some factors affecting infection. *Annals of Applied Biology* **125,** 97-104.

Phillips, A.J.L. (1994b). Influence of fluctuating temperatures and interrupted periods of plant surface wetness on infection of bean leaves by ascospores of *Sclerotinia sclerotiorum*. *Annals of Applied Biology* **124,** 413-427.

Postgate, J.R. (1969). Viable counts and viability. In: *Methods in Microbiology*, vol. 1. (ed. J. R. Norris and D. W. Ribbons) pp. 611-623. Academic Press, London.

Pratella, G.C. (1995). Notes on the bio-pathology and techniques of storage-transport of fruits. Kiwifruit (part 2). *Rivista di Frutticoltura e di Ortofloricoltura* **57,** 80-83.

Pratt, H.K. & Reid, M.S. (1974). Chinese gooseberry: seasonal patterns in fruit growth and maturation, ripening, respiration and the role of ethylene. *Journal of the Science of Food and Agriculture* **25,** 747-757.

Price, K. & Colhoun, J. (1975a). Pathogenicity of isolates of *Sclerotinia sclerotiorum* (Lib.) to several hosts. *Phytopathologische Zeitschrift* **83,** 232-238.

Price, K. & Colhoun, J. (1975b). A study of variability of isolates of *Sclerotinia sclerotiorum* (Lib.) de Bary from different hosts. *Phytopathologische Zeitschrift* **83,** 159-166.

Purdy, L.H. (1958). Some factors affecting penetration and infection by *Sclerotinia sclerotiorum*. *Phytopathology* **48,** 605-609.

Purdy, L.H. (1979). *Sclerotinia sclerotiorum*: History, disease and symptomatology, host range, geographic distribution, and impact. *Phytopathology* **69,** 875-880.

Purdy, L.H. & Bardin, R. (1953). Mode of infection of tomato plants by the ascospores of *Sclerotinia sclerotiorum*. *Plant Disease Reporter* **37,** 361-362.

Pyke, N.B. & Alspach, P.A. (1986). Inter-relationships of fruit weight, seed number and seed weight in kiwifruit. *New Zealand Journal of Agricultural Science* **20,** 153-156.

Rainbow, A.F. (1970). *Sclerotinia* disease in dwarf tomatoes. *New Zealand Journal of Agriculture* **121,** 58-62.

Rajender, S., Tripathi, N.N. & Singh, R. (1996). Effect of soil type on apothecial production of *Sclerotinia sclerotiorum* (Lib) de Bary. *Indian Journal of Agricultural Research* **30,** 191-196.

Reid, M.S., Heatherbell, D.A. & Pratt, H.K. (1982). Seasonal patterns in chemical composition of the fruit of *Actinidia chinensis*. *Journal of the American Society for Horticultural Science* **107,** 316-319.

Roberts, C.A., Marquardt, R.R., Frohlich, A.A., McGraw, R.L., Rotter, R.G. & Henning, J.C. (1991). Chemical and spectral quantification of mold contaminated barley. *Cereal Chemistry* **68,** 272-275.

Roberts, D.A. & Boothroyd, C.W. (1984). Rots of plant products. In: *Fundamentals of plant pathology*. (ed. D. A. Roberts and C. W. Boothroyd) pp. 219-229. W.H. Freeman & Co, New York.

Saito, I. (1977). Studies on the maturation and germination of sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary, a causal fungus of bean stem rot. *Report of Hokkaido Prefectural Agricultural Experiment Stations* **26,** 106.

Sale, P. (1993). Spray programme guidelines for kiwifruit 1993-94. *New Zealand Kiwifruit* **October/November**. p. 9.

Sale, P.R. (1981). Kiwifruit: diseases and non-pathogenic damage. *AgLink* HPP 237, Ministry of Agriculture and Fisheries. Wellington, New Zealand. pp. 4.

Sale, P.R. & Lyford, P.B. (1990). Cultural, management and harvesting practices for kiwifruit in New Zealand. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 247-296. Ray Richards, Auckland.

Sansford, C.E. & Coley-Smith, J.R. (1992). Production and germination of sclerotia of *Sclerotinia sclerotiorum*. *Plant Pathology* **41,** 154-156.

Sargent, G.P. (1980). Computation of vapour pressure, dew point and relative humidity from dry- and wet- bulb temperatures. *Meteorological Magazine* **109,** 551-571.

Savage, S.D. & Sall, M.A. (1981). Radioimmunosorbent assay for *Botrytis cinerea*. *Phytopathology* **71,** 411-415.

Schein, R.D. (1964). Design, performance and use of a quantitative inoculator. *Phytopathology* **54,** 509-513.

Scherm, H. & van Bruggen, A.H.C. (1994). Effects of fluctuating temperatures on the latent period of lettuce downy mildew (*Bremia lactucae*). *Phytopathology* **84,** 853-859.

Schmid, R. (1978). Reproductive anatomy of *Actinidia chinensis* (Actinidiaceae). *Botanische Jahrbucher fur Systematik Pflanzengeschichte und Pflanzgeographie* **100,** 149-195.

Schnurer, J. (1993). Comparison of methods for estimating the biomass of three food-borne fungi with different growth patterns. *Applied Environmental Microbiology* **59,** 552-555.

Schoolfield, R.M., Sharpe, P.J.H. & Magnuson, C.E. (1981). Non-linear regression of biological temperature-dependant rate models based on absolute reaction-rate theory. *Journal of Theoretical Biology* **88,** 719-731.

Schots, A., Dewey, F.M. & Oliver, R.P. (ed.). (1994). *Modern assays for plant pathogenic fungi: identification, detection and quantification*. CAB International, Oxford. 267 p.

Schwartz, H.F. & Steadman, J.R. (1978). Factors affecting sclerotium populations of, and apothecia production by, *Sclerotinia sclerotiorum*. *Phytopathology* **68,** 383-388.

Schwarzbach, E. (1979). A high throughput jet trap for collecting mildew spores on living leaves. *Phytopathologische Zeitschrift* **94,** 165-171.

