EPIDEMIOLOGY AND MANAGEMENT OF FLOWER DISEASES OF PYRETHRUM

By

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This thesis contains no material which has previously been accepted for the award of any other degree or diploma at any other University, and to the best of my knowledge this thesis contains no material published previously or written by another author except where reference has duly been made.

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Abstract

Pyrethrum *(Tanacatum cinerariifolium)* is cultivated worldwide and in southern Australia for the extraction of insecticidal esters or pyrethrins contained within the achenes of flowers. Producing a significant proportion of the worlds botanical pyrethrins, Australian crops may suffer reduced yields from annual flower disease epidemics caused by pathogenic fungi *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Little is known regarding (i) the epidemiology of flower blights caused by *B. cinerea* and *S. sclerotiorum* in pyrethrum, (ii) the efficacy of current control methods, (iii) whether there is evidence of fungicide resistance in the fungal population, (iv) whether alternative fungicides can provide improved control over those currently used, (v) whether other fungi could be involved in annual flower disease epidemics and, (vi) the loss in flower yield and pyrethrin assay from flower diseases.

A survey of the incidence of *B. cinerea* and *S. sclerotiorum* in flowers was undertaken in ten commercial pyrethrum crops in one year. Flowers from non-fungicide treated areas in commercial crops were periodically sampled throughout flowering prior to being surface sterilised and incubated under high humidity to encourage fungal growth. *B. cinerea* and *S. sclerotiorum* were prevalent, with both occurring in flowers from all 10 crops. The mean incidence of *B. cinerea* in flowers sampled between 10-11 December across all crops was 56%, significantly higher (P = 0.024) than *S. sclerotiorum* (29.4%), while between 16-18 December mean incidence of *B. cinerea* was 75.7%, again significantly higher (P = 0.026) than incidence of *S. sclerotiorum* (51.8%) at this time.

The main means of managing flower blights of pyrethrum is currently through a fungicide program over flowering involving tebuconazole and carbendazim. The efficacy of the flowering fungicide program for controlling *B. cinerea* and *S. sclerotiorum* flower blights and promoting benefits in terms of increased yield was evaluated in nontreated and fungicide treated plots in 10, 10 and 17 commercial pyrethrum crops during the flowering period over three years, respectively. In each of two years, fungicide treatment resulted in a mean incidence of *B. cinerea* near to harvest which bordered on being significantly lower ($0.05 < P < 0.1$) than no treatment. In the third year, fungicide treatment significantly (P = 0.038) reduced mean fungal incidence from 75% to 53.3% in nontreated close to harvest. The incidence of *Sclerotinia sclerotiorum* in flowers from nontreated was significantly higher ($P < 0.001$) than from fungicide treated in year one (62.8% and 29.2% respectively) and again significantly higher (P = 0.019) in year two (26% and 8.1% respectively). Across all fields, fungicide applications in year one resulted in no significant yield improvement. During year two, mean pyrethrin yield (across all sites) was significantly increased from 90.4kg/ha in

nontreated areas to 108.4 kg/ha in fungicide treated plots (*P* = 0.032) and during the final year fungicide treatment significantly increased dry flower yield by 216 kg/ha (*P* = 0.018) in comparison to nontreated. However, while fungal incidence was often reduced within individual fields or across all sites by fungicides, few measurable benefits in yield quantity or quality were measured. *In vitro* sensitivity testing of 49 and 96 isolates of *B. cinerea* and *S. sclerotiorum* respectively to fungicides commercially used for control was conducted. A proportion of *B. cinerea* isolates appeared sensitive to carbendazim at low concentrations i.e. 39% had EC_{50} less than 1.05 µg a.i./ml. However a high proportion (61%) of *B. cinerea* isolates had EC_{50} values to carbendazim greater than 100 µg a.i./ml. This provided evidence for potential resistance amongst the population of *B. cinerea* and may explain the inconsistency of yield responses from the fungicide program. By comparison, 96.9% of *S. sclerotiorum* isolates had EC_{50} values <2.4 µg a.i./ml to carbendazim with a mean of 0.5 µg a.i./ml. However, a small number (3.1%) of *S. sclerotiorum* isolates were highly insensitive to carbendazim with EC_{50} of >1000 µg a.i./ml. All isolates of *B. cinerea* and *S. sclerotiorum* were sensitive to tebuconazole at low concentrations with mean EC_{50} of 0.64 and 0.18 µg a.i./ml respectively,

Alternative fungicides for flower disease control were evaluated in replicated field trials to determine suitability for inclusion into the flowering fungicide program as replacements for the potentially ineffective and now deregistered fungicide carbendazim. Boscalid and iprodione showed greater benefits in terms of yield from statistical analysis than other treatments, and significantly reduced fungal incidence of flowers equal to, or better than, commercial treatments. The mean incidence of *B. cinerea* from nontreated flowers sampled on 4 December of 30% was significantly (*P* = 0.007) higher than boscalid (8.5%) and iprodione (10%). Mean incidence of *S. sclerotiorum* of flowers from nontreated plots sampled on 4 December was 26.5%, and significantly (*P* <.001) higher than boscalid (7%) and iprodione (12%). In *vitro* fungicide sensitivity of 46 isolates of *B. cinerea* and *S. sclerotiorum* to iprodione indicated no evidence of reduced sensitivity with mean EC_{50} (and maximum) values of 1.62 (8.48 µg a.i./ml) and 0.19 (0.61 µg a.i./ml) respectively.

Sclerotinia minor, a previously undocumented pathogen of pyrethrum flowers, was consistently isolated from diseased, surface-sterilised pyrethrum flowers over multiple years. Fungal identity was confirmed with morphological, genetic and phylogenetic evaluation. Occurrence in-field of mature, sporulating, apothecia of *S. minor* were documented; while sclerotia of 8 of 10 isolates conditioned in the laboratory successfully underwent carpogenic germination *in vitro* demonstrating the ability of endemic isolates of *S. minor* to produce air borne inoculum necessary to achieve flower infection in pyrethrum and other crops. The relative sensitivity of isolates of *S. minor* to the

fungicides carbendazim, tebuconazole and iprodione was evaluated. Mean (and maximum) EC_{50} values of *S. minor* were 1.92 (2.62 µg a.i/ml) for carbendazim, 0.1 (0.13 µg a.i./ml) for tebuconazole and 0.3 (1.23 µg a.i/ml) for iprodione.

The effect of *B. cinerea* and *S. sclerotiorum* flower inoculation on measured yield attributes and pyrethrum flower development were evaluated with glasshouse studies. Inoculation of immature flowers with *Botrytis cinerea* resulted in highly significant reductions in dry weight (P <.001) and pyrethrin yield (P <.001) of mature flowers compared to non inoculated. Inoculation with *S. sclerotiorum* in two of four varieties resulted in significantly higher flower development stages after 21 days (P = 0.021; P <.001) and significantly faster flower senescence (P = 0.001; P = 0.008) in comparison to non inoculated. Inoculation of flowers with ascospores of *S. minor* led to symptoms of flower disease indistinguishable from those of *S. sclerotiorum* flower blight, significantly lower fresh flower weights in replicated experiments (P = <.001; 0.032) and significantly higher developmental stage of flowers (P = <.001) after only two weeks in comparison to non inoculated. These studies indicated that flower infection with *B. cinerea* and *S. minor* could significantly reduce flower yield and pyrethrin yields. Furthermore, significantly faster flower development; senescence and desiccation may result from flower infection with *S. sclerotiorum* and *S. minor.* The completion of Koch's postulates additionally confirmed pathogenicity toward pyrethrum flowers and demonstrated pure cultures of *S. minor* could be reisolated from flowers inoculated on living host plants.

The study has provided new knowledge in the epidemiology of known flower blights of pyrethrum caused by *B. cinerea* and *S. sclerotiorum*. Furthermore the study has described for the first time *S. minor* as a pathogen of pyrethrum flowers and documented a rare example of carpogenic germination of sclerotia being involved in the epidemiology of a disease caused by *S. minor*. The study has also provided insights into the effect of flower blights on pyrethrum yield and into improving the management of flower diseases by fungicides.

Introduction

Valued at around $25 million annually (DPIWE, 2010a), commercial cultivation of pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.) in Australia occurs mainly in Tasmania. Approximately 2000 ha of land on Tasmania's northwest coast are cropped each year, for production of insecticidal esters known as pyrethrins. Pyrethrins are contained within the seeds of the flower head, and following extraction and refining are incorporated into a broad range of arthropod pest control products used worldwide. Roughly 1700 ha was cultivated during the 2009-2010 season (Sarah Pethybridge, *Personal* communication).

A number of economically important fungal pathogens exist in Tasmanian pyrethrum production, including flower blights caused by *Botrytis cinerea* Pers. and *Sclerotinia sclerotiorum* (Lib.) de Bary. (Pethybridge *et al,* 2008a). *Botrytis* flower blight and *Sclerotinia* flower blight both occur during the flowering period and are favoured by cool, damp weather and infect flowers via windblown spores. While both fungi are known to cause blights during flower, information about the epidemiology of these diseases is lacking.

Sclerotinia sclerotiorum and another species of *Sclerotinia, S. minor,* also cause of crown rot disease of pyrethrum in Tasmania. Crown rot causes wilting and death of plants throughout the year following mycelogenic germination of sclerotia in the soil. While existing in pyrethrum crops, *S. minor* has not previously been documented to contribute to flower disease epidemics in Tasmania.

Control of flower diseases depends on fungicides, presently tebuconazole and carbendazim, applied throughout the flowering period in Tasmanian pyrethrum crops. Quantitative information however, on the effect of flower infection on yield attributes, and the efficacy of fungicide applications on reducing pathogen incidences and improving yield and quality is at this time unknown. However there is some preliminary evidence to suggest reduced sensitivity of *B. cinerea* to currently used fungicides based on *in vitro* studies (Pethybridge and Cole, *Unpublished data*). Reliance on only two fungicides for flower disease control may be of particular concern if reduced

sensitivity was detected. Alternative fungicides from different resistance groupings could be required to provide effective flower disease control in future and should therefore be investigated.

There is currently a gap of information regarding flower disease onset and intensity through the susceptible flowering period; efficacy of fungicides presently used at reducing pathogen incidence in the field and enhancing yield qualities; and whether unidentified phytopathogenic fungi are also associated with flower diseases.

Objectives of this study were therefore to:

- Provide more detailed epidemiological information on flower blights of pyrethrum caused by *Botrytis cinerea* and *Sclerotinia sclerotiorum*, and investigate whether *S. minor* or other fungi play a role in flower disease epidemics.
- Evaluate the efficacy of the current flowering fungicide program.
- Determine if alternative fungicides can provide improved control of flower diseases in Tasmanian pyrethrum crops.
- Assess the sensitivity of known causal agents of flower diseases to fungicides.

Quantitatively assess the effect of flower infection with known flower disease causing fungi on aspects of flower developments, flower yield and pyrethrin assay.

Literature review

Pyrethrum

Cultivated pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch.Bip.) is an angiosperm and member of the family *Asteraceae*. This family comprises approximately 1,100 genera and 25,000 species, and is characterised morphologically by its floral components, in which the 'capitula' or 'heads' bear ray and disc florets inserted on a flattened receptacle surrounded by bracts (Curtis and Morris, 1956; Toothill and Blackmore, 1984). A number of species belonging to genus *Tanacetum* have been identified as possessing insecticidal properties, including *T. cinerariifolium* and *T. coccineum* (Katsuda, 1999).

Pyrethrum is a herbaceous plant that grows to a height of approximately 1 m. Leaves are pinnately lobed/narrowly lanceolate to oblong lanceolate alternate (Brown and Menary, 1994). Plant habit is slender and tufted, the flowers are borne on a solitary capitula comprised of 18-22 white pistillate ray florets surrounding 40 to 100 yellow bisexual disc florets atop convex to sub-globose receptacles (Plate I; Zito, 1994). The involucre diameter varies between 12 to 18 mm, while 3 to 10 ribbed achenes are found between floret and receptacle of all florets (Heywood, 1976).

Plate I - (A) Pyrethrum flower with yellow disc florets encircled by white ray florets, **(B)** *Tanacetum cinerariifolium* (Source: Freeland, 2001)

Pyrethrum was endemically distributed in Dalmatia (formerly Yugoslavia) along the Adriatic coast east of Italy (Katsuda, 1999; Cupac *et al,* 2002). The inherent insecticidal properties possessed by pyrethrum have been long recognised, and referred to in Chinese literature as early as the first century AD (Mocatta, 2003). In the early 19th century in Europe, insecticidal preparations combining extracts of numerous *Chysanthemum* species (*C. roseum, C. coccineum, C. corneum* and *C. balsamita*) were widely used, with these crude refinements referred to as 'Persian insect powders' (Casida and Quistad, 1995; Duval, 1993). However, after pyrethrum's superior insecticidal properties were discovered, this species dominated the natural insecticide market (Katsuda, 1999). Kerosine extraction of pyrethrins active ingredients during 1919, development of gas aerosol sprays in 1941 as well as the use of synergists proved other notable enhancements of pyrethrum efficacy (Winney, 1976). Other species known to contain pyrethrins include *Dysodia tenuifolius, Ageratum conzoides, Tagetes minuta* and *T. patula,* but at lower concentrations than *T. cinerariifolium* (Kamal and Mangla, 1987; Kamal and Mehra, 1991). Chemical precursors to pyrethrins have been obtained from *Artemisia annua,* however relative insecticidal properties are not reported (Allen *et al,* 1977).

Improved species identification within genus *Chrysanthemum* during the 1840's marked the beginning of documented pyrethrum cultivation. For example, Dalmatian 'insect powders' were probably formulations based on extracts of pyrethrum, and originated from the Dalmatia' region of present day Croatia (Glynne-Jones, 2001). Introduction and cultivation of *T. cinerariifolium* in the USA, Japan, Switzerland, France, Australia as well as a number of African nations led to the formation and development of the pyrethrum industry (Casida and Quistad, 1995).

While the defining features of *Tanacetum* genus members were documented by C. H. Schultz Bipontinus in 1844, within the literature, pyrethrum has been referred to as *C. cinerariifolium* and *T. cinerariifolium.* Both botanical names appear correct until genus *Chrysanthemum* itself was more clearly defined in 1987, when numerous perennials including *Tanacetum* were reclassified to separate genera based on capitula structure (Anderson, 1987). Variation in species nomenclature has existed also, with both *T. cinerariaefolium* and *T. cinerariifolium* used throughout literature until changes to connecting vowels in 2009 (Anonymous, 2011).

Pyrethrins and pyrethroids

Pyrethrum is cultivated for the production of pyrethrins; a group of six closely related esters possessing non-persistent insecticidal properties, low mammalian toxicity and limited photo stability (Zito *et al*, 1983). Pyrethrin degradation can also be caused by acid or base, heat and microbial activity (Otieno and Pattenden, 1979). These six esters are collectively referred to as 'pyrethrins' and are separated into two groups of three, pyrethrins I and pyrethrins II (Plate II).

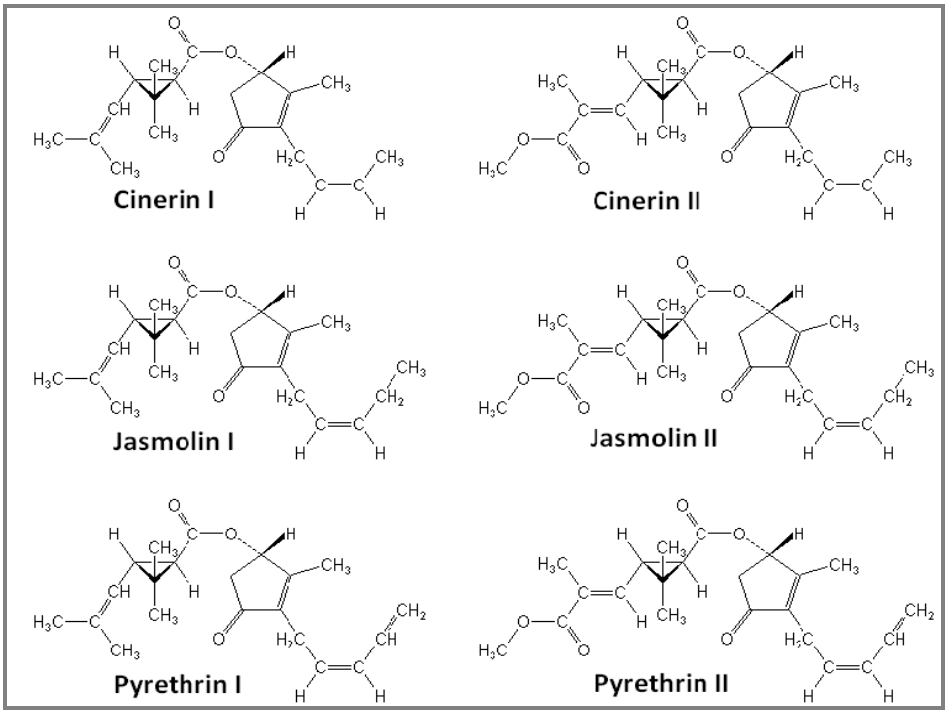


Plate II - Structural formulae of pyrethrins. (Source: www.alanwood.net/pesticides/pyrethrins.html)

Pyrethrin I (C21 H28 O3), cinerin I (C20 H28 O3) and jasmolin I (C21 H30 O3) are components of chrysanthemic acid, and make up the pyrethrin I fraction. Products of pyrethric acid comprise the pyrethrin II fraction, being pyrethrin II (C22 H28 O5), cinerin II (C21 H28 O5) and jasmolin II (C22 H30 O5) (Brown, 1992; Glynne-Jones, 2001). Pyrethrins I and pyrethrins II were chemically defined and isolated in 1924, followed by cinerin I and cinerin II in 1944, and finally jasmolin I and jasmolin II in 1965 (Beevor *et al,* 1965; Godin *et al,* 1966).

Small amounts of pyrethrins are found inside oil glands within secretory ducts in leaf, stem and root tissue, while most (94%) are located within oil glands of the achenes comprising mature pyrethrum flower heads (Zito *et al,* 1983). When mixed with the synergist piperonyl butoxide arthropod detoxification is competitively inhibited enhancing pyrethrin efficacy by 6.5-18.7% in some species (Glynne Jones and Green, 1959; Vashkov *et al,* 1976; Maciver *et al* 1997). Essential oil extracts from *Pongamia glabra, Blumera lacera* and *B. malcomii* as well as sesame oil have also shown the ability to synergise natural pyrethrins, but to a lesser extent than piperonyl butoxide (Parma, 1977; Joffe *et al,* 2011).

Defining the chemical features of the six pyrethrin fractions allowed synthesis of analogues of these esters, the pyrethroids. The first of these, allethrin, developed in 1949, was a crude reproduction of its botanical counterpart. The addition of a chlorine molecule to pyrethroid structure led to improvements including photo stability, with isomers such as permethrin and deltamethrin developed during 1973-74 (Casida and Quistad, 1995). Originating as simple copies of a complex natural molecule, contemporary pyrethroids have diverged significantly in structure and increasing complexity, bearing little resemblance to their botanical templates (Katz, 1989). Pyrethroids typically have a faster knock-down time for arthropods but lower mortality rate 24 hours after treatment than pyrethrins (Anonymous, 1997a). Another characteristic unique to pyrethrins is insect repellence at below lethal doses (Winney, 1976).

Pyrethrins and pyrethroids have been extensively used worldwide in public health for control of numerous important disease bearing arthropods for over sixty years. For example, pyrethrin based insecticides may have saved more lives during World War two than penicillin (Anonymous, 1948; Isman, 2006). Despite the evolving chemistry of pyrethroids allowing broader environmental usage, aerial applications of synergised pyrethrins have provided successful control of *Anopheles albimanus,* the predominant vector of malaria in El Salvador (Pal, 1953; Hobbs, 1976; Megaw, 1984). Moreover, domestic insecticidal preparations and products incorporating pyrethrins as active ingredients include pyrethrin impregnated mosquito nets and coils thermal fogs, mists, bug bombs or foggers for indoor use, fabric protectants, protective food packaging, human head and body lice treatments as well as pet flea collars and sprays (Rajan, 1975; Bry *et al,* 1977; Highland *et al,* 1977; Robinson and Shepherd, 1984).

Despite widespread use of synergised pyrethrins since the 1930's, only four cases of resistance were reported worldwide by 1963, and there is little evidence of resistance documented without previous pyrethroid exposure (Fine, 1963; Megaw, 1984). Conversely, reports of reduced sensitivity via prolonged use of pyrethroids are extensive. Resistance to pyrethroids by the prevalent insect vector of malaria, *Anopheles gambiae* has been reported in Côte d'Ivoire, Benin, Burkina Faso (Chandre *et al,* 1999), Kenya (Hemingway and Ranson, 2000) and western Uganda (Rubaihayo *et al,* 2008); while organophosphate resistant populations of two-spotted spider mite *Tetranychus urticae* of hop in the Czech Republic has shown cross resistance to multiple pyrethroids (Hurkova, 1984). Pyrethroids have substantially diverged from early isomers and now have very little structural

resemblance to pyrethrins, some more closely resembling the chlorinated hydrocarbons and have similar resistance mechanisms and patterns as DDT (Adams and Miller, 1980; Katz, 1989). Pyrethroids have shown greater evidence of reduced efficacy from resistance and cross resistance than natural pyrethrins, which when mixed with a synergist are used at lesser concentrations attracting lower ecological risk of resistance development than other insecticide classes (Fine, 1963; Gaaboub, and Shaker, 1971; Cichy, 1971).

Cultivation

Prior to the First World War, the predominant producer of pyrethrum worldwide was its endemic region of origin, now present day Croatia (Wainaina, 1995). Japan succeeded as the leading producer after this, while pyrethrum was introduced into the highlands of numerous east African countries such as Zaire, Tanzania, Rwanda and Kenya during the late 1920's. The Second World War enabled Kenya to become the foremost producing nation by 1941, and during 1996 Kenya still ranked as the largest producer, contributing over 70% of total world production (Tuikong, 1984; Casida and Quistad, 1995; Wanjala, 1997).

Issues with reliability of supply, stemming from political pressures in other countries and the development of superior varieties and more mechanised means of production have allowed Australia to become the main producer of natural pyrethrins worldwide, contributing approximately 60% of total production during 2009 from cultivation in Tasmania (Sarah Pethybridge, Botanical Resources Australia, *Personal communication*). Other major pyrethrum producing nations in order of current market contribution are Kenya, Rwanda, Tanzania, China and Papua New Guinea (Pethybridge *et al,* 2008a).

Grown at altitudes of 1800-2900 m in Kenya, Rwanda and Tanzania on predominantly small scale plots less than 2.0 hectares in size, crops are established with transplanted clones before periodic hand harvesting of flowers when mature (Wainaina, 1995). Agronomic inputs are often not fiscally viable despite notable research on pest, disease and weed control, climatic effects, plant breeding, fertiliser application and mineral deficiencies being documented throughout the Kenya based African pyrethrum industry journal "Pyrethrum Post" amongst others (Pinkerton, 1970;

Parlevliet, 1970a and 1970b, 1975a and 1975b; Mwakha, 1974; 1979a and 1979b; Wainaina, 1995; Wanjala, 1997).

Australia

In Australia, pyrethrum is currently grown in Tasmania and Victoria. Most production of pyrethrum in Australia occurs in the island state of Tasmania, which is approximately 68,000 km^2 in size and situated off the southern coast of mainland Australia, between latitude 40-43° South and longitude 144-149° East. Notable variation in the cool temperate climate exists across the island, caused by the moderating sea influence and prevailing north westerly winds. Severe frosts are common during winter in the midlands, while the highlands and the west coast are colder and damper than the east coast and lower lying areas. Victoria is the southernmost state of mainland Australia, approximately 227,600 km^2 in size and located between latitude 34-39° South and longitude 141-150° East. Having a temperate climate which varies between highland, coastal and inland regions, Victoria receives more rainfall annually than all states and territories except Tasmania.

In contrast to the lack of sophistication and limited agronomic inputs in most pyrethrum production systems worldwide, a highly mechanised approach to all aspects of crop agronomy is utilised in Tasmania pyrethrum cultivation (MacDonald, 1995). Following renewed interest over the preceding fifteen years elsewhere at cultivating botanical pyrethrins, research in Tasmania was initiated in 1978 to determine its prospects as a lucrative horticultural crop by Professor R. C. Menary of the University of Tasmania. Overseas plant material and expertise was sought and obtained, followed by selection of plants with higher yields, lower levels of lodging in addition to synchronous flower development (Bhat and Menary, 1984). Selection trials also successfully produced progeny which could grow with sufficient vigour under cultivation at low altitude and high latitude in Tasmania, contrasting the typical low latitudes and high altitudes pyrethrum has been commercially cultivated at previously (Bhat and Menary, 1984).

Pyrethrum cultivation in Tasmania was initiated by the British Oxygen Company (BOC) in 1981, after successful plant breeding trials developed ‘Hypy’, a higher yielding clone (Fulton, 1998).

After restructuring within the industry during 1996, Botanical Resources Australia Pty. Ltd (BRA) has been the largest producer of pyrethrins in Australia, cropping approximately 1700 hectares in Tasmania and Victoria for refinement in Ulverstone, Tasmania annually. Valued at approximately $25 million per year, Australian production now supplies around 60% of the world demand for natural pyrethrins having almost doubled market share within the last four years (DPIWE, 2010a; Sarah Pethybridge, Botanical Resources Australia*, Personal communication*).

Australian crops are sown with seed between July and September each year, and cut for harvest the following December when flowers are mature. Crops are harvested thereafter annually for up to five consecutive flower harvests, or until plant mortality and high weed pressure lead to crop termination. Controlling weed species over a period of years in pyrethrum crops remains challenging, with herbicide applications the predominant means of management (Rawnsley *et al*, 2006). During cutting, crop material is pushed into windrows and left to dry for around two weeks. Flowers and achenes are then mechanically separated from trash by modified headers and taken for refinement (Anonymous, 2004a).

Fungal diseases of pyrethrum

Worldwide

Fungal pathogens documented to cause disease in pyrethrum overseas include crown and root rots caused by *Armillaria mellea*, bud and flower blights caused by *Coniothyrium* and *Microdiplodia* species, flower and leaf blights caused by *Diplodia chrysanthemella* and *Helminthosporium* species, ray blight and bud disease from *Ramularia bellunensis* infection, bud disease due to *Phyllosticta* sp., vascular wilting, flower blights as well as crown, root and stem rots attributed to *Sclerotinia* sp. and leaf spot caused by *Septoria chrysanthemella* (Natrass, 1947; Robinson, 1963; Anonymous, 1987). *Alternaria solani, A. tenuissima* and *Stemphyllium* sp. were found in association with diseased *Chysanthemum* sp. symptomatic of flower blight, however causal relations were not demonstrated (Cox, 1969).

Fusarium, *Sclerotinia*, *Ascochyta* and *Rhizoctonia* species have been reported as causing significant root rot diseases in Kenya, while wilt diseases caused by *Phytophthora cambivora* and *R. solani* and root rot caused by *F. solani* anastomosis group four (AG-4) have challenged cultivation in India (Warui *et al,* 1991; Alam *et al,* 2004). *S. minor* is responsible for wilting and crown rot disease of pyrethrum in Kenya, symptoms also believed related to damage from numerous phytopathogenic nematodes, whereby *Meloidogyne* sp. feeding sites allow easy infection for fungi including *F. solani* (Natrass, 1950; Bullock, 1961; Wanjala, 1991).

Numerous plant pathogens including nematodes, viruses, and fungi have been identified and described in Tasmania since pyrethrum cultivation began (Pethybridge *et al,* 2004c; Pethybridge *et al,* 2008a). Despite over six decades of African pyrethrum cultivation research there are few disease reports and fewer disease control measures documented which provide information useful in Tasmania. This suggests the significantly lower altitudes and higher plant densities pyrethrum is cultivated at in Tasmania provide may more favourable conditions for disease development. This, in addition to economics and differing levels of industry mechanisation may often render disease control unwarranted, unaffordable or impractical in low intensity production systems (Pethybridge *et al,* 2008a).

Australia

The most significant fungal diseases affecting pyrethrum cultivation in Tasmania can broadly be grouped into three categories. Crown rot, ray blight and flower blight disease epidemics can occur progressively throughout the year, requiring the timely application of fungicide programs to optimise disease control and yield (Table I; O'Malley *et al,* 2008; Pethybridge *et al,* 2006b, 2008a and 2008b). *Fusarium* sp. have been reported to significantly reduce seedling emergence in Tasmania; while *Aureobasidium pullulans, Alternaria alternata, A. tenuissima, B. cinerea, Cladosporium cladosporioides, C. herbarum, Microsphaeropsis* sp., *Mucorales* sp., *Penicillium* sp., *P. ligulicola, P. epicoccina, Stemphylium botryosum* (teleomorph: *Pleospora herbarum*), *Ulocladium atrum, Rhizoctonia* sp. are often identified on diseased leaves (Aldaoud *et al,* 1995; Pethybridge *et al,* 2006a). The pathogenicity of *A. alternata, A. tenuissima, S. botryosum, C. cladosporioides and U. atrum* to several pyrethrum cultivars was evaluated. *Alternaria tenuissima* and *S. botryosum* inoculation resulted in necrotic ring and spot development on foliage. *Alternaria alternata, C.*

cladosporioides and U. atrum were deemed saprophytic in the absence of disease symptoms (Pethybridge *et al,* 2004a). More recently a new fungal species, *Microsphaeropsis tanaceti* has also been demonstrated pathogenic toward pyrethrum in the field. Brown, necrotic leaf spots may be observed during winter when cool, wet weather favors fungal growth and dissemination of conidia (Pethybridge *et al,* 2008c). Many of these fungi are believed to contribute to the loss of green leaf area over winter.

Pathogen/s	Disease	Fungicide/active ingredient (a.i.)	Activity group
Sclerotinia sp.	Crown rot	Filan^{R} (boscalid)	7
		Sumisclex^{R} (procymidone)	2
P. ligulicola	Ray blight	Amistar^{R} (azoxystrobin)	11
		Bravo^{R} (chlorothalonil)	M5
		Filan^{R} (boscalid)	7
B. cinerea &	Flower blights	Rovral^{R} (iprodione)	1
S. sclerotiorum		Folicur^{R} (tebuconazole)	3

Table I - Fungicides used in Tasmanian in pyrethrum cultivation (Source: O'Malley *et al,* 2008; Pethybridge *et al,* 2008b).

Crown rot diseases caused by S. minor and S. sclerotiorum

Crown rot disease is caused by both *S. minor* and *S. sclerotiorum,* and seriously effects pyrethrum production in Tasmania (Casida and Quistad, 1995). Occurring during crop establishment and growth, symptoms begin with pale, bleached lesions on stems prior to wilting and rapid plant death (Plate III). Crown infection is believed to occur via myceliogenic germination of sclerotia residing in soil when conditions are favourable, often during autumn, winter and early spring. Sclerotia develop on and inside stems of infected plants. Sclerotia of *S. sclerotiorum* may also form on or within infected flowers.

Plate III - Crown rot caused by *Sclerotinia* spp. during December in Tasmania.

Ray Blight disease caused by Phoma ligulicola

Ray blight disease of pyrethrum, caused by *Phoma ligulicola,* has been reported in numerous countries (Robinson, 1963; Shaw, 1984) as well as being one of the most problematic diseases of Australian production (Pethybridge *et al,* 2007b; 2008a). The causal agent of Tasmanian ray blight disease is *P. ligulicola* var. *inoxydabilis,* varying from the type variety by inability to produce the antibiotic 'E' and having typically smaller conidia (Boerema *et al,* 2004). *Phoma ligulicola* is a Coelomycete fungus and the anamorph of *Didymella ligulicola*, the causal agent of ray blight disease in the closely related *chrysanthemum* genus (Horst and Nelson, 1997). Annual epidemics of ray blight disease in spring in Tasmanian pyrethrum have been recognised since 1999, causing shoot dieback and reductions in green leaf area and flower number (Plates VI-V) if not managed (Pethybridge and Hay, 2001).

Plate IV - Flagged plot at Wesley Vale trial site which received no spring fungicide applications for ray blight disease control showing reduced production of white flowers in comparison to surrounding commercial crop, flags superimposed.

Plate V - Necrotic, withered stem and flower typical of *Phoma ligulicola* var. *inoxydabilis* flower infection (Source: Sarah Pethybridge, Botanical Resources Australia, *Personal communication*).

Inoculum may be present in soil as resting structures (pseudosclerotia) or be harboured in planting material such as transplants or seed, with only anamorphic reproduction documented in Tasmanian pyrethrum fields (Pethybridge *et al,* 2006a). Fungicide applications are the predominant means of controlling ray blight disease in Tasmania. Site specific risk factors associated with ray blight disease development such as aspect and elevation have been investigated, leading to the avoidance of higher risk areas or fields (Pethybridge and Hay, 2001). Fungicide seed treatment with fludioxonil has demonstrated some efficacy at reducing seedborne inoculum of *P. ligulicola* var. *inoxydabilis* (Pethybridge *et al,* 2006a; O'Malley, 2007).

Flower diseases of pyrethrum

Pyrethrum flower diseases in Tasmania are commonly caused by two fungi, *S. sclerotiorum* and *B. cinerea* (Pethybridge *et al,* 2008a), with both often occurring together in Tasmanian pyrethrum crops.

Botrytis cinerea

Belonging to genus *Botryotinia* of the *Sclerotiniaeceae* family, *B. cinerea* Pers. Fr. is a necrotrophic fungus of order *Helotiales* (Williamson *et al,* 2007). Genus *Botryotinia* is known to be comprised of twenty two species and one hybrid through detailed phylogenetic analysis (Staats *et al,* 2005). *B. cinerea* is the anamorph stage of this fungus, with mycelia grey in colour, extensive, branched and septate.

Species of *Botrytis* are found in many environmental niches, and are some of the most prolific disease causing fungi worldwide (Agrios, 1997). Manifesting predominantly as floral blights with decaying fruiting bodies, *B. cinerea* causes plant diseases affecting over two hundred predominantly Dicotyledon plants of ornamental horticulture, perennial fruit cultivation and vegetable cropping in both greenhouse and field environments (Coley-Smith *et al,* 1980; Myresiotis *et al,* 2007). *B. cinerea* can also cause rotting of roots and other below ground storage organs, damping off of seedlings as well as stem rot diseases (Agrios, 1997). *B. cinerea* causes bunch rot of grapes throughout Australian viticulture, in addition being responsible for grey moulds of berry fruit cultivation on Tasmania's northwest coast (Essling *et al,* 2010; Craig Morris, Turners Beach Berry Patch, *Personal communication*).

Survival of *B. cinerea* and dispersal of inoculum is achieved through formation of propagules including chlamydospores, sclerotia, conidia, apothecia and ascospores (Holz *et al,* 2004). Asexual chlamydospores and microconidia produced by *B. cinerea* have been noted in association with older cultures, microbial contamination or during dry periods; and these structures may permit limited dissemination during periods unfavourable for conidia (Holz, 1999). Little information exists on the

survival of *B. cinerea* as mycelia under field conditions, indeed differentiating mycelium from microsclerotia or chlamydospores may itself be problematic (Holz *et al,* 2004).

Considered the most important survival means, sclerotia of *B. cinerea* may be black, grey or white in colour and very small or variable in size, with diameters reported to range between 1-18 mm (Clarkson and Whips, 2002; Kulakiotu *et al,* 2004; Schumann and D'arcy, 2010). The shape of sclerotia may also be variable, with formation occurring over a wide range of climatic conditions (Holz *et al,* 2004). Providing protection from adverse environmental conditions and microbial penetration, the melanised rind or wall of the sclerotia encases a central bundle of hyphae, this central mycelial mass also enclosed within a sheath of β-glucans (Backhouse and Willets, 1984; Holz *et al,* 2004). Sclerotia of *B. cinerea* may germinate myceliogenically and give rise to successive generations of conidia, or result in apothecial development and discharge of ascopsores (Nair and Nadtotchei, 1987). Germination (mycelia) rates of sclerotia of *B. cinerea* of ≥50% were reported after twelve months storage (Araújo *et al,* 2005). Asexual sporulation of *B. cinerea* is considered the most widespread means of reproduction, with conidia allowing transport over long distances to environments where carpogenic germination may be unlikely. While much genetic variation is noted across populations, the perfect stage of this fungus, apothecia are seldom found in the field suggesting that while necessary, sexual reproduction is not common (Beever and Weeds, 2004).

Profuse conidial development leading to the characteristic 'gray mould' of *B. cinerea* is favoured under high relative humidity (>90%) within a temperature range of 9-22°C, and with an optimum between 17-18°C (Sosa-A´ lvarez *et al,* 1995). Ultra violet light stimulates development of conidia; however some isolates will sporulate in complete darkness (Williamson *et al,* 2007). Conidia measure approximately 6 × 7.5 µm in size, having a dual layered wall 263 nm thick prior to germination (Plate VI A; Gull and Trinci, 1971). Once initiated, formation of determinate and highly branched conidiophores and consecutive generations of conidia may continue for up to three months (Nair and Nadtotchei, 1987). Considered to be short lived propagules of dissemination, the survival of conidia of *B. cinerea* after release from conidiophores is reported to vary considerably depending on factors such as light exposure, temperature, humidity and mycoparasitism (Backhouse and Willets, 1984; Holz *et al,* 2004). Once conidia are present, discharge occurs early each day as rising temperature and falling humidity cause conidiophores to contract and distort (Jarvis, 1962a; Williams *et al,* 2007). Within this daily pattern of mechanical release, the concentration during the

day of airborne conidia was shown to be greatest between 6am-5pm, with a noticeable spike observed between 9am-2pm in field cropped strawberries (Blanco *et al,* 2006). Spore viability after discharge has been shown to range from several minutes to longer than one year, with conidia still able to germinate and cause lesion formation on ray florets of gerbera flowers after 14 months storage at low humidity (Salinas *et al,* 1989; Elad *et al,* 2004). After deposition onto plant surfaces, conidia require the presence of free water to germinate and begin infection (Coertze and Holz, 2002).