Seager, N.G. (1993). Temperature effects on kiwifruit maturation. PhD. thesis, Massey University. 295 p.

Sedun, F.S. & Brown, J.F. (1987). Infection of sunflower leaves by ascospores of *Sclerotinia sclerotiorum*. *Annals Applied Biology* **110,** 275-285.

Sexton, R. & Woolhouse, H.W. (1984). Senescence and abscission. In: *Plant Physiology*. (ed. M. B. Wilkins) pp. 469-497. Pitman Publishing Ltd, London.

Shaul, O., Elad, Y. & Zieslin, N. (1995). Suppression of Botrytis blight in cut rose flowers with gibberellic acid: effects of postharvest timing of the gibberellin treatment, conidial inoculation and cold storage period. *Postharvest Biology and Technology* **6,** 331-339.

Singh, B. & Srivastava, J.S. (1993). Studies on ascospore germination on *Sclerotinia sclerotiorum* causing stem and pod rot of pea. *International Journal Tropical Plant Diseases* **11,** 61-68.

Singh, D. (1991). Development of white rot of pea in relation to meterological factors. *Indian Journal Mycology and Plant Pathology* **21,** 192-195.

Singh, U.P. & Singh, R.B. (1987). Effect of light of different wavelengths on apothecium formation in *Sclerotinia sclerotiorum*. *Zeitschrift-fur-Pflanzenkrankheiten-und-Pflanzenschutz* **94,** 500-508.

Sirjusingh, C. & Sutton, J.C. (1996). Effects of wetness duration and temperature on infection of geranium by *Botrytis cinerea*. *Plant Disease Reporter* **80,** 160-165.

Sirjusingh, C., Sutton, J.C. & Tsujita, M.J. (1996). Effects of inoculum concentration and host age on infection of geranium by *Botrytis cinerea*. *Plant Disease Reporter* **80,** 154-159.

Spotts, R.A. & Cervantes, L.A. (1996). Sclerotinia rot of pears in Oregon. *Plant Disease* **80,** 1262-1264.

Stahl, P.D. & Parkin, T.B. (1996). Relationship of soil ergosterol concentration and fungal biomass. *Soil Biology and Biochemistry* **28,** 847-855.

Steadman, J.R. & Cook, G.E. (1974). A simple method for collecting ascospores of *Whetzelinia sclerotiorum*. *Plant Disease Reporter* **58,** 190.

Steadman, J.R., Marcinkowska, J. & Rutldge, S. (1994). A semi-selective medium for isolation of *Sclerotinia sclerotiorum*. *Canadian Journal of Plant Pathology* **16,** 68-70.

Sutton, B.C. (1980). *The Coelomycetes, fungi imperfecti with pycnidia, acervuli and stromata*. Commonwealth Mycological Institute, Kew. 696 p.

Sutton, D.C. & Deverall, B.J. (1983). Studies of infection of bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) by ascospores of *Sclerotinia sclerotiorum*. *Plant Pathology* **32,** 251-261.

Tariq, V.N., Gutteridge, C.S. & Jeffries, P. (1985). Comparative studies of cultural and biochemical characteristics used for distinguishing species within *Sclerotinia*. *Transactions of the British Mycological Society* **84,** 381-397.

Tariq, V.N. & Jeffries, P. (1984). Appressorium formation by *Sclerotinia sclerotiorum:* scanning electron microscopy. *Transactions British Mycological Society* **82,** 645-651.

Tate, K.G., Beresford, R.M., Wood, P.N. & Manktelow, D.W.L. (1996). Infection period forecasting for improved apple black spot management in Hawke's Bay. In: Proceedings of the forty ninth New Zealand Plant Protection Conference, Nelson. **49,** 90-95.

Tores, J.A. (1990). *In vitro* variability of a number of characteristics of *Sclerotinia sclerotiorum*. *Phytoparasitica* **18,** 321-329.

Tourneau, D.L. (1979). Morphology, cytology, and physiology of *Sclerotinia* species in culture. *Phytopathology* **69,** 887-890.

Tourvielle, D., Guillaumin, J.J., Vear, F. & Lamarque, C. (1978). Role of ascospores in infection of sunflower by *Sclerotinia sclerotiorum* (Lib.) de Bary. *Annales de Phytopathologie* **10,** 417-431.

Trapero-Casas, A. & Kaiser, W.J. (1992). Influence of temperature, wetness period, plant age, and inoculum concentration on infection and development of Ascochyta Blight of Chickpea. *Phytopathology* **82,** 589-596.

Trutmann, P., Keane, P.J. & Merriman, P.R. (1982). Biological control of *Sclerotinia sclerotiorum* on aerial parts of plants by the hyperparasite *Coniothyrium minitans*. *Transactions of the British Mycological Society* **78,** 521-529.

Tu, J.C. (1989). Modes of primary infection caused by *Sclerotinia sclerotiorum* in navy bean. *Microbios* **57,** 85-91.

Turkington, T.K. & Morrall, R.A.A. (1993). Use of petal infestation to forecast *Sclerotinia* stem rot of canola: The influence of inoculum variation over the flowering period and canopy density. *Phytopathology* **83,** 682-689.

Turkington, T.K., Morrall, R.A.A. & Gugel, R.K. (1991). Use of petal infestation to forecast sclerotinia stem rot of canola: evaluation of early bloom sampling, 1985-90. *Canadian Journal of Plant Pathology* **13,** 50-59.

Twengstrom, E., Kopmans, E., Sigvald, R. & Svensson, C. (1998). Influence of different irrigation regimes on carpogenic germination of sclerotia of *Sclerotinia sclerotiorum*. *Journal of Phytopathology* **146,** 487-493.

Upsdell, M.P. (1994). Bayesian smoothers as an extension of non-linear regression. *New Zealand Statistician* **29,** 66-81.

van der Plank, J.E. (1963). *Plant diseases: Epidemics and control*. Academic Press, New York. 349 p.

van Doorn, W.G. & Stead, A.D. (1997). Abscission of flowers and floral parts. *Journal of Experimental Botany* **48,** 821-837.

Vear, F. & Tourvielle, D. (1984). Recurrent selection for resistance to *Sclerotinia sclerotiorum* in sunflowers using artificial infections. *Agronomie* **4,** 789-794.

Vincelli, P.C. & Lorbeer, J.W. (1989). BLIGHT-ALERT: A weather-based predictive system for timing fungicide applications on onion before infection periods of *Botrytis squamosa*. *Phytopathology* **79,** 493-498.

Vingnana, S.V. & Long, P. (1998). Estimating biomass of *Botrytis cinerea*. In: Proceedings of the fifty first New Zealand Plant Protection Conference, Hamilton. **51,** 262.

Walton, E.F. & Fowke, P.J. (1993). Effect of hydrogen cyanamide on kiwifruit shoot flower number and position. *Journal of Horticultural Science* **68,** 529-534.

Walton, T. & Sommerville, E. (ed.). (1998). *New Zealand Agrichemical Manual*, Master edition. WHAM Chemsafe Ltd., Wellington, New Zealand. 652 p.

Wang, S.Y.C. & Tourvielle, D. (1971). Carbon sources, growth, sclerotium formation and carbohydrate composition of *Sclerotinia sclerotiorum*. *Archiv fur Mikrobiologie* **80,** 219-233.

Warrington, I.J. (1990). Areas and trends of kiwifruit production in New Zealand and around the world. In: *Kiwifruit: science and management*. (ed. I. J. Warrington and G. C. Weston) pp. 511-525. Ray Richards, Auckland.

Weiss, A., Hipps, L.E., Blad, B.L. & Steadman, J.R. (1980a). Comparison of within-canopy microclimate and white mold disease (*Sclerotinia sclerotiorum*) development in dry edible beans as influenced by canopy structure and irrigation. *Agricultural Meteorology* **22,** 11-21.

Weiss, A., Kerr, E.D. & Steadman, J.R. (1980b). Temperature and moisture influences on development of white mold disease (*Sclerotinia sclerotiorum*) on Great Northern beans. *Plant Disease Reporter* **64,** 757-759.

Welham, S.J. & Thompson, R. (1992). REML likelihood ratio tests for fixed model terms. Royal Statistical Society Conference. University of Sheffield. pp. 9-11.

Whetzel, H.H. (1945). A synopsis of the generea and species of the Sclerotiniaceae, a family of stromatic inoperculate discomycetes. *Mycologia* **37,** 648-714.

Willetts, H.J. (1971). The survival of fungal sclerotia under adverse environmental conditions. *Biological Review* **46,** 387-407.

Willetts, H.J. & Wong, J.A.-L. (1980). The biology of *Sclerotinia sclerotiorum*, *Sclerotinia trifoliorum* and *Sclerotinia minor* with emphasis on specific nomenclature. *Botanical Review* **46,** 101-165.

Williams, G.H. & Western, J.H. (1965). The biology of *Sclerotinia trifoliorum* Erikss. and other species of sclerotium-forming fungi. II The survival of sclerotia in soil. *Annals Applied Biology* **56,** 261-268.

Williams, J.R. & Stelfox, D. (1979). Dispersal of ascospores of *Sclerotinia sclerotiorum* in relation to sclerotinia stem rot of rapeseed. *Plant Disease Reporter* **63,** 395-399.

Williamson, B., Duncan, G.H., Harrison, J.G., Harding, L.A., Elad, Y. & Zimand, G. (1995). Effect of humidity on infection of rose petals by dry-inoculated conidia of *Botrytis cinerea*. *Mycological Research* **99,** 1303-1310.

Windholz, M., Budavari, S., Stroumtsos, L. & Fertig, M.N. (ed.). (1976). *The Merck Index*, Ninth edition. Merck & Co. Inc., Rahway, New Jersey. 1937 p.

Winston, P.W. (1960). Saturated solutions for the control of humidity in biological research. *Ecology* **41,** 232-237.

Woodson, W.R. & Handa, A.K. (1987). Changes in protein patterns and *in vivo* protein synthesis during presenescence and senescence of hibiscus petals. *Journal of Plant Physiology* **128,** 67-75.

Xu, X.-M. (1996). On estimating non-linear response of fungal development under fluctuating temperatures. *Plant Pathology* **45,** 163-171.

Young, J.M., Cheesmur, G.J., Welham, F.V. & Henshall, W.R. (1988). Bacterial blight of kiwifruit. *Annals of Applied Biology* **112,** 91-105.

Young, J.M., Garden, L., Ren, X.-Z. & Hu, F.-P. (1997). Genomic and phenotypic characterization of the bacterium causing blight of kiwifruit in New Zealand. *Plant Pathology* **46,** 857-864.

Zadoks, J.C. & Schein, R.D. (1979). *Epidemiology of plant disease*. Oxford University Press, Oxford. 427 p.