Regarded as the most important structure involved in spreading inoculum of many *Botrytis* species including *B. cinerea,* conidia are hydrophobic and may be disseminated by wind, rain or via insect vectors (Holz *et al,* 2007). After mechanical ejection from conidiophores, airborne conidia may fall through still air under gravity alone or be impacted onto plant surfaces by wind (Holz *et al,* 2007). Conidial adhesion was demonstrated to be greater when inoculum was applied as a suspension in liquid to dry surfaces, or to wet plant surfaces as dry inoculum (Holz *et al,* 2007). Jarvis (1962b) reported an association between high density of airborne *Botrytis* conidia and rainfall, and that most conidia of many *Botrytis* species adhere to rain droplet surfaces with few penetrating the droplet itself (Jarvis 1962a; 1962b). Rainfall may therefore aid dispersal of conidia of *B. cinerea*, transporting conidia on droplet surfaces throughout precipitation events and during runoff. Vectoring of *B. cinerea* conidia by retention in or on bodily structures of numerous arthropods has been summarised by Holz *et al* (2007), with viable inoculum also remaining in excreta post evacuation.

Infection of plant tissue by *B. cinerea* may proceed either rapidly, with expanding lesions evident and be aggressive, or remain inactive or dormant for a period of time and be quiescent (Williamson *et al,* 2007). Quiescent infection of floral structures by *B. cinerea* such as carpels, stamens, styles, filaments, sepals, pollen tubes and ovules has been widely documented across many plant species, while plant host secretions including insect repelling exudates and stigmatic fluids are reportedly used by the fungus during development (Holz *et al,* 2004). Germination assessment of conidia of *B. cinerea* on varying organs of raspberry plants showed stamens to be the most conducive tissue for infection; up to 98% of conidia germinating after 8 hours while colonisation of leaf and stem tissues were reported at rates of up to 17 and 88% respectively (Yu and Sutton, 1997). Floral organs and tissues are recognised as important sites of primary infection for *B. cinerea;* however infection may be quiescent for a period of time after colonisation (Williamson *et al,* 2007). After infection of raspberry and strawberry inflorescences by *B. cinerea*, hyphal growth proceeded

until reaching the ovule. The fungus then survived as a saprophyte until fruit maturity, at which time destruction of host plant tissues resumed (McNicol *et al,* 1985). Infection of grape flower receptacles has indicated evidence host plant defences are involved in maintaining this latent period (Williamson *et al,* 2007).

Direct penetration of *B. cinerea* through open plant wounds often leads to infection when conidia are dry and recently deposited, but may be affected by host plant defences and climatic conditions (Holz *et al,* 2004). Penetration through suitable host plant tissue by *B. cinerea* may also be achieved with specialised structures known as appressoria, which are formed by developing conidial germ tubes (Kars and van Kan, 2004).

Physical and chemical signalling processes between host and pathogen are required for inoculum penetration, invasive growth, morphogenesis and inducing plant stress responses; these processes involved in determining the host range of disease causing agents (Tudzynski and Gronover, 2004). More specifically, the cyclic adenosine monophosphate (cAMP) and conserved mitogen-activated protein kinase (MAP) signalling pathways have been demonstrated as important processes necessary for pathogenesis of *B. cinerea* to occur (Mitchel and Dean, 1995; Xu, 2000).

After adhesion to and germination of conidia of *B. cinerea* on plant tissue, enzymes such as lipases and cutinases may assist penetration of the wax layer and cuticle, while mycotoxins, oxalic acid and the induction of active oxygen species (AOS) are known to be associated with inducing host cell mortality during pathogenesis (Kars and van Kan, 2004). A significant constituent of plant cell walls, pectin, is subject to decay after cell death by numerous cell wall degrading enzymes (CWDEs) produced by *B. cinerea* during conversion to fungal biomass of host plant tissues (Kars and van Kan, 2004). Pectinolytic CWDEs with known involvement in pathogenesis are pectin methylesterase, endopolygalacturonase, exopolygalacturonase, pectin lyase and pectate lyase as well as rhamnogalacturonan hydrolase, while xylanase and arabinase have been shown to target the hemicelluloses component of primary cell walls (Kars and van Kan, 2004). Other enzymes likely to be playing roles in the pathogenesis of *B. cinerea* include aspartic proteases and laccases (Kars and van Kan, 2004). Host plant defences generate a number of secondary metabolic products in response to

infection by *B. cinerea*, such as proanthocyanidins, cucurbitacins, saponins, α-tomatine in addition to resveratrol and other stilbenes (van Baarlen *et al,* 2004).

A number of phytohormones able to affect disease development may also be produced during pathogenesis of *B. cinerea*. Ethylene, gibberellic acid, abscisic acid as well as auxins can be synthesised by either host or pathogen, while cytokinins are produced only by host plants (Sharon *et al,* 2004). High levels of ethylene production have been observed by *B. cinerea* in culture, reported as a primary response by plants to fungal attack, and appeared to enhance disease development (Sharon *et al,* 2004). Furthermore; tissue of *chrysanthemum*, leaves of *pelargonium*, ruscus and rose plants in addition to blackcurrant, carnation and rose flowers inoculated with conidia of *B. cinerea* produced higher levels of ethylene compared to no treatment (Williamson, 1950; Smith *et al,* 1964; McNicol *et al,* 1989; Sharon *et al,* 2004). The rate of ethylene generation by *B. cinerea* was shown to be correlated with grey mould disease severity (Sharon *et al,* 2004). Inoculation of developing blackcurrant flowers showed significant ethylene production could also result from asymptomatic infection, and confirmed ethylene generated by plant defence mechanisms plays a significant role in causing premature flower senescence and abscission (McNicol *et al,* 1989). Artificial infection of grape flowers at full bloom with conidia of *B. cinerea* resulted in higher disease incidence than inoculation at earlier stages of flower development (Keller *et al,* 2003).

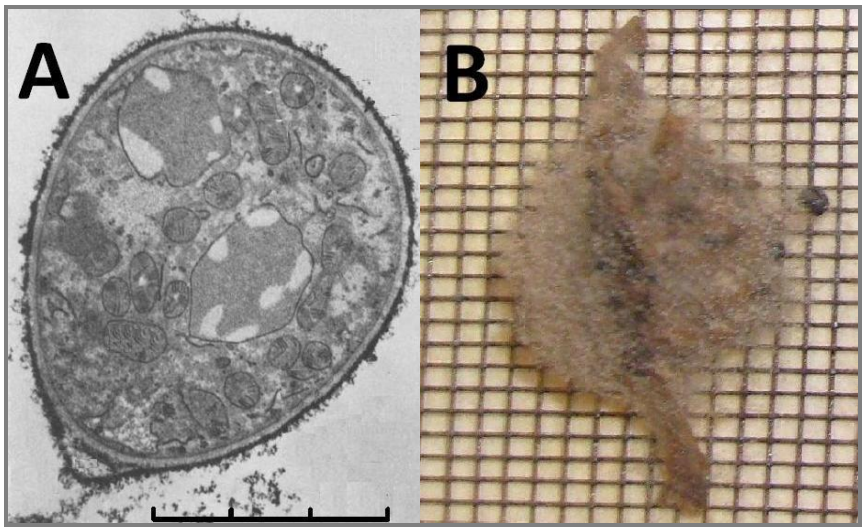


Plate VI - (A) Scanning electron micrograph of conidia (incremented scale bar in µm) and **(B)** masses of conidium of *B. cinerea* and sclerotia on incubated pyrethrum flower, mesh = 1 × 1 cm (Source: A, Gull and Trinci, 1971).

Botrytis flower blight

Since at least 1992 premature browning of flower heads from *Botrytis* flower blight has been recognised in Tasmanian pyrethrum crops, contributing to disease epidemics during the flowering period (MacDonald, 1995). Wet conditions and irrigation during December of 2004 resulted in significant epidemics of *B. cinerea* flower blight in many Tasmanian fields, notably reducing pyrethrin yields (Mark Raspin, Botanical Resources Australia, *Personal communication*). Unpublished data from 2006-07 showed the incidence of *B. cinerea* in maturing flowers averaged 87.7% and 76% without fungicides and in the presence of commercial spray applications respectively (Helen Cole, Botanical Resources Australia, *Personal communication*). Pyrethrum cultivation overseas reports no flower disease epidemics from this pathogen, however petal blights caused by *B. cinerea* are known to severely affect many ornamental species from the closely related *Chrysanthemum* genus (Horst and Nelson, 1997), cause significant disease in sunflower (Williams *et al,* 2007), head rot of safflower (Oelke *et al,* 1992) as well as mould of geranium flower crops (Scheuerell and Mahaffe, 2006).

Disease symptoms

Infections of flowers petals by *B. cinerea* are reported to cause discreet lesions or larger areas of necrotic or rotting tissue, often preceding formation of spore laden conidiophores (Williamson *et al,* 2007; Plate VI B). Symptoms typical of flower blight of *B. cinerea* in pyrethrum include necrotic disc florets which may become matted and fused together, with noticeable grey mycelium present on the flower surface (Pethybridge *et al,* 2007a). Flower development may be aborted prematurely, or result in flowers appearing to brown or senesce more rapidly leaving many disc florets unopened while few or no ray florets remain (Plate VII).

Plate VII - (A) Flowers showing symptoms of *Botrytis* blight infection and **(B)** Diseased flowers with matted, necrotic ray florets and unopened disc florets (Source: A, Pethybridge *et al,* 2007a).

Primary infection of *B. cinerea* of pyrethrum flowers in Tasmania occurs during late spring from windblown conidia, with anamorphic reproduction assumed to be the sole reproductive method of this fungus in Tasmania. Apothecial ascomycetes are uncommon; indeed few *Botrytis* species are known to produce a teleomorph or *Botryotinia* perfect stage, and even then only seldomly (Agrios, 1997). *B. cinerea* commonly overwinters in crop detritus and senescent plant tissue of perennial blueberry (Elmer and Michailides, 2004), boysenberry (Walter *et al,* 1997), raspberry (Williamson and McNicol, 1986), strawberry (Braun and Sutton, 1987) and grape cultivation (Essling *et al,* 2010), providing a primary source of inoculum to infect floral tissues the following season. Airborne conidia in pyrethrum fields may originate from infield inoculum from diverse sources including but not limited to: sclerotia, and mycelia sporulating in or on senescing foliage of pyrethrum and weeds, or other crop debris remaining from previous cultivation. Post harvest plant regrowth may provide damp, covered conditions and senescent foliage susceptible to direct mycelial re-infection with *B. cinerea* (Agrios, 1997). Beans, onions and garlic are grown in close proximity and rotation with pyrethrum in Tasmania, are also susceptible to diseases caused by *B. cinerea* and therefore possible sources of primary inoculum (DPIWE, 2010a and 2010b). The temperate spring climate and temperature range of conidia development of *B. cinerea* of 9-22°C reported by Sosa-A´ lvarez *et al* (1995) suggest sporulation is occurring prior to flower disease onset, infection itself possibly mediated by pathogen-host interactions involving chemical and physical signalling processes, enzyme production, phytohormones (Mitchel and Dean, 1995; Xu, 2000; Kars and van Kan, 2004; Sharon *et al,* 2004) and the developmental stage of flowers (McNicol *et al,* 1989; Keller *et al,* 2003).

Many plant species report quiescent infection of *B. cinerea* in floral tissues, becoming aggressive once stigmatic fluids or other exudates produced by maturing flowers are available for utilisation by the fungus in metabolic processes associated with pathogenesis (Holz *et al,* 2004). Periods of high relative humidity at suitable temperature can allow rapid completion of consecutive disease cycles of *B. cinerea* (Williamson *et al,* 2007), allowing masses of conidia to develop on flowers which may become matted together and exacerbate lodging. Conidia may continue to be produced and dispersed on flowers and infected tissue until harvest through this summer disease cycle (Fig. I; Agrios, 1997; O'Malley *et al,* 2008). Release of conidia may continue after mowing crops into windrows providing suitable conditions, as observed with above average December rainfall during 2004 in Tasmania (Mark Raspin, Botanical Resources Australia, *Personal communication*). Decomposing flowers and other decaying plant tissue may provide a substrate for *B. cinerea* to overwinter saprophytically as sclerotia or mycelia, the following spring contributing to primary flower disease inoculum in perennial cultivation of pyrethrum in Tasmania.

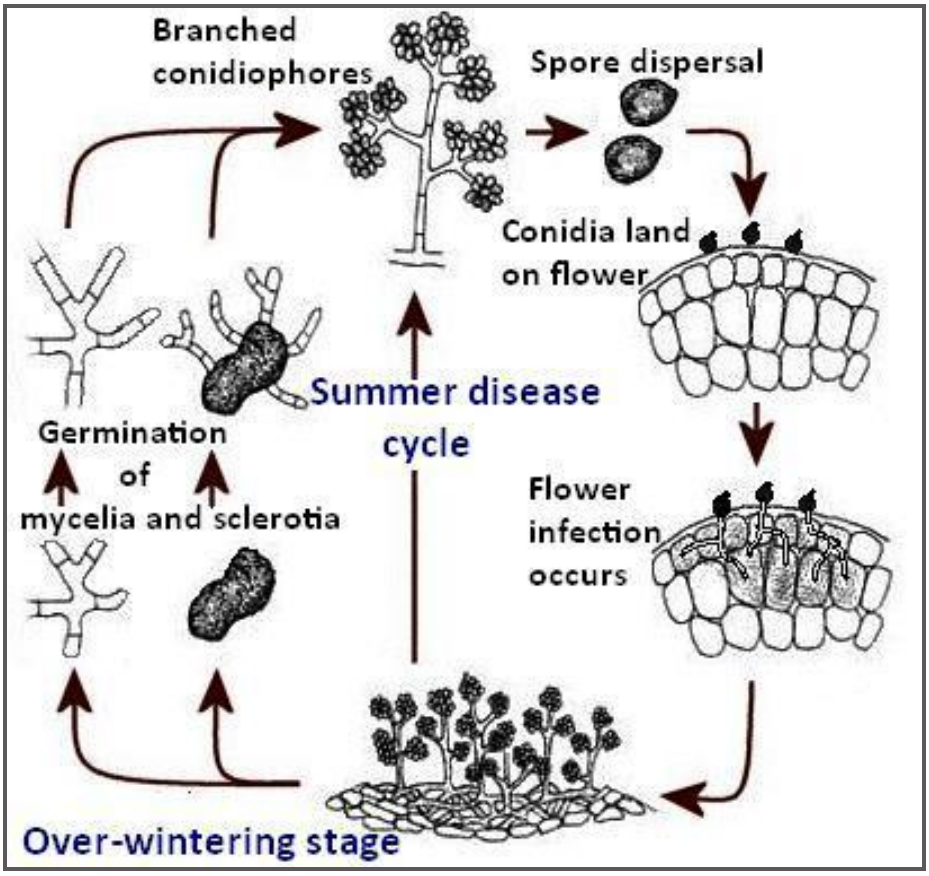


Figure I - Generalised life cycle of *B. cinerea* in Tasmanian pyrethrum crops (Source: Anonymous, 2010a. http://www.thewinedoctor.com/images/sweetbclifecycle.gif)

Management

Fungicides are commonly used to control bunch rot disease of grapes in Australia caused by *B. cinerea*, and include boscalid, azoxystrobin, chlorothalonil, fenhexamid, pyrimethanil, cyprodinil, fludioxinil and iprodione (Essling *et al,* 2010). In pyrethrum, disease intensity can be reduced by ceasing irrigation three weeks prior to harvesting, however management of *B. cinerea* flower blight is based almost exclusively on the timely application of fungicides (Mark Raspin, Botanical Resources Australia, *Personal communication*). Fungicides are applied from late spring during the latter stages of flowering at intervals of approximately 10-14 days, with up to a maximum of four spray applications per year. Currently the fungicides tebuconazole (as Folicur^{R}) and iprodione (as Rovral^{R}) are used in alternation, with tebuconazole usually the first applied (O'Malley *et al,* 2008). Carbendazim was previously used in place of iprodione, however review of usage of this chemical in Australia has led to restricted use (http//www.apvma.gov.au/news media/chemicals/carbendazim.php) and required alternative fungicides to be sought for flower disease control.

Biological control of plant diseases caused by *B. cinerea* has been demonstrated with species of *Trichoderma* fungi, acting by interfering with signal transduction, host recognition and penetration as well as combating plant defence mechanisms such as ethylene production (Elad, 2000a and 2000b; Tudzynski and Gronover, 2004). When dispersed with bumble and honey bees, isolates of *Trichoderma harzianum* provided control of *B. cinerea* comparable with fungicides applied at bloom in field cropped strawberries and vineyards (Elad, 1994; Kovach *et al,* 2000; Freeman *et al,* 2002). *Ulocladium atrum* demonstrated greater saprophytic capacity than *B. cinerea,* with colonisation of senescent lily leaves reducing leaf area covered with *B. cinerea* conidiophores compared to leaves not inoculated with *U. atrum* (Köhl *et al,* 1995). This provides very limited evidence to suggest *U. atrum* may be suitable for the lengthy process of evaluation as a potential biocontrol agent, this fungus also consistently identified from pyrethrum flowers in Tasmania with high disease incidence (O'Malley, 2007).

Sclerotinia sclerotiorum

S. sclerotiorum (Lib.) de Bary is a necrotrophic phytopathogen responsible for plant diseases in hundreds of species from the *Asteraceae, Chenopodiaceae, Crusiferae, Leguminosae, Solonaceae*

and *Umbelliferae* families (Willets and Wong, 1980). *S. sclerotiorum* is a Discomycete fungus of the *Helotiales* order, belonging to the same family *(Sclerotinaceae)* as the other known agent of flower disease, *B. cinerea* (Bolton *et al,* 2006). Mycelium is predominantly white and fluffy in appearance, having branched, multinucleate and septate hyphae. Culture colour may also vary to light brown or beige (Willets and Wong, 1980; Bolton *et al,* 2006).

Cultivated plants with measured economic losses from diseases caused by *S. sclerotiorum* are numerous and include alfalfa *(Medicago sativa)*, beans *(Phaseolus vulgaris)*, celery *(Apium graveolens)*, lettuce *(Lactuca sativa)*, peanut *(Arachis hypogaea*), potato *(Solanum tuberosum),* sunflower *(Helianthus annuusi)*, tomato *(Lycopersicon esculentum)*, groundnut *(Arachis hypogaea),* apricot *(Prunus Armeniaca),* citrus species *(Citrus spp.),* cabbage *(Brassica oleracea* var.*)*, eggplant *(Solanum melongena),* coriander *(Coriandrum sativum)* and cucumber *(Cucumis sativus)* (Purdy, 1979; Saharan and Mehta, 2008). *S. sclerotiorum* is also reported as the causal disease agent in numerous species of *chrysanthemum* (Horst and Nelson, 1997), also causing head rot in safflower (Oelke *et al,* 1992) as well as Australian sunflower production (Ekins *et al,* 2002).

S. sclerotiorum is an air and soil borne pathogen, infecting and causing disease from sclerotial and myceliogenic germination in addition to windblown ascospores able to infect above ground plant structures (Abawi and Grogan, 1979). Cottony soft rots, white moulds and watery soft rots of many vegetable crops prior to and after harvest are typical of infection by *S. sclerotiorum* (Saharan and Mehta, 2008), most crop diseases beginning with ascospore infection following carpogenic germination and completion of the sexual stage of this fungus (Bolton *et al,* 2006). Ascospores may only achieve a relatively short range of localised dispersal within a crop or district, wider pathogen dissemination aided by inadvertent transport of sclerotia or mycelia within soil or trash by machinery (Kora *et al,* 2003). Once present, inoculum of *S. sclerotiorum* may lead to disease development on an annual basis, with sclerotia known to remain viable for three years or more while buried in soil (Agrios, 1997). Within individual fields, disease distribution patterns of *S. sclerotiorum* flower blight were similar over subsequent crop rotations in temperate Canadian vegetable production (Adams and Ayers, 1979). Many plant disease epidemics caused by *S. sclerotiorum* such as white mould of bean only occur after flowering, when senescent floral tissues provide external nutrients allowing ascospores to begin infection (Abawi and Grogan, 1979).

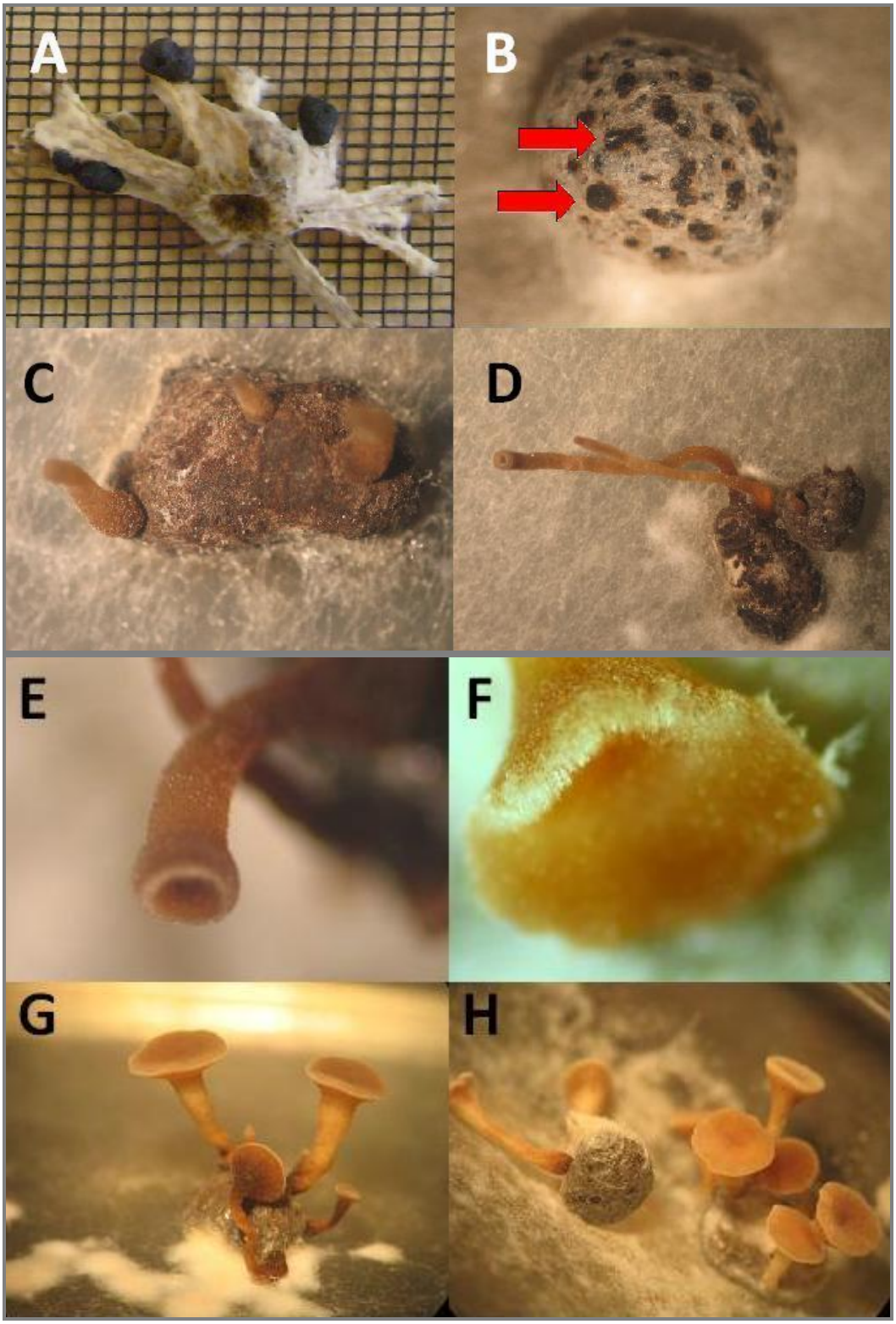
Survival and dispersal of inoculum of *S. sclerotiorum* is achieved through formation of propagules such as sclerotia, microconidia apothecia and ascospores (Willets and Wong, 1980). These structures allow *S. sclerotiorum* to undergo developmental stages of dormancy as sclerotia, saprophytism as mycelia, and parasitism as mycelia or ascospores (Kora *et al,* 2003). Microconidia are commonly produced by *S. sclerotiorum* in field and culture, however poor viability indicates ascospores to be the only spores produced by *S. sclerotiorum* capable of infection (Willets and Wong, 1980). Important survival structures known as sclerotia are formed at an optimum temperature range of 15-22°C, as mycelium aggregates into defined structures on plant tissue between 4-13 mm in size (Ekins *et al,* 2002). Over a period of days these masses melanise and harden becoming mature sclerotia capable of surviving in soil for a period of years (Plate VII A) (Willets and Wong, 1980). The melanised outer layer of the sclerotia provides protection from mycoparasitism and the environment, and is composed of β-1,3 glucans, chitin and pigments (Bullock *et al,* 1980). The central medullary bundle of hyphae of *S. sclerotiorum* is comprised of chitin, β-1,3 glucans and proteins and surrounded by a matrix of β-1,3 glucans and protein, while polyphosphates build up in vacuoles of fully developed sclerotia (Saito, 1974).

Under favourable conditions carpogenic germination of sclerotia will occur, producing airborne ascosporic inoculum. An optimum temperature of 15°C is reported for *S. sclerotiorum* to undergo carpogenic germination and reproduce sexually (Hao *et al,* 2003), beginning with projections or stipe initials forming on the surface of sclerotia (Plate VIII B). Stipe initials lengthen into photosensitive stipes (Plate VIII C-E) and open into disc shaped apothecia (Plate VIII F-H), which when mature are capped by a hymenium composed of paraphyses and ascospores sheathed within asci (Willets and Wong, 1980). Ascospore dimensions of *S. sclerotiorum* range between 10.2-14 µm in length and 6.4-7.7 µm in width (Purdy, 1955). Similar to *B. cinerea, S. sclerotiorum* follows a diurnal pattern of ascospore release driven by reduced humidity, ascospores being forcibly ejected up to 1cm from contracting asci to reach more turbulent air above the surface stratum (Abawi and Grogan, 1979). In the presence of adequate moisture, sclerotia of *S. sclerotiorum* infecting temperate bean crops produced apothecia continuously throughout spring and summer causing disease of cool, temperate bean production (Abawi and Grogan, 1979). Apothecia carpogenically germinating in the field were shown to discharge consistent volumes of ascospores over a seven day period, after which release rate of spores declined significantly (Willets and Wong, 1980). Providing a film of water is present, ascospores will germinate on suitable plant tissue within 3-6 hours of discharge (Willets and Wong, 1980), and can survive for a period of time after release under dry

conditions (Abawi and Grogan, 1975). High temperature and relative humidity can reduce ascospore survival after release from asci (Clarkson and Whips, 2002), declining from 21 days at 7% relative humidity to less than five days at 100% relative humidity (Abawi and Grogan, 1979).

An adhesive, mucilaginous secretion is released with and on ascospores, possibly assisting dispersal and in binding to host plant tissue (Abawi and Grogan, 1979). Single appressoria are given rise to by ascospores germinating on plant tissue, allowing enzyme assisted mechanical penetration of the host plant cuticle with multiple infection pegs (Willets and Wong, 1980; Kora *et al,* 2003). Early studies of enzymes produced during fungal pathogenesis were undertaken over a century ago on *S. sclerotiorum*, a pathogen armed with an indiscriminate mode of attack often allowing host invasion before plant defences mechanisms are active (de Bary, 1887; Kora *et al,* 2003). Cell wall degrading enzymes (CWDEs) and oxalic acid are produced by *S. sclerotiorum* during pathogenesis, oxalic acid potentially having a role in acidifying pH which enhances the action of pectic enzymes (Lumsden, 1979). A defined order of production is evident for CWDEs, beginning with polygalacturonases and concluding with cellulase synthesis (Bauer *et al,* 1977). Water soaked lesions of macerated, soft rotted tissue resulting in cell damage and death are caused by the action of pectolytic enzymes (Bateman and Basham, 1976). Other enzymes potentially active during pathogenesis of *S. sclerotiorum* are proteinases and phospholiases, believed to interfere with plant membrane structure (Willets and Wong, 1980).

Plate VIII - (A) Pyrethrum flower with white, floccose mycelium and sclerotia of *S. sclerotiorum* (10× magnification), **(B)** Stipe initials on mature sclerotia (30× Mag.), **(C)** Stipes beginning to lengthen (30× Mag.), **(D,E)** Apothecia starting to form on extended stipe (30,60× Mag.), **(F)** Mature apothecia (60× Mag.) and **(G,H)** Sclerotia with numerous apothecia protruding (30× Mag.).



Sclerotinia flower blight

S. sclerotiorum has been recognised since the early 1990's as an agent of serious wilting or crown rot diseases Tasmanian pyrethrum fields (MacDonald, 1995). More recently it has been identified as a major contributor to disease epidemics during the flowering period and requiring management with fungicides each year (Pethybridge *et al,* 2007a and 2008a). Fungicides confer protection during periods of greater risk such as ascospore liberation, but are unlikely to purge soil of sclerotia (Pung and Cross, 2004).

Disease symptoms

Necrotic lesions may be visible on flowers a week after successful colonisation and infection (Plate IX A), while characteristic white, floccose mycelia of *S. sclerotiorum* may also be observed developing on flowers (Pethybridge *et al,* 2010). Ray florets may senesce prior to unfurling of any disc florets (Plate IX B) while infected flowers may appear darkened and necrotic with capitula remaining upright (Pethybridge *et al,* 2007a). Systemic plant infection can result in a wilted and silvery appearance of stems and foliage.

Plate IX - (A) Necrotic disc florets following inoculation with ascospores, and **(B)** Shrivelled ray florets ringing mostly unopened disc florets.

Primary flower infection by *S. sclerotiorum* occurs in Tasmanian pyrethrum fields during late spring or early summer when airborne ascospores land on developing flowers. A brief incubation period after initial infection followed by development of flower disease symptoms on florets may be observed, also reported in head rot of sunflower in Australia (Ekins *et al,* 2002). Airborne inoculum may originate infield, from sclerotia at ground level producing apothecia and ascospores. Due to protection afforded by melanised sclerotia as resting structures, *Sclerotinia* spp. are persistent soil borne fungi which can be difficult to control. Many field vegetables including beans, brassicas, carrots, lettuce, peas and potatoes are grown in rotation with pyrethrum in Tasmania and also susceptible to diseases caused by *S. sclerotiorum* and potential contributors of primary inoculum toward flower disease epidemics (DPIWE, 2010c). Weed species observed in Tasmania including narrow leafed plantain *(Plantago major* and *P. lanceolata), Raphanus* species (radish, wild radish), hedge mustard *(Sisymbrium officinale),* dandelion *(Taraxacum officinale),* fennel *(Foeniculum vulgare)* and wild turnip *(Raphistrum rugosum)* are also reported as hosts of *Sclerotinia* sp., and also potential sources of primary inoculum (Melzer *et al,* 1997). Agronomic practices such as tilling, sowing, fertilising, cutting and harvesting of pyrethrum and host crops grown in rotation with pyrethrum may facilitate longer distance dissemination of inoculum of *S. sclerotiorum* (Kora *et al,* 2003). The temperate climate during flowering and reported temperature range of apothecial development and sporulation of *S. sclerotiorum* suggest inoculum is present in Tasmanian fields before flower infection occurs. Continual development of apothecia from sclerotia over a period of months has been observed in temperate vegetable cropping, and ascospores of *S. sclerotiorum* are known to require an external energy source to drive infection that senescent floral tissue of pyrethrum may provide (Abawi and Grogan, 1979). In addition to flower development, pathogen-host interactions involving environmental, physical and enzymatic processes appear fundamental for infection to begin and pathogenesis to proceed (Lumsden, 1979; Bauer *et al,* 1977; Willets and Wong, 1980; Kora *et al,* 2003). Fluctuating humidity drives the daily release of ascospores, while overlapping generations of apothecia have been reported to release ascospores continuously for up to one week over a period of five months (Abawi and Grogan, 1979). While this might give the appearance of continuous inoculum production and multiple disease cycles being completed during the flowering period, it would seem more likely a staggered release of ascospores from a population of sclerotia in the soil with different time requirements for conditioning. Ascospore release may continue after cutting crops into windrows for drying given conducive conditions. Diseased stems and leaves of pyrethrum, as well as flowers disintegrating prior to or during cutting and collection may harbour mycelium and give rise to sclerotia able to produce infective inoculum over successive flower harvests.

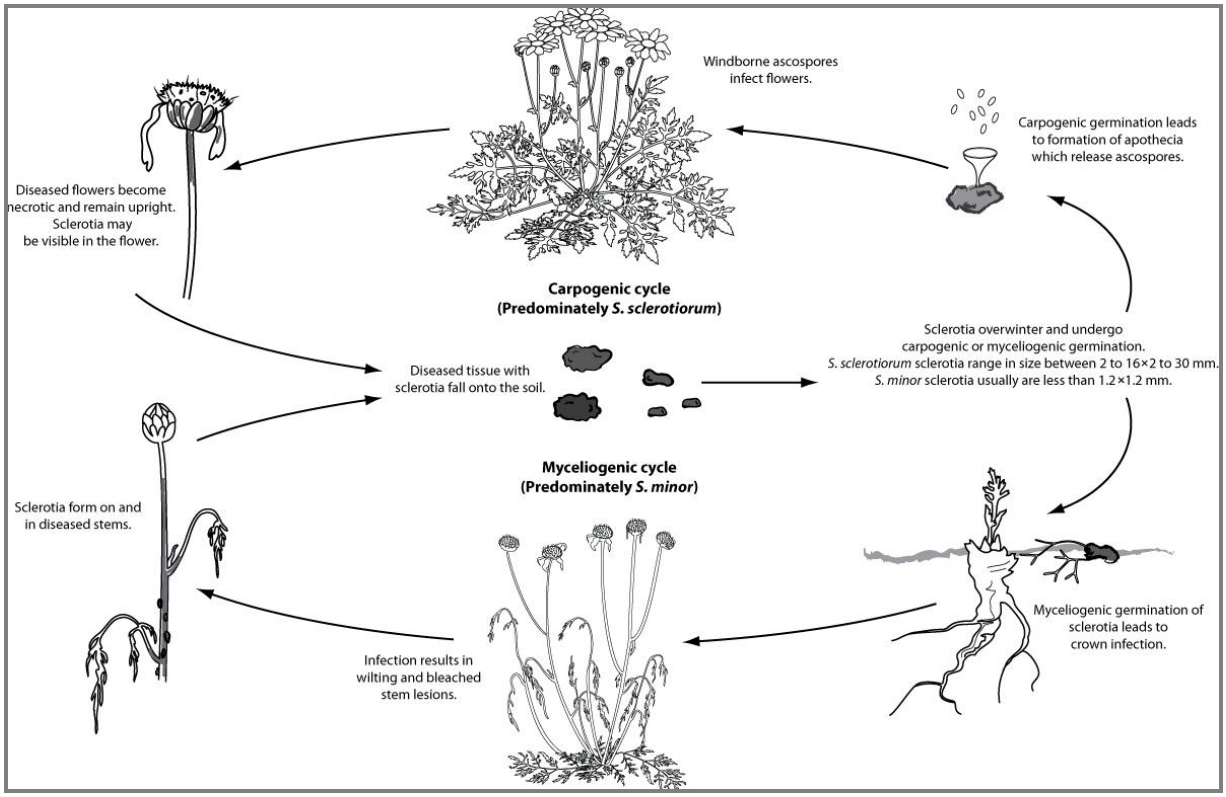


Figure II – Life cycles of *S. sclerotiorum* and *S. minor* in Tasmanian pyrethrum fields (Source: Pethybridge *et al,* 2008a).

Management

Like flower blight caused by *B. cinerea* in Tasmanian pyrethrum fields, management of flower disease caused by *S. sclerotiorum* relies predominantly on fungicide applications; however halting irrigation several weeks prior to cutting can restrict disease development (Mark Raspin, Botanical Resources Australia, *Personal communication*). The fungicides tebuconazole and carbendazim are applied in alternation from late spring until near harvest, aiming to reduce inoculum levels and limit *Sclerotinia* flower disease progression. More recently carbendazim has been replaced by iprodione in the flowering fungicide program (O'Malley *et al,* 2008). Epidemiological and morphological similarities between the two recognised agents of flower disease result in the flowering fungicide spray program targeting both pathogens in Tasmanian pyrethrum crops. Managing *Sclerotinia* sp. diseases in alternative host crops grown in rotation with pyrethrum also requires fungicide applications. Procymidone has previously been used for disease control in bean and lettuce crops, while more recently boscalid has been used for control of diseases caused by *Sclerotinia* sp. in Tasmania (Pung and Cross, 2004).

Effective biological control of plant diseases caused by *S. sclerotiorum* with species of *Trichoderma* fungi has been demonstrated by affecting signal transduction, host recognition and penetration (Elad, 2000; Tudzynski and Gronover, 2004). Strains of *T. harzianum* have provided effective biocontrol of glasshouse diseases of cucumber and tomato caused by *S. sclerotiorum,* as well as controlling disease in the perennial pathosystem of viticulture (Elad, 1994; 2000a; 2000b). *Coniothyrium minitans* showed efficacy in field trials as a mycoparasite of sclerotia of *S. sclerotiorum* in potato, bean and carrot; reducing apothecial numbers by up to 90% the year following treatment (Gerlagh *et al,* 1999). Biocontrol agents incorporating *C. minitans* as the active ingredient are now commercially available for control of white mould diseases caused by *S. sclerotiorum* and *S. minor*, such as the biological fungicide Contans^{R} WG (Plant Products Co. Ltd. Ontario, Canada. www.contans.ca/). The relative success of biological control agents however is often unpredictable and unreliable in broad acre agriculture, with limited shelf life of preparations and climatic variations limiting applications of such products in commercial cultivaltion (Agrios, 1995).

Sclerotinia minor

While carpogenic germination of *S. minor* has not been documented in Tasmanian agriculture, predictive modelling by Ekins *et al* (2005) showed it was most likely to occur in Australia's southern regions. Pyrethrum is a known host plant of *S. minor* (Melzer *et al,* 1997), and it is susceptible to crown rot disease caused by this fungus in Tasmania (Pethybridge *et al,* 2008).