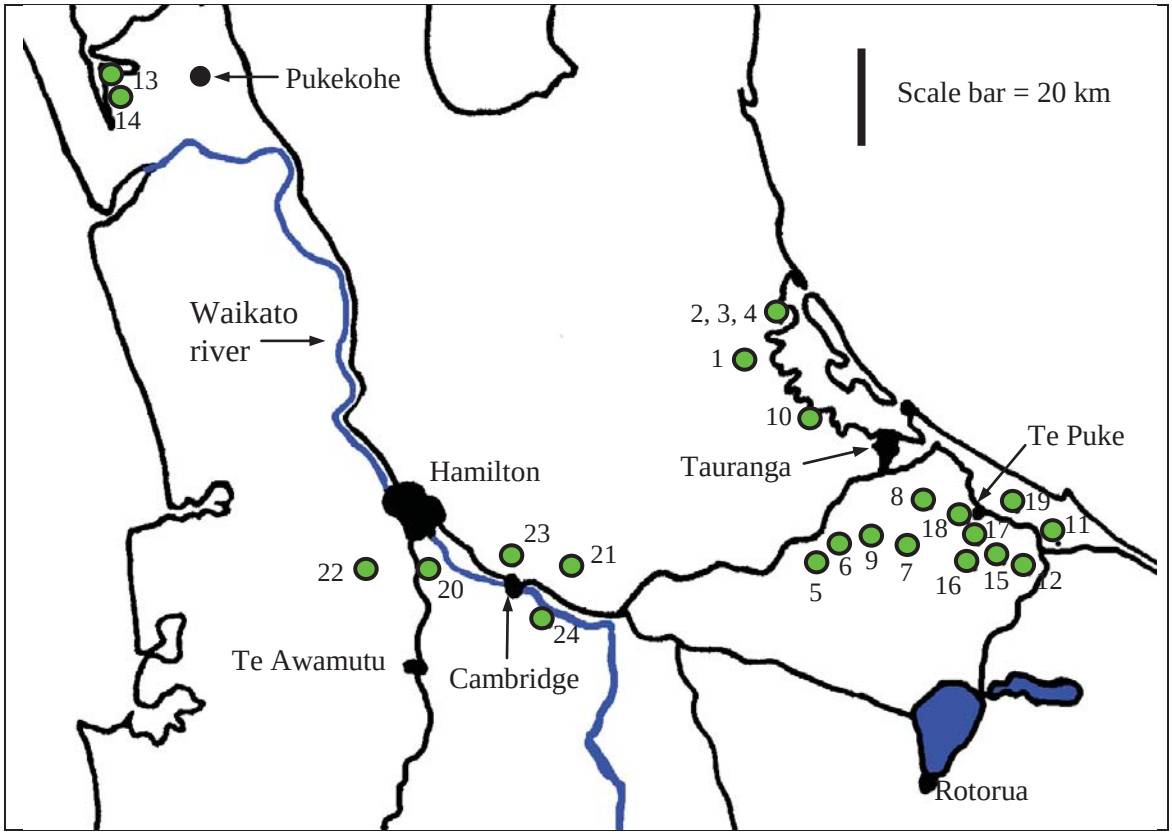
Zhou, T. & Reeleder, R.D. (1991). Colonization of bean flowers by *Epicoccum purpurascens*. *Phytopathology* **81,** 774-778.

Appendix 1: Kiwifruit orchards used in this study

Orchard (see map next page)	Names of blocks used	Region	Training system
1	Goat and Shade	Katikati	Pergola
2	Gate and Shed	Katikati	T-Bar
3	Drain and House	Katikati	T-Bar
4	Avocado	Katikati	Pergola
5	Pine	Lower Kaimai	T-Bar
6	Car and Spa	Lower Kaimai	Pergola
7	D block and Strawberry	Lower Kaimai	Pergola
8	Pig	Lower Kaimai	Pergola
9	Block 19 and Waterfall	Lower Kaimai	Pergola
10 *	Grass and Water tank	Omokoroa	Pergola
11	Golf	Paengaroa	Pergola
12	Kaituna and Ridge	Paengaroa	Pergola
13	Early and Late	South Auckland	Pergola
14	3rd block and Corner	South Auckland	Pergola
15	Front and Gully	Te Puke	Pergola
16	Big block and Packhouse	Te Puke	Pergola
17	Entomology and Office	Te Puke	T-Bar
18	Lawn and Pool	Te Puke	Pergola
19	Drive and Poplar	Te Tumu	Pergola
20	Block 10 and Block 12B	Waikato	T-Bar
21	Hill	Waikato	Pergola
22	Nashi and Pond	Waikato	T-Bar
23	Bee and Willow	Waikato	T-Bar
24 *	Fence and Sheep	Waikato	Pergola

***** Certified Organic orchard for at least 5 years.

NB. Orchard 17 and 20 are the Te Puke Research Orchard and Waikato Research Orchard, respectively. The latter is previously known as Blands.



Map of South Auckland, Bay of Plenty and Waikato regions, showing the locality of New Zealand orchards used during the course of this study from 1996–1999, numbers refer to the table on the previous page.

Appendix 2: Sclerotia and ascospore production from *S. sclerotiorum* isolate Sc1

2.1 Sclerotia production

Sclerotia were produced from isolate Sc1 by a minor modification to the method of Sansford and Coley-Smith (1992). Substrate was prepared by screening commercial Perlite (grade C400, New Zealand Perlite Ltd) through a 3.5 mm sieve. Five grams of Perlite retained on the sieve was added to each of twelve 250 ml conical flasks. A layer of whole wheat grains (10 g per flask) and 25 ml distilled water was added to the flasks without mixing. The flasks were capped with aluminium foil and autoclaved (121^{o}C for 20 minutes).

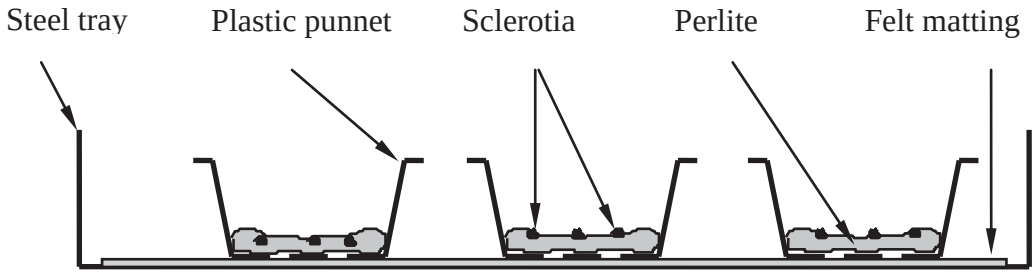
Three mycelial plugs (5 mm dia.) were taken 10 mm from the growing margin of 3 day-old cultures of *S. sclerotiorum* and added to each flask. Flasks were incubated at 20^{o}C for 4 weeks in the dark. After 2 and 4 days the flasks were shaken vigorously to mix the wheat, Perlite and mycelium to prevent the development of a dense mycelial mat. Flasks were shaken again after approximately 6, 10 and 21 days to mix the culture and dislodge sclerotia in contact with the glass surface which fail to develop a complete rind.

Most sclerotia were produced within 10–14 days followed by a second flush usually during the third week. The contents of the flasks were removed after 4 weeks incubation and placed onto a 1 mm sieve. Sclerotia were loosened from the wheat chaff and Perlite by irrigating with water and rubbing gently with fingers. The contents remaining in the sieve was placed into a 2 ℓ beaker of tap water and the floating material was discarded. The sclerotia, which sink in water, and remaining wheat chaff was collected in the 1 mm sieve and transferred to a plastic tray (150 × 300 mm) lined with a paper towel. After air drying in the laboratory for 3–4 days this was transferred into a larger plastic tray (300 × 600 mm) and the sclerotia were separated from the dried wheat chaff by slightly tilting the tray and shaking it sideways while blowing to force the wheat chaff to the top of the tray and allow the sclerotia to roll to the base of the tray.

Sclerotia were surface-sterilised in 1% sodium hypochlorite solution (amended with 0.1% Tween80 and 0.1% acetic acid) for 2 minutes, followed by three rinses in sterile distilled water. Sclerotia were air dried for 2–3 days before treatment for inducing apothecia formation.

2.2 Apothecia formation

A layer of Perlite (7–10 mm) was placed into plastic vegetable punnets (150 × 110 × 55 mm) with holes in the base to allow free passage of water. Approximately 50 surface-sterilised sclerotia were transferred onto the Perlite of each punnet and partially covered with additional Perlite. Twenty punnets with sclerotia were placed on felt matting laid into the base of a steel tray (1000 × 600 mm). Distilled water was added to the tray until the water level reached the base of the punnets. The water was quickly absorbed by the felt matting and Perlite by capillary action providing free moisture to the sclerotia. The tray was placed on a shelf in a walk-in coolstore (4–6^{o}C) and a sheet of black plastic was laid over the top to exclude light.



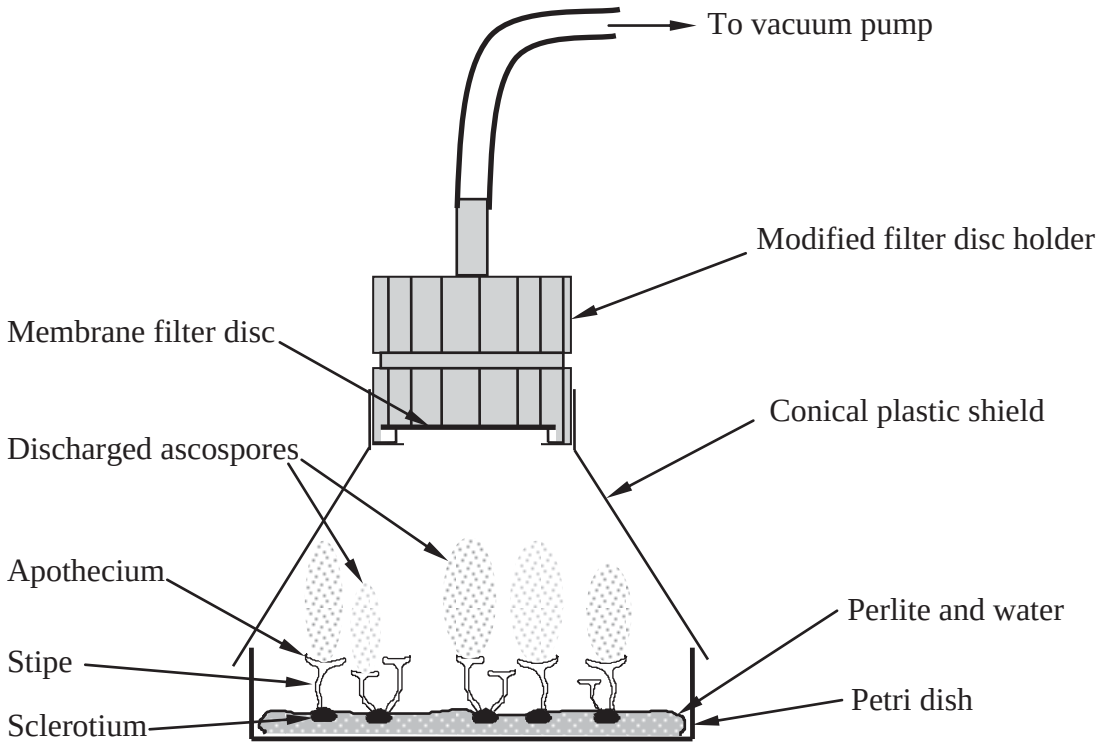
Cross-section of plastic punnets containing Perlite and sclerotia on felt matting inside a steel tray used for induction of stipes at 4–6^{o}C.

Stipes developed within 2–3 months, after which sclerotia were either removed to induce apothecia formation or left in the coolstore until required. However, sclerotia with stipes could not be kept longer than 4–5 months as the stipes decayed.

Approximately 10 sclerotia with stipes were transferred with tweezers into a 25 mm deep Petri dish containing a single layer of coarse Perlite and enough distilled water to keep them moist. The lids were replaced and they were placed onto a large plastic tray positioned outside the laboratory but were protected from direct sunlight. Apothecia developed at the tip of stipes and matured in 8–12 days.

2.3 Ascospore collection and storage

Ascospores were collected from mature apothecia following methods described by (Steadman & Cook, 1974; Hunter *et al*., 1982). Petri dishes containing mature apothecia were placed in a biohazard hood, with the air circulation fan turned off to provide a clean working space without any air flow which might have interfered with ascospore collection. In a quick motion the Petri dish lid was replaced with the conical plastic shield connected to a modified polycarbonate in-line filter holder (SM 16508 B, Sartorius) connected to a vacuum pump (ODI/2 Dynavac).



Modified membrane filter disc holder with conical plastic shield connected to vacuum pump, and Petri dish with mature apothecia discharging ascospores.

The membrane filters were mixed cellulose ester filters with 9 mm^2 grid markings (GN-6 0.45 μm grid, Gelman Sciences) and the vacuum pump operated at approximately 5–6 ℓ/minute.