S. minor Jagger is a necrotrophic plant pathogen and Discomycete fungus of order *Heliotales* and family *Sclerotinaceae* (Willets and Wong, 1980; Bolton *et al,* 2006). At least 94 plant species are reported as host plants of *S. minor*, many from the *Asteraceae* family, indicating a host range significantly smaller than *S. sclerotiorum* (Melzer *et al,* 1997). Mycelium is generally fluffy and white in appearance with branched, multinucleate and septate hyphae, culture colour varying from light brown or beige (Willets and Wong, 1980; Bolton *et al,* 2006).

With the characteristic 'eruptive' mycelial germination of *S. minor* distinctive amongst *Sclerotinia* species and few reports of carpogenic germination in culture or field cropping, mycelium is assumedly the primary inoculum source of many plant diseases caused by *S. minor* (Abawi and Grogan, 1979; Melzer *et al,* 1997). Plant diseases caused by *S. minor* predominantly infect crown and upper root tissue during disease development often leading to wilting, collapse and plant mortality (Melzer *et al,* 1997); in contrast to the plethora of flower, fruit and vegetable diseases caused by ascospores infection by *S. sclerotiorum* (Saharan and Mehta, 2008).

Along with numerous reports of pyrethrum, species reported as hosts of *S. minor* in a compilation by Melzer *et al* (1997) were tulip (*Tulipa* sp.), sunflower *(Helianthus annus)* and carnation *(Dianthus carophyllus).* Plant mortality caused by *S. minor* has also been reported in Kenyan pyrethrum production (Natrass, 1950). Crown rot disease of pyrethrum in Tasmania caused by *S. minor* is recognised, assumedly originating from the eruptive mycelial germination typical of this pathogens predominantly asexual reproduction (Abawi and Grogan, 1979). Few records in field agriculture exist of significant disease caused from *S. minor* infecting aerial plant parts with ascospores, most epidemics starting from asexual reproduction and eruptive mycelial germination onto host tissue (Abawi and Grogan, 1979). Carpogenic germination of *S. minor* and discharge of airborne inoculum has reportedly caused flower blight of peanut (Wadsworth, 1979) in addition to being associated with diseased canola petals in New South Wales (Fuhlbohm *et al,* 2003). Ascospores from Australian isolates of *S. minor* cultured *in vitro* were demonstrated to be pathogenic and cause sunflower head rot disease (Ekins *et al,* 2002). Apothecia and ascospore production has also been reported to occur in field cropping of lettuce in New Zealand between September and early November (Hawthorne, 1976), while more recently Ekins *et al* (2002) suggested carpogenic germination of *S. minor* in Australia's southern regions is likely to occur using predictive modelling. Despite the common nature of *S. minor* as a plant pathogen, these reports constitute the limited evidence available worldwide that this pathogen can develop carpogenically in the field.

Survival and dispersal propagules produced by inoculum of *S. minor* include sclerotia, microconidia, apothecia and ascospores (Willets and Wong, 1980). Microconidia are often cultured *in vitro,* but viability is generally low and these spores are not considered effective reproductive means in the field (Willets and Wong, 1980). *S. minor* forms sclerotia in culture which are scattered, abundant, occasionally in clusters or groups (Kohn, 1979), and reported to range from 0.5-2.0 mm in

diameter (Melzer *et al,* 1997; Ekins *et al,* 2002). Outer hyphal layers of sclerotia are melanised and composed of pigment, chitin and β-1,3 glucans providing protection from other soil borne mycoflora and the elements (Bullock *et al,* 1980). Within lies an extracellular matrix made of β-1,3 glucans and an unidentified polysaccharide, surrounding a central bundle of glycogen-rich medullary hyphae (Bullock *et al,* 1980).

Mycelial germination, the predominant infection mode of *S. minor,* is reported to occur within a temperature range of 5-25°C, with an optimum of 15°C (Hao *et al,* 2003). Carpogenic germination of *S. minor* is reported to occur between 11-17°C, also with an optimum temperature of 15°C (Hawthorne, 1976). *S. minor* has tetranucleate ascospores with outer exipulum at apothecial borders made up of globose cells, allowing differentiation from *S. sclerotiorum* which has binucleate ascospores and exipulum perimeters comprised of prosenchyma cells (Kohn, 1979; Wong and Willets, 1979).

Similarly to *S. sclerotiorum,* the production of pH altering oxalic acid and formation of pectic and pectolytic CWDEs including polygalacturonases and cellulose are involved during pathogenesis of *S. minor,* while proteinases and phospholiases are believed to effect plant membrane structure (Bauer *et al,* 1977; Lumsden, 1979; Willets and Wong, 1980).

Plants species reported as susceptible hosts of *S. minor* and cropped in rotation or within close proximity to pyrethrum in Tasmania and potentially able to contribute soil borne inoculum and airborne ascospores if present include tulip *(Tulipa* sp.), carrot *(Daucus carota)*, lettuce *(Latuca sativa)*, several species of *Brassica* (cauliflower, cabbage, brussel sprouts), bean *(Phaseolus vulgaris)*, broad bean *(Vicia faba),* pea *(Pisum sativum)*, several clover *(Trifolium)* species of pasture, tomato *(Lycopersicum esculentum),* potato *(Solanum tuberosum)* in addition to orchard cropping of apples *(Malus sylvestris)* and pears *(Pyrus communis)* (Melzer *et al,* 1997). *S. minor* has also been reported to cause white mould of green beans in Tasmania (DPIWE, 2010b). However, 150 isolates of *Sclerotinia* sp. collected from diseased bean pods in 55 commercial bean fields in northern Tasmania during 2008 and 2009 were all identified as *S. sclerotiorum* (Jones *et al*, 2011). Common weed species found in Tasmania also noted as hosts of *S. minor* include fennel *(Foeniculum vulgare)*,

dandelion *(Taraxacum officinale),* hedge mustard *(Sisymbrium officinale), Raphanus* species (radish, wild radish), wild turnip *(Raphistrum rugosum)* as well as common and narrow leafed plantain *(Plantago major* and *P. lanceolata)* (Melzer *et al,* 1997).

1. Epidemiology of flower diseases in Tasmanian pyrethrum fields.

1.1 Introduction

During the flowering period in Tasmanian pyrethrum fields, from November until the conclusion of crop cutting in January, flower blights caused by *B. cinerea* and *S. sclerotiorum* can occur in crops (MacDonald, 1995). Premature necroses of ray and tubular disc florets, with ray florets at times fusing together are indicative symptoms of flower infection by *B. cinerea* (Plate 1.1 A-B). Noticeable signs of this pathogen include grey coloured mycelium and profuse growth of conidiophores present on flower surfaces (Pethybridge *et al,* 2007a). Plants infected by *S. sclerotiorum* appear generally wilted, with foliage appearing less verdant and having a more silvery colour. Infected flowers may have obvious necrotic lesions and be darker in appearance, with ray florets often desiccating and withering prior to the opening of disc florets (Plate 1.1 C-E; Pethybridge *et al,* 2008a). Signs of flower infection by *S. sclerotiorum* include white, cottony mycelial growth noticeable on flower surfaces (Pethybridge *et al,* 2010).

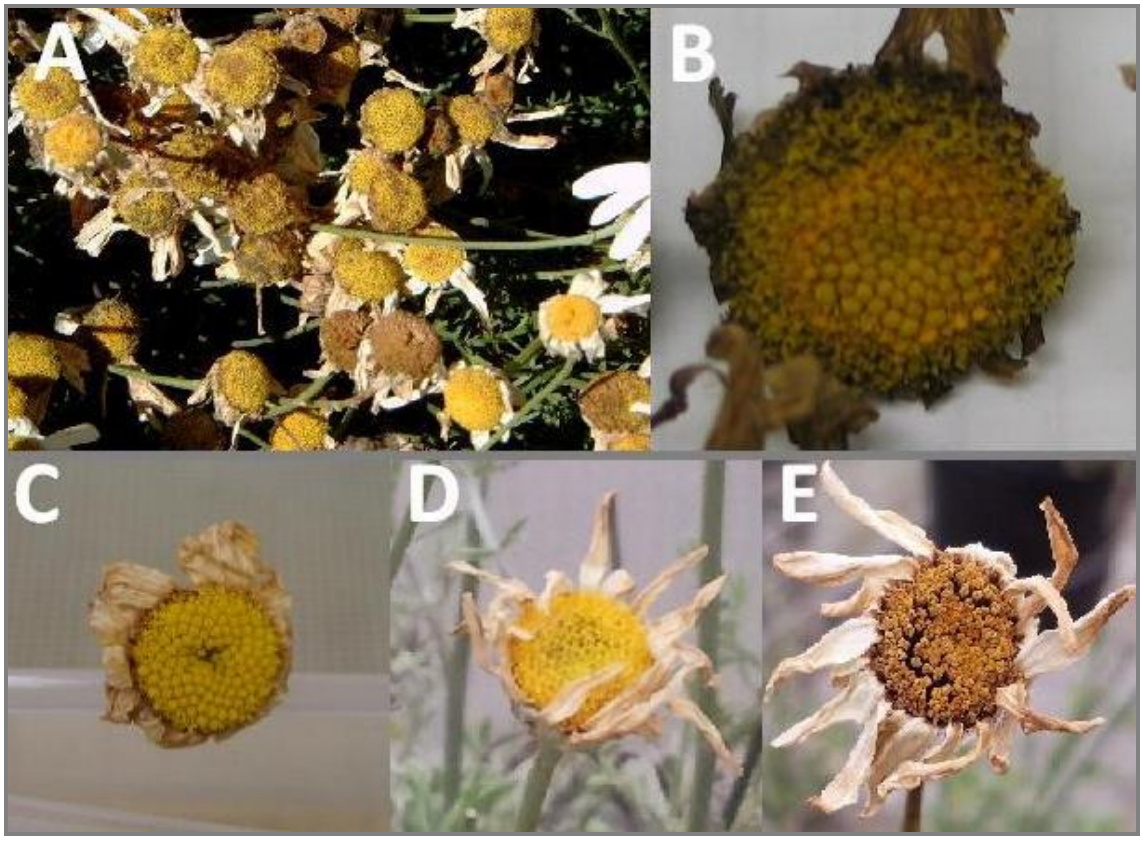


Plate 1.1 - (A, B) Flowers showing symptoms of *B. cinerea* flower disease, and **(C-E)** flowers displaying disease symptoms typical of flower infection by *S. sclerotiorum* (Source: A, Pethybridge *et al,* 2008a).

Encompassing the distribution, incidence and management of a disease epidemic, epidemiology provides information on variances of disease intensity within a host population over a defined time period (Madden *et al,* 2007). A graphical representation of disease intensity against time is possible to generate if three or more measurements of disease incidence (or amount) are recorded over a given duration, and is referred to as the *disease progress curve,* (Campbell and Madden, 1990). Disease progress curves are considered uniquely descriptive of an epidemic, and are utilised to describe epidemics in addition to assist making informed decisions to achieve effective management (Madden *et al,* 2007).

A plant disease epidemic may develop from inoculum present infield, producing one cycle of infection per growing season (monocyclic), or achieve multiple infection cycles increasing inoculum levels within a growing season (polycyclic) (Madden *et al,* 2007). Numerous models may be fitted to disease progress curves to determine whether a plant disease epidemic is monocyclic or polycyclic in nature; including exponential, monomolecular, logistic, Gompertz, log-logistic, Richards or Weibull models (Madden *et al,* 2007).

The exponential, monomolecular and logistic models provided the foundations for some of the most thorough studies in epidemiology to date (Madden *et al,* 2007). The exponential model has been used to describe population growth for over two hundred years, and can be useful for representing polycyclic epidemics during their early stages. Chemical reactions, animal growth as well as plant disease epidemics have been described by the monomolecular model, also referred to as the restricted or negative exponential model and often suggestive of monocyclic plant disease epidemics (Campbell and Madden, 1990). The logistic model borrows aspects from both monomolecular and exponential models, and is recognised as a superior descriptor of polycyclic plant diseases throughout the course of an epidemic (Madden *et al,* 2007). The Gompertz model was originally devised for measuring animal growth, and is considered to be less constraining than the logistic model when describing polycyclic plant disease epidemics (Campbell and Madden, 1990; Madden *et al,* 2007). The Richards model represents another modification of the Logistic model, and has been used to draw objective comparisons between models (Madden *et al,* 2007).

Model	**Absolute rate of disease increase or decrease (*dy/dt*)**	**Rate *(r)* or other parameter**
exponential	$r_E y$	r_E
monomolecular	$r_M(1-y)$	r_M
logistic	$r_L y(1-y)$	r_L
Gompertz	$r_G y[\ln(1)-\ln(y)]$	r_G
Richards	$r_R y(1-y^{\eta-1})/\eta-1$	r_R, η(unitless parameter)

Table 1.1 - Differential equations of selected models where *y* = measured disease incidence or severity, adapted from Madden *et al* (2007).

Presently, little information is available pertaining to epidemiology of *B. cinerea* in Tasmanian pyrethrum fields. In a recent study, development of *S. sclerotiorum* flower blight was best described as a monocyclic disease; with 92% of Tasmanian fields surveyed between 2007-2009 having epidemics most aptly being described by the monomolecular model (Pethybridge *et al,* 2010). Additionally, there has been no comprehensive study of mycoflora associated with pyrethrum flowers in Tasmania. Whether or not other pathogenic fungi potentially contribute to flower disease in pyrethrum in Tasmania is presently unknown (MacDonald, 1995). Determining if mycoflora other than *B. cinerea* and *S. sclerotiorum* are associated with diseased pyrethrum flowers and potentially contributing to flower disease epidemics in Tasmania would therefore be of great interest.

The objective of this experiment was therefore to assess the fungal incidence of flowers through the flowering period to:

- Investigate and describe epidemiology of *B. cinerea* and *S. sclerotiorum* flower blights of pyrethrum in Tasmanian fields.
- Provide more detailed information on approximate time of initial infections of *B. cinerea* and *S. sclerotiorum* in flowers in the field.
- Determine periods when disease incidence is ascending rapidly late during the flowering period to enable more effective timing of control measures/fungicide applications.
- To determine if fungal species other than known pathogens of pyrethrum flowers were regularly associated with diseased pyrethrum flowers, and potentially contributing to flower disease.

1.2 Materials and methods

Plots to receive no flowering fungicide applications (Nontreated plots) were established in ten commercial pyrethrum fields approaching first flower harvest across numerous cultivation districts during late October of 2007 (Table 1.2.1). Fields were located across the North West coast up to approximately 75km apart and up to 20km back from the coast. Plots measured between approximately 10m × 10m to 20 × 20m (depending on spray boom and spray run widths at field sites) and were centred across adjacent spray runs (Figure 1.2.1). Marker pegs tied with brightly coloured flagging tape clearly identified plots, with signs reading 'no flowering fungicides' affixed at each end of nontreated plots. Growers were requested not to apply flowering fungicides to these areas, while the remainder of each field received the industry standard applications of the flowering fungicide program.

Site	District
07-1	Barrington
07-2	Sassafras
07-3	Forth
07-4	Forth
07-5	Kindred
07-6	Penguin
07-7	Table Cape
07-8	Table Cape
07-9	Table Cape
07-10	Wesley Vale

Table 1.2.1 - Field sites and cultivation districts (2007-08 flower harvest).

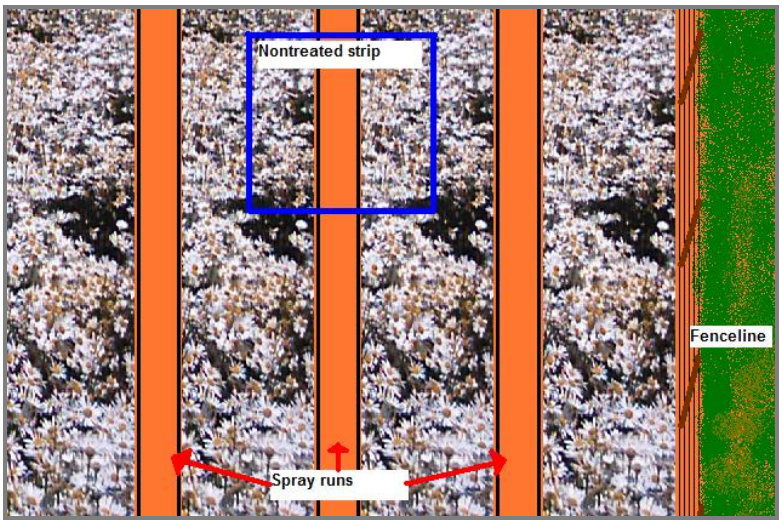


Figure 1.2.1 - Nontreated trial plot layout, approximately 15 × 15 m.

Sampling flowers for pathogen incidence

Flowers were sampled from nontreated plots for assessment of fungal incidence on five occasions during the flowering period (Table 1.2.2). At each sampling time, 100 flowers were randomly chosen from within each of the nontreated plots at each trial site. Fifty flowers were collected from along two transects on each side of the spray run and at least 2m into the crop from the edge of the spray run. Flowers were chilled during transit, and stored at 5°C for no longer than two days prior to processing. Flowers were placed inside mesh bags and surface sterilised in a solution of 2% sodium hypochlorite (Bleach, White King ™) in distilled water for five minutes. Flowers were subsequently rinsed with sterile distilled water three times to remove residual bleach. Flowers were spread out on filter paper inside a laminar flow cabinet for at least three hours to dry. Flowers were then placed on fibreglass insect mesh cut to fit sealable plastic trays (Genfac Plastics, Melbourne, Australia) suspended over a damp, folded tissue with 5 mm Gutterguard ™ (Boddingtons Australia Pty. Ltd., Melbourne) mesh. Five flowers were positioned separately in each tray (Plate 1.2.1 A). Trays were sealed and incubated at room temperature (18-22°C day/ 10-15°C night) for 15-25 days. After incubation and development of fungal growth (Plate 1.2.1 B) each flower was individually assessed under a Zeiss stereomicroscope (63×).

B. cinerea was identified by occurrence of distinctive conidiophores, conidia and sclerotia (Plate 1.2.1 C). Sclerotia of *B. cinerea* are described as very small or variable in size with diameters ranging between 1-18 mm and colour being black, grey or white (Clarkson and Whipps, 2002; Kulakiotu *et al,* 2004; Schumann and D'arcy, 2010). During this study, sclerotia of *B. cinerea* ranged from approximately 5-10 mm in size, and were generally smaller than those of *S. sclerotiorum*. Sclerotia of *B. cinerea* often had a distinctive pock marked 'golf ball' appearance on the upper surface; and were also distinctly concave in shape on the underside, as opposed to *S. sclerotiorum* in which sclerotia were convex in shape, with a smooth exterior. Sclerotia of *S. sclerotiorum* were black in colour once mature, while *B. cinerea* gave rise to grey, dark grey or black sclerotia. Sclerotia of *B. cinerea* often had conidiophores growing directly from surfaces, thus providing several means to distinguish between sclerotia of both genera. *S. sclerotiorum* was identified by typical white, floccose mycelium and distinctive sclerotia (Plate 1.2.1 D).

Plate 1.2.1 - Pyrethrum flowers; before **(A)** and after **(B)** incubation, profusely covered with conidia of sclerotia of *B. cinerea* **(C)**, and with mycelial growth and sclerotia of *S. sclerotiorum* **(D)**.

	Flower sampling dates in 2007				
Site	**1st**	**2nd**	**3rd**	**4th**	**5th**
07-1	15-Nov	30-Nov	11-Dec	16-Dec	21-Dec
07-2	15-Nov	30-Nov	11-Dec	16-Dec	21-Dec
07-3	16-Nov	4-Dec	11-Dec	16-Dec	21-Dec
07-4	15-Nov	29-Nov	11-Dec	16-Dec	21-Dec
07-5	15-Nov	4-Dec	11-Dec	16-Dec	21-Dec
07-6	15-Nov	4-Dec	11-Dec	18-Dec	21-Dec
07-7	13-Nov	30-Nov	10-Dec	18-Dec	21-Dec
07-8	13-Nov	30-Nov	10-Dec	18-Dec	21-Dec
07-9	13-Nov	30-Nov	10-Dec	18-Dec	21-Dec
07-10	15-Nov	29-Nov	11-Dec	16-Dec	21-Dec

Table 1.2.2 - Flower sampling dates of trial sites late in flowering period.

In addition, at each time of flower sampling the flower maturity stage (FMS) of one hundred flower samples from plots at all field sites were assessed. Flowers were categorised into one of eight stages based on maturity (Plate 1.2.2) as follows:

1. Flowers in bud, green and still unopened.

2. Buds just beginning to open ray florets upright.

3. Ray florets wide open and with less than one third of disc florets being open.

4. Ray florets wide open and between one third and two thirds of disc florets open.

5. Ray florets wide open and between two thirds and all disc florets open.

6. Ray florets open and intact but shrivelling, all disc florets open.

7. Ray and disc florets desiccated or missing, flower drying out.

8. Flower dead.

Plate 1.2.2 - Stages of flower development as described by the flower maturity stage (FMS).

Data analysis

A mixed model approach was employed to describe temporal changes of incidence of *B. cinerea* and *S. sclerotiorum* over the flowering period using SAS Version 9.1 (SAS Institute, Cary, NC, USA). The incidence of fungi in surface sterilised flowers sampled on five occasions during the season was fitted to exponential, Gompertz, linear, logistic and monomolecular models to evaluate which population growth model most accurately described changes in the incidences of *B. cinerea* and *S. sclerotiorum* (Nutter, 1997). The goodness of fit of each models linear forms were compared using the root mean square error, coefficient of determination (R^2), the F-statistic for linearity, visual comparison plotting predicted against residual values, and by comparing back transformed fitted disease values against original incidence data (Nutter, 1997). The mean incidence in nontreated plots at all five sampling times for *B. cinerea* and *S. sclerotiorum* were also compared against each other, using a paired sample Student's *t*-test using GenStat Version 10.1 (VSN International Ltd.).

Survey of other mycoflora associated with pyrethrum flowers

These flower samples were collected as part of further studies described in chapter 2, with all flower sampling, surface sterilisation, incubation and microscopic assessment methodology used here described in chapter 2 materials and methods (Chapter 2.2 p55-57). Twenty five pyrethrum fields in total (Table 2.2.2) were surveyed throughout the flowering period over consecutive years for the presence or absence of fungi displaying sclerotial morphology consistent with *S. minor* on incubated flower samples. Flowers were sampled from non fungicide treated plots in fields during the flowering period as described previously (Fig. 1.2.1), and were sampled three times from ten fields during 2008-09 and twice from 15 fields in 2009-10 (Table 2.2.2, app. 2.2.2). Flower samples collected during 2008-09 were additionally surveyed for all identifiable mycoflora associated with flowers other than *B. cinerea, S. sclerotiorum* and fungi suspected of being *S. minor.* Flower samples were surface sterilised and incubated as described previously in materials and methods for this chapter.

Collection of fungal isolates for further study

During assessment of pathogen incidence in flowers, 73 isolates of *S. sclerotiorum* (Appendix 1.3.2 A) and 49 isolates of *B. cinerea* (Appendix 1.3.2 B) were collected from flower samples for further study. Each isolate was obtained from separate incubation boxes each containing 5 flowers (Plate 1.2.1 A-B), with all flowers sampled from nontreated plots in commercial fields.

Sclerotia of *S. sclerotiorum* were picked off flowers with sterile implements and surface sterilised in a solution of 2% sodium hypochlorite (Bleach, White King™) in distilled water for five minutes before being rinsed with two consecutive washes of distilled water to remove excess bleach. After drying thoroughly overnight on filter paper in a laminar flower cabinet, sclerotia were bisected and plated onto petri plates containing PDA and incubated in the dark (20°C). Hyphal tips were removed from colonies after two days and placed onto fresh PDA plates. The resultant cultures were then incubated (20°C) for further production of mycelium and sclerotia. Cork plugs of mycelium 5 mm in diameter were cut from the actively growing outer edge of each isolate, dried overnight on sterile petri plates before being stored in cryogenic vials at -80°C under glycerol. Approximately two dozen dried mycelial plugs were stored under glycerol, with an equal number also stored as dry plugs in cryogenic vials at -80°C. Once mature, all sclerotia from one PDA plate per isolate were collected with aseptic implements onto sterile filter paper and air dried in a laminar flower cabinet overnight. Sclerotia were then transferred to autoclaved, air dried 5 ml glass jars and stored at 4°C.

Individual conidia of *B. cinerea* from masses of conidiophores present on incubating flowers (Plate 1.2.1 C) were removed with a sterile scalpel, plated onto PDA and incubated at 20° C until mycelial growth covered most of plate surface. Cork plugs 5 mm in diameter were then removed from the actively growing mycelial front of each isolate and dried overnight in a laminar flow cabinet, before storage at -80°C in sterile 5 ml cryogenic vials.

1.3 Results

1.3.1 Disease intensity and modelling

The incidence of *B. cinerea* throughout the flowering period in nontreated plots at field trial sites was best fitted to the Gompertz and logistic models at two and six fields respectively, indicative of a polycyclic disease epidemic (Table 1.3.1 A). The measured floral incidence of *S. sclerotiorum* over time was best explained by the exponential, logistic and Gompertz models at three, one, and three fields respectively (Table 1.3.1 A). Residual values were examined during regression analysis to determine the model of best fit for incidence changes of *B. cinerea* and *S. sclerotiorum* at each field trial site.

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Initial flower infection and time of incidence in flowers reaching 50% was estimated from rising disease intensity and model analysis. Incidence of *B. cinerea* and *S. sclerotiorum* could not be fitted to models at 2 and 3 fields respectively due to decreasing disease incidence approaching the end of the flowering period. From sites where models could be fitted, initial infection by *B. cinerea* was estimated to have occurred between 11-16 November, while infection by *S. sclerotiorum* was estimated between 15 November and 1 December. Incidence of *B. cinerea* from flowers sampled between 13-16 November was ≤1% at 3 fields, while between 29 November and 4 December was ≥1% at 8 of 10fields. Across field sites where models were fitting, *B. cinerea* was estimated to reach 50% disease incidence on 11 December, with this time ranging between 30 November and 18 December among fields. Most fields had ≤1% incidence of *S. sclerotiorum* between 15-29 November. *S. sclerotiorum* was estimated to reach 50% disease incidence between 10-28 December, with a mean date of 18 December across fields. Changing incidences of both fungi in addition to flower maturity (FMS) over the flowering period for each site are shown (Fig. 1.3.1 A-J).

Pathogen	Site	Model of best fit	P	Slope	Y Intercept	R^2	Back transformed R^2	RMSE	Coef. of variation	50% incidence reached (≈)
B. cinerea	07-1	Gompertz	0.008	0.066	-22.87	0.88	0.93	0.078	27.06	18-Dec
	07-2	logistic	<.0001	0.186	-64.71	0.99	0.99	0.024	5.77	12-Dec
	07-3	logistic	0.002	0.210	-71.86	0.98	0.97	0.082	14.73	7-Dec
	07-4	logistic	0.0002	0.175	-59.56	0.99	0.99	0.036	6.59	5-Dec
	07-5	logistic	0.008	0.198	-66.27	0.96	0.93	0.119	16.99	30-Nov
	07-6	logistic	0.008	0.120	-42.10	0.76	0.93	0.088	23.25	14-Dec
	07-7	logistic	0.055	0.137	-46.87	0.89	0.76	0.197	37.93	*
	07-8	logistic	0.006	0.125	-43.91	0.59	0.94	0.077	23.48	16-Dec
	07-9	logistic	0.053	0.117	-39.32	0.73	0.76	0.187	29.30	*
	07-10	Gompertz	0.004	0.060	-20.97	0.88	0.96	0.061	20.26	17-Dec
S. sclerotiorum	07-1	Gompertz	0.001	0.162	-56.52	0.93	0.98	0.061	18.76	15-Dec
	07-2	Gompertz	0.04	0.121	-41.58	0.99	0.80	0.173	45.97	10-Dec
	07-3	Gompertz	0.029	0.050	-17.74	0.80	0.84	0.075	38.55	28-Dec
	07-4	exponential	<.0001	0.119	-42.75	0.99	0.99	0.018	6.96	19-Dec
	07-5	exponential	0.017	0.060	-22.24	0.72	0.89	0.057	26.54	25-Dec
	07-6	exponential	0.08	0.003	-1.49	0.009	0.68	0.015	2.63	*
	07-7	Gompertz	0.24	0.031	-10.86	0.52	0.41	0.129	49.49	*
	07-8	logistic	0.015	0.147	-50.91	0.98	0.90	0.124	29.73	12-Dec
	07-9	logistic	0.093	0.066	-23.13	0.85	0.66	0.136	34.82	*
	07-10	exponential	<.0001	0.111	-39.85	0.99	0.99	0.018	6.34	18-Dec

*Incidence declines at final flower sampling, suggest invalid to fit to model.

Table 1.3.1 A - Summary of modelling including model of best fit to pathogen incidence over time, estimated time of flower infection and 50% incidence.

Flower assessment	Sampling date	Mean incidence across sites		F pr.	*t*-statistic
		B. cinerea	*S. sclerotiorum*		
1st	13-16 Nov	3.5	2.4	0.084	1.94
2nd	Nov 29-4 Dec	9.5	2.1	0.072	2.04
3rd	10-11 Dec	56	29.4	0.024	2.71
4th	16-18 Dec	75.7	51.8	0.026	2.64
5th	22-Dec	81.4	62.8	0.07	2.05

Table 1.3.1 B - Differences between incidences of flower disease causing fungi within flowers at different sampling times across sites.

Changes in pathogen incidence and average flower maturity stage (FMS) at sampling times throughout the flowering period were plotted for each trial site and are shown below (Figures 1.3.1 A-J).

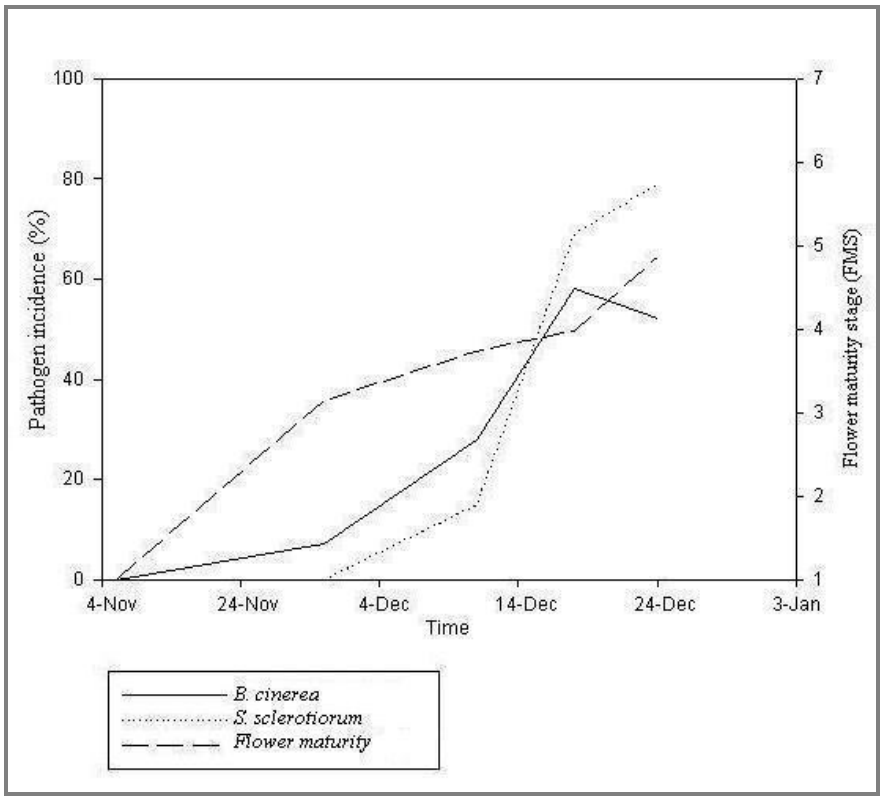


Figure 1.3.1 A - Site 07-1, Barrington.

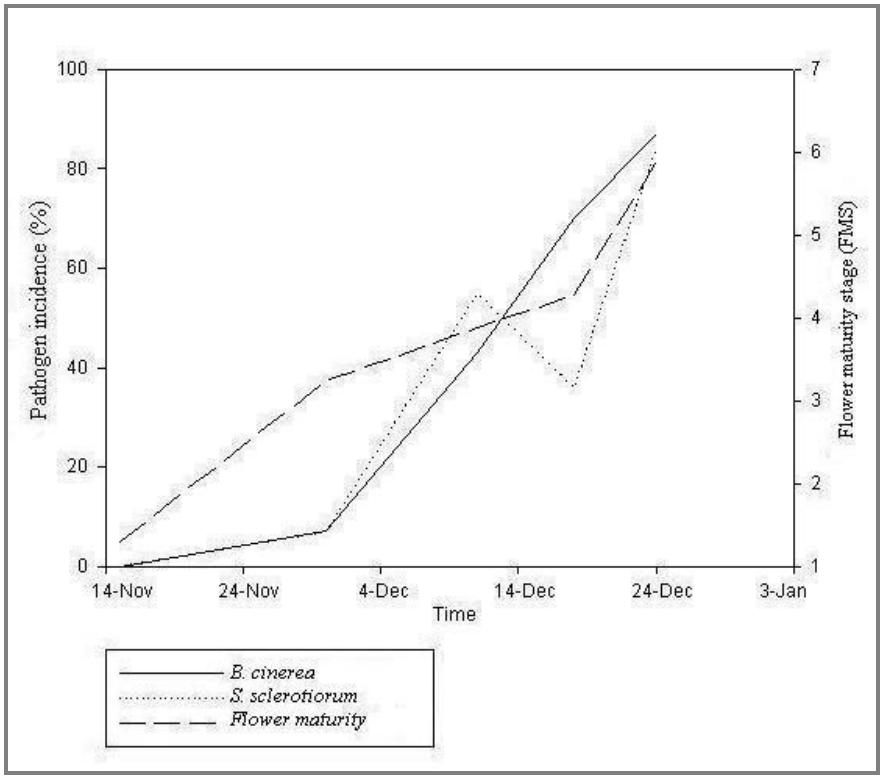


Figure 1.3.1 B - Site 07-2, Sassafras.

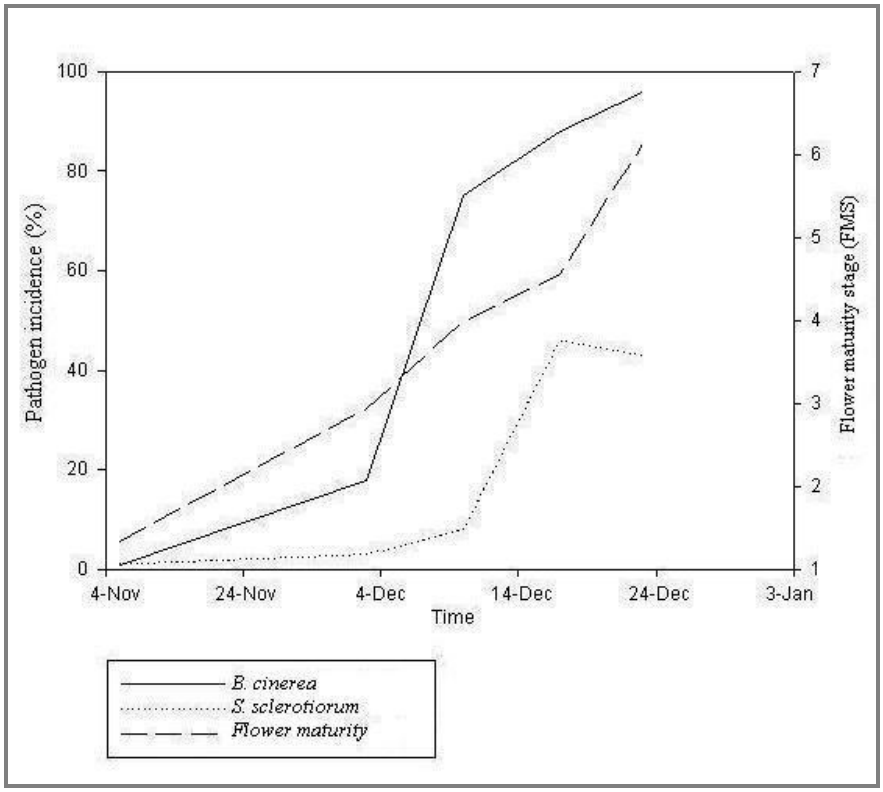


Figure 1.3.1 C - Site 07-3, Forth.

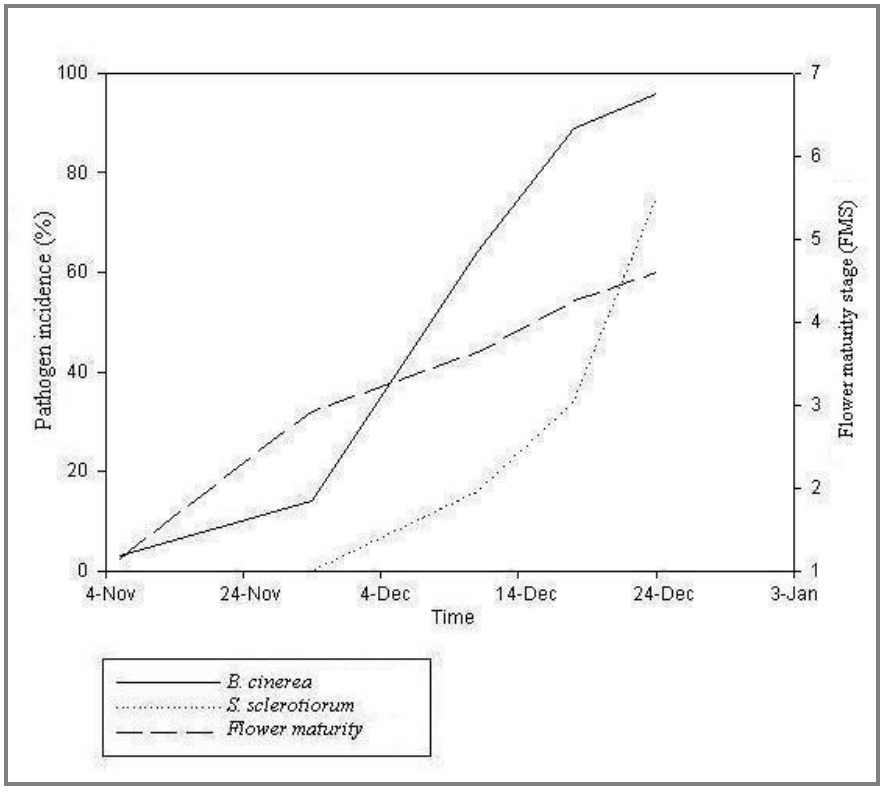


Figure 1.3.1 D - Site 07-4, Forth.