Typically ascospores discharged *en mass* within 1–2 seconds of removing the Petri dish lid and were immediately swirled up and sucked onto the membrane filter. The membrane filter disc was then replaced and the procedure could be repeated for other Petri dishes with apothecia. Membrane filters discs with ascospores were transferred to sterile Petri dishes and placed into a glass desiccator vessel and kept at 4–6^{o}C.

Ascospore viability was checked every 6 months by sampling a 1 cm^2 section from at least two membrane filter discs. Ascospores were washed from the sections by shaking in 10 ml SDW for 1 minute. A 50 μl aliquot of this suspension was placed onto each of three Petri dishes of water agar and spread with a sterile glass rod. The percentage germination of ascospores was determined with a microscope (200 ×) after 18 h incubation at 20°C in the dark, by counting the number of germinated ascospores from a sample of 100 ascospores on each Petri dish. Germination was considered to have taken place when germ tubes were greater than the length of the ascospore. Batches of membrane filter discs with ascospores were discarded if the germination rate fell below 80%.

2.4 Density of ascospores on membrane filter discs

The number of ascospores on 20 membrane filter discs, collected from 7–15 February 1996, was estimated by counting ascospores along a transect (22.5 μm × 3 mm) within each of five randomly selected grids, with a compound microscope (100 ×). This was done by scrolling the microscope stage whilst counting ascospores within a fixed field of view, aided by an eyepiece graticle. The number of ascospores/disc was calculated by multiplying the mean number of ascospores per transect by the number of transect widths per grid and the number of grids per disc. Ascospores were observed on the surface of membrane filter discs as densely packed clusters, each with approximately 4–10 ascospores. Total number of ascospores/disc ranged from 6×10^5–5×10^6 with an overall mean of 2.4×10^6 (SE ± 3×10^5). This equals 1.3×10^5 (SE ± 1.6×10^4) ascospores on 16 grids of membrane filter disc (144 mm^2), the standard area used for each inoculation batch.

Ascospore numbers were not counted for subsequent collections of ascospores. However, each disc was visually checked with the same microscope to confirm that the density of ascospores were similar to the original 20.

Appendix 3: *Sclerotinia sclerotiorum* selective medium (John Knight's)

This selective medium, supplied by Dr G. Bourdot, was developed by John Knight of AgResearch, Lincoln for use in myco-herbicide trials investigating *S. sclerotiorum* inoculum within New Zealand pastures.

3.1 Recipe

For each litre:

- **Basal Medium**

49g Czapek-Dox solution agar (DIFCO)

15g Potassium chlorate or potassium chloride

1g Yeast extract

1g Casamino acids

Measure above ingredients into a 1 litre beaker. Make up to 800 ml with hot tap water and stir until dissolved. Pour into Schott bottle and make up to 1 litre with tap water. Autoclave. While autoclaving make up stock solutions as follows:

- **Terrachlor** - In a 100 ml volumetric flask add 21.5 ml of 95% ethanol and make up to 100 ml with sterile distilled water (SDW) giving a 20% ethanol solution. Add Terrachlor and shake.
- **Copper sulphate** - In a 100 ml volumetric flask add 10g copper sulphate and make up to line with SDW. Put in waterbath to dissolve into saturated solution.

NB. Make up fresh stock solution for each batch of media. Keeps only a few days.

After autoclaving:

Pour lactic acid, Terrachlor (shake well first), and copper solution into small beakers. Pour agar into 1 litre beaker and with syringes add:

- **7 ml Copper sulphate solution (0.7 g/l)**
- **7 ml Terrachlor solution (70 mg/l)**
- **Final pH 3.10**

While stirring agar with pH meter, adjust pH with **Lactic acid** using syringe (approx. 4–5 ml) until final reading of **pH 3.10.**

Pour thickly into sterile petri dishes for field use because the agar dehydrates readily - gives approx. 25–30 plates per litre. Refrigerate until required. Life of agar is unknown but it may deteriorate after several days in storage. Therefore it is recommended that fresh media be made up for each field sampling.

The following explanatory notes were requested to be included when I first published research which utilised the selective medium.

> "In order to measure the dispersal of spores from a treated thistle stand a selective medium for *S. sclerotiorum* was developed. This was based on a medium used by (Ben-Yephet & Bitton, 1985) to study the dispersal of *S. sclerotiorum* spores from a wheat-field in Israel. However, it was found that this medium was not sufficiently selective to be exposed for long periods of time under New Zealand pasture conditions. *S. sclerotiorum* is very tolerant to $CuSO_4$ even at a very low pH and this characteristic was used to increase the selectivity of the medium. It was found that 73% of spores from an homogenous spore suspension germinated on this $CuSO_4$/pH modified medium compared with 76% on Ben-Yephet's medium but there was a higher level of selectivity." (G. Bourdot pers comm.).

3.2 Colony characteristics

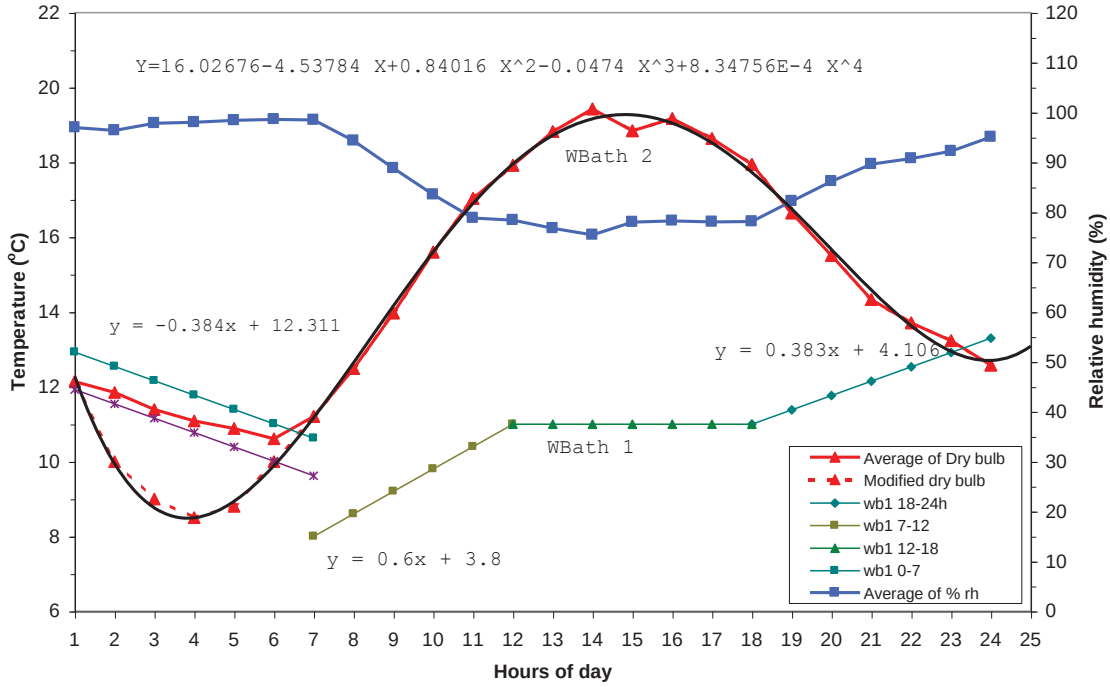
A selectivity test was carried out to check that colonies of *S. sclerotiorum* (isolate Sc1) could be distinguished from four fungal species which commonly occur within kiwifruit orchards. One isolate of *B. cinerea*, *Cladosporium cladosporioides*, *E. purpurescens* and *Alternaria alternata*, isolated from floral and leaf tissues in kiwifruit orchards, was supplied by Dr Phil Elmer, Hort Research, Lincoln. A 50µl aliquot of a conidial suspension (approximately 5×10^4 conidia ml^{-1}) of each of the above five isolates, was spread onto three replicate Petri dishes of JK selective medium. These were incubated on the laboratory bench and observed after 2 and 4 weeks.

S. sclerotiorum ascospores produce small white colonies with a dense and slightly raised centre (2–3 mm) and a similar sized beige coloured area on the underside of the colony after 2 weeks incubation on JK selective medium. After 4 weeks a slowly spreading flat colony sometimes developed, particularly when few colonies were present on the medium. These colonies of *S. sclerotiorum* were readily counted from

the underside of the Petri dish. Sclerotia formed on only a few colonies and could not be used to quantify colony formation.

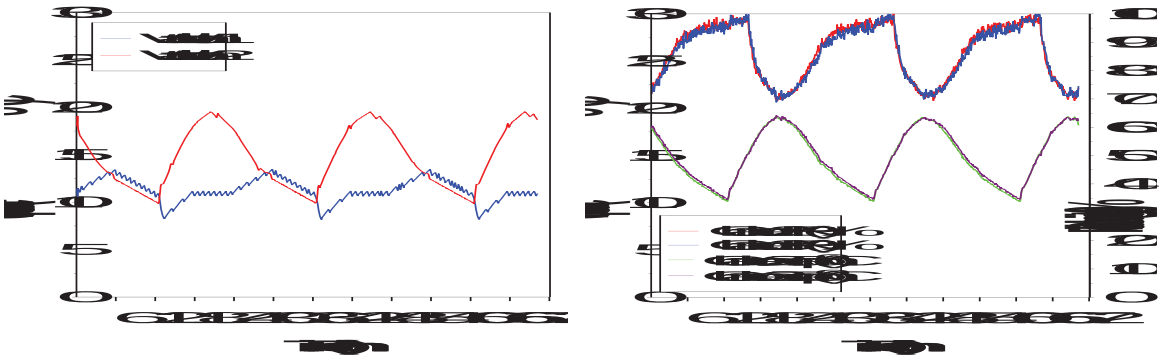
B. cinerea produced raised dome-like grey colonies which sporulated after 2–3 weeks. *C. cladosporioides* produced dark green/black colonies <3 mm diameter after 2 weeks, expanding to form raised irregular shaped colonies (5–8 mm diameter) after a further 2 weeks. *E. purpurescens* and *A. alternata* had not formed colonies after 4 weeks incubation. *S. sclerotiorum* could therefore easily be distinguished from these four common fungal species.

Appendix 4: Temperature and relative humidity within water chambers during diurnally fluctuating conditions



Mean 24-hourly temperature and relative humidity (RH) at Te Puke Research Orchard meteorological site from 1 October–31 December 1996, modified temperature profile used for fitting polynomial and plots of functions used for controlling the set points of waterbath 1 and 2 to achieve diurnal fluctuation of temperature and RH.

A B



(A) Temperature profile of waterbath 1 and 2 and (B) air temperature and relative humidity (RH), within waterbath chambers 2 and 3 during first run of treatment 6 (diurnally fluctuating temperature and RH) in Experiment 5-10.

Appendix 5: Curve fitting results for the thermodynamic growth rate model

Estimates of parameter coefficients for the thermodynamic growth rate model (equation 1, section 5.4.4) following non-linear regression of mycelial growth rate (Figure 5.11), percentage area of lesions (Figure 5.13) and colony forming units (cfu)/petal (Figure 5.14) against temperature, in Origin.

Factor	Figure	Parameters						curve
		p_{25}	ΔH_A	ΔH_H	$\Delta T_{1/2H}$	ΔH_L	$\Delta T_{1/2L}$	optimum
Mycelial growth	5.11	1.2	1176	143099	300.6	-33444	287.4	24.2^{o}C
% area lesions	5.13	116.2	5094	77573	302.3	-89900	288.7	24.0^{o}C
cfu/petal	5.14	2908	5308	80130	301.3	-79950	290.0	24.2^{o}C

Appendix 6: Publications during thesis

Hoyte, S.M. (1996). Does Sclerotinia infect your floral tissue? New Zealand Kiwifruit **November**, 11.

Hoyte, S.M. (1997). Sclerotinia - the pathogen and its management. Technical Updating Seminar HortResearch Internal Report 97/53, pp. 5-10.

Hoyte, S.M., Pak, H.A. & Manning, M.A. (1997). Pathways of Sclerotinia infection in kiwifruit. In: Proceedings of the 11th Biennial Australasian Plant Pathology Society Conference, Perth, Australia. pp. 94.