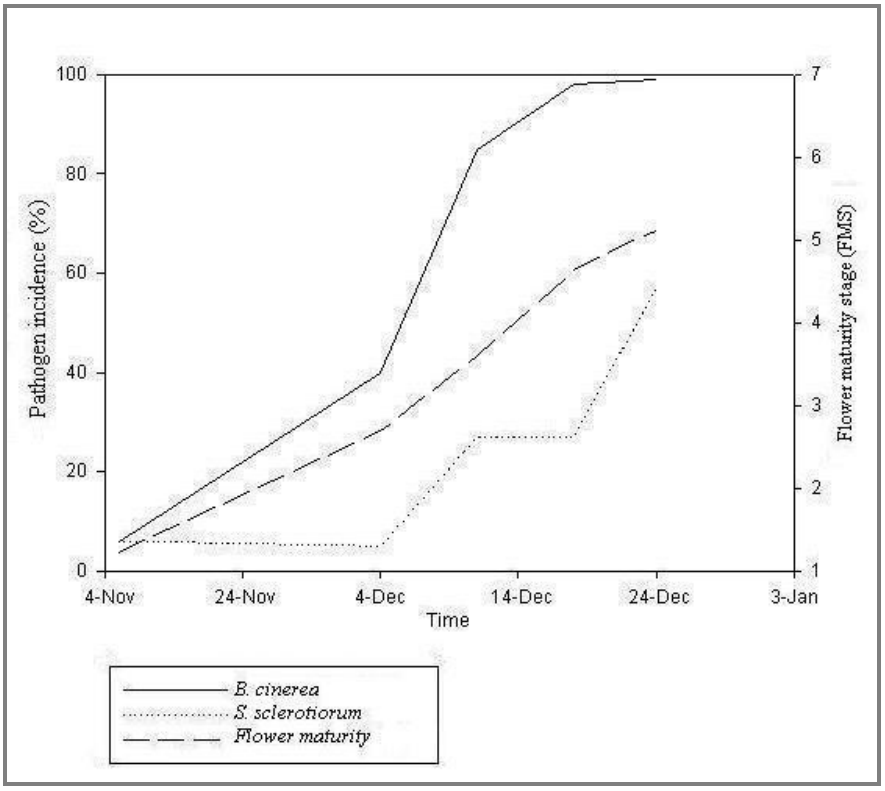


Figure 1.3.1 E - Site 07-5, Kindred.

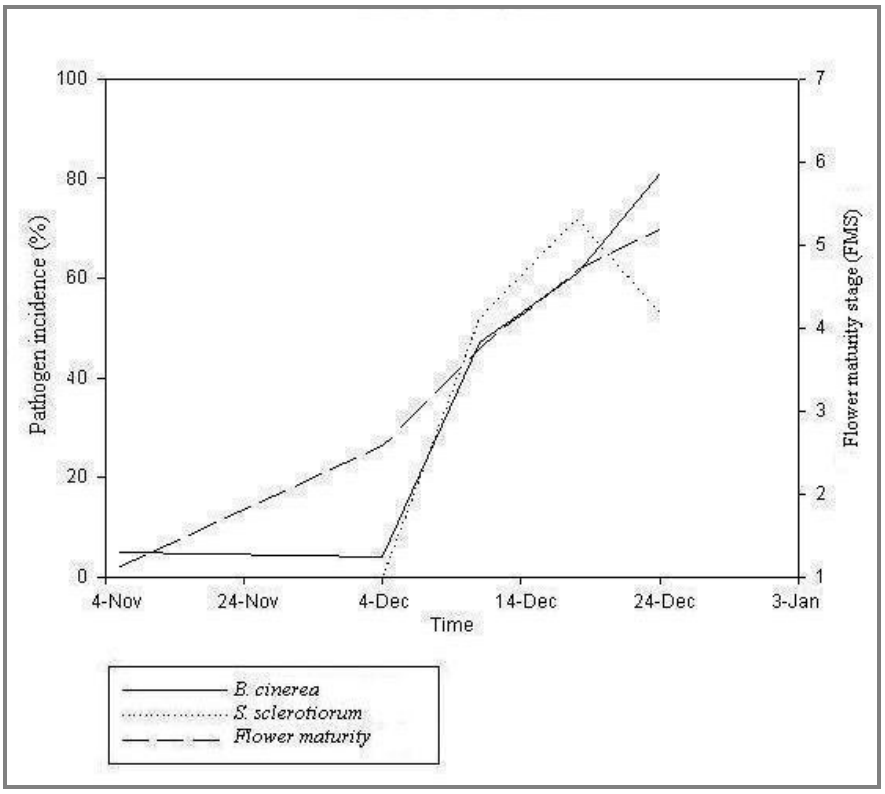


Figure 1.3.1 F - Site 07-6, Penguin.

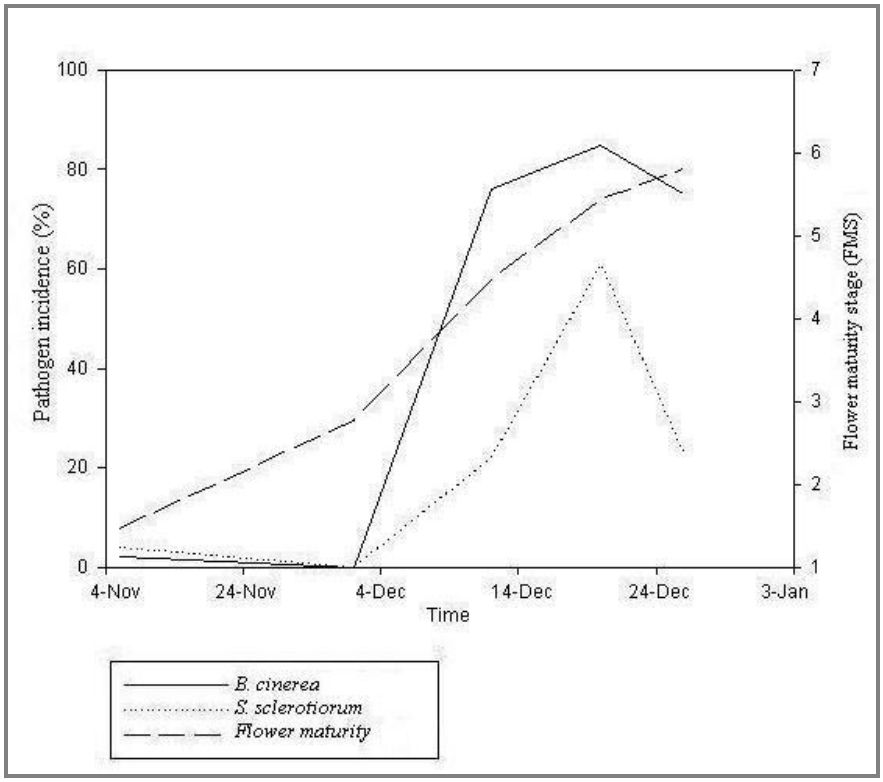


Figure 1.3.1 G - Site 07-7, Table Cape.

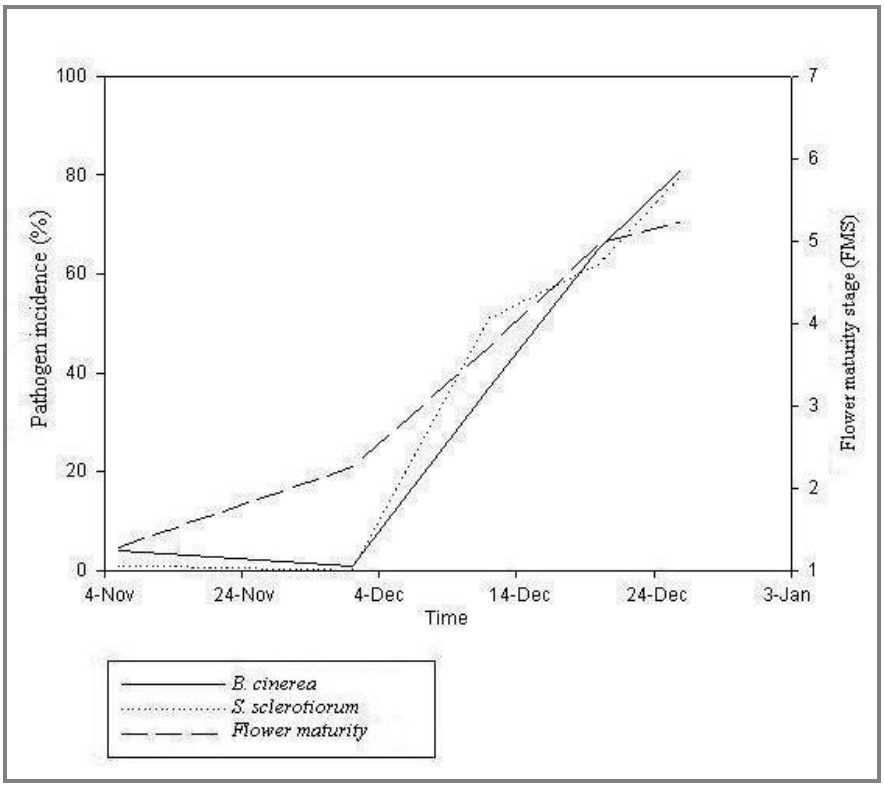


Figure 1.3.1 H - Site 07-8, Table Cape.

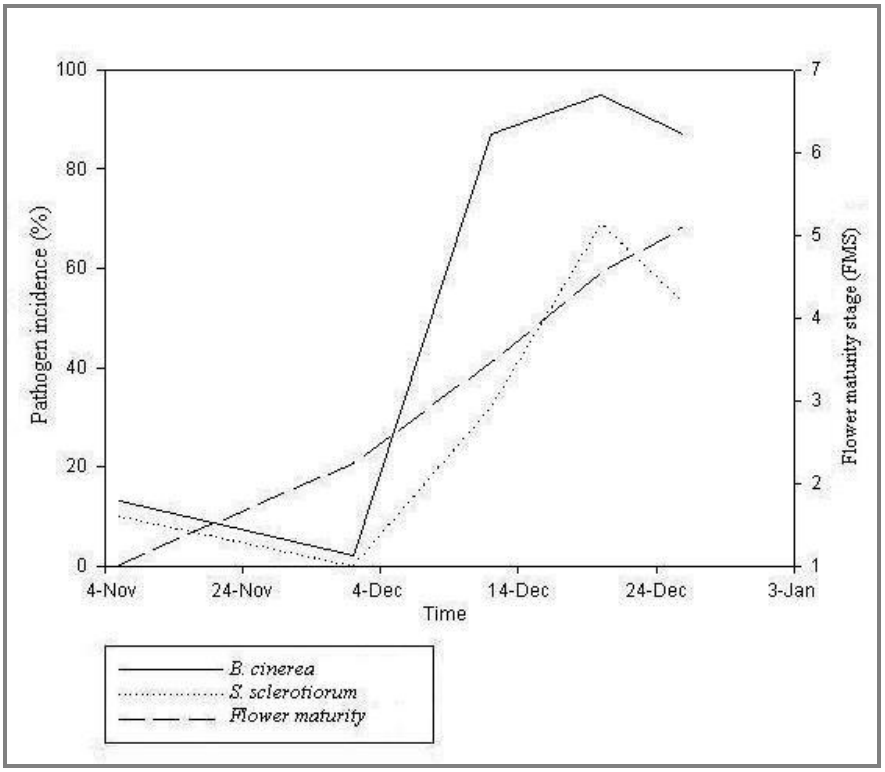


Figure 1.3.1 I - Site 07-9, Table Cape.

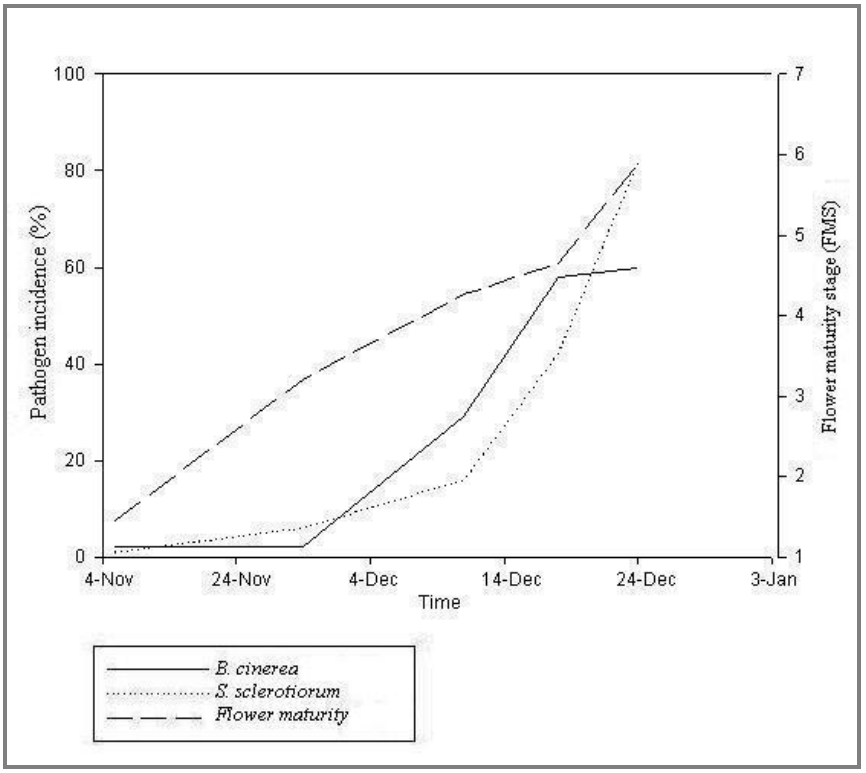


Figure 1.3.1 J - Site 07-10, Wesley Vale.

1.3.2 Survey of mycoflora associated with diseased pyrethrum flowers

Forty five fungal isolates tentatively identified as *S. minor* on the basis of sclerotial size (Plate 1.3.2) were isolated from diseased flowers and foliage collected from field sites during 2008-09 and 2009-10 (Appendix 1.3.2 C). Isolates were stored for further study as described previously in materials and methods of this chapter for *S. sclerotiorum.*

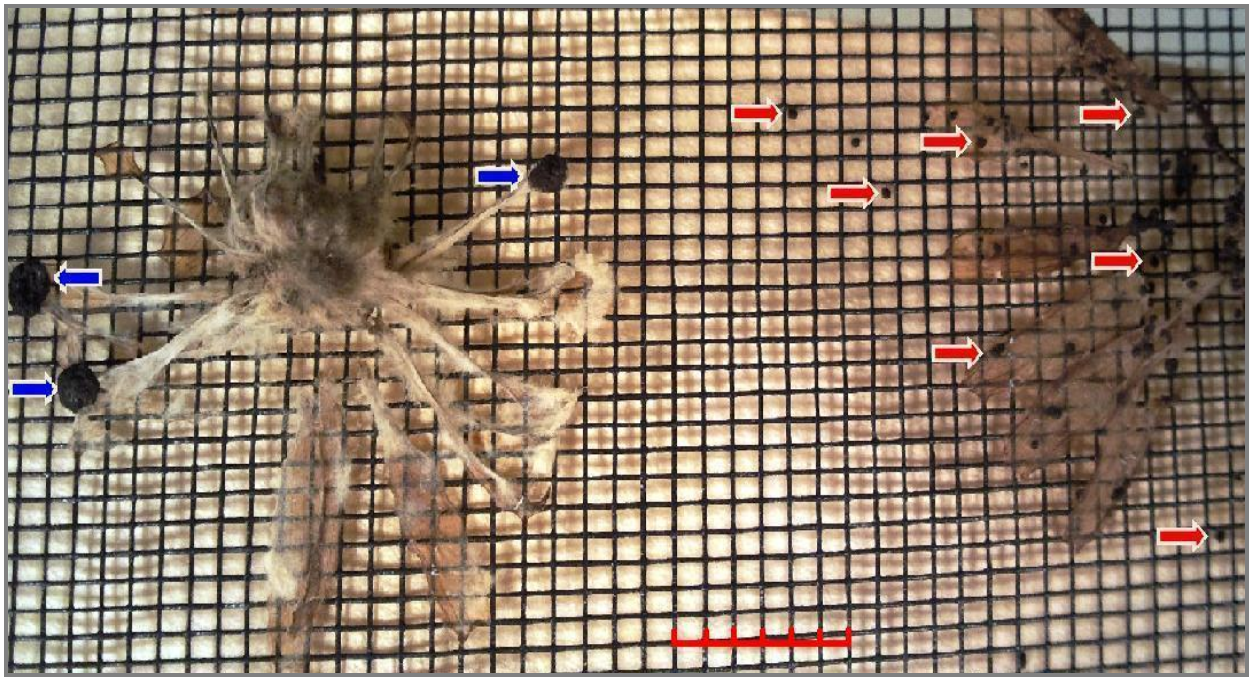


Plate 1.3.2 - Mature sclerotia grown on diseased, incubated pyrethrum flowers consistent in size with *S. sclerotiorum* (denoted by blue arrows) and *S. minor* (red arrows), scale in 5 mm increments.

Alternaria alternata and *A. tenuissima* in addition to *Penicillium* and *Stemphyllium* species were microscopically identified from flowers after surface sterilisation and incubation. Incidences are summarised as mean values across ten fields from Table Cape, Kindred, Moriarty Stowport and Forth districts (Appendix 1.3.2 D).

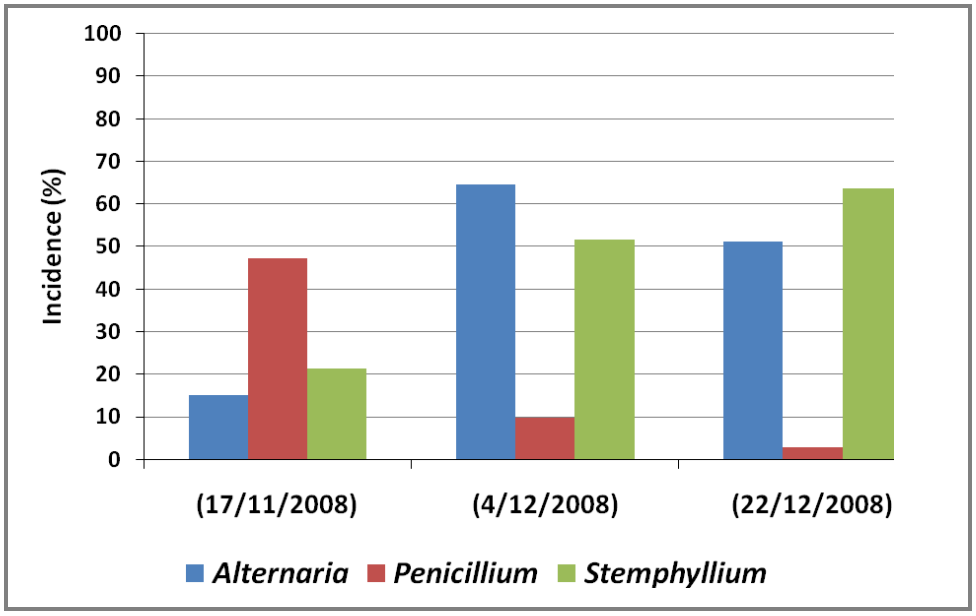


Figure 1.3.2 - Mycoflora identified from flower samples during 2008-09 (All data shown in Appendix 1.3.2 D).

1.4 Discussion

Flower blights caused by both recognised fungal pathogens of pyrethrum flowers in Australia, *B. cinerea* and *S. sclerotiorum,* were suggested from predictive modelling in these studies to follow patterns of polycyclic disease epidemic development. Studies by Nicot *et al* (1996) indicated more than 15 million conidia of *B. cinerea* could be produced on a 2 cm stem of tomato within seven days, showing the potential for rapid disease development of this pathogen. Although *B. cinerea* has shown the ability to survive for longer on rose petals than other plant tissue (Araújo *et al,* 2005), inoculum of *B. cinerea* is likely originating from reservoirs of greater availability at time of infection other than flowers (leaves and stems, crop trash or alternative hosts) , with conidia infecting flowers of crops through the flowering period. In turn, these new infections are capable of producing new conidia and complete numerous cycles of asexual reproduction during the latter stages of flowering accumulating further inoculum.

While modelling suggested *S. sclerotiorum* flower blight in Australia follows a polycyclic epidemic pattern of development, these findings are contrary to those of Pethybridge *et al* (2010)

where monocyclic epidemic progression was suggested. However, the biology of *S. sclerotiorum* reproduction may explain this disparity, as ascospore release from individual apothecia may occur over a period of seven days or more, and not simultaneously (Newton and Sequira, 1972). Relative humidity has been shown as a factor affecting ascospore release, with ascocarps observed to desiccate when exposed to less than 98% relative humidity, recovering once humidity again climbed to 100% (Partyka and Mai, 1962). After ascospore release, moisture availability may affect when ascospore germination of *S. sclerotiorum* occurs. With optimum conditions this may occur between 3-6 hours after discharge (Willets and Wong, 1980), while at 60%, 80% and 98% relative humidity ascopsores were still viable after 119, 94 and 64 hours respectively (Partyka and Mai, 1962). Ascospores may therefore be discharged sporadically, with localised germination of such spores occurring intermittently when moisture permits, giving the appearance of a polycyclic disease. Furthermore, sclerotia in the soil may require different periods of conditioning before undergoing carpogenic germination. *S. sclerotiorum* is therefore likely to be infecting flowers throughout the season from staggered ascospore release, with flower infections unlikely to contribute to secondary spread within the season. Furthermore in some crops development of apothecia is synchronous with crop flowering. Therefore more apothecia may be formed during flowering leading to greater amounts of ascospores and potential infection events over flowering. The effect of environmental conditions on flower disease development caused by *S. sclerotiorum* during the one season evaluated in this study can only be speculated. The staggered release of ascospores of *S. sclerotiorum* and the effect of humidity on survival and germination of ascospores has been demonstrated (Newton and Sequira, 1972; Willets and Wong, 1980), but whether climate was particularly conducive for continuous carpogenic germination and release of ascospores during flowering in this study is unknown.

Flower blights caused by *S. sclerotiorum* were best fitted to exponential, logistic and Gompertz disease progress curves suggesting polycyclic disease epidemiology. This was probably an artefact of ascospore release from sclerotia germinating sequentially over the flowering period and of apothecia releasing ascospores over periods of days. The life cycle of *S. sclerotiorum* suggests the nature of disease progression through the flowering period should be monocyclic. *S. sclerotiorum* follows monocyclic epidemiology in white mould diseases of legumes, sunflowers and canola (Heffer Link and Johnson 2007), while crown rot of chickpea caused by *S. trifolium* was shown also to be a monocyclic disease (Njambere, 2009). Monocyclic epidemiology of *S. sclerotiorum* flower blight was recently demonstrated by Pethybridge *et al* (2010) in 92% of pyrethrum fields surveyed in Tasmania.

This more detailed study was conducted over multiple flower harvests with increased replicate fields and therefore likely to provide a more accurate description of disease epidemiology than predictive modelling provided in my study.

During the mycofloral survey of diseased pyrethrum flowers, *Alternaria* species observed were predominantly *A. alternata* (data not shown), reported to have minimal pathogenicity toward pyrethrum foliage and assumed to be a saprophytic agent only (Pethybridge *et al,* 2004a). *Stemphyllium* species are reported to cause ray speck diseases of *Chrysanthemum spp.* (Horst and Nelson, 1997) while *S. botryosum* has been demonstrated as pathogenic to pyrethrum foliage (Pethybridge *et al,* 2004a). *S. botryosum* has also been identified in association with diseased pyrethrum flowers harvested for seed in Tasmania (O'Malley, 2007). Further investigation into *S. botryosum* pathogenicity toward pyrethrum flower tissue could prove worthy of investigation, with mean incidence from flowers across all fields observed in this study greater than 60% during late December approaching flower harvest (Figure 1.3.2; App. 1.3.2 D). With generally low incidences and no reports of eliciting flower disease in the *Tanacetum* or *Chrysanthemum* genera, *Penicillium* sp. was assumed to be saprophytic only.

1.5 Conclusions

Gompertz and logistic disease progress curves best described disease progression of *B. cinerea* flower blight in Tasmanian pyrethrum fields, suggesting polycyclic epidemiology as has been shown in other pathosystems including grape (Elmer and Michailides, 2004) rose (Araújo *et al,* 2005) and greenhouse tomato (Decognet *et al,* 2009).

While predictive modelling suggested *Sclerotinia* flower blight to follow polycyclic epidemic development, monocyclic epidemiology appears more plausible and the sexual stage of the life cycle of *S. sclerotiorum* in addition to more detailed studies of this flower pathogen in Australian pyrethrum fields indicating the models may have been overwhelmed in this study.
The incidences of *B. cinerea* in flowers bordered on or were significantly higher than *S. sclerotiorum* at all occasions of sampling. Across all fields at the final sampling on 22 December, mean incidences of 81.4% and 62.8% were reached for *B. cinerea* and *S. sclerotiorum* respectively.

Predictive modelling suggested *B. cinerea* initiated flower infection between 11-16 November, reaching 50% pathogen incidence in flowers on 11 December on average. Where models were could be fitted, *S. sclerotiorum* was estimated to begin flower infection later than *B. cinerea* (between 15 November and 1 December), with 50% disease incidence also occurring after *B. cinerea* on 18 December on average.

Fungal cultures tentatively identified as *S. minor* were isolated from diseased flowers. This may suggest *S. minor* is involved in annual flower disease epidemics, and potentially a contributor of crown rot disease inoculum in Tasmanian pyrethrum fields. Further work on *S. minor* is reported later in this thesis. *Alternaria, Penicillium* and *Stemphyllium* fungal species were also regularly identified from diseased flowers. However, the status of these fungi as pathogens of pyrethrum flowers is currently unknown and worthy of investigation.

2. Evaluation of the efficacy of current control methods for *B. cinerea* and *S. sclerotiorum* flower blights of pyrethrum.

2.1 Introduction

Flower blights caused by *B. cinerea* and *S. sclerotiorum* in Australian pyrethrum crops are controlled solely with fungicides. These industry recommended sprays are referred to as the 'flowering fungicide program', applied through the latter stages of flowering during November and December. Two fungicides (tebuconazole and carbendazim) have been used in alternation as the flowering fungicide program, tebuconazole (applied as Folicur^{R}) belongs to the triazole group, and carbendazim (applied as Spinflo^{R} or Bavistin^{R}) is a benzimidazole fungicide. Carbendazim belongs to activity group 1 (formerly A), and tebuconazole is a group 3 (previously C) activity fungicide (Anonymous, 2009). Carbendazim has protecant and eradicant properties, and is a systemic methyl benzimidazole carbamate fungicide, taken up through root, stem and leaf tissues. Germ tube formation and mycelial growth are inhibited by contact with carbendazim, which acts to interfere with β-tubilin and DNA synthesis resulting in irregular spore germination, cell division and fungal growth (Ware, 1994; Anonymous, 2007). Tebuconazole belongs to the triazole chemical class and is a demethylation inhibiting (DMI) fungicide possessing protective and eradicative properties. The mode of action of DMI fungicides involves disrupting the synthesis of sterols necessary in formation of fungal cell membrane structures (Ware, 1994). These fungicides have been used through the flowering period for over 10 years in Australian pyrethrum fields. Fungicides are routinely applied at 10-14 days intervals through the flowering period, up to a maximum of four spray applications (two of each fungicide). Over the life of a pyrethrum crop, fungicides for flower disease control may total fifteen or more applications.

Numerous vegetable crops commonly grown in rotation with pyrethrum in Tasmania are also susceptible to *B. cinerea* and *S. sclerotiorum. Sclerotinia* spp. are known pathogens of pea, potato, lettuce, brassica, carrot, and green bean cultivation in Tasmania (Wardlaw, 2004). *B. cinerea* often causes fruit rots and flower blights, in addition to diseases in vegetable crops rotated with pyrethrum, such as beans, brassicas and lettuce (Persley *et al,* 2010). Controlling vegetable diseases caused by *Sclerotinia* spp. in Tasmania is achieved with fungicides including boscalid and iprodione, while management of *Botrytis* diseases in Tasmania is often obtained with chemicals such as boscalid, azoxystrobin, chlorothalonil, pyrimethanil and iprodione. These chemicals belong to activity/resistance groups 7 (formerly G), 11 (formerly K), M5 (formerly Y), 9 (formerly I) and 2 (known formerly as group B) respectively. Therefore there is potential for pathogen populations in

fields used for pyrethrum production to have had significant exposure to fungicides prior to pyrethrum.

No data currently exists regarding the efficacy of the flowering fungicides tebuconazole and carbendazim in the field, for reducing pathogen incidence of *B. cinerea* and *S. sclerotiorum* in pyrethrum flowers or the effect of the flowering fungicide on pyrethrum yield. Information on the efficacy of these fungicides is therefore of vital importance in making informed management decisions to reduce the economic impact of flower disease. Furthermore, it is unknown whether use of fungicides representing only two resistance or activity groupings for control of flower blights in Tasmanian pyrethrum crops has led to the development of resistance. The relative sensitivity of local isolate populations of *B. cinerea* and *S. sclerotiorum* to carbendazim and tebuconazole would therefore be of significant interest, to determine if prolonged use of these fungicides has translated to resistance in field populations of either pathogen. Cross resistance of *B. cinerea* between benzimidazole fungicides is recognised, and likely to have been eroding the efficacy of this chemical class for over twenty years (Leroux, 2004). Reduced sensitivity of *B. cinerea* to benzimidazole fungicides has been documented in greenhouse and ornamental crops (LaMondia and Douglas, 1997; Yourman and Jeffers, 1999), while resistance to carbendazim in vegetable cropping has also been reported (Myresiotis *et al,* 2007). Reduced sensitivity of *B. cinerea* to carbendazim was shown to be associated with isolates adapted to colder temperatures (Dewey and Yohalem, 2004), suggesting Tasmania's cool and temperate climate may provide an environment suitable for this to occur. Resistance of *S. sclerotiorum* to carbendazim has been reported in field cropped oilseed rape in China (Ma *et al,* 2009).

Objectives of these studies were therefore to:

- Assess the efficacy of the current fungicide program at reducing the incidence of *B. cinerea* and *S. sclerotiorum* in flowers.
- Determine whether the flowering fungicide program currently provides economic benefits in terms of improved pyrethrin assay and flower yields.
- Measure, with *in vitro* sensitivity testing, the relative efficacy of carbendazim and tebuconazole at inhibiting mycelial growth of isolates of *B. cinerea* and *S. sclerotiorum* obtained from diseased flowers in Tasmanian pyrethrum fields.

2.2 Materials and methods

Establishment of trial sites

Field trials were conducted over three successive flower harvests from 2007-2009 to determine the efficacy of the flowering fungicide program in Tasmanian pyrethrum fields during flowering. Between 10 to 15 crops were randomly chosen each year across all cultivation localities in Tasmania (Fig. 2.2). All sites were situated within 40km of Bass Straight from approximately east of Table Cape (40° 94' S, 145° 67' E) to Shearwater (41° 15' S, 146° 54' E) and were subject to recommended industry weed control, plant nutrition and irrigation strategies. Fields approaching their first harvest were used on most occasions (Tables 1.2.1; 2.2).

Figure 2.2 - Map of Cradle coast, Tasmania with trial sites represented by triangles.

Year	Site	District
2008-09	08-1	Table Cape
	08-2	Kindred
	08-3	Kindred
	08-4	Forth
	08-5	Forth
	08-6	Penguin
	08-7	Penguin
	08-8	Moriarty
	08-9	Moriarty
	08-10	Stowport
2009-10	09-1	North Motton
	09-2	North Motton
	09-3	North Motton
	09-4	North Motton
	09-5	North Motton
	09-6	Penguin
	09-7	Penguin
	09-8	Forth/Don
	09-9	Forth/Don*
	09-10	Forth/Don*
	09-11	Forth
	09-12	Forth
	09-13	Forth*
	09-14	Lillico*
	09-15	Forth*
	09-16	Forth*
	09-17	Forth*

*Sites approaching third harvest, all others approaching first harvest.

Table 2.2 - Trial site information.

Two treatment plots were established within each pyrethrum field and centred across adjacent spray runs, a nontreated plot (See Fig. 1.2.1), which was to receive no fungicides through the flowering period, and a commercial plot, which was to receive the standard industry recommended fungicides. In each field, commercial and nontreated plots were located approximately 30 m apart, were 10 × 10 m in size, and had 4 m buffers around which had received the same treatment. Marker pegs, coloured flagging tape and signs reading 'no flowering fungicides' were again used to denote nontreated plots as described in chapter 1. Growers were requested not to apply flowering fungicides to these areas, while the remainder of each field received the industry standard recommendations of the flowering fungicide program. Watchdog model 450 data loggers (Spectrum Technologies, Plainfield Illinois) were used in all growing districts each year, recording temperature, relative humidity, leaf wetness and rainfall/irrigation. Weather data is shown (Appendix 2.5). Fungicide applications proceeded as per the commercial recommendations on each

farm, with standard tractor mounted boom spray apparatus used. Fungicide was applied at a rate of 300 l/ha under approximately 200 kpa of pressure to crops each year throughout the flowering period. Folicur^{R} applications were either 350 ml/ha of Folicur^{R} 430SC or 750 ml/ha of Bavistin^{R}, with application dates and product details for each site shown (Appendix 2.2.1).

Sampling for pathogen incidence

One hundred flowers were sampled from nontreated and commercial plots within all fields for assessment of pathogen incidence at five times during 2007, three times in 2008 and twice during 2009 (Appendix 2.2.2). All flowers samples were collected, surface sterilised, air dried, assessed for maturity using the flower maturity stage (FMS), incubated and microscopically assessed for incidences of *B. cinerea* and *S. sclerotiorum* as described (Chapter 1).

Sampling for yield/assay

Each season, nontreated and commercially treated plots were assessed for flower yield and pyrethrin content of flowers (assay). During 2007-08 and 2008-09, plots were assessed by hand harvesting all flowers from within a 0.7 × 0.7 m (0.49 m^2) quadrat constructed from 30 mm polyethylene pipe. Quadrats stood approximately waist high with legs affixed, and were randomly placed inside plots. During 2007-08, flowers were harvested from four replicate quadrats within each of the nontreated and commercial plots per field site, and from six quadrats during 2008-09.

For yield determination, flowers from each hand-picked quadrat were weighed to obtain fresh weight per quadrat on a Masscal balance in the laboratory to an accuracy of ± 0.005 kilograms. A subsample comprising approximately 20% by weight was reweighed on an Ohaus balance (±0.01 g), dried (55°C/24 h) in a fan forced incubator (Binder 720/115, Germany) and reweighed to determine dry matter (%), which was used to convert the fresh weight per quadrat to dry weight. The mean weight of flowers of quadrats in each plot was used in statistical analysis. The dried subsample from each quadrat was bulked together and was stored frozen (-20°C) until determination of pyrethrin content (% by weight) by HPLC (Botanical Resources Australia Pty. Ltd.). Flower samples were finely ground for hexane extraction, followed by agitation and a 16 hour rest period. Samples were then

passed through a 0.45 µm filter before normal phase high performance liquid chromatography (HPLC) was used to analyse samples, on a Shimadzu HPLC system (McEldowney and Menary, 1998). Standardised reference samples were compared to field samples, with pyrethrin detection by ultra violet (UV) light at 223 nm. Further details of High Pressure Liquid Chromatography extraction of pyrethrins are commercial in confidence and unavailable. A bulked subsample of material from all quadrats in each of the nontreated and commercial plots was used to obtain one pyrethrin assay value for each of the nontreated and commercial plots at each site during 2007-08. The single pyrethrin assay from each of the commercial and nontreated plots was combined with the dry weight of flowers from each quadrat within the commercial and nontreated and used to estimate pyrethrin yield of each quadrat. In 2008-09 bulked subsamples of quadrats 1-3 and quadrats 4-6 were used to obtain two assay values per treatment at each field site. Again the pyrethrin assay obtained from the bulked sample was combined with the dry weight of flowers obtained from respective individual quadrats to obtain an estimate of the pyrethrin yield per quadrat. Individual assays could not be conducted on all replicates due to cost constraints.

In addition, 100 flowers from one quadrat within each plot were weighed (± 0.01) and assessed visually for Flower Maturity Stage (FMS) (Plate 1.2.2), and for disease. Diseased flowers were defined as flowers with one third of tubular disc florets closed with white outer ray florets discoloured, desiccated, dying or absent. Smaller, brown or dry flowers were additionally classed as 'diseased', often with stems having an obvious crook shape characteristic of infection by *Phoma ligulicola*. Fresh weight of 50 flowers was converted to dry weight from the dry matter (%) of the oven dried subsample, and used to estimate dry weight per flower (g). In addition the following yield, and yield component calculations were estimated for each quadrat:

(i) Yield of dried flowers (kg/ha) = (mean dry weight of flowers per 0.49 m^2 quadrat ÷ 0.49 m^2) × 10,000 m^2 per ha
(ii) Pyrethrin yield (kg/ha) = Pyrethrins content (%) of bulked sample corrected for optical density ÷ 100%) × yield of dried flowers (kg/ha).
(iii) No. flowers per quadrat = weight of flowers per 0.49 m^2 quadrat (g) ÷ dry weight per flower (g).

The mean values of quadrats were used for statistical analysis (below).

During 2009-10, an alternative method of harvesting flowers for yield evaluation was used, specifically a hand held hedge trimmer (Husqvarna 226 HD 60S) fitted with a safety guard over the cutting blades, and a catching tray to collect most flower and stem material. Plants were cut at approximately half the height of the plant and flowers and stalks from each quadrat were individually bagged, with flowers falling to the ground during cutting picked up afterwards. Six replicate quadrats were harvested for each of the nontreated and commercial plots. Each quadrat consisted of two swaths, each measuring 2.4 × 0.5 m (2.4 m^2).

Fresh yield per unit area was determined by weighing flowers from 2.4m^2 quadrats on a Masscal balance (± 0.005 kg). Several grab samples totalling approximately 300 g (~10% of the total weight) were arbitrarily taken from fresh material from each quadrat, and weighed on an Ohaus balance (± 0.01 g). The numbers of flowers in each 300 g sample were counted. Samples were oven dried and reweighed (as above) and dry matter (%) was calculated. In addition, stems were removed from 100 flowers per quadrat and weighed (± 0.01 g) to obtain fresh weight per flower (g) without stalks. Flower samples were oven dried (as above). Samples of 100 flowers were reweighed and weight divided by 100 to give dry weight per flower (g), before storing frozen (-20 °C) for analysis of pyrethrins by HPLC as described above. Pyrethrins assay was conducted on four of six replicate quadrats from each of the nontreated and commercial plots at every site due to cost considerations.

A further 50 flowers from material harvested from each quadrat were removed and assessed visually for FMS and flower disease incidence as described above.

The following measured data was therefore obtained: (i) fresh weight of harvested material per 2.4 m^2, (ii) No. flowers in 300 g fresh harvested material, (iii) fresh weight of 100 flowers, (iv) dry weight of 100 flowers (used to calculated dry weight per flower), pyrethrin content (%) of dried 100 flower sample, (v) FMS of 50 flowers and (vi) disease status of 50 flowers.

From this the following calculations were made for each quadrat:

(i) No. flowers per 2.4 m^2 = No. flowers in 300 g fresh material ÷ 300g × total fresh weight fresh material from 2.4m^2. This was converted to no. flowers/m^2 for each quadrat.

(ii) Fresh weight of flowers (kg/2.4 m^2) = (Fresh weight of 100 flowers ÷ 100 flowers) × no. flowers per 2.4 m^2.

(iii) Dry weight of flowers (kg/2.4 m^2) = (Dry weight of 100 flowers ÷ 100 flowers) ×no. flowers per 2.4 m^2. This was converted to dry weight of flowers (kg/ha) for analysis.

(iv) Pyrethrin yield (kg/2.4 m^2) = (Pyrethrin content of bulked sample corrected for optical density (%) ÷ 100%) × dry weight of flowers per 2.4 m^2. This was converted to pyrethrin yield (kg/ha) for each quadrat.

The mean value of quadrats for each plot was used for statistical analysis.

While evaluating the flowering fungicide program during this study, efforts were made over successive years at optimising flower sampling from field plots. Quadrats were hand harvested during the first two years, with a modified hedge trimmer used in the final season of field trials. Increased numbers of assay samples and larger treatment areas were assessed each year, to limit variability and allow more detailed data analysis (Appendix 2.2.3).

Data analysis

For each year, the effect of fungicides on incidence of *B. cinerea* and *S. sclerotiorum* was analysed within trial sites at each stage of flower sampling by Analysis of Variance (ANOVA) using Gens tat Version 10.1. Five replicates of 20 flowers were used per treatment, with each replicate consisting of four sealed plastic incubation trays containing five flowers apiece. In 2007-08 ANOVA was conducted on the fresh and dry weight of flowers (kg/ha) using each of the four harvested quadrats as replicates. As only a single pyrethrin assay was conducted on a bulked sample from each of the nontreated and commercial, the pyrethrin yield from each quadrat was estimated as:

Pyrethrin yield = Dry weight (g) of flowers per quadrat × Optical Density (proportion) × Pyrethrins assay from bulked sample (% by dry weight).

In 2008-09, ANOVA was conducted on the fresh and dry weight of flowers (kg/ha) using six harvested quadrats as replicates. The pyrethrin yield from each quadrat was estimated as:

Pyrethrin yield of quadrats 1-3 = Dry weight (g) of flowers per quadrat × Optical Density (proportion) × Pyrethrins assay from bulked sample of quadrats 1-3 (% by dry weight).

Pyrethrin yield of quadrats 4-6 = Dry weight (g) of flowers per quadrat × Optical Density (proportion) × Pyrethrins assay from bulked sample of quadrats 4-6 (% by dry weight).

In 2009-10 ANOVA was conducted on the fresh and dry weight of flowers (kg/ha) using six harvested quadrats as replicates, and four replicates for pyrethrin assay and estimated pyrethrin yield per quadrat. The effect of fungicide treatment across sites was also analysed by ANOVA (GenStat version 10.1) using the mean incidence values of *B. cinerea* and *S. sclerotiorum*, flower yield, pyrethrins assay and pyrethrins yield for each treatment at each site as a replicate.

In vitro fungicide sensitivity testing

Tebuconazole and carbendazim were screened in agar plate tests to evaluate efficacy at inhibiting mycelial growth of seventy three isolates of *S. sclerotiorum* and forty nine isolates of *B. cinerea* obtained from diseased pyrethrum flowers. Isolates of *S. sclerotiorum* and *B. cinerea* were obtained from diseased flowers sampled within nontreated plots from all trial sites and districts district during 2007-08 (Figure 2.2; App. 1.3.2 A-B). A further 25 isolates of *S. sclerotiorum* were collected from diseased bean pods in Tasmanian bean fields were also evaluated during this fungicide sensitivity screening, with green beans often grown in rotation with pyrethrum in Tasmania.

Eight concentrations of each fungicide (0, 0.01, 0.05, 0.5, 5, 50, 100 and 500 µg a.i./ml) were tested, using three replicate petri plates per concentration of fungicide. Commercial fungicides Folicur^{R} 430 SC® (430 g/l tebuconazole) and Bavistin® (500 g/l carbendazim) were dissolved in sterile distilled water and made up to a concentration of 70% ethanol (absolute, 200 proof, Sigma-Aldrich) producing a sterile stock fungicide solution from which a dilution series was prepared under aseptic conditions. Sufficient aliquots from the dilution series were then added to 400 ml bottles of

autoclaved Potato Dextrose Agar (PDA) (Amyl Media, Dandenong, Victoria) maintained at 60°C in a water bath to obtain fungicide concentrations. All plates were inoculated with a cork boring taken from the edge of an actively growing colony of *B. cinerea, S. sclerotiorum* or *S. minor* on PDA. Plates were incubated at 20°C for 2-4 days or until at least one control (0 µg a.i./ml concentration) plate had approached the edge of the plate. Colony diameter and radius was then measured. The estimated concentration of the active ingredient required to inhibit 50% of fungal growth (EC_{50}) for each isolate and fungicide was calculated by PROBIT analysis using SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA).

2.3. Results

2.3.1 - Year 1 (2007-08)

Effect of fungicides on pathogen incidence of B. cinerea

At the final flower sampling immediately prior to commercial harvest, plots receiving commercial fungicide applications had significantly ($P < 0.05$) lower incidence of *B. cinerea* than nontreated plots at 6 of 10 field sites (Table 2.3.1 A). Incidence of *B. cinerea* at the first sampling ranged between 24-96% in commercial and 52-99% in the nontreated plots (Table 2.3.1 A). Fungicide applications resulted in significantly lower incidences of *B. cinerea* on at least one occasion at every site over the flowering period. During the third and fourth flower sampling times, there was a significantly lower incidence of *B. cinerea* in commercial compared to nontreated plots at 5 trial sites. The four sites in which there was no significant difference among nontreated and commercial during the final sampling did show lower incidences on previous occasions, but incidence of *B. cinerea* increased rapidly near harvest. Incidence of *B. cinerea* at all sampling times are listed in Appendix 2.3 A.

Site	Commercial (incidence)	Nontreated (incidence)	F pr.	LSD	CV %
07-1	24	52	0.02	22.18	40
07-2	25	87	<.001	14.86	18.2
07-3	73	96	0.004	13.15	10.7
07-4	76	96	0.006	12.52	10
07-5	96	99	0.29	-	4.3
07-6	81	81	1	-	11
07-7	81	75	0.172	-	8.1
07-8	38	81	<.001	16.31	18.8
07-9	87	87	1	-	9.6
07-10	24	60	<.001	13.84	22.6

Table 2.3.1 A - Incidence of *B. cinerea* at trial sites at final flower sampling.

Effect of fungicides on pathogen incidence of S. sclerotiorum

Flowers sampled on the final occasion from commercial plots had significantly ($P < 0.05$) lower incidence of *S. sclerotiorum* than nontreated at 9 of 10 trial sites (Table 2.3.1 B). The incidence of *S. sclerotiorum* at all sites during the first and second sampling times was low, ranging between 0-10% and 0-18% respectively with no statistical differences between commercial and nontreated at any site at these times. At the third and fourth evaluation, the incidence of *S. sclerotiorum*, ranged between 15-72% in nontreated plots at trial sites. The incidence of *S. sclerotiorum* in commercial plots was significantly lower than nontreated at six and five sites during the third and fourth assessment times respectively (Table 2.3.1 B). The incidence of *S. sclerotiorum* within the commercial plot at site (07-10) approached being statistically higher ($P = 0.051$) than that of nontreated flowers on the second sampling occasion (Appendix 2.3 B). This may indicate incidences *B. cinerea* increased at a more rapid rate than *S. sclerotiorum*.

Site	Commercial (incidence)	Nontreated (incidence)	F pr.	LSD	CV %
07-1	41	79	<.001	15.38	17.6
07-2	28	82	<.001	12.74	15.9
07-3	20	43	0.039	21.45	46.7
07-4	36	76	<.001	11.41	14
07-5	21	57	0.002	18.59	32.7
07-6	22	53	0.001	14.22	26
07-7	22	23	0.883	-	46.1
07-8	46	80	<.001	5.65	6.1
07-9	12	53	<.001	12.2	25.7
07-10	44	82	0.003	20.63	22.4

Table 2.3.1 B - Incidence of *S. sclerotiorum* at trial sites at final flower sampling.

Incidence of both *B. cinerea* and *S. sclerotiorum* at all stages of flower sampling in treatments at all field trial sites are displayed (Fig. 2.3.1 A-J), with data summarised (Appendices 2.3.1 A-B). Across all sites on average, fungicide applications resulted in significantly lower incidences of *B. cinerea* and *S. sclerotiorum* at the final sampling stage, approaching harvest (Table 2.3.1 C).

Figure 2.3.1 - Effect of fungicide application on incidence of *B. cinerea* and *S. sclerotiorum* in pyrethrum flower at different times over flowering (Bars are LSD at *P* = 0.05).

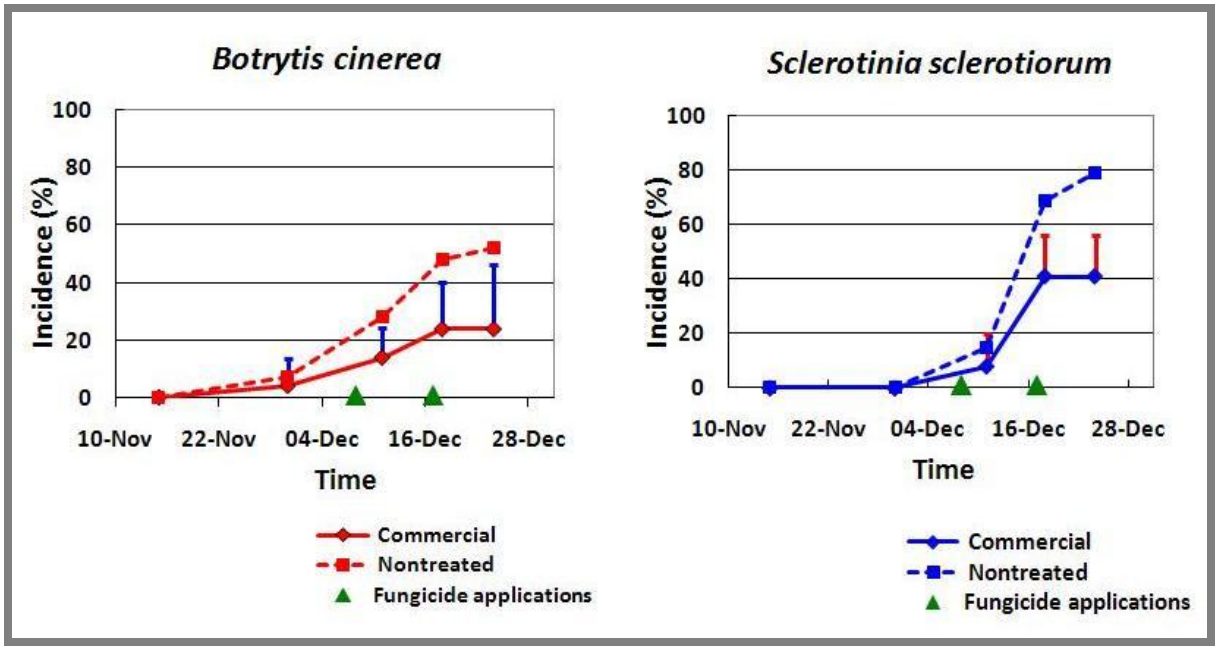


Figure 2.3.1 A - Site 07-1, Forth.

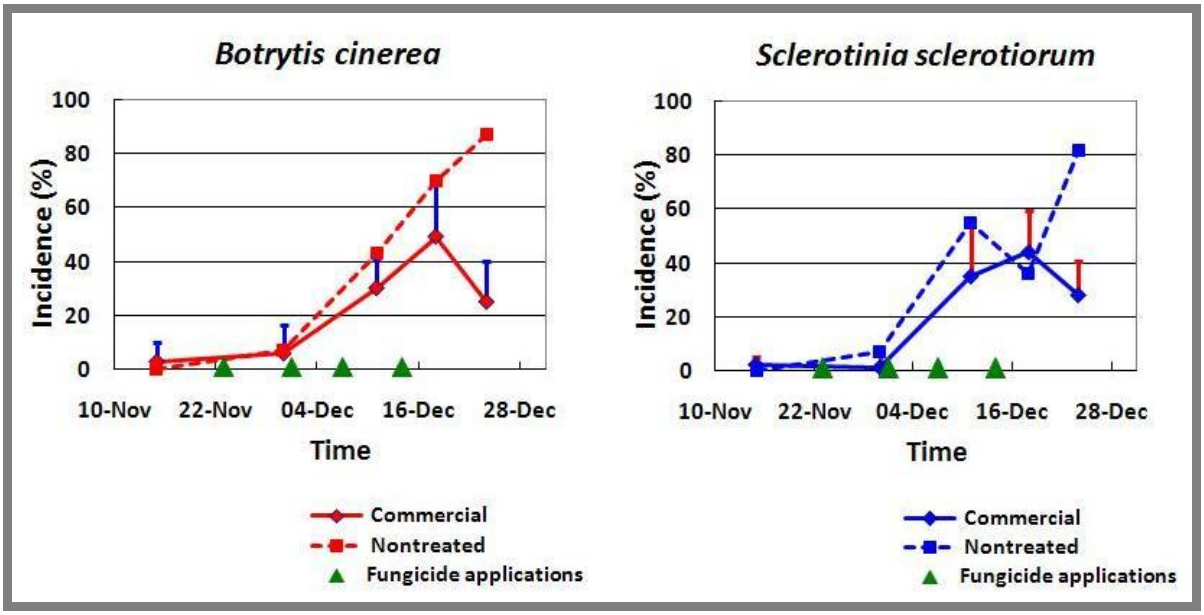


Figure 2.3.1 B - Site 07-2, Sassafras.

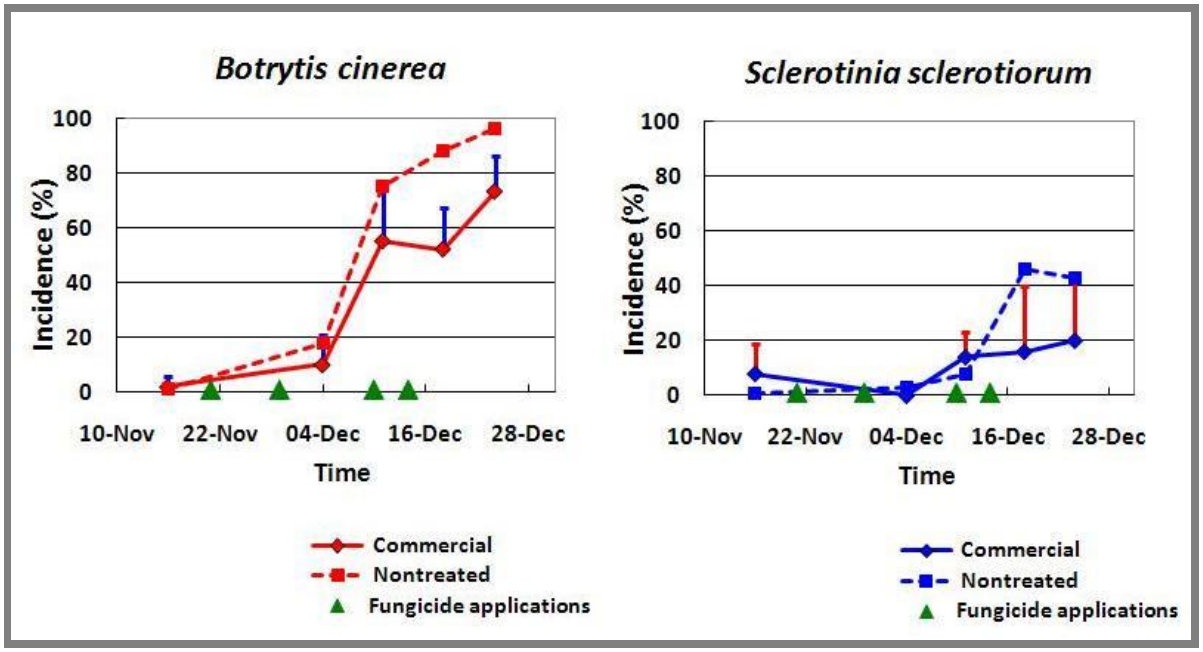


Figure 2.3.1 C - Site 07-3, Forth.

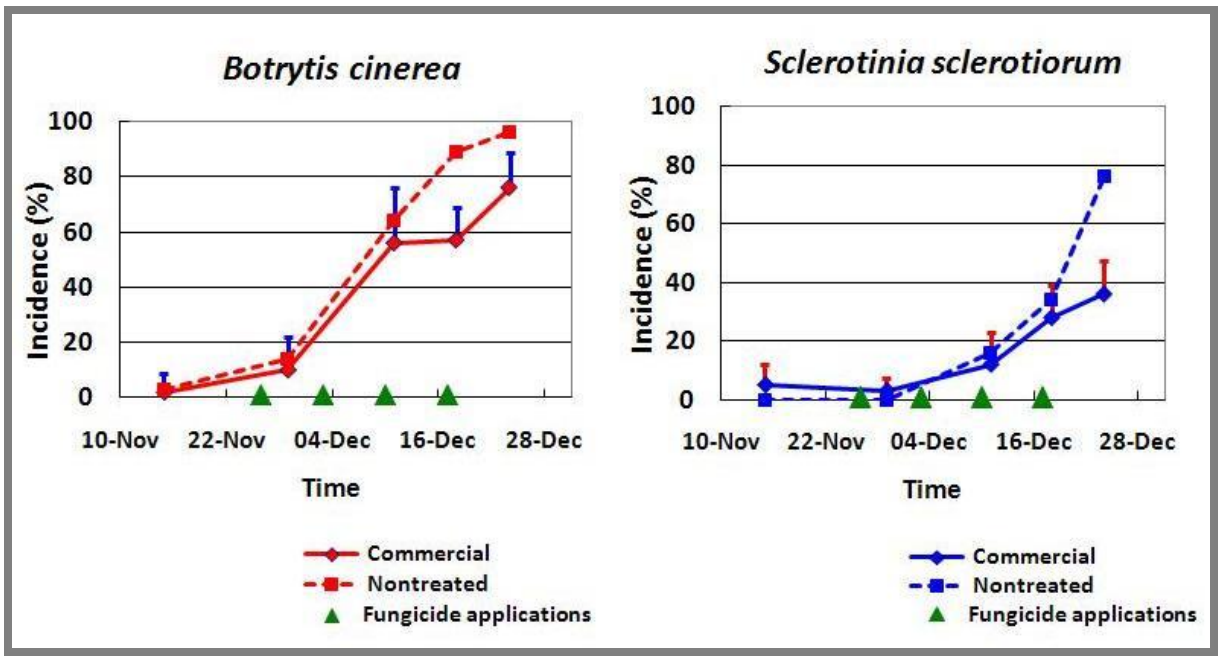


Figure 2.3.1 D - Site 07-4, Forth.

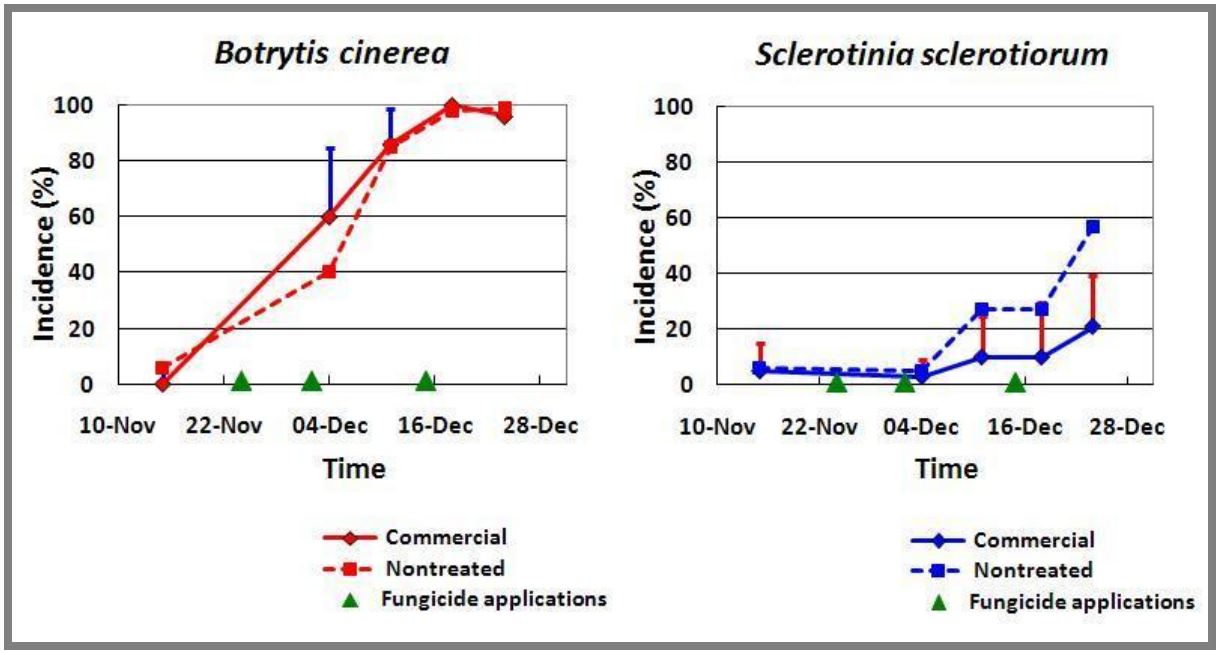


Figure 2.3.1 E - Site 07-5, Kindred.

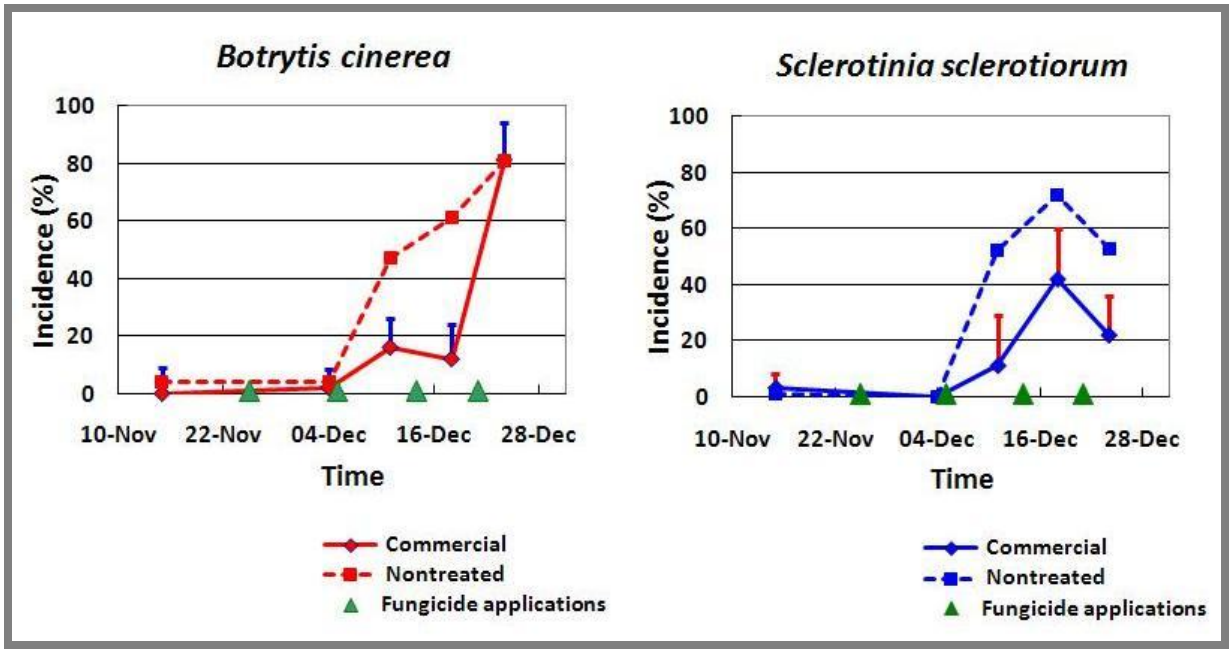


Figure 2.3.1 F - Site 07-6, Penguin.

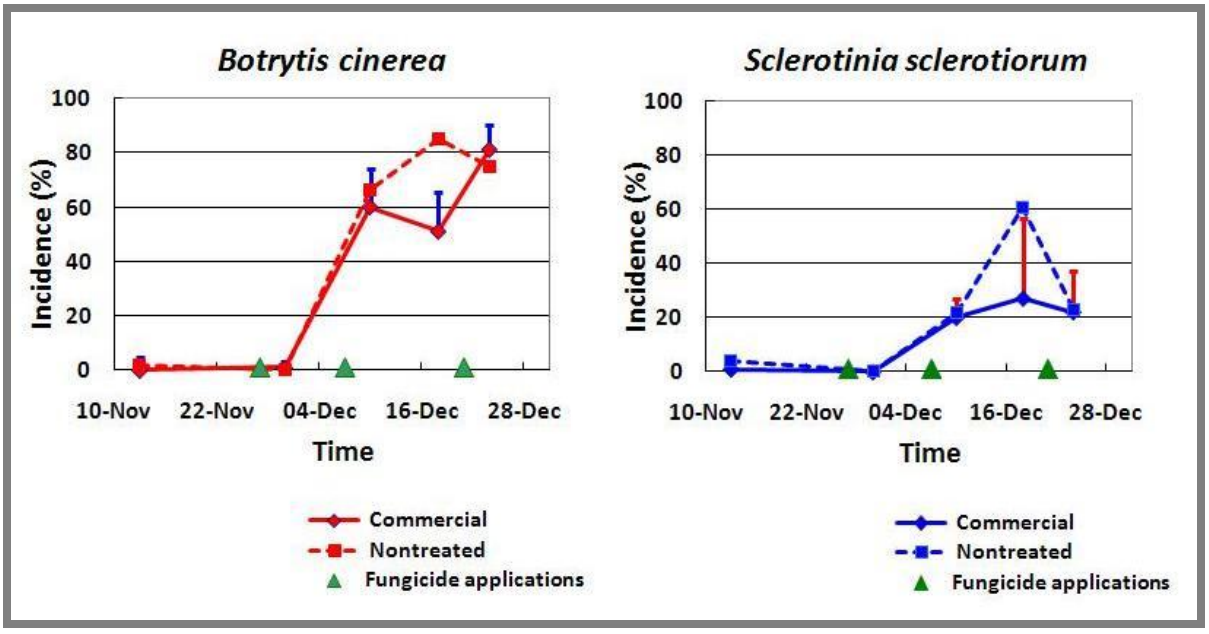


Figure 2.3.1 G - Site 07-7, Table Cape.

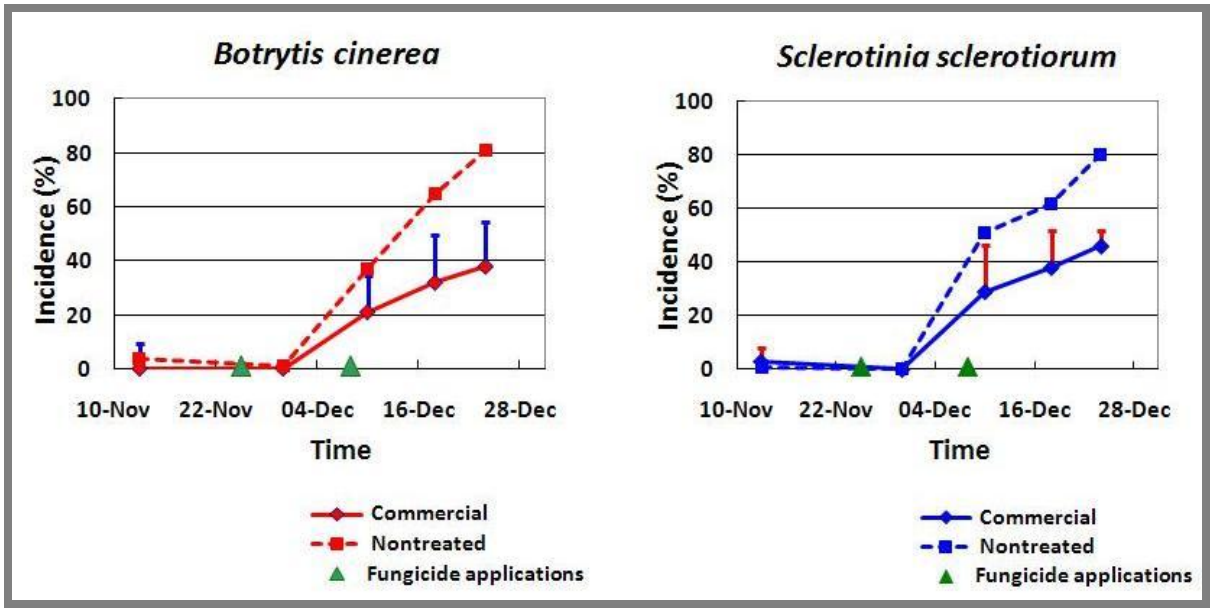


Figure 2.3.1 H - Site 07-8, Table Cape.

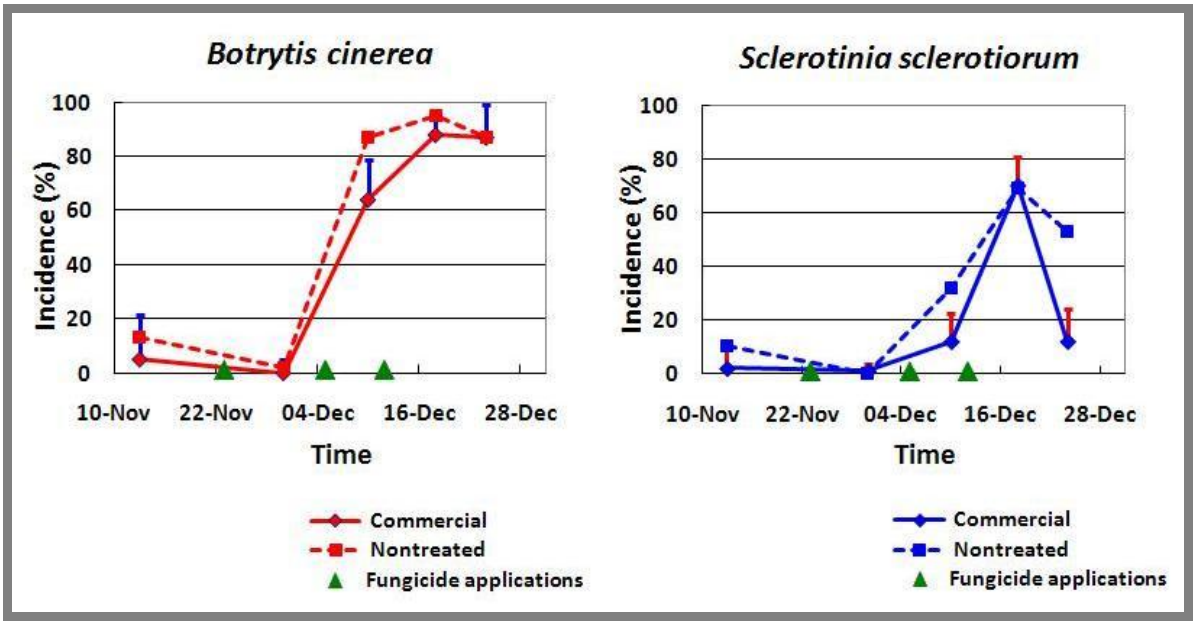


Figure 2.3.1 I - Site 07-9, Table Cape.

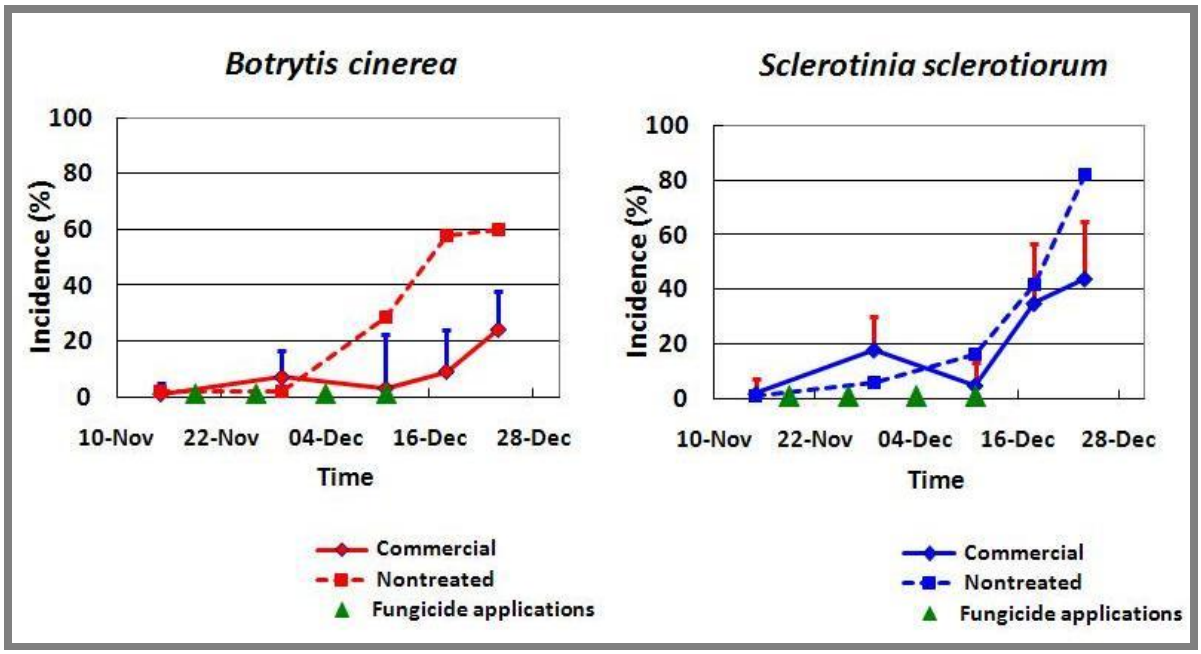


Figure 2.3.1 J - Site 07-10, Wesley Vale.

Effect of fungicides on yield and assay

Within trial sites, commercial treatments resulted in a significantly higher (P = 0.045) yield of dry flowers (kg/ha) than nontreated at only one site (07-10) (Table 2.3.1 C). At a second site (07-3) the nontreated had significantly higher (P = 0.026) yield of dried flowers than the commercial treatment (Table 2.3.1 C). Pyrethrin yields in commercially treated plots bordered on being significantly greater ($0.05 < P < 0.1$) than nontreated lots at two sites (07-5 and 07-10) (Table 2.3.1 D). However, at one site (07-3) the nontreated had significantly greater yield of pyrethrins than the commercial treatment (Table 2.3.1 D).

Site	Dry flower yield (kg/ha)		F pr.	LSD	CV %
	Commercial	Nontreated			
07-1	2865.06	2887.22	0.943	-	12.3
07-2	4229.23	4767.07	0.304	-	12.4
07-3	3960.44	4497.48	0.026	432.3	4.5
07-4	3745.46	4204.37	0.563	-	22.4
07-5	3956.73	3525.89	0.204	-	9.3
07-6	3924.85	3730.90	0.569	-	10
07-7	4254.47	3522.32	0.416	-	25.4
07-8	4071.15	3641.63	0.337	-	12.5
07-9	3497.64	3332.91	0.82	-	24.3
07-10	2928.36	2356.44	0.045	552.4	9.2

Table 2.3.1 C - Effect of fungicide treatment on dry flower yields at different trial sites.

Site	Pyrethrin yield (kg/ha)		F pr.	LSD	CV%
	Commercial	Nontreated			
07-1	52.46	51.55	0.87	-	12.3
07-2	94.17	106.85	0.282	-	12.4
07-3	86.44	96.18	0.044	9.30	4.5
07-4	92.56	95.33	0.875	-	21.6
07-5	89.00	73.10	0.066	17.59	9.6
07-6	85.28	79.20	0.421	-	10.1
07-7	87.31	68.39	0.317	-	26.1
07-8	88.07	73.57	0.157	-	12.7
07-9	73.38	73.11	0.986	-	24.5
07-10	58.45	48.05	0.06	-	9.2

Table 2.3.1 D - Effect of fungicide treatment on pyrethrin yields at different trial sites.