Pak, H.A., Manning, M.A. & Hoyte, S.M. (1997). Apothecial density and disease incidence in Sclerotinia on kiwifruit. In: Proceedings of the 11th Biennial Conference of the Australasian Plant Pathology Society Conference, Perth, Australia. pp. 96.

Hoyte, S.M., Elmer, P.A.G., Reglinski, T. & Perry, J. (1998). Biological Suppression of Sclerotinia: A future option for Disease Control. Kiwifruit Technical Updating Seminar, HortResearch Internal Report 98/48, pp. 39-44.

Elmer, P.A.G., Hoyte, S.M. & Reglinski, T. (1999). Biological suppression of *Sclerotinia sclerotiorum* in kiwifruit. In: Proceedings of the 12th Biennial Australasian Plant Pathology Society Conference, Canberra, Australia. pp. 63.

Hoyte, S.M., Pak, H.A. & Maning, M.A. (1999a). Development of a disease risk prediction system for Sclerotinia. In: Proceedings of the Kiwifruit New Zealand Research Ltd. Conference. "Creating Wealth Through Research", Rotorua. pp. 52-57.

Hoyte, S.M., Pak, H.A. & Manning, M.A. (1999b). Sclerotinia Disease Risk Prediction 1998/99. Report to Kiwifruit New Zealand 165/99, HortResearch client report No. 2000/84. 25 p.

Hoyte, S.M. (2000). The role of adhering floral tissues in sclerotinia disease of kiwifruit. New Zealand Plant Protection 53, 138-142

Revised section for the KiwiGreen Manual

MANAGEMENT PRACTICES FOR THE PREVENTION OF FUNGAL DISEASES

DISEASE DEVELOPMENT

Sclerotinia disease is caused by spores released from apothecia (small beige coloured mushrooms) which form at the soil surface beneath vines. During flowering and petal-fall floral tissues become infected and flower blight can occur, particularly with wet weather. These floral tissues are retained on fruit and are the main source of fruit disease (scarring and soft rot) during wet weather from December to late February.

DISEASE RISK

Disease risk in an orchard block is determined by previous levels of Sclerotinia disease, the presence of apothecia within orchard blocks, and prevailing weather conditions, especially during flowering and petal-fall. Fungicide application is only warranted under moderate to high disease risk.

MANAGEMENT PRACTICES

Fungicide application(s) are the main form of disease control at flowering/petal-fall and during high risk periods up until late-February. Benlate must be applied prior to first petal-fall, and because Rovral acts as a protectant, it should be applied before disease symptoms become evident i.e. before rain rather than after.

Cultural control options include:

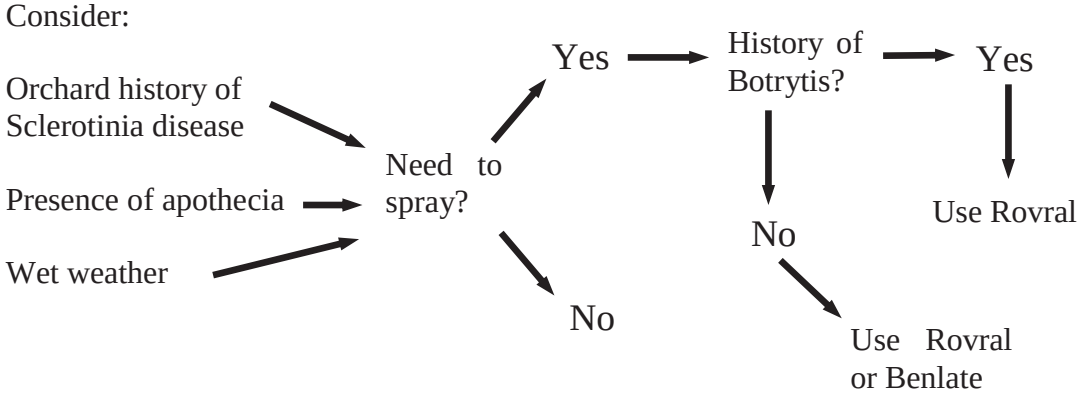
- ◊ Maintain adequate air circulation and sunlight into the vines by; selecting high quality moderate vigour canes after winter pruning; summer pruning to avoid the development of a dense canopy; pruning males early, and trimming and topping shelter.
- ◊ Remove male vine prunings from blocks.
- ◊ Thin the crop early, as infection can also develop where fruit are touching.

CHOICE OF FUNGICIDE

Benlate treated properties tend to have more Botrytis compared with properties left untreated or treated with dicarboximide (Rovral). Rovral is therefore the preferred fungicide to prevent Sclerotinia disease in orchards with a history of Botrytis stem-end rot.

DETERMINING THE NEED FOR CONTROL

Consider:



Appendix 7: CD-ROM electronic copy of thesis, CR10 datalogger programme, conference papers and powerpoint presentations

Contents of CD-ROM

Return to Conceptual_Model_of_Disease_Development

Apothecial Formation:

Effect_of_size_and_source_of_sclerotia

Production_of_apothecia

Return to Conceptual_Model_of_Disease_Development

Sclerotial Formation:

Production_from_whole_vines

Effects_of_fruit_length

Sclerotial_size_grades

Return to Conceptual_Model_of_Disease_Development

Primary infection of floral tissues:

Detached_petal_assay_method

Evaluation_of_detached_petal_assay

Effects_of_flower_age

Effects_of_temperature

Effects_of_relative_humidity

Psychrometric_plots

Return to Conceptual_Model_of_Disease_Development

Secondary spread to flowers:

Type_of_inoculum

Effects_of_misting_duration

Return to Conceptual_Model_of_Disease_Development

Adhering floral tissues:

Survey_of_AFT

Fruit_scarring_with_and_without_AFT

Categories_of_AFT

Removal_of_AFT

Return to Conceptual_Model_of_Disease_Development

Secondary spread to fruit:

Inoculum_type_and_misting

Effects_of_misting_in_December

Effects_of_misting_in_January