Across all trial sites, there was no statistical difference among treatments in flower yields or pyrethrin assay (Table 2.3.1 E). The mean incidence of *S. sclerotiorum* across all sites at the final time of sampling was significantly higher (P <.0001) in nontreated plots (62.8%) in comparison to commercial treatments (29.2%) (Table 2.3.1 E). Across all trial sites commercial treatment had a mean incidence of *B. cinerea* which bordered on being significantly lower than nontreated (P = 0.06). However the mean incidence of *B. cinerea* in commercially treated flowers still remained high at 60.5%. Flowers sampled on the final occasion from commercial plots had significantly (P < 0.05) lower incidence of *S. sclerotiorum* than nontreated at 9 of 10 trial sites (Table 2.3.1 B). The incidence

of *S. sclerotiorum* at all sites during the first and second sampling times was low, ranging between 0-10% and 0-18% respectively with no statistical differences between commercial and nontreated at any site at these times. At the third and fourth evaluation, the incidence of *S. sclerotiorum*, ranged between 15-72% in nontreated plots at trial sites. The incidence of *S. sclerotiorum* in commercial plots was significantly lower than nontreated at six and five sites during the third and fourth assessment times respectively (Table 2.3.1 B). The incidence of *S. sclerotiorum* within the commercial plot at site (07-10) showed a strong trend of being higher (P = 0.051) than that of nontreated flowers on the second sampling occasion (Appendix 2.3.1 B). This may indicate that incidence of *B. cinerea* increased at a more rapid rate than *S. sclerotiorum,* or that more inoculum of *B. cinerea* was present to begin with.

Variate	Commercial	Nontreated	F pr.	LSD	CV %
Pyrethrins (I) (% of dry weight)	1.29	1.20	0.17	-	11.4
Pyrethrins (II) (% of dry weight)	1.00	0.97	0.516	-	10.7
Ratio: P(I)/P(II)	1.30	1.25	0.58	-	15.2
Pyrethrins (total) (% of dry weight)	2.30	2.22	0.364	-	7.5
Pyrethrin yield (kg/ha)	80.7	76.5	0.585	-	21.4
Dry weight flowers (kg/ha)	3743	3647	0.731	-	16.7
Incidence *B. cinerea* (%)	60.5	81.4	0.06	-	32.9
Incidence of *S. sclerotiorum* (%)	29.2	62.8	<0.001	15.52	35.9

Table 2.3.1 E - Effect of fungicide treatment on yield, pyrethrins assay and pathogen incidence at final flower sampling across all sites.

The commercial flowering fungicide program was shown to reduce the incidence of *B. cinerea* and *S. sclerotiorum* at several sites throughout sampling in the flowering period, increasingly so with rising disease incidence. Initial sampling was prior to the commencement of fungicide applications and incidences of *B. cinerea* and *S. sclerotiorum* were both low, averaging 3.5% or less in both treatments across all sites. At the second sampling time, only four of ten sites had received the first flowering fungicide application and no improvements from commercial treatments were noticed. At the third sampling time, nine of ten sites had received at least two spray applications, and the commercial fungicide program provided significantly reduced incidences of both pathogens in six fields. At the fourth stage of flower sampling, incidence of *B. cinerea* and *S. sclerotiorum* in commercial and nontreated plots averaged 47.4% and 75.7% respectively across all sites. Fungicide

applications significantly reduced frequency of *B. cinerea* at eight sites, but incidences remained high ranging between 50-100% in commercial plots at five sites. Mean incidence of *S. sclerotiorum* across all sites was generally lower at this time, averaging 35% and 52% in commercial and nontreated plots respectively. At the final sampling of flowers from field trials, only four days after the previous occasion, average incidence of *B. cinerea* over all sites had risen to 60.5% in commercial treatments and 81.4% in nontreated plots. While commercially recommended fungicides provided significantly lower incidences at six sites, frequency of *Botrytis* flower blight was high, ranging between 73 - 96% in commercially treated areas at six fields and exceeding 80% in nontreated strips at seven sites. Incidence of *S. sclerotiorum* at this time averaged 62.8% across all nontreated strips, while since the previous assessment commercial plots showed a declining average frequency of 29.2% across all sites. While incidences were high within some nontreated plots and exceeded 75% at five sites, fungicide applications resulted in incidences of 22% or less at five sites with no site having incidence of *S. sclerotiorum* greater than 46% in the commercial treatment.

2.3.2 - Year 2 (2008-09)

Effect of fungicides on incidence of B. cinerea and S. sclerotiorum

At the first flower sampling, the incidence of *B. cinerea* was low across all sites, ranging between 0-5% (Appendix 2.3.2 A). At two sites (08-1 and 08-3) *B. cinerea* was not detected at this time. While incidence was low there was a significantly lower incidence in the fungicide treated plot at one site (08-7). Frequency of *S. sclerotiorum* at the first sampling ranged between 17-36% in nontreated plots, with significantly lower incidences reported in fungicide treated plots at 3 sites (08-3, 08-5 and 08-8; Appendix 2.3.2 B).

Incidences of *B. cinerea* in flowers ranged between 3-26% in nontreated plots at the second flower sampling. Plots receiving commercial fungicide applications had significantly lower ($P < 0.05$) incidences of *B. cinerea* at three sites (08-7, 08-8, and 08-10) in comparison to nontreated (Appendix 2.3.1 A). Flowers sampled on the second occasion also had low incidences of *S. sclerotiorum*, ranging between 8-27% in nontreated areas. Fungicide treatments resulted in significantly ($P < 0.05$) lower incidence compared to no treatment at two sites (08-6 and 08-7) and reductions bordering on

statistical significance ($0.05 < P < 0.1$) at three additional sites (08-4, 08-5 and 08-9; Appendix 2.3.1 B).

The incidence of *B. cinerea* in flowers sampled on the final occasion, nearest to harvest, ranged between 35-97% in nontreated plots, with six sites having incidence between 71-97%. Significant ($P < 0.05$) reductions in incidence of *B. cinerea* from fungicide treatments were reported at six sites (08-1, 08-3, 08-5, 08-8, 08-9 and 08-10; Appendix 2.3.1 A). The incidence of *S. sclerotiorum* in flowers at this time ranged from 2-59% in nontreated plots, while fungicide treated plots at all sites had incidences of 20% or less. Significantly ($P < 0.05$) lower incidence of *S. sclerotiorum* were observed in nontreated plots at five sites (08-3, 08-5, 08-6, 08-7 and 08-10; Appendix 2.3.1 B).

In the 2008/09 season the commercial fungicide treatment resulted in borderline ($0.05 < P < 0.1$) or significant ($P < 0.05$) reductions in mean incidence of both *B. cinerea* and *S. sclerotiorum* over all field sites in comparison to the nontreated at the final sampling time (Table 2.3.2 B). In fungicide treated and nontreated plots the incidence of *B. cinerea* was 56.4 and 78.1% respectively and of *S. sclerotiorum* was 8.1 and 26% respectively (Table 2.3.2 B).

Across sites, the mean incidence of *S. sclerotiorum* was 26.6%, 15.8% and 24.9% and that of *B. cinerea* was 1.1%, 10.9% and 75.0% on 17 November, 4 and 22 December respectively (Table 2.3.2 B). The incidence of *S. sclerotiorum* was significantly ($P < 0.001$) higher than that of *B. cinerea* on 17 November and significantly ($P < 0.001$) lower than that of *B. cinerea* on 22 December.

Effect of fungicides on yield and assay

Two fields (08-4, 08-9) showed significantly higher and borderline increases respectively from pyrethrin yields in nontreated areas than fungicide treated plots (Table 2.3.2 A). With doubt over possible overspray of nontreated plots with fungicides at these fields existing, these sites were removed as outliers from data analysis. Commercial fungicide treatment resulted in a significant ($P <$

0.05) increase in dry weight of flowers harvested at 3 sites (08-8, 08-10 and 08-1), and two sites (08-1, 08-8) had significantly ($P < 0.05$) higher pyrethrin yields than nontreated. Commercial fungicides treatment at site 08-10 resulted in an increase bordering on statistical significance (Table 2.3.2 A). Across all field sites this season, there was a significant difference between commercial and nontreated mean pyrethrin yields, being significantly improved from commercial fungicide applications and averaging 108.4 kg/ha in commercial plots and 90.7 kg/ha in nontreated areas ($P = 0.032$,; Table 2.3.2 B). The dry weight of flowers yielded from treated plots bordered on being significantly greater than nontreated strips, averaging 4676 and 4101 kg/ha in commercial and nontreated plots respectively after removing sites 08-4 and 08-9 from analysis ($P = 0.086$) (Table 2.3.2 B).

Yield	Site	Commercial	Nontreated	F pr.	LSD	CV%
Dry flowers (kg/ha)	08-1	5662	3846	<0.001	768.4	12.6
	08-2	3648	3962	0.235	-	11.3
	08-3	4257	3905	0.414	-	17.5
	08-4	4315	5659	0.003	767.1	12
	08-5	4354	4327	0.943	-	14.8
	08-6	5170	5499	0.417	-	12.6
	08-7	4192	3787	0.235	-	13.9
	08-8	5544	4458	0.028	942.6	14.7
	08-9	4188	4833	0.133	-	15.1
	08-10	4171	3641	0.046	517.8	10.3
Pyrethrins (kg/ha)	08-1	134.1	88	<.001	17.43	12.2
	08-2	100.7	105.2	0.556	-	12.6
	08-3	96.8	93	0.7	-	17.5
	08-4	99.8	133.5	0.003	18.81	12.5
	08-5	99.3	85.5	0.126	-	15.5
	08-6	116.2	111.8	0.609	-	12.5
	08-7	95.2	86.5	0.305	-	15.3
	08-8	138.9	112.4	0.039	24.93	15.4
	08-9	89	107.2	0.068	-	15.7
	08-10	75.1	64.9	0.078	-	12.8

Table 2.3.2 A - Within site analysis of treatment effect on yield qualities.

Variate	Commercial	Nontreated	F pr.	LSD	CV%
Pyrethrins (I) (% of dry weight)	1.39	1.37	0.918	-	17.2
Pyrethrins (II) (% of dry weight)	1.04	1.00	0.428	-	10.2
Ratio: P(I)/P(II)	1.33	1.38	0.689	-	17.5
Pyrethrins (total) (% of dry weight)	2.43	2.37	0.712	-	12.5
Pyrethrin yield (kg/ha)	4676	4101	0.086	-	20.9
Dry weight flowers (kg/ha)	108.4	90.7	0.032	16.06	22.3
Incidence of *B. cinerea* (%)	56.4	78.1	0.078	-	34
Incidence of *S. sclerotiorum* (%)	8.1	26	0.041	17.04	93.1

Table 2.3.2 B - Effect of fungicide applications on yield qualities and pathogen incidence of flowers (at final sampling occasion) across all sites.

Initial flower sampling and assessment began on 17 November, prior to the commencement of the flowering fungicide program. By the second assessment of flower samples on 4 December all sites had received the first flowering fungicide application of tebuconazole, while five of ten sites had also received the second spray which at all trial sites this year was carbendazim. Incidence of *B. cinerea* was low and averaged just 8.4% and 10.9% in commercial and nontreated plots respectively, while the mean incidence of *S. sclerotiorum* was also low, averaging 8.3% and 15.8% across commercial and nontreated plots respectively. By the final sampling assessment on 22 December, nine of ten sites had received three spray applications and eight of ten fields had received four fungicide applications. The third and final fungicide applications were tebuconazole and carbendazim respectively at all sites where applied. Incidence of *B. cinerea* had risen to a mean of 75% in nontreated plots and 53.3% in commercial plots by this time.

2.3.3 - Year 3 (2009-10)

Effect of fungicides on FMS, and incidence of B. cinerea and S. sclerotiorum

Flowers harvested on the first sampling occasion (2-Dec) showed significantly ($P < 0.05$) higher flower maturity stage (FMS) from nontreated at 9 of 17 sites, with two additional sites showing borderline ($0.05 < P < 0.1$) increases in flower maturity in the nontreated in comparison to the fungicide treated (Appendix 2.3.3 A). However, no significant differences in FMS were noted during the final sampling of flowers from fields between 14-18 December.

Flowers sampled on the first occasion (2-Dec) from fungicide treated plots showed significantly ($P < 0.05$) lower incidence of *B. cinerea* than nontreated plots at 9 sites (09-8, 09-1, 09-2, 09-3, 09-4, 09-16, 09-6, 09-11, 09-17), with 3 other sites (09-5, 09-12, 09-14) showing statistically borderline ($0.05 < P < 0.1$) reductions (Appendix 2.3.3 B). Incidences ranged from 22-79% in nontreated plots, with 10 sites having ≥50% incidence. In fungicide treated plots, the incidence of *B. cinerea* ranged between 9-63%, with five sites having incidence of ≥42% from flowers sampled at this time (Appendix 2.3.3 B). The incidence of *S. sclerotiorum* from flowers sampled from nontreated plots on the first occasion was significantly ($P < 0.05$) higher than fungicide treated plots at nine sites (09-9, 09-10, 09-8, 09-2, 09-3, 09-4, 09-15, 09-17, 09-14; Appendix 2.3.3 C). The incidence of *S. sclerotiorum* was generally low at this time, with nontreated plots at only two sites having greater than 50% incidence and commercial treatment at 11 sites having incidence of 10% or less (Appendix 2.3.3 C).

Flowers sampled on the final occasion (14-18 December) near to harvest had mean incidences of *B. cinerea* between 23-87% in nontreated plots, with 6 sites having pathogen incidences of 77% or higher. Significant ($P < 0.05$) reductions in flower incidence of *B. cinerea* were noted in fungicide treated plots in comparison to nontreated at 7 sites (09-9, 09-10, 09-2, 09-6, 09-7, 09-11, 09-12), while incidence at 9 sites was 50% or higher at this time (Appendix 2.3.3 B). The incidence of *S. sclerotiorum* in flowers nearest to harvest was significantly ($P < 0.05$) lower in fungicide treated plots at 7 sites (09-8, 09-4, 09-15, 09-16, 09-7, 09-13, 09-11), with 2 additional sites (09-10 and 09-2) showing reductions from fungicide applications approaching statistical significance ($0.05 < P < 0.1$; Appendix 2.3.3 C). Across all sites and treatments, the incidence of *S. sclerotiorum* was generally low at this time. Commercial treatments at 12 sites had incidence of 18% or less and only 2 nontreated strips had greater than 50% incidence (Appendix 2.3.3.1 C).

Across sites, flowers sampled on the first occasion from commercially treated plots reported significantly lower incidences of both fungal pathogens (Table 2.3.3). Across all trial sites at the final sampling, incidence of *B. cinerea* bordered on being significantly lower ($0.05 < P < 0.1$) in commercially treated compared to nontreated plots, averaging 48.9% and 62.9% in each treatment respectively (Table 2.3.3). Similarly, the incidence of *S. sclerotiorum* bordered on being significantly lower ($0.05 < P < 0.1$) in the commercial treatments in comparison to nontreated strips, averaging

22.7% and 11.9% from each treatment respectively (Table 2.3.3). On both sampling occasions, flowers from nontreated plots had significantly higher incidences of *B. cinerea* than *S. sclerotiorum*. During initial sampling the mean incidence of *B. cinerea* was 53.1%, and for *S. sclerotiorum* was 23.2% (P <0.001, LSD = 11.94, CV% = 44.8). Upon the final sampling of field material, incidence of *B. cinerea* and *S. sclerotiorum* in flowers was 62.9% and 22.7% respectively (P <0.001, LSD = 14.11, CV% = 42.2). Mean incidence of *B. cinerea* from nontreated flowers across all sites was greater than 50% upon initial sampling, showing high incidences of this pathogen may be present in flowers prior to when estimated via predictive modelling (11 December) as discussed in chapter 1.

Effect of fungicides on yield and assay

Commercial fungicides resulted in a significantly greater yield of flowers (kg/ha) at one field (Site 09-1; P <0.008, LSD = 437.9, CV% = 9.2), while site 09-10 showed an increase bordering on being significant (Appendix 2.3.3 D). Yield of pyrethrins (kg/ha) was also significantly increased from fungicide treatments at site 09-1 (P <0.014, LSD = 14.26, CV% = 12.9), with a borderline increase at site 09-11 (Appendix 2.3.3 D). The pyrethrin II (PII) assay fraction from commercial plots at sites 09-8 and 09-12 were significantly greater than nontreated plots, while at site 09-10 the PII assay fraction from nontreated strips was significantly greater than the commercially treated area (Appendix 2.3.3 E). Total pyrethrins assayed from nontreated plots at sites 09-3, 09-4 and 09-15 were significantly higher than commercially treated plots, while bordering on being significantly lower than fungicide treated plots at site 09-11 (Appendix 2.3.3 E). No statistical differences in the pyrethrin I (PI) assay fraction were noted within sites, while significant or borderline differences in the pyrethrin ratio (PI/PII) at three sites were observed (Appendix 2.3.3 F).

Across all field trial sites, the mean dry weight of flowers (kg/ha) was significantly (P = 0.018) higher in plots treated with commercial fungicide than nontreated, by some 216 kg/ha (Table 2.3.3). However, treatment had no significant effect on yield of pyrethrins (kg/ha) or percentage concentration of pyrethrins in flowers (Table 2.3.3).

Variate	Commercial	Nontreated	F pr.	LSD	CV%
Pyrethrins (I) (% of dry weight)	1.196	1.224	0.566	-	10.9
Pyrethrins (II) (% of dry weight)	1.066	1.082	0.699	-	10.3
Ratio: P(I)/P(II)	1.134	1.144	0.763	-	8.0
Pyrethrins (total) (% of dry weight)	2.262	2.306	0.347	-	11.1
Pyrethrin yield (kg/ha)	52.5	48.7	0.122	-	25.8
Dry weight flowers (kg/ha)	2491	2275	0.018	178.4	20.7
Incidence of *B. cinerea* 14-18 Dec (%)	48.9	62.9	0.094	-	39.3
Incidence of *S. sclerotiorum* 14-18 Dec (%)	11.9	22.7	0.052	-	84.8
Incidence of *B. cinerea* 2-Dec (%)	32.0	53.1	0.001	12.29	41.3
Incidence of *S. sclerotiorum* 2-Dec (%)	9.1	23.2	0.004	9.14	81.0

Table 2.3.3 - Effect of fungicide applications on yield qualities and pathogen incidence in flowers at (final sampling occasion) across all sites, 2009-10.

Modest decreases in incidence of *B. cinerea* were noted at most sites where fungicides yielded statistically significant reductions, and generally incidences of *S. sclerotiorum* observed were low across all treatments and trial sites. Indeed, the mean incidence of *B. cinerea* in commercially treated plots was more than double that of *S. sclerotiorum* from nontreated plots across all sites. This led to a significant increase in harvested dry weight of flowers (kg/ha) at only one field from recommended fungicide applications during the flowering period. Across all sites on average during 2009 fungicide applications did result in a significant increase (P = 0.02) in dry weight of flowers harvested in comparison to nontreated, however no increase in pyrethrins (kg/ha) was measured.

2.3.4 *In vitro* fungicide sensitivity screening

Most isolates of *B. cinerea* tested (96%) reported EC_{50} to tebuconazole of between 0.10-1.00 a.i./ml. The mean EC_{50} value of 0.43 µg a.i./ml was observed when excluding one isolate with a higher EC_{50} value of 10.48 µg a.i./ml (Appendix 2.3.4 B), while including this value resulted in a mean EC_{50} of 0.64 µg a.i./ml. Of the isolates of *B. cinerea* tested, 60% had EC_{50} values of 100 µg a.i./ml or higher to carbendazim (Appendix 2.3.4 B). Only 35% of *B. cinerea* isolates had EC_{50} values <1 µg a.i./ml to carbendazim, suggesting the higher EC_{50} values reported of >100 µg a.i./ml were much less sensitive and that this insensitivity is removed from natural levels of fungal sensitivity to this fungicide.

All isolates of *S. sclerotiorum* tested reported EC_{50} between 0.01-1.00 µg a.i./ml to tebuconazole, with a mean EC_{50} value of 0.18 µg a.i./ml across all isolates (Appendix 2.3.4 A). Of isolates of *S. sclerotiorum* tested, 97.2% had EC_{50} of between 0.01-2.00 µg a.i./ml to carbendazim and an overall mean EC_{50} value of 606.52 µg a.i./ml was estimated (Appendix 2.3.4 A). Three isolates had EC_{50} of greater than 1000 µg/a.i. per ml, and a mean EC_{50} value of 0.50 µg a.i./ml was calculated when excluding these values (Appendix 2.3.4 A). Three isolates reported EC_{50} values of greater than 1000 µg/a.i. per ml, and a mean EC_{50} value of 0.5 µg a.i./ml was calculated when excluding these values (Appendix 2.3.4 A). Sensitivity profile graphs for both fungal species to tebuconazole and carbendazim are shown below (Fig. 2.3.4 A-D).

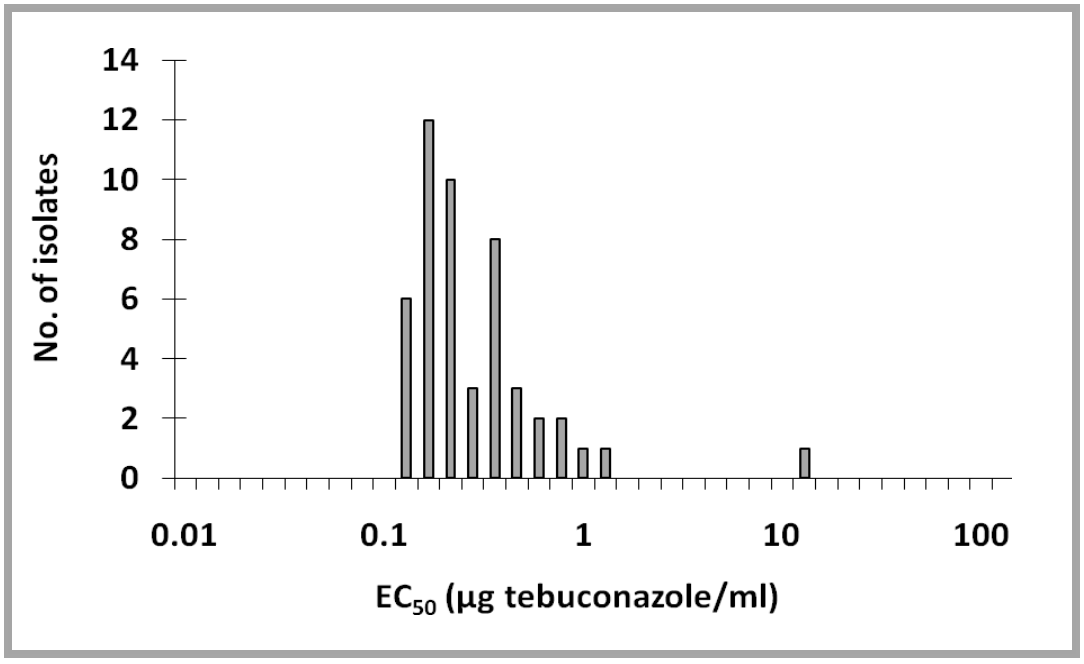


Figure 2.3.4 A - Sensitivity of isolates of *B. cinerea* to tebuconazole.

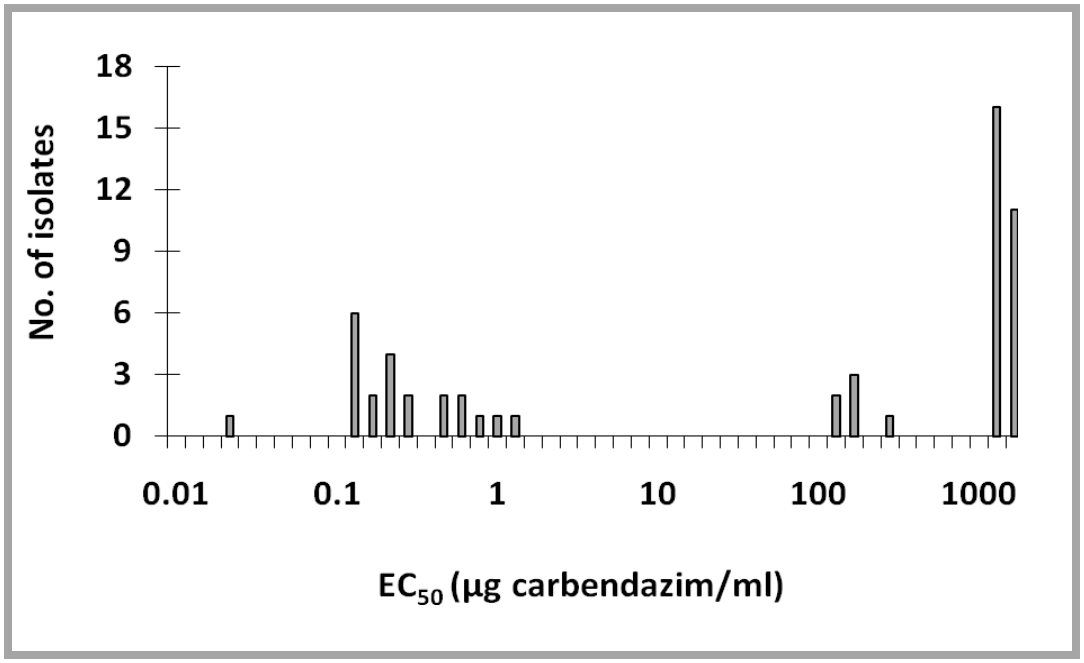


Figure 2.3.4 B - Sensitivity of isolates of *B. cinerea* to carbendazim.

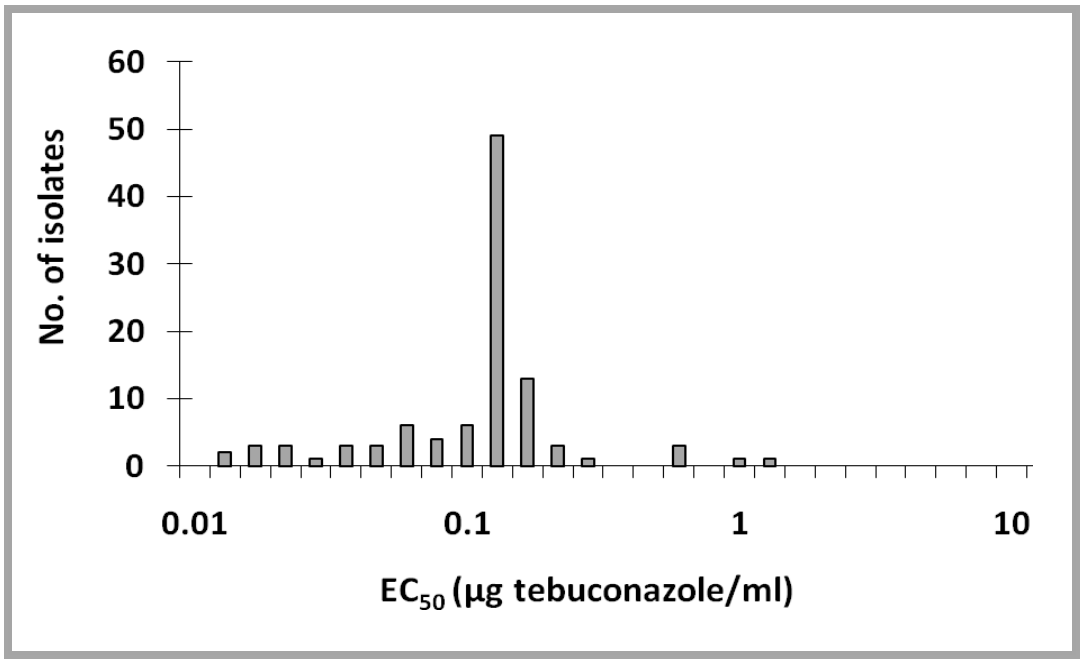


Figure 2.3.4 C - Sensitivity of isolates of *S. sclerotiorum* to tebuconazole.

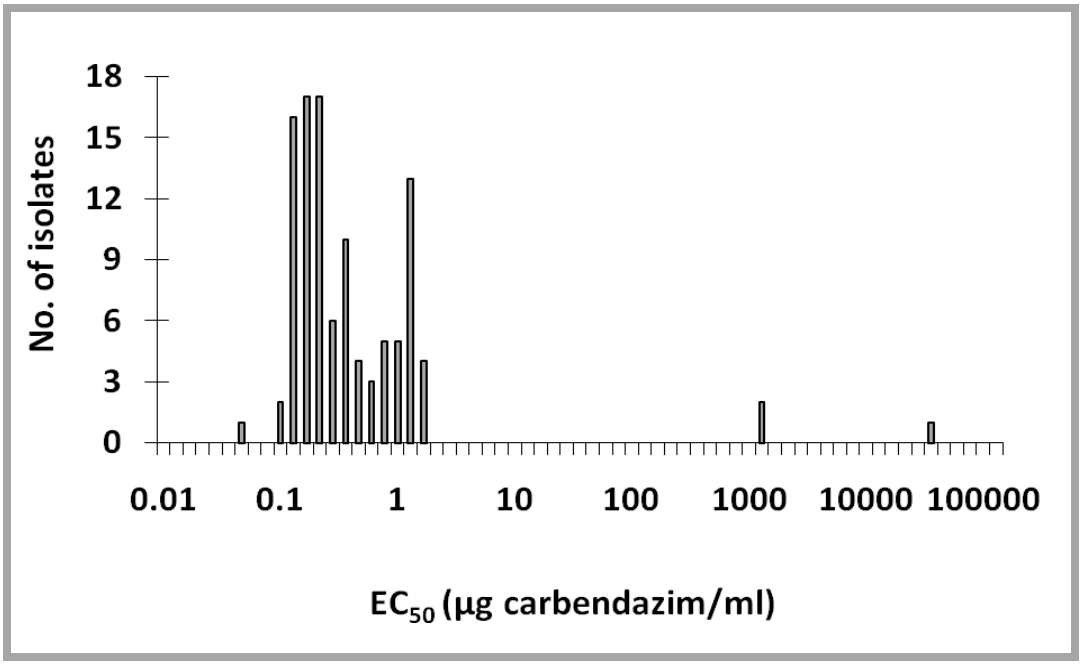


Figure 2.3.4 D - Sensitivity of isolates of *S. sclerotiorum* to carbendazim.

2.4 Discussion

Year 1 (2007-08)

Between the second and fourth assessments between 29 November and 17 December, disease incidence increased rapidly, with *S. sclerotiorum* rising from a mean of 2.1 to 51.8% across nontreated plots from all sites. Incidence of *B. cinerea* also rose sharply during this period, increasing from 9.5 to 75.7% across nontreated strips and still averaging almost 50% over commercially treated areas. Nine sites received fungicide applications of carbendazim as a second spray during this period, between 26 November and 14 December. Four of the six sites showing significant or highly significant reductions in incidence of *B. cinerea* from fungicides at the final assessment received their third spray application 5-11 days prior to the other sites, with the fungicide being tebuconazole at all but one site. Furthermore, sites 07-2 and 07-10 received the third spray application between 4-7 December, 8 days earlier than other sites on average aside from site 07-1, which received the second application of tebuconazole on 8 December. These were the only commercially treated areas

to record incidences of *B. cinerea* of 25% of less. Remaining sites received the third application between 10-21 December, with commercial treatments across these sites averaging 76% of incidence *Botrytis* flower blight. While carbendazim proved effective at reducing incidence of *S. sclerotiorum* during periods of high flower disease pressure, it appeared reasonably ineffective at reducing the frequency of *B. cinerea* where resistance to this fungicide has previously been reported (Beever *et al,* 1989; Elad *et al,* 1992). Adequate control of flower disease incidence caused by *B. cinerea* appeared to be obtained only at sites where an application of tebuconazole was applied during the first week of December.

Similarly to the pathogen incidences of *B. cinerea* and *S. sclerotiorum* in developing pyrethrum flowers discussed in chapter 1, mean pathogen incidence in flowers of *B. cinerea* (all fields) rose from 10.9-75.0% between 4-22 December. This rapid increase of floral pathogen incidence, often in the presence of commercial fungicide applications, may be indicative of multiple infection cycles occurring through this period and further supports the polycyclic nature of *B. cinerea* noted in chapter 1. The ability of *B. cinerea* to sporulate rapidly and profusely has been demonstrated, with production of an estimated 15 million conidia from a 2 cm piece of tomato stem within a seven day period (Nicot *et al,* 1996). The absence of rapid increases in pathogen incidences of *S. sclerotiorum* in flowers observed this season support the suggestion this disease epidemic is monocyclic, with staggered dispersal and ascospore release as apothecia form and mature as previously reported by Newton and Sequira (1972).

Year 2 (2008-09)

While fungicides provided significantly lower incidence of *B. cinerea* in flowers at six sites, high incidences ranging between 62-85% remained in commercially treated areas at six fields. Furthermore, six sites which received the third spray (of tebuconazole) on a mean date between 3-4 December averaged 45% incidence of *B. cinerea* in flowers. The remaining sites received the penultimate spray application of carbendazim on a mean date of 17 December, and reported an average incidence in commercial plots of 70% across these sites. This suggests that final pathogen incidences of *B. cinerea* were better controlled by earlier application of tebuconazole as a third spray, during rising levels of this pathogen in early December. Incidence of *S. sclerotiorum* was low in

nontreated, averaging only 24.9% across all sites. Fungicides did however provide significantly decreased incidences at five sites, with an average across all sites of 9.6% *S. sclerotiorum* in commercial plots approaching harvest.

Year 3 (2009-10)

A significant increase in dry weight flowers harvested across all sites, few notable yield responses within sites, significantly higher incidence of *B. cinerea* than *S. sclerotiorum* across all sites at both occasions of flower sampling, and high incidence of *B. cinerea* at many sites despite commercial fungicide treatments are the main points of interest from the findings of field trials during 2009. Lower total rainfall and irrigation, in addition to shorter periods of prolonged leaf wetness on average indicate conditions less conducive for proliferation of *S. sclerotiorum* may have existed this year, when compared to conditions in the first year of field trials during 2007 (Appendices 2.5.4 A-B). The generally low incidence of *S. sclerotiorum* across all sites led to little response from fungicides. Conversely, lack of statistical decline in incidence of *B. cinerea* due to fungicides across all sites seemed due to inability of fungicides to sufficiently reduce incidence and control *B. cinerea* flower disease. This again raises questions over the efficacy of the current flowering fungicide program at providing control of flower blights and tangible yield benefits in Tasmanian pyrethrum crops.

During this final year of field trials, quadrats harvested for yield measurements were larger in area, and more assay samples per treatment at each trial site were analysed than in previous years (Appendix 2.2.3). This appeared to reduce sample variability, however while dry flower yield was significantly increased on average across all sites from fungicide treatments, no other yield attributes were. Although frequency of *S. sclerotiorum* flower disease this year was generally low, the commercial fungicides came closer to significantly reducing incidence of this pathogen than *B. cinerea* across all sites on average. The current efficacy of the flowering fungicide program particularly at controlling flower blights caused by *B. cinerea* in Tasmanian pyrethrum crops therefore appears questionable, and a cause of concern for cost effective flower disease management.

The baseline sensitivities of *B. cinerea* to carbendazim and tebuconazole were reported as 0.03 µg a.i./ml and 0.3 µg a.i./ml respectively for inhibition of mycelial growth (Leroux, 2004). *S. sclerotiorum* has a reported intrinsic toxicity (EC_{50}) value to carbendazim of approximately 0.16 µg a.i./ml (Qian and Fox, 1994), while *Sclerotinia* sp. have reported baseline sensitivities ranging from 0.013 to 0.08 µg a.i./ml to tebuconazole (Hsiang *et al,* 1997; Ferry and Davis, 2010). *In vitro* fungicide sensitivity testing of carbendazim (Spinflo^R or Bavistin^R) and tebuconazole (Folicur^R) against endemic isolates of *B. cinerea* and *S. sclerotiorum* revealed that reduced sensitivity of *B. cinerea* to carbendazim was common amongst isolates collected from diseased pyrethrum flowers. Resistance to carbendazim by *B. cinerea* has been previously reported on numerous occasions (Beever *et al,* 1989; Elad *et al,* 1992). High EC_{50} values (>100) of most (60%) isolates evaluated in this study to carbendazim indicates widespread reduced sensitivity amongst the isolate population of *B. cinerea* collected to this fungicide, which may be reducing the efficacy of flower disease control in Tasmanian pyrethrum fields (Appendix 2.3.4 B). Tebuconazole provided effective reductions in mycelial growth of both fungi, with no significant variation from baseline sensitivities of mean EC_{50} values for each fungal species. Most isolates (90%) of *S. sclerotiorum* reported EC_{50} values <1 µg of carbendazim per ml, while 3% had higher EC_{50} values of >1000 µg a.i./ml (Appendix 2.3.4 A). With a reported baseline sensitivity of *S. sclerotiorum* to carbendazim of 0.16 µg a.i./ml (Hsiang *et al,* 1997) this indicates a degree of reduced sensitivity amongst a small percentage of the fungal population tested during this study.

Climate data

All climate data is shown (Appendix 2.5). Over three successive commercial flower harvests and series of field trials, average daily temperature and relative humidity values varied little among sites and years (Appendix 2.5.3 B). Accumulated rainfall and prolonged leaf wetness periods appeared more prominent during the first year of field trials in 2007 (Appendix 2.5.4 B). November rainfall during 2007, 2008 and 2009 averaged 71.1 mm, 33.3 mm and 50.1 mm respectively across trial sites where Watchdog 450 weather stations were installed. Mean December rainfall during the first year of field trials was 49.3 mm, in comparison to less than 20 mm during the same period in the second and third years of crop monitoring. Periods of leaf wetness during the 2007 flowering period were also greater than successive years. Weather stations indicated the average period of prolonged leaf wetness per day for November and December in 2007 was 7.8 and 6.4 hours respectively. During 2008, maximum daily periods of leaf wetness for November and December

averaged 4.5 and 3.6 hours respectively, with 2009 reporting averages of 3.4 hours during November and 1.7 hours in December. Also, the longest consecutive average daily period of leaf wetness with a value of 7 or more during 2007, exceeded field site averages of 2008 and 2009. For the months of November and December 2007, mean daily hours of leaf wetness (peak readings >7) were 4.7 and 4.2 hours respectively. During 2008, November and December averaged 3.0 and 2.2 hours respectively, while in 2009 average maximum periods of leaf wetness per day of 7 or higher for these months equalled 2.0 and 1.1 hours respectively.

Sample variation and sampling technique

Across all sites during 2007 little difference in dry flower yield existed among fungicide treatments and control strips, with both yielding averages between 3640 and 3750 kg/ha. Dry flower yields did vary widely across both treatments at all fields, nontreated yields ranging between 2356 - 4767 kg/ha and commercial areas between 2865 - 4254 kg/ha. Variation was apparent across LSD values also during this first year, with a difference exceeding 1195.2 kg/ha required to constitute a significant increase in flower yield across all sites (Appendix 2.3.3 G). This would equate to a nontreated quadrat (0.49 × 0.49 m) with a dry flower weight of 178 g needing an increase of 58.6 g (31.5%) or more to show significant improvement from fungicides.

During 2007-08, treatment plots flagged in fields averaged approximately 15 × 15 m in size across all sites, four 0.7 × 0.7 m quadrats were hand harvested from plots at each site, and pyrethrum fields averaged 6.23 ha in size. Treatment plots were estimated to occupy 0.36% of each field, and plots were practically quite large in size (15 × 15 m) with an estimated 0.87% of total plot area hand harvested for yield assessment. Aggregated inoculum distribution of *S. sclerotiorum* in pyrethrum fields has been demonstrated by Pethybridge *et al* (2010), requiring a greater sized sample area to accurately assess fungal incidence of flowers than the predominantly random and non localised spatial distribution of inoculum reported from plant disease epidemics caused by *Botrytis* species (Carisse *et al,* 2008). Variation may indicate sampling sensitivity was less than satisfactory, so increased area was harvested per treatment and more numerous assay samples analysed in subsequent years (Appendix 2.2.3). During the second year in 2008, area harvested within each treatment strip was increased from 1.96 to 2.94 m, and two samples per treatment were assayed. Increased replicate numbers appeared to limit variation, and reduce the difference among

treatments necessary to demonstrate increased dry flower yields. Least significant difference (LSD) values averaged 775.1 kg/ha across all sites, this representing a 17.7% yield increase required to show improvement from commercial fungicide applications and less variation than the previous year. In the final year of field trials during 2009, areas harvested for yield assessment were increased to 9.6 m^2 per treatment at each site. This represented an increase to 4.3% of average total treatment plot area harvested per field, allowed by changing from hand harvesting to mechanical cutting (Appendix 2.3.3 G). Increasing assay sample numbers each year also showed declining average LSD values separating flower yields of both treatments across all sites, falling from 25.6 kg/ha on average in 2007 to 13.7 kg/ha during the final year (Appendix 2.3.3 G). Mean coefficients of variation across all fields each year of dry flower yields were also reduced from increasing sample (plot) size and number over successive years of field trials, estimated at 68.55 kg/ha in 2007-08 and 14.55 kg/ha during 2009-10 (Appendix 2.3.3 G).

Variation between years (effect of site on yield)

Over three consecutive flowering periods in Tasmanian pyrethrum crops, frequency of *B. cinerea* in flowers averaged 71.6% in the absence of fungicides and incidence did not vary significantly between years (Appendix 2.3.3 H). Mean incidence of *S. sclerotiorum* was significantly higher across nontreated strips in 2007 than other years, and higher mean rainfall and longer periods of peak (>7) leaf wetness across trial sites during November and December this year suggest the first year of trials may have provided the best opportunity to determine effect of fungicides on disease incidence and yield qualities than subsequent years. Climate data suggested 2007 may have had more conducive conditions for disease development than latter years, however sampling for estimation of yield attributes this year were less sensitive than during 2008 and 2009 assessments. Dry flower weights and pyrethrin yields per hectare also showed significant differences between years, with 2009 reporting the lowest on average across commercially treated plots (Appendix 2.3.3 H). This might be partially explained by one third of fields during this year being sites approaching third flower harvest, while all fields during 2007 and 2008 were nearing first flower harvest. It is generally noted that, yields decline and become more variable across fields with increasing crop age.

Variation between the three years of field trials proved significant in numerous instances. Average flower maturity during 2007 and 2009 was significantly higher in commercial plots at harvest time than the second year (Appendix 2.3.3 H). The presence of higher disease levels during 2007, particularly *Sclerotinia* flower blight, may have hastened flower senescence. Mean FMS values near harvest across all commercial treatments in 2007 and 2009 were 5.1 and 4.1 respectively (Appendix 2.3.3 H). Three of fifteen fields during 2009 were approaching third harvest, opposed to other years where sites nearing first flower harvests were utilised. Aging pyrethrum crops generally suffer declining annual yields, and it is possible crop age has an effect on ultimate flower maturity.

Conclusions

During 2007-08 significant reductions in fungal incidence of *B. cinerea* and *S. sclerotiorum* on numerous occasions within and across trial sites were noted from commercial applications of the flowering fungicide program. However dry flower weights were significantly increased from fungicides at only one site, and across all sites there was no significant improvement in measured yield properties.

Despite providing numerous significant reductions in fungal incidence in flowers compared to no treatment during 2008-09, commercial fungicide applications resulted in no significant improvements in yield qualities across all field sites and increased dry flower weights harvested at only three sites.

Across all field trial sites during 2009-10 the dry weight of flowers (kg/ha) harvested was significantly higher in plots treated with commercial fungicide applications, while dry flower yield and the pyrethrin (PII) assay fraction were only significantly greater than no treatment at one and two sites respectively.

Laboratory tests showed widespread reduced sensitivity amongst the population of *B. cinerea* tested to carbendazim, and a small number of isolates of *S. sclerotiorum* showing reduced sensitivity to this fungicide. There was no evidence of reduced sensitivity of isolates of *B. cinerea* or *S. sclerotiorum* to tebuconazole.

Results suggest carbendazim (Bavistin^{R} & Spinflo^{R}) may not provide the most effective control of flower disease caused by *B. cinerea* and *Sclerotinia* sp. in Tasmanian pyrethrum crops. Tebuconazole (Folicur^{R}) may continue to provide control of flower disease caused by *B. cinerea* and *Sclerotinia* species.

3. Evaluation of alternative fungicides for the control of flower diseases in Tasmanian pyrethrum fields.

3.1 Introduction

Flower blights due to *B. cinerea* and *S. sclerotiorum* are able to cause reduced flower yields in Tasmanian pyrethrum crops. Control of flower blight is based upon timely fungicide applications over flowering. Presently and for some years preceding, two fungicides have been used to accomplish this, tebuconazole (Folicur^{R}) and carbendazim (Bavistin^{R} or Spinflo^{R}). After recent changes in the nomenclature of fungicide groupings, tebuconazole and carbendazim now belong to fungicide activity groups three and one respectively (Anonymous, 2009). These fungicides are applied in alternation through November and December at intervals of at least ten days in Tasmanian pyrethrum crops, tebuconazole often being the first chemical applied and with a maximum of two sprays per fungicide each year. As pyrethrum crops in Tasmania may have a productive life of 4-5 years, this can result in up to ten applications of fungicide activity groups three and one from the flowering fungicide program alone. Prolonged use of fungicides representing two activity groups is therefore a concern regarding development of resistance and reduced efficacy in chemical control of flower diseases.

Previously questions have been raised about the efficacy of the flowering fungicide program, and evaluation of its performance over three successive flowering periods (Chapter 2) has proven it to be less than ideal. While showing modest yield improvements in some instances and consistently lowering the pathogen incidence in flowers of both fungi within and across trial sites, incidence of *B. cinerea* generally remained high after the application of fungicides (Chapter 2). Limited evidence showed inability to sufficiently reduce incidence of *B. cinerea* may have been associated with carbendazim applications, and *in vitro* studies also suggested a high proportion of isolates of *B. cinerea* to be relatively insensitive to carbendazim (Chapter 2). Chemical practices are continually scrutinised, with recent review of carbendazim usage in Australia leading to suspension of label approvals of products containing carbendazim (Anonymous, 2007 and 2010b). With the questionable efficacy of current flowering fungicide recommendations, rapidity of documented resistance development of *B. cinerea* to benzimidazole fungicides and the imminent withdrawal of carbendazim, it would seem imperative to investigate potential alternative fungicides for control of flower diseases in Tasmanian pyrethrum fields.

Tebuconazole is a demethylation inhibiting or DMI fungicide and member of the triazole chemical class, possessing protective and eradicative properties. The mode of action of these fungicides involves disrupting the synthesis of necessary fungal cell membrane elements, the sterols (Ware, 1994). To date, resistance of *B. cinerea* to DMI fungicides has been reported in vegetable crops, with no resistance of *S. sclerotiorum* reported within the literature (Anonymous, 2008).

Carbendazim is a methyl benzimidazole carbamate fungicide, acting to interfere with β-tubilin and DNA synthesis resulting in irregular spore germination, cell division and fungal growth (Ware, 1994). Benzimidazole fungicides have been used against a wide range of plant pathogens including *Botrytis* and *Sclerotinia* species since its introduction in 1968 (Ware, 1994). *Botrytis* species have displayed more rapid development of resistance to benzimidazole fungicides than other plant pathogenic fungi, and have also reported more instances of reduced sensitivity than *Sclerotinia* species to fungicides of this activity grouping. To date, resistance of *Botrytis* species to benzimidazoles has been documented in numerous vegetable crops as well as grapes, cyclamen, lily, lisianthus and tulip (Brent and Hollomon, 2007; Anonymous, 2008). Reduced sensitivity of *S. sclerotiorum* is reportedly widespread in oilseed rape cultivation regions of China (Ma *et al,* 2009).

Fungicides selected for evaluation in field trials for suitability in pyrethrum flower disease control were boscalid (Filan^{R}), cyprodinil/fludioxinil (Switch^{R}), iprodione (Rovral^{R}), prothioconazole/tebuconazole (Prosaro^{R}) and pyrimethanil (Scala^{R}).

Boscalid is a pyridine carboximide fungicide from activity grouping seven, with a mode of action inhibiting enzymatic respiration of mitochondrial electron transport chains and tricarboxylic cycle at complex II of succinate-dehydrogenase (Anonymous, 2008). Boscalid is currently applied to pyrethrum crops in spring for control of ray blight disease caused by *P. ligulicola* (Pethybridge *et al,* 2008a). Control of *S. sclerotiorum* with boscalid in carrot has also been reported (McDonald *et al,* 2008). Instances of field resistance to carboximide fungicides have been documented, including reduced sensitivity of *Alternaria alternata, Corynespora cassiicola* and *Ustilago nuda* in pistachio, cucumber and barley cultivation respectively (Anonymous, 2008).

Cyprodinil, one active ingredient of Switch[R], belongs to the anilinopyrimidine chemical family and fungicide activity group nine. Chemicals of this class are believed to act via impeding biosynthesis of amino acids, also being known as AP fungicides (Anonymous, 2008). Pyrimethanil also belongs to the anilinopyrimidine chemical class and activity group nine (Anonymous, 2008). Fludioxinil, the second active ingredient of Switch[R], belongs to the phenylpyrrole chemical class and fungicide activity group twelve (Anonymous, 2009). This class of fungicides disturbs activity of MAP/Histidine-kinase in osmotic signal transduction (os-2, HOG1) and are also known as PP fungicides (Anonymous, 2008). Fludioxinil has been shown to provide control of foliar disease in lettuce caused by *S. sclerotiorum* and *S. minor* (Matheron and Porchas, 2004). The combination of active ingredients cyprodinil and fludioxinil in Switch[R] represent activity groups nine and twelve, and both AP and PP fungicide classes. Switch[R] has previously displayed efficacy as a seed treatment for reducing transmission of ray blight disease *(P. ligulicola)* in Tasmanian pyrethrum seed during limited testing (O'Malley, 2007). Cyprodinil (as Chorus[R]) is currently used in spring for the control of ray blight disease in pyrethrum.

Iprodione belongs to the dicarboximide chemical family and is a member of fungicide activity group two. Chemicals of this family interfere with the enzymatic activity of MAP/Histidine-kinase in osmotic signal transduction (os-1, Daf1). Dicarboximide fungicides including iprodione and procymidone have been widely used since the 1970's in plant protection for control of fungal diseases caused by *Botrytis* and *Sclerotinia* species (Brent and Hollomon, 2007). Iprodione is a contact fungicide with both protectant and eradicant properties, and has shown particular efficacy against *Botrytis* and *Sclerotinia* species. Procymidone is systemic and currently used (as Fortress[R]) for control of crown rot in Tasmanian pyrethrum crops, caused by *S. minor* and *S. sclerotiorum.*

Prosaro[R] is also a DMI triazole fungicide and member of activity group three, also having a broad spectrum of activity and consists of active ingredients prothioconazole and tebuconazole - the latter being the active ingredient of Folicur[R] currently used in the flowering fungicide program (Anonymous, 2009). Prothioconazole, boscalid and iprodione have demonstrated increased yields and significant reductions of infield frequency of *Sclerotinia* stem rot of canola *(Brassica napus)* caused by *S. sclerotiorum* (Bradley *et al,* 2006). Filan[R], Rovral[R], Scala[R], and Switch[R] have been widely used to achieve control of bunch rot of grapes caused by *B. cinerea* in Australian viticulture (Essling *et al,* 2010).

The objectives of this study were to evaluate the relative performance of these fungicides against present commercial recommendations and no fungicide treatment:

- For reducing the incidence of *B. cinerea* and *S. sclerotiorum* flower disease pathogens.
- For improvement of flower and pyrethrin yields.
- To determine suitability for inclusion into the flowering fungicide program as potential replacements for carbendazim.
- To determine in agar plate tests, the relative sensitivity of isolates of *B. cinerea* and *S. sclerotiorum* to fungicides showing efficacy in significantly reducing fungal incidence in flowers or improving yields.

3.2 Materials and methods

Field trial sites

Replicated plot field trials were conducted in commercial pyrethrum crops during 2007 and 2008 at sites 07-3 and 08-4 respectively. Both fields were on Werrin Farm near Braddon's lookout, Forth (41° 19' S, 146° 25' E) and approaching the first harvest of flowers. During 2009, fifteen commercial fields were used to evaluate a modified fungicide program against the commercial recommendations. Ten of these sites were first harvest crops, while five were older crops and approaching third harvest (Appendix 2.2.2).

Figure 3.2.1 - Field trial at site 08-4 during 2008-09 on Werrin Farm.

Replicated field trial at site 07-3 (2007-08)

This trial was established in a field of variety 'Polycross' nearing first harvest at Werrin farm, Forth (Site 07-3) during late October. Sixteen plots measuring 8 × 13.5 m in size separated by a 1 m buffer were pegged out between two spray runs, with coloured flags denoting treatment (Fig. 3.2.2). Four treatments were each randomly allocated to four replicate plots within the trial area. The treatments were as follows: Nontreated (no fungicides), Commercial (alternating applications of Folicur^{R} and Bavistin^{R}), Switch^{R} (alternating sprays of Folicur^{R} and Switch^{R}) and Filan^{R} (alternating applications of Folicur^{R} and Filan^{R}). Fungicides were applied with a tractor mounted boom spray apparatus. Irrigation of 25 mm was applied on 26 October and 9, 17 and 29 November by linear move irrigator.

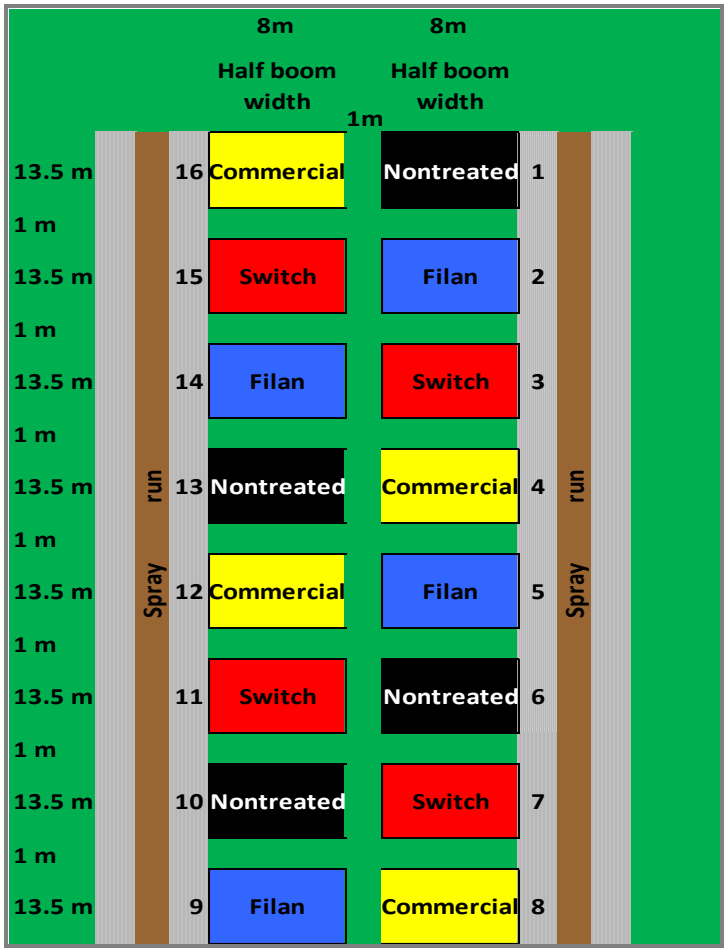


Figure 3.2.2 - Trial site layout (2007-08).

The entire trial area received the first commercial fungicide application of tebuconazole on 21 November. Thereafter fungicide treatments were applied at rates specified (Table 3.2.1) on four occasions during the flowering period (Table 3.2.2).

Fungicide	Active ingredients	Activity group/s	Rate (per ha)
Folicur[R]	tebuconazole 430 g/L	3	350 ml
Bavistin[R]	carbendazim 500 g/L	1	750 ml
Switch[R]	cyprodinil 375 g/kg fludioxinil 250 g/kg	9 12	480 g 320 g
Filan[R]	boscalid 500 g/L	7	1 kg

Table 3.2.1 - Fungicide active ingredients and application rates (2007-08).

Treatment	Application/date			
	21-Nov	29-Nov	10-Dec	14-Dec
Nontreated	tebuconazole*	-	-	-
Commercial	tebuconazole	carbendazim	tebuconazole	carbendazim
Switch[R]	tebuconazole	cyprodinil fludioxinil	tebuconazole	cyprodinil fludioxinil
Filan[R]	tebuconazole	boscalid	tebuconazole	boscalid

*Accidental overspray of nontreated plots with Folicur[R].

Table 3.2.2 - Treatment application dates (2007-08).

Flowers were sampled for disease incidence and yield/assay as described in chapter 1, with the exception that disease incidence assessment was conducted on 50 flowers per treatment plot. The incidence of fungi in flowers was assessed on five occasions during the flowering period prior to harvest, i.e. 16 November and 4, 11, 18 and 22 of December. After cutting into windrows on 2 January, flower samples were taken from the windrow on 10 and 17 January. Flower samples for yield and assay were collected on 22 of December. For yield determination, flowers were handpicked from each of 3, 0.49 m^2 quadrats within each plot and bagged individually. Flowers from each quadrat were weighed; a subsample comprising approximately 20% by weight was reweighed, dried (55°C/24 h) and reweighed to determine dry matter (%). The dried subsample from each of the

three quadrats was bulked together and was stored frozen until determination of pyrethrin content (% by weight) by HPLC. The fresh weight of flowers from each quadrat was converted to dry weight and the mean used in statistical analysis. In addition, 50 flowers from one quadrat within each plot were assessed visually for Flower Maturity Stage (FMS) as described previously (Plate 1.2.2). Flowers were also assessed for disease and the fresh weight of 50 flowers recorded. All flags were removed before commercial harvest and replaced with yellow star picket post caps to determine plot location after cutting. Flower samples from windrows (5 × 10 lots of flowers) were randomly taken from the centre of each plot at least 2 m from the border.

Replicated field trial at site 08-4 (2008-09)

This trial was established during late October on Werrin Farm, Forth (site 08-4). The crop was approaching first flower harvest and was cultivar Pyrite. Twenty four plots measuring 10 × 12 m were pegged out, either side of two spray runs. Plots were separated by a 1 m buffer or spray run, with coloured flags denoting treatment (Fig. 3.2.3). Six treatments were each randomly allocated to four replicate plots within the trial area. The treatments were: Nontreated (no fungicides), Switch^{R}, Filan^{R}, Rovral^{R}, Prosaro^{R} and Scala^{R} (Table 3.2.3). Commercial fungicide treatments (alternating applications of Folicur^{R} and Bavistin^{R}) were applied to the remainder of the field, with flower samples for this treatment taken from plots adjacent to and approximately 30 m away from the trial area. Commercial fungicide applications and trial treatments were applied four times through November and December (Table 3.2.4). Fungicides were applied with tractor mounted boom spray apparatus. The trial site received 25 mm of irrigation by linear irrigator applied on 1, 12 and 20 November and 2 December.

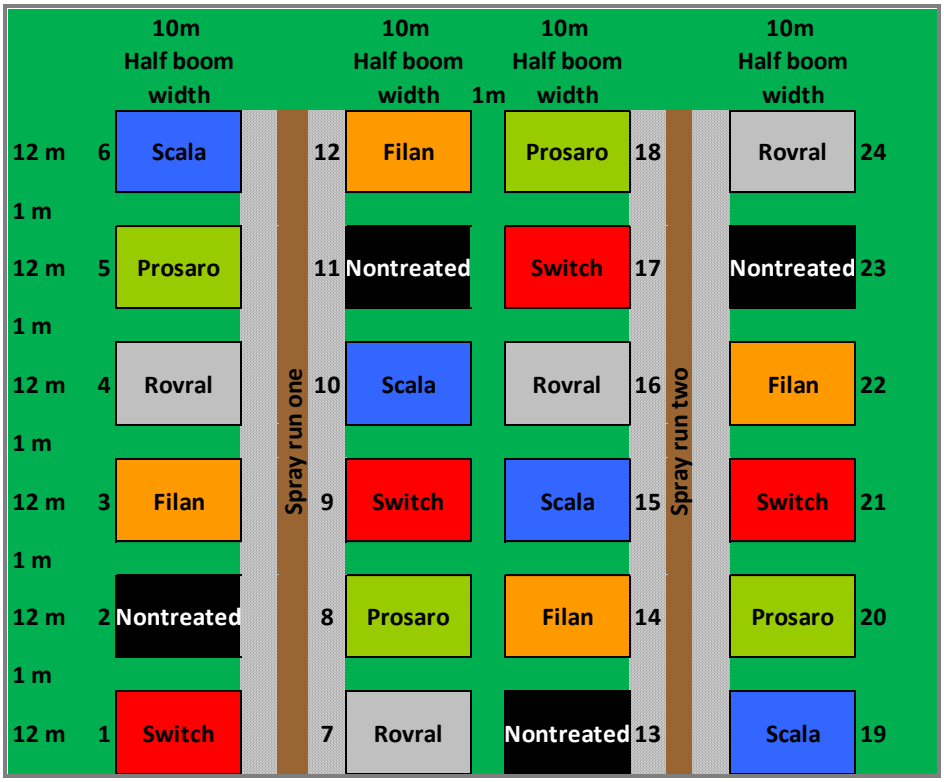


Figure 3.2.3 - Trial site layout (2008-09).

Fungicide	Active ingredients/Concentration	Activity group	Rate (per ha)
Folicur[R] SC	tebuconazole 430 g/L	3	350 ml
Bavistin[R] FL	carbendazim 500 g/L	1	750 ml
Switch[R] WG	cyprodinil 375 g/kg fludioxinil 250 g/kg	9 12	480 g 320 g
Filan[R] WG	boscalid 500 g/L	7	1 kg
Rovral[R] SC	iprodione 500 g/L	2	1 L
Prosaro[R] SC	prothioconazole 210 g/L tebuconazole 210 g/L	3 3	105 g 105 g
Scala[R] SC	pyrimethanil 500 g/L	9	2 L

Table 3.2.3 - Active ingredients and application rates of fungicides (2008-09).

Treatment	Application/date			
	17-Nov	4-Dec	17-Dec	23-Dec
Nontreated	-	-	-	-
Commercial	tebuconazole	carbendazim	tebuconazole	carbendazim
Switch[R]	cyprodinil fludioxinil	cyprodinil fludioxinil	cyprodinil fludioxinil	cyprodinil fludioxinil
Filan[R]	boscalid	boscalid	boscalid	boscalid
Rovral[R]	iprodione	iprodione	iprodione	iprodione
Prosaro[R]	prothioconazole tebuconazole	prothioconazole tebuconazole	prothioconazole tebuconazole	prothioconazole tebuconazole
Scala[R]	pyrimethanil	pyrimethanil	pyrimethanil	pyrimethanil

Table 3.2.4 - Treatment application dates (2008-09).

Flowers were sampled for disease incidence and yield as described in chapter 1 materials and methods. Fifty flowers per treatment plot were sampled at each assessment of disease incidence. Incidence of *B. cinerea* and *S. sclerotiorum* in flowers was assessed on five occasions, on 17 November, 4 and 22 December as well as 14 and 21 January. Cutting of this field into windrows occurred on 29 December, with the last two disease assessments done on samples obtained from windrows. Windrow plots were marked and samples taken as described from 2007-08 Werrin Farm replicated field trial. Flower samples for assay and yield evaluation were harvested on 22 of December. Flowers were handpicked from each of three 0.49 m^2 quadrats within each plot and assessed as described for the 2007-08 Werrin Farm trial (above).

Field trial sites (2009-10)

During the final year of field trials, one modified fungicide program was compared to the current commercial recommendations for flower disease control. Larger plots were used than in previous replicated field trials during 2007-08 and 2008-09, and establishment of treatment plots in numerous commercial pyrethrum fields allowed comparison of fungicide treatment effect within and among sites. Modified fungicide treatments were applied to one whole spray run within each trial site, with coloured flags at each end clearly marking plot boundaries. Nontreated plots were marked with flags and established across adjacent spray runs as described previously (Chapter 1; Fig. 1.2.1) and were located at least one spray run away from the modified treatment strip at each site.

The remainder of the field was treated with the commercial recommendations for flower disease control (Fig. 3.2.4).

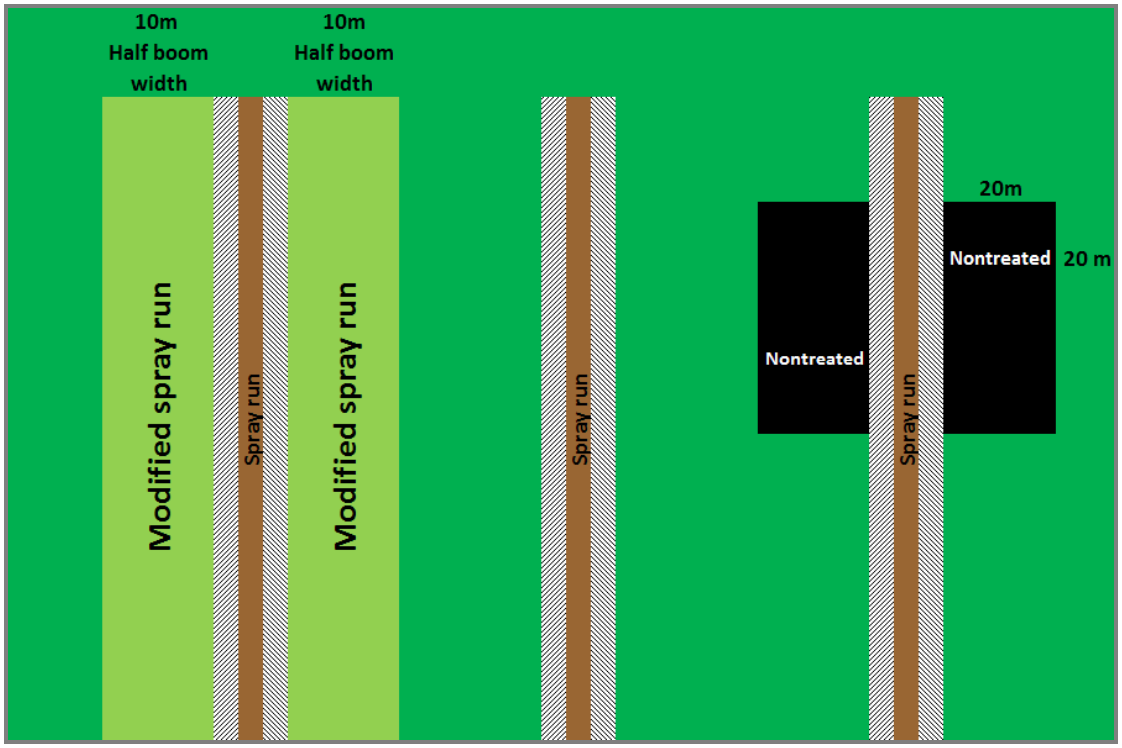


Figure 3.2.4 - Trial site layout of demonstration strips (2009-10).

This year, the effect of a modified fungicide program consisting of alternating applications of Folicur[R] and Filan[R] was compared against standard commercial recommendations and no treatment (Table 3.2.5). Pyrethrum crops were of varying age and from numerous growing districts in North West Tasmania (Table 3.2.6). Treatments received four spray applications at each site. Some fields received Folicur[R] as the first commercial application and some Bavistin[R], while modified treatment applications at all fields began with Folicur[R] and concluded with Bavistin[R]. Modified fungicide sprays were applied on the same date as commercial treatments at each site. One hundred flower samples for assessment of disease incidence were randomly taken from within plots on two occasions late in the flowering period before cutting, with samples for yield measurement obtained upon the latter occasion (Table 3.2.6).

Treatment	Fungicide	Active ingredient	Rate (per ha)	Activity group
Nontreated	-	-	-	-
Commercial	Folicur[R] Bavistin[R]	tebuconazole carbendazim	350 ml 750 ml	3 1
Modified	Folicur[R] Filan[R]	tebuconazole boscalid	350 ml 1 kg	3 7

Table 3.2.5 - Fungicide treatment details (2009-10).

Site code	District	Harvest	Commercial fungicide application dates				Sampling dates	
			1st	2nd	3rd	4th	1st	2nd
09-1	North Motton	1st*	19-Nov	30-Nov	7-Dec	18-Dec	2-Dec	15-Dec
09-2	North Motton	1st*	19-Nov	30-Nov	7-Dec	18-Dec	2-Dec	15-Dec
09-3	North Motton	1st*	19-Nov	30-Nov	7-Dec	18-Dec	2-Dec	15-Dec
09-4	North Motton	1st*	19-Nov	30-Nov	7-Dec	18-Dec	2-Dec	15-Dec
09-5	North Motton	1st*	19-Nov	30-Nov	7-Dec	18-Dec	2-Dec	16-Dec
09-6	Penguin	1st*	19-Nov	29-Nov	2-Dec	16-Dec	2-Dec	16-Dec
09-7	Penguin	1st*	19-Nov	29-Nov	8-Dec	16-Dec	2-Dec	16-Dec
09-8	Forth/Don	1st	21-Nov	1-Dec	8-Dec	15-Dec	2-Dec	14-Dec
09-9	Forth/Don	3rd	19-Nov	1-Dec	8-Dec	15-Dec	2-Dec	14-Dec
09-10	Forth/Don	3rd	20-Nov	1-Dec	8-Dec	15-Dec	2-Dec	18-Dec
09-11	Forth	1st	20-Nov	1-Dec	8-Dec	15-Dec	2-Dec	14-Dec
09-12	Forth	1st	20-Nov	1-Dec	8-Dec	15-Dec	2-Dec	14-Dec
09-13	Forth	3rd	20-Nov	30-Nov	8-Dec	15-Dec	2-Dec	14-Dec
09-14	Lillico	3rd	20-Nov	1-Dec	9-Dec	15-Dec	[na]	[na]
09-15	Forth	3rd	20-Nov	1-Dec	9-Dec	16-Dec	2-Dec	18-Dec
09-16	Forth	3rd	19-Nov	1-Dec	9-Dec	16-Dec	2-Dec	16-Dec
09-17	Forth	3rd	20-Nov	1-Dec	8-Dec	16-Dec	[na]	[na]

* Crops received Bavistin[R] as first commercial fungicide application; order of fungicides applied was carbendazim (C), tebuconazole (T), carbendazim (C), tebuconazole (T). Remaining sites received in opposing order; ie. T, C, T, C.

[na] Crops terminated from study due to poor plant density.

Table 3.2.6 - Site details, fungicide application and flower sampling dates (2009-10).

Flowers sampled during 2007-08, 2008-09 and 2009-10 for disease incidence, flower yield and pyrethrin assay measurement were surface sterilised, incubated and microscopically assessed for incidence of *B. cinerea* and *S. sclerotiorum* as described in chapter 1.

Statistical analysis

During each year of trials, the mid-point method of calculating area under the disease progress curve (AUDPC) (Madden *et al,* 2007) was used to calculate an area under the fungal incidence curve (AUFIC) between sampling dates for each fungicide treatment for *B. cinerea* and *S. sclerotiorum*. Due to the difficulty in visually differentiating symptoms of flower blight caused by *B. cinerea* and *S. sclerotiorum*, the incidence of fungi in surface sterilised flowers was measured rather than assessing disease.

$$\text{AUFIC} = \frac{\text{AUDPC}}{(t_F - t_0)}$$

t_0 Start time
t_F Finishing time

Values of AUFIC were compared with a mixed model analysis using SAS Version 9.1 and replicate as a random block effect. At all sites fungicide treatment effect on incidence of *B. cinerea* and *S. sclerotiorum* at all sampling times, and treatment effect on flower yield and assay qualities were evaluated with a completely randomised block design analysis of variance (ANOVA) using GenStat (Version 10.1).

Correlations between disease incidence (and AUFIC values) against yield measurements were also investigated using GenStat (Version 10.1). Pearson's coefficient of correlation (two tailed test) was used to determine associations between 16, 21 and 37 pairs in field trials during 2007-08, 2008-09 and 2009-10 respectively. Relationships between yield, treatment and disease were evaluated using simple linear regressions with SAS (Version 9.2), as well as dry matter yields among fungicide treatments compared with Tukey's adjusted pair wise probabilities.

In vitro fungicide sensitivity screening

The relative efficacy of iprodione at inhibiting mycelial growth of *B. cinerea* and *S. sclerotiorum* was evaluated in laboratory agar plate tests. Forty six isolates of each fungal species obtained from diseased pyrethrum flowers were screened (Appendix 3.3.4) against eight

concentrations of 0, 0.01, 0.05, 0.5, 5, 50, 100 and 500 µg iprodione per ml of agar, with three replicate plates per concentration used. Commercial Rovral^{R} Aquaflo (500 g/L iprodione) fungicide was used for sensitivity testing. Plates were prepared, inoculated, incubated, measured and data subsequently analysed as previously described (Chapter 2, materials and methods).

3.3 Results

3.3.1 Year 1 - Replicated field trial at site 07-3 (2007-08)

Commercial fungicide applications of Folicur^{R}/Bavistin^{R} resulted in mean flower maturity stage (FMS) values which bordered on being significantly lower than nontreated plots during initial flower sampling on the 19 November (P = 0.056, Appendix 3.3.1 A). No other differences among treatments were noted in flower maturity at any sampling stage. Flowers were sampled once for FMS assessment after the crop had been cut into windrows, on 10 January.

Disease incidence and Area under the fungal incidence curves (AUFIC)

The mean incidence of *B. cinerea* in nontreated plots was low on 4 December (3%), but increased to 66.5% by 11 December and 85.5% by 22 December, immediately prior to cutting (Table 3.3.1 A). During the final assessments of windrowed flower samples on 10 and 17 January, nontreated plots had mean incidence of *B. cinerea* of 97.5% and 92% respectively. Fungicide treatments resulted in no statistically significant reductions in incidence of *B. cinerea* compared to nontreated plots before the crop was cut into windrows (Table 3.3.1 A). However at the penultimate assessment on 10 January, all fungicide treatments had significantly lower mean incidences of *B. cinerea* than nontreated plots (Table 3.3.1 A). From the flower assessments preceding cutting, the AUFIC for Folicur^{R}/Filan^{R} and Folicur^{R}/Switch^{R} treatments was significantly lower than nontreated plots (Table 3.3.1 A). Commercial flowering fungicide applications of Folicur^{R}/Bavistin^{R} provided no statistical reduction in AUFIC of *B. cinerea* compared to nontreated plots before cutting. By the final

flower assessment on the 17 January, mean AUFIC values of all three fungicide treatments were significantly lower than nontreated plots (Figure 3.3.1).

S. sclerotiorum was not detected in nontreated strips upon the first flower assessment, and mean incidence was only 0.5% by the second evaluation on 3 December (Table 3.3.1 A) Thereafter the mean incidence of *S. sclerotiorum* in nontreated plots increased rapidly to 55.5%, by 11 December declining to 19.5% on 18 December and reached a mean incidence of 47.5% at the final flower sampling (22 December) before cutting (Table 3.3.1 A). Within the windrow the mean incidence ranged from 27.5-33.0%. At the third sampling occasion (11 December), all three fungicide treatments had significantly lower mean incidences of *S. sclerotiorum* than nontreated (Table 3.3.1 A). At the final flower assessment (22 December) before cutting of the crop into windrows, the commercially recommended fungicide treatments of Folicur^{R}/Bavistin^{R} had significantly lower incidences of *S. sclerotiorum* than nontreated plots, with Folicur^{R}/Switch^{R} applications in turn providing a significantly lower incidence than commercial fungicide sprays (Table 3.3.1 A). In the windrow, all treatments had significantly lower incidences of *S. sclerotiorum* than no treatment on 10 January. However by the final flower evaluation, no differences between any treatments were noted (Table 3.3.1 A). All fungicide treatments had significantly lower AUFIC values than control plots, both prior to harvest on 2 January and after two weeks drying in windrows, with no statistical differences among fungicide treatments (Table 3.3.1 A, Fig. 3.3.2).

Yield and assay

Commercial fungicide applications of Folicur^{R}/Bavistin^{R} had a significantly higher total pyrethrin assay than nontreated and Folicur^{R}/Switch^{R} plots (Table 3.3.1 B). The commercial fungicide treatment also had the highest pyrethrin (PII) assay, which bordered on statistical significance (Table 3.3.1 B). However, fungicide treatment had no significant effect on dry weight of flowers (kg/ha) or pyrethrin yield (kg/ha).

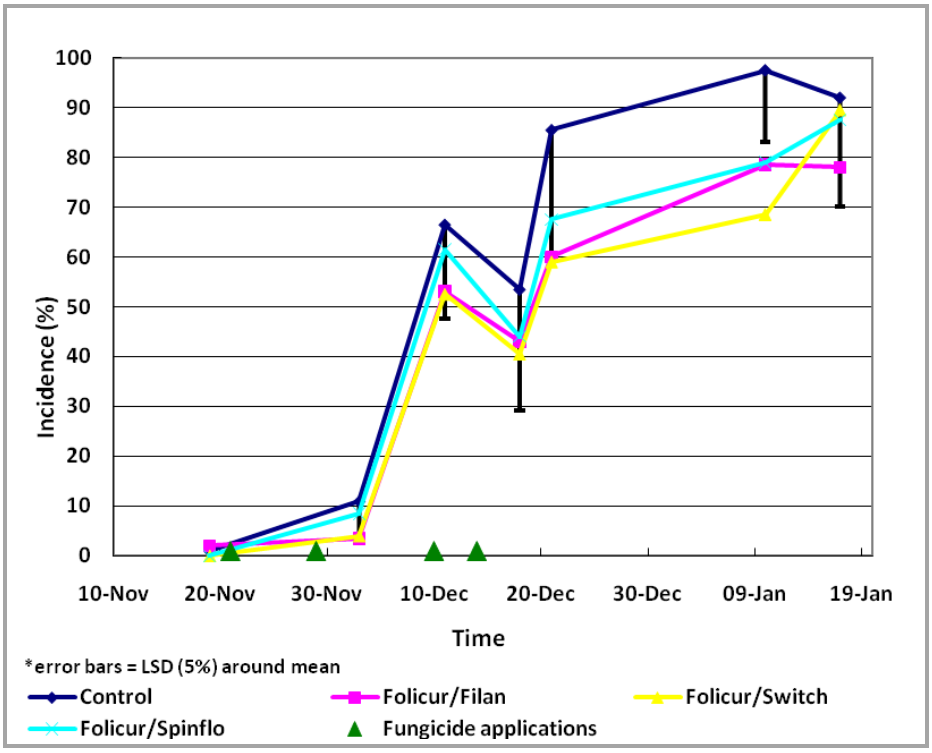


Figure 3.3.1 - Mean incidence of *B. cinerea* in pyrethrum flowers from different treatments throughout sampling (2007-08).

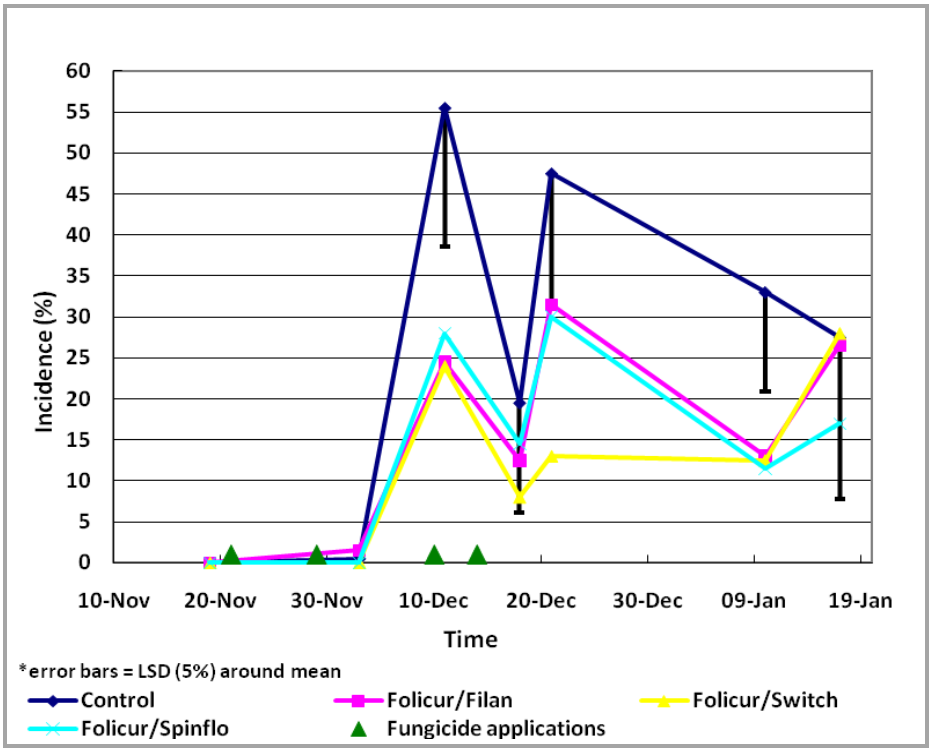


Figure 3.3.2 - Mean incidence of *S. sclerotiorum* in pyrethrum flowers from different treatments throughout sampling (2007-08).

AUFIC/fungal incidence of *B. cinerea* in flowers (%)									
Treatment	**16-Nov**	**4-Dec**	**11-Dec**	**18-Dec**	**22-Dec**	**AUFIC^{BH}**	**10-Jan**	**17-Jan**	**AUFIC^{WR}**
Nontreated	1	11	66.5	53.5	85.5	905.3a	97.5a	92	2434a
Folicur^{R}/Filan^{R}	2	3.5	53	43	60	671.3b	78.5b	78	1907b
Folicur^{R}/Bavistin^{R}	0	8.5	61.5	44	67.5	765.5ab	79b	87.5	2048b
Folicur^{R}/Switch^{R}	0	4	52.5	40.5	59	643.3b	68.5b	89.5	1803b
F pr.	0.55	0.13	0.34	0.67	0.14	0.01	0.007	0.55	0.022
LSD	-	-	-	-	-	149.3	14.5	-	283.4
AUFIC/fungal incidence of *S. sclerotiorum* in flowers (%)									
Treatment	**16-Nov**	**4-Dec**	**11-Dec**	**18-Dec**	**22-Dec**	**AUFIC^{BH}**	**10-Jan**	**17-Jan**	**AUFIC^{WR}**
Nontreated	0	0.5	55.5a	19.5	47.5a	502a	33.0a	27.5	1025a
Folicur^{R}/Filan^{R}	0	1.5	24.5b	12.5	31.5ab	273b	13.0b	26.5	547b
Folicur^{R}/Bavistin^{R}	0	0	28.0b	14.5	30.0b	286.5b	11.5b	17	508b
Folicur^{R}/Switch^{R}	0	0	24.0b	8	13.0c	198b	12.5b	28	453b
F pr.	-	0.22	0.004	0.35	0.006	0.001	0.006	0.59	<0.001
LSD	-	-	16.9	-	16.5	122.3	12.1	-	100.2

^{BH} Before harvest/cutting of pyrethrum crop.
^{WR} Crop cut into windrows.

Table 3.3.1 A - The effect of fungicide treatment on incidence and area under fungal incidence curve (AUFIC) of *B. cinerea* and *S. sclerotiorum* in pyrethrum flowers prior to and post harvest (2007-08).

	Nontreated	**Folicur^{R}/Filan^{R}**	**Folicur^{R}/Bavistin^{R}**	**Folicur^{R}/Switch^{R}**	**F pr.**	**LSD**
Dry weight flowers (kg/ha)	3984	4342	4059	4013	0.71	-
Pyrethrin yield (kg/ha)	84.8	96	91.8	85.5	0.32	-
Pyrethrin assay (%)	2.28b	2.36ab	2.44a	2.29b	0.01	0.09
Ratio (PI/PII)	1.39	1.37	1.37	1.52	0.23	-
PI (% of dry weight)	1.32	1.37	1.41	1.38	0.08	-
PII (% of dry weight)	0.96	1	1.03	0.91	0.34	-

Table 3.3.1 B - Effect of fungicide treatments on yield and assay of total pyrethrins and constituent pyrethrins PI and PII (2007-08).

3.3.2 Year 2 - Replicated field trial at site 08-4 (2008-09)

Flower maturity stage (FMS) was assessed twice during this trial, on 4 and 22 December. Mean FMS from samples gathered on the 4th of December varied little, ranging between 2.77 (Switch^{R}) and 2.95 (Nontreated), with no significant difference between treatments (Appendix 3.3.2 A). At the final sampling, mean FMS bordered ($0.05 < P < 0.1$) on statistical significance (Appendix 3.3.2 A).

Disease incidence

At the first assessment of flowers on 4 December, samples from nontreated plots had a significantly ($P = 0.007$) higher mean incidence of *B. cinerea* than all fungicide treatments except the commercial applications (Appendix 3.2.2 A). Scala^{R}, Rovral^{R}, Filan^{R} and Switch^{R} had significantly lower incidence of *B. cinerea* than the commercial and nontreated (Appendix 3.3.2 A). At the second evaluation one week prior to flower cutting, nontreated plots had the highest mean incidence of *B. cinerea* (76.5%), however treatment had only a borderline statistical effect on incidence at this time ($0.05 < P < 0.1$). By the penultimate assessment, cut crop material had been drying in windrows for 17 days and mean incidence of *B. cinerea* had risen to 89.5% in nontreated plots. Prosaro^{R}, Filan^{R} and Switch^{R} had significantly lower incidences of *B. cinerea*, with Switch^{R} treatments in turn resulting in significantly lower incidences than Prosaro^{R} (Appendix 3.2.2 A). By the final evaluation, the incidence of *B. cinerea* in nontreated plots was 20.5%, with Switch^{R} and Scala^{R} treatments having significantly lower fungal incidence of *B. cinerea* than the nontreated (Appendix 3.3.2 A).

At the first assessment (4 December) all fungicide treatments, aside from commercially recommended applications, had significantly lower incidences of *S. sclerotiorum* than nontreated (Table 3.3.2 A). At the second evaluation approaching cutting (22 December), only the commercial flowering fungicide applications showed significantly lower incidence of *S. sclerotiorum* than nontreated. Scala^{R} had a significantly higher mean incidence of *S. sclerotiorum* than all other fungicide treatments at this time, and was not statistically different from nontreated plots (Table 3.3.2 A). At the third assessment of flower samples on 14 January, Rovral^{R}, Scala^{R}, Filan^{R}, and Prosaro^{R} had significantly lower incidence of *S. sclerotiorum* than nontreated and commercial plots (Table 3.3.2 A). Only Prosaro^{R} and Switch^{R} treatments had significantly lower incidences of *S.*

sclerotiorum than nontreated at the final assessment (21 January), after flowers had been lying in windrows (Table 3.3.2 A).

Yield and assay

Fungicide treatment had no significant effect on flower yield, pyrethrin yield, pyrethrin assay or constituent pyrethrins (PI, PII) (Table 3.3.2 B). There was a borderline statistical difference ($0.05 < P < 0.1$) in pyrethrins ratio (Table 3.3.2 B).

Comparison of flower yields from treatments with Tukey's adjusted pair wise probabilities showed Filan^{R} applications yielded significantly greater dry weight than nontreated, commercial and Switch^{R}. Rovral^{R} treatment had a statistically borderline increase in dry matter yield compared with commercial and Switch^{R} treatments, while also having significantly higher dry flower yield per hectare than no treatment (Figure 3.3.2 C, App. 3.3.2 B-C). This provided a limited means to rate and separate the relative performance of different fungicide treatments, based on dry flower yield and reductions in fungal incidence of flowers compared to no treatment.

Fungal incidence of *Botrytis cinerea* in flowers (%)				
Treatment	**4-Dec[BH]**	**22-Dec[BH]**	**14-Jan[WR]**	**21-Jan[WR]**
Nontreated	30.0a	76.5	89.5a	20.5a
Switch[R]	7.0c	63.5	68.0c	11.5bc
Filan[R]	8.5c	57.5	72.0bc	13.0ab
Rovral[R]	10.0c	65.5	84.5ab	17.5ab
Prosaro[R]	14.0bc	63.5	77.5b	20.5a
Scala[R]	10.5c	65.0	82.5ab	7.0bc
Commercial	25.0ab	51.5	84.5ab	13.5ab
F pr.	0.007	0.094	<.001	0.008
LSD	12.92	-	9.47	7.96
Fungal incidence of *Sclerotinia sclerotiorum* in flowers (%)				
Treatment	**4-Dec[BH]**	**22-Dec[BH]**	**14-Jan[WR]**	**21-Jan[WR]**
Nontreated	26.5a	22.0ab	21.0ab	24.5a
Switch[R]	10.5bc	15.5bcd	14.5abc	10.0d
Filan[R]	7.0c	13.5bcd	10.0c	19.0abc
Rovral[R]	12.0bc	15.0bcd	13.0c	18.5abc
Prosaro[R]	6.0c	15.0bcd	7.5c	14.0cd
Scala[R]	6.5c	25.0a	11.0c	23.0ab
Commercial	15.5ab	10.0d	22.0a	19.0abc
F pr.	<.001	0.025	0.005	0.025
LSD	6.97	8.85	7.66	8.33

[BH] Before harvest/cutting of pyrethrum crop.
[WR] Crop cut into windrows.

Table 3.3.2 A - Effect of fungicide treatments on fungal incidence of *B. cinerea* and *S. sclerotiorum* in flowers (2008-09).

Treatment	**Dry weight flowers (kg/ha)**	**Pyrethrin yield (kg/ha)**	**Pyrethrin assay (%)**	**Ratio (PI/PII)**	**PI (%)**	**PII (%)**
Scala[R]	4343	110.8	2.71	1.82	1.75	0.96
Filan[R]	4577	115.9	2.68	1.63	1.66	1.02
Switch[R]	4458	114.1	2.72	1.81	1.75	0.97
Rovral[R]	4329	105.3	2.58	2.01	1.73	0.86
Prosaro[R]	4858	125.0	2.74	1.72	1.73	1.01
Commercial	3915	98.7	2.70	1.73	1.70	0.99
Nontreated	4560	112.3	2.62	1.79	1.68	0.94
F pr.	0.776	0.621	0.582	0.074	0.695	0.145
LSD	-	-	-	-	-	-

Table 3.3.2 B - Effect of fungicide treatments on yield and assay (2008-09).

3.3.3 Year 3 - Field trial sites (2009-10)

Effect of fungicide treatment on flower maturity stage (FMS)

At the first assessment, fungicide treatments resulted in significantly lower FMS at ten sites during the first evaluation compared to no treatment. Differences from commercial and modified treatments occurred at nine sites each. Both treatments caused significantly decreased FMS at eight sites in comparison to flowers in nontreated strips, while FMS values did not vary significantly between fungicide treatments at these sites (Appendix 3.3.3 A). A non significant but strong trend suggesting a decrease in FMS from commercial treatment was noted at one additional site (09-2, Appendix 3.3.3 A) in comparison to no fungicide treatment. Mean FMS on 2 December across all sites was 3.29, 3.30 and 3.54 in commercial, modified and nontreated plots respectively with no significant difference among treatments. At the second assessment of flower maturity, modified fungicide treatment resulted in significantly lower FMS than commercial at one site (09-15) and a borderline significant difference between the same treatments at one other site (09-4, Appendix 3.3.3 B). Mean FMS varied little among treatments across sites at the second assessment, with mean values of 4.73, 4.79 and 4.70 in commercial, modified and nontreated respectively.

Effect of fungicide treatment on fungal incidence in flowers

At the first disease assessment, modified and commercial fungicide treatments significantly reduced the incidence of *B. cinerea* at 15 and 10 sites respectively compared to nontreated (Appendix 3.3.3 C). The modified fungicide treatment also had significantly lower incidence of *B. cinerea* than commercial at four sites. Across all sites at the first assessment, commercial treatment resulted in significantly lower incidence of *B. cinerea* than nontreated, while modified treatment in turn had a significantly a lower mean incidence than commercial treatments (Appendix 3.3.3 C).

The incidence of *S. sclerotiorum* in flowers at the first disease assessment was significantly lower in modified and commercial treatments at nine and eight sites respectively in comparison to nontreated (Appendix 3.3.3 C). Reduced incidences of this pathogen bordered on significance at one and two further sites for modified and commercial treatments respectively. Mean incidence of *S. sclerotiorum* for nontreated plots at this time was low at 23.2%, with only two fields having

nontreated plots with incidence of *S. sclerotiorum* of 50% or higher. Both fungicide treatments decreased incidence significantly compared to nontreated areas.

At the second and final evaluation of flowers for fungal incidence, modified and commercial fungicide treatments resulted in significantly lower incidences of *B. cinerea* at twelve and seven sites respectively (Table 3.3.3 A). At five sites both fungicide treatments provided significantly lower fungal incidences compared to nontreated strips, while modified applications resulted in significantly lower incidences of *B. cinerea* than commercial treatments at seven sites. Across all sites at this time, modified treatments resulted in significantly lower fungal incidence of *B. cinerea* compared to no treatment, while mean incidence of *B. cinerea* flower blight across commercial plots was not statistically different to nontreated or modified strips.

During the last flower assessment, flowers from modified fungicide treatment plots had significantly lower incidences of *S. sclerotiorum* at eleven sites while commercial applications significantly decreased incidence at six sites in comparison to nontreated (Table 3.3.3 A). Modified treatments at nine sites also had significantly lower incidences of *S. sclerotiorum* compared to commercial fungicide applications, which at two additional sites had higher disease incidence than nontreated. The incidence of *S. sclerotiorum* only exceeded 50% in nontreated at two sites, with a mean of 26.5% across all nontreated strips. Commercial fungicide applications had a notably lower mean incidence of 17.9%, with the modified fungicide applications in turn demonstrating significantly lower incidences than commercial treatments across all sites (Table 3.3.3 A).

Effect of fungicide treatment on yield and assay

Within individual fields, commercial fungicide treatments resulted in a significant increase in dry flower yield (kg/ha) and pyrethrin yield (kg/ha) compared to no treatment at only one site (09-1), with the modified intermediate between and statistically indistinguishable between the commercial and nontreated (Appendix 3.3.3 D). There were some statistically borderline differences among treatments in flower and pyrethrin yield (Appendix 3.3.3 D).

There was no significant difference among treatments in the content of P(I) at any site, however the P(II) content was significantly affected by treatment at two sites (Appendix 3.3.3 E). At site 09-10, the modified had significantly higher P(II) content than the commercial, with the nontreated intermediate between and statistically indistinguishable from each of the other treatments. At site 09-8, the commercial had higher P(II) content than the modified and nontreated which in turn were not statistically different from each other (Appendix 3.3.3 E). The total pyrethrins content (% dry weight) was significantly higher in the nontreated in comparison to the commercial and modified at two sites (09-15 and 09-7) (Appendix 3.3.3 F). In addition, the nontreated had significantly higher total pyrethrin content than the commercial, which in turn had significantly higher pyrethrin content than the modified at one site (09-3) (Appendix 3.3.3 F).

Averaged across all sites, fungicide applications demonstrated significant improvements in a number of measured yield qualities. The dry weight flowers harvested (kg/ha) was significantly greater in modified treatment than nontreated, while commercial was intermediate between and statistically indistinguishable from other treatments (Table 3.3.3 B). The commercial and modified treatments had significantly higher numbers of flowers per m^2 (Table 3.3.3 B) than the nontreated. Fungicide treatment had a statistically borderline effect on pyrethrin yield per hectare, with no effect on total pyrethrins, P(I) or P(II) assay fractions (Table 3.3.3 B).

Pyrethrum fields approaching first and third harvests were utilised for evaluation of alternative fungicides during this year, with lower measured yield attributes associated with increasing crop age. Sites 09-9 and 09-13, both third harvest fields, reported significantly lower dry flower weights and pyrethrin yields (kg/ha) than ten and eight other sites respectively (Table 3.3.3 C). First harvest crops at sites 09-6 and 09-7, located at Penguin, had significantly lower total pyrethrin assays than eleven other fields (Table 3.3.3 C). Both fields at Penguin also reported pyrethrins one (PI) and pyrethrins two (PII) assay fractions which were significantly lower than six and eight other trial sites respectively (Table 3.3.3 D).

Incidence of *B. cinerea* in flowers (%)					
Site	**Commercial**	**Modified**	**Nontreated**	**F pr.**	**LSD**
09-9	20b	26b	50a	0.001	13.89
09-10	64b	45c	87a	<.001	15.41
09-8	75a	37b	86a	<.001	11.6
09-1	51a	22b	56a	<.001	14.18
09-2	40b	6c	58a	<.001	11.93
09-3	67a	39b	62a	0.004	15.04
09-4	56a	24b	54a	<.001	11.32
09-5	56	40	58	0.106	-
09-15	70	72	77	0.542	-
09-16	84	77	87	0.255	-
09-6	5b	4b	44a	<.001	6.77
09-7	4b	2b	23a	<.001	8.25
09-13	42a	21b	37a	0.036	16.06
09-11	48b	43b	84a	<.001	16.06
09-12	52b	48b	80a	<.001	14.4
Mean	**48.9ab**	**33.7b**	**62.9a**	**0.003**	**16.27**
Incidence of *S. sclerotiorum* in flowers (%)					
Site	**Commercial**	**Modified**	**Nontreated**	**F pr.**	**LSD**
09-9	7	10	6	0.658	-
09-10	24a	4b	25a	0.004	12.2
09-8	9b	9b	60a	<.001	8.34
09-1	35a	6b	33a	<.001	12.58
09-2	18a	5b	20a	0.013	9.98
09-3	18a	2b	9a	0.037	11.8
09-4	10b	14b	57a	<.001	15.15
09-5	15b	3b	30a	0.005	14.23
09-15	17b	0c	35a	<.001	4.536
09-16	27b	11c	48a	<.001	15.04
09-6	16a	0b	18a	0.007	10.89
09-7	25a	0b	8b	<.001	10.37
09-13	12b	9b	28a	0.001	9.16
09-11	24a	11b	10b	0.014	9.66
09-12	11	11	10	0.96	-
Mean	**17.9b**	**6.3c**	**26.5a**	**<.001**	**8.46**

- No significant difference among treatments

Table 3.3.3 A - Incidence of *B. cinerea* and *S. sclerotiorum* in flowers at final sampling occasion, between 14-18 December (2009-10).

Yield attribute	Commercial	Modified	Nontreated	F pr.	LSD
Dry weight flowers (kg/ha)	2491ab	2557b	2275a	0.017	201.3
Flowers per m^2	1553b	1564b	1386a	0.025	142.8
Pyrethrins (kg/ha) [1]	52.46	53.79	48.74	0.109	-
Total pyrethrins (%)	2.26	2.29	2.31	0.219	-
PII (%)	1.07	1.08	1.08	0.723	-
PI (%)	1.20	1.21	1.22	0.382	-
Ratio (PI/PII)	1.13	1.15	1.14	0.926	-

Table 3.3.3 B - Effect of fungicide treatments across different sites on pyrethrum yield components (2009-10).

Site	DW flowers (kg/ha)	Site	Pyrethrins (kg/ha)	Site	Total pyrethrins (%)
09-9 [3]	1490 a	09-9 [3]	28.63 a	09-7 [1]	1.81 a
09-13 [3]	1810 ab	09-13 [3]	37.06 ab	09-6 [1]	2.01 ab
09-10 [3]	2233 bc	09-7 [1]	41.37 abc	09-9 [3]	2.08 bc
09-3 [1]	2273 bc	09-10 [3]	45.88 bcd	09-13 [3]	2.21 bcd
09-15 [3]	2345 bc	09-6 [1]	46.72 bcd	09-10 [3]	2.23 cde
09-7 [1]	2463 cd	09-3 [1]	49.79 bcde	09-12 [1]	2.25 cde
09-8 [1]	2464 cd	09-15 [3]	50.94 bcdef	09-2 [1]	2.29 cdef
09-6 [1]	2530 cd	09-12 [1]	54.35 cdef	09-11 [1]	2.35 defg
09-2 [1]	2552 cd	09-2 [1]	54.47 cdef	09-5 [1]	2.35 defg
09-12 [1]	2585 cd	09-8 [1]	55.34 def	09-15 [3]	2.36 defg
09-11 [1]	2653 cd	09-11 [1]	57.89 def	09-3 [1]	2.37 defg
09-16 [3]	2682 cd	09-4 [1]	61.81 ef	09-8 [1]	2.44 efg
09-4 [1]	2687 cd	09-16 [3]	62.46 ef	09-4 [1]	2.48 fg
09-1 [1]	2714 cd	09-1 [1]	63.89 f	09-1 [1]	2.53 g
09-5 [1]	2965 d	09-5 [1]	64.37 f	09-16 [3]	2.55 g
F pr.	<.001		<.001		<.001
LSD	342.5		7.706		0.119
Mean	2430		51.67		2.287

[1, 3] Age of crop (years) in parentheses

Table 3.3.3 C - Variation between site in yield of dried flowers (kg/ha), pyrethrin yield (kg/ha) and total pyrethrins (% dry weight) across all treatments (2009-10).

Site	PI (%)	Site	PII (%)	Site	Ratio (PI/PII)
09-7 [1]	0.94 a	09-7 [1]	0.87 a	09-10 [3]	1.04
09-9 [3]	1.07 ab	09-6 [1]	0.93 ab	09-9 [3]	1.08
09-6 [1]	1.07 ab	09-9 [3]	1.01 abc	09-5 [1]	1.08
09-10 [3]	1.12 bc	09-12 [1]	1.05 bc	09-11 [1]	1.09
09-13 [3]	1.15 bcd	09-13 [3]	1.06 bc	09-7 [1]	1.09
09-12 [1]	1.20 bcde	09-2 [1]	1.07 bc	09-13 [3]	1.09
09-11 [1]	1.21 bcdef	09-15 [3]	1.08 bc	09-3 [1]	1.11
09-5 [1]	1.22 bcdef	09-10 [3]	1.11 c	09-2 [1]	1.14
09-2 [1]	1.22 bcdef	09-8 [1]	1.12 c	09-12 [1]	1.15
09-3 [1]	1.24 cdef	09-4 [1]	1.12 c	09-6 [1]	1.16
09-15 [3]	1.29 defg	09-3 [1]	1.13 c	09-1 [1]	1.19
09-8 [1]	1.32 efg	09-11 [1]	1.13 c	09-8 [1]	1.19
09-4 [1]	1.36 efg	09-16 [3]	1.13 c	09-15 [3]	1.21
09-1 [1]	1.37 fg	09-5 [1]	1.13 c	09-4 [1]	1.22
09-16 [3]	1.41 g	09-1 [1]	1.17 c	09-16 [3]	1.26
F pr.	<.001		<.001		0.095
LSD	0.089		0.097		-
Mean	1.21		1.08		1.14

[a] Age of crop (years) in parentheses

Table 3.3.3 D - Variation among sites of pyrethrin ratio (PI/PII), PI and PII assay fractions across all treatments (2009-10).

3.3.4 Relationships between fungal incidence/AUFIC and yield qualities

Over three consecutive years of field trials evaluating alternative fungicides for control of flower blights in Tasmanian pyrethrum fields, a number of significant or borderline significant negative correlations between fungal incidence/AUFIC and measured or estimated yield qualities were found. During each year (2007-08, 2008-09 and 2009-10) relationships between declining yield attributes and floral incidence of *B. cinerea* and *S. sclerotiorum* were observed. Stronger correlations were revealed pooling data from replicated plot trials at sites 07-03 (2007-08) and 08-4 (2008-09) and including year as a random effect in statistical analysis. Both sites were located on the same farm and were separated by approximately 500 metres. Fungal incidence and yield assessments were conducted on similar or identical dates during both years, while data sets from successive years had similar variances and no obvious qualitative differences between years. All borderline and significant correlations are shown (Table 3.3.4).

During the replicated plot trial at site 07-3 (2007-08), total pyrethrin assay values bordered on being significantly negatively correlated with area under the fungal incidence curve (AUFIC) values for *S. sclerotiorum* between 11-21 December, the third and fifth occasions of flower sampling for disease incidence (r = - 0.4, $0.05 < P < 0.1$; Figure 3.3.4.1, Tab. 3.3.4).

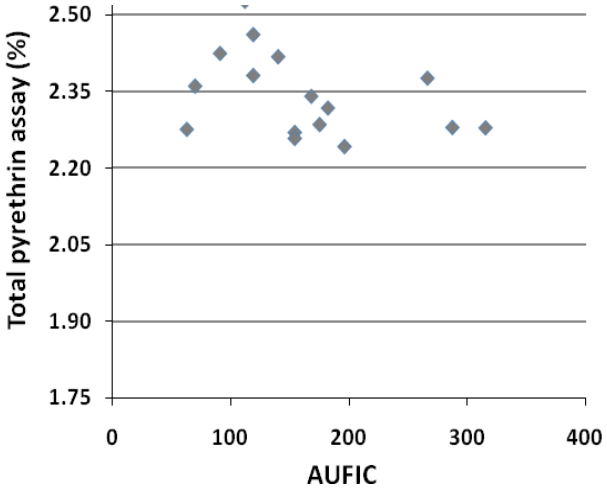


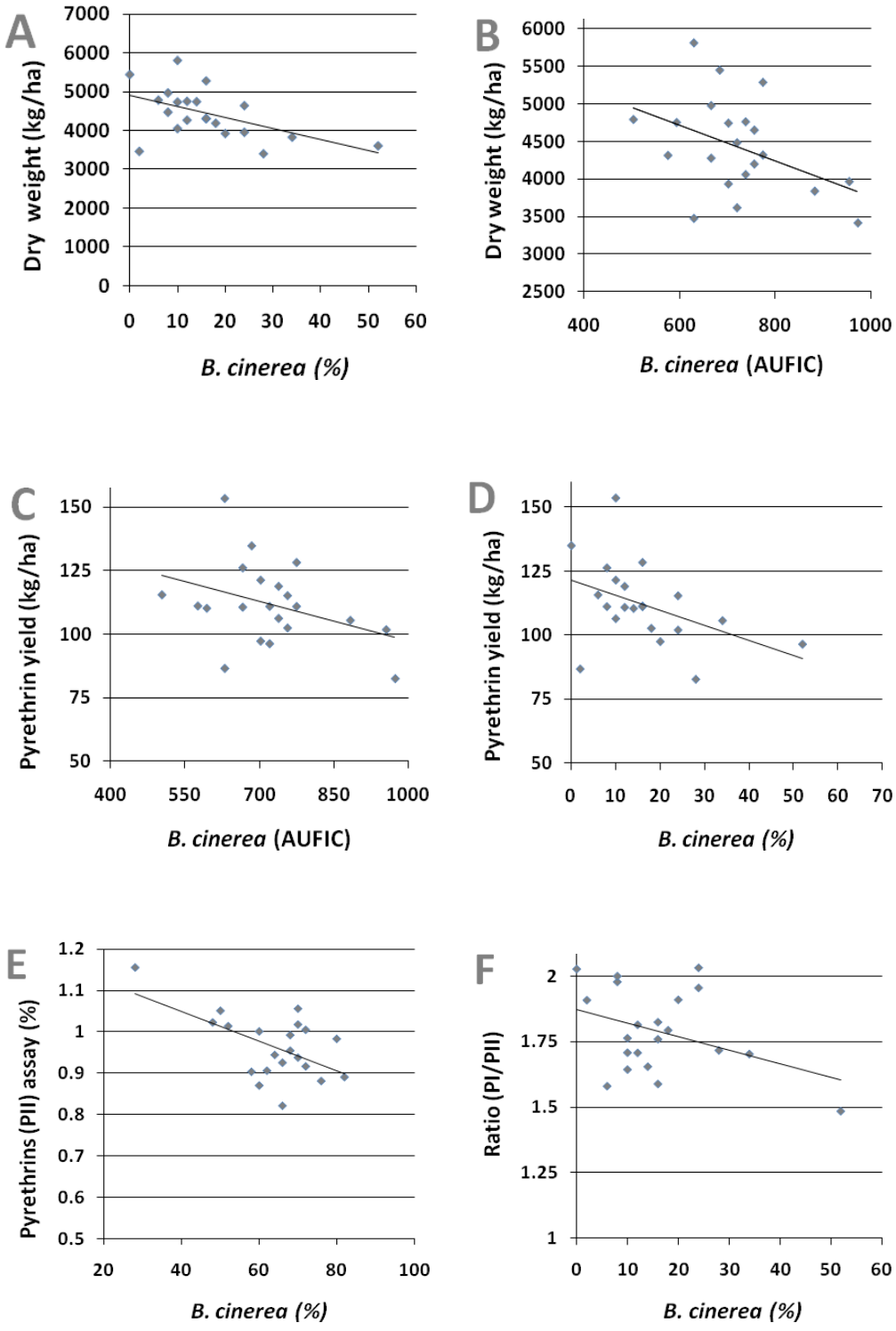
Figure 3.3.4.1 - Relationship between Area Under the Fungal Incidence curve (AUFIC) of *S. sclerotiorum* between 11-21st December and total pyrethrin (P total) assay (% of dry weight), site 07-3 (2007-08).

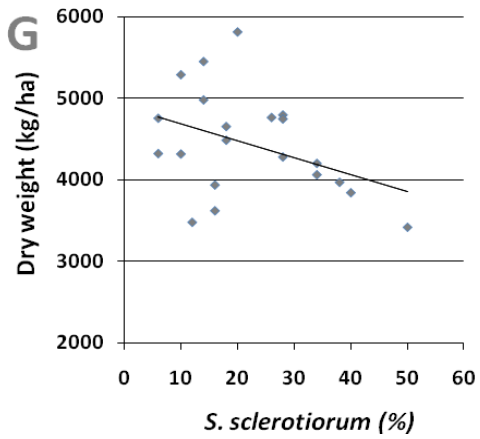
From the replicated fungicide trial at site 08-4 during 2008-09, numerous relationships between fungal incidence measurements and yield qualities were described. At the first instance of flower sampling on 4 December, there was a significant negative correlation between the floral incidence of *B. cinerea* and dry weight of flowers harvested from treatment plots (r = -0.517, $0.01 < P < 0.02$). The AUFIC of *B. cinerea* prior to harvest between 4-22 December was significantly negatively correlated with dry flower yield, however not to the same degree as disease incidence (r = -0.423, $0.02 < P < 0.05$). During this period also, the AUFIC of *Botrytis* flower blight bordered on being significantly negatively correlated with pyrethrin yield (r = -0.37, $0.05 < P < 0.1$). Incidence of *B. cinerea* isolated from flower samples on 4 December 2008 also showed a significant negative correlation with fresh weight of flowers yielded per plot (r = -0.44, $0.02 < P < 0.05$) and pyrethrins per hectare yielded (r = -0.43, $0.02 < P < 0.05$). The pyrethrin ratio (PI/PII) bordered on being

significantly negatively correlated with incidence of *B. cinerea* at the first disease assessment of flowers (r = -0.385, 0.05 < P < 0.1). The incidence of *B. cinerea* at the final disease evaluation prior to harvest on 22 December was highly significantly negatively correlated with the pyrethrins (PII) assay fraction (r = -0.56, P < 0.01). The mean incidence of *S. sclerotiorum* at the first sampling stage bordered on being significantly negatively correlated with dry weight (kg/ha) of flowers yielded (r = -0.39, 0.05 < P < 0.1). Correlation data is displayed in Table 3.3.4.

Numerous significant linear regressions were also established from the replicated fungicide trial at site 08-4 during 2008-09, including between dry matter (kg/ha) and incidence of *B. cinerea* on 4 December (Figure 3.3.4.2 A; P = 0.016, R^2 = 0.27). A maximum yield of 4892.4 kg/ha DM was estimated with zero disease incidence, predicted to decline by 28.3 kg/ha for each percent increase in *Botrytis* frequency reported on 4 December (y = -28.3x + 4892.4). Regressions bordering on significance were noted between the AUFIC of *B. cinerea* and both dry flower weight (Figure 3.3.4.2 B; P = 0.056, R^2 = 0.179) and pyrethrin yield (Figure 3.3.4.2 C; P = 0.098, R^2 = 0.137), as well as incidence of *B. cinerea* on 4 December and pyrethrin yield (Figure 3.3.4.2 D, App. 3.3.3 G; P = 0.052, R^2 = 0.185). Regressing the fresh weight of material harvested (kg/ha) against incidence of *B. cinerea* on 4 December showed a significant relationship (P = 0.046, R^2 = 0.194). Maximum fresh weight yielded was estimated at 10,889 kg/ha with zero *Botrytis* flower blight incidence, being reduced by 54 kilograms per percent rise in recorded disease incidence at this time (y = -54x + 10,889, Appendix 3.3.3 G). The incidence of *B. cinerea* on 22 December was significantly linearly related to pyrethrins (PII) assay (Figure 3.3.4.2 E; P = 0.0078, R^2 = 0.317). A minimum *Botrytis* incidence of 28% in flowers resulted in a PII concentration of 1.19% at this time, with an estimated 0.036% of assay lost for every 10% increase in incidence of *Botrytis* up to approximately 80% (Y = -0.0036x + 1.1936, Appendix 3.3.3 H). Further regressions from this replicated trial bordering on significance between incidence of *B. cinerea* on 4 December and the ratio (PI/PII) of both pyrethrin assay fractions (Figure 3.3.4.2 F; P = 0.08, R^2 = 0.15) and between fungal incidence of *S. sclerotiorum* on 4 December and dry matter yield were observed (Figure 3.3.4.2 G, App. 3.3.3 G; P = 0.078, r^2 = 0.155).

Figure 3.3.4.2 - Plotted linear regressions from fungicide trial (2008-09) between **(A)** Fungal incidence in flowers of *B. cinerea*/dry weight ($P = 0.016$, $R^2 = 0.27$), **(B)** AUFIC of *B. cinerea/*dry flower yield ($P = 0.0558$, $R^2 = 0.1793$), **(C)** AUFIC of *B. cinerea*/pyrethrin yield ($P = 0.0984$, $R^2 = 0.137$), **(D)** Fungal incidence *B. cinerea*/pyrethrin yield ($P = 0.0517$, $R^2 = 0.185$), **(E)** Fungal incidence of *B. cinerea*/pyrethrin (PII) assay fraction ($P = 0.0078$, $R^2 = 0.317$), **(F)** Incidence *B. cinerea*/ratio (PI/PII), and **(G)** Fungal incidence of *S. sclerotiorum*/dry weight ($P = 0.078$, $r^2 = 0.155$).





During the 2009-10 season, the fungal incidence of *B. cinerea* across all sites at the first stage of flower sampling bordered on being significantly negatively correlated with the dry weight of flowers harvested (Figure 3.3.4.3 A; r = -0.272, 0.05 < P < 0.1). The AUFIC of *B. cinerea* between 2-16 December was significantly negatively correlated with dry flower yield per hectare (Figure 3.3.4.3 B; r = -0.321, 0.02 < P < 0.05). The correlation matrix between all assessment times and yield components is given (Table 3.3.4).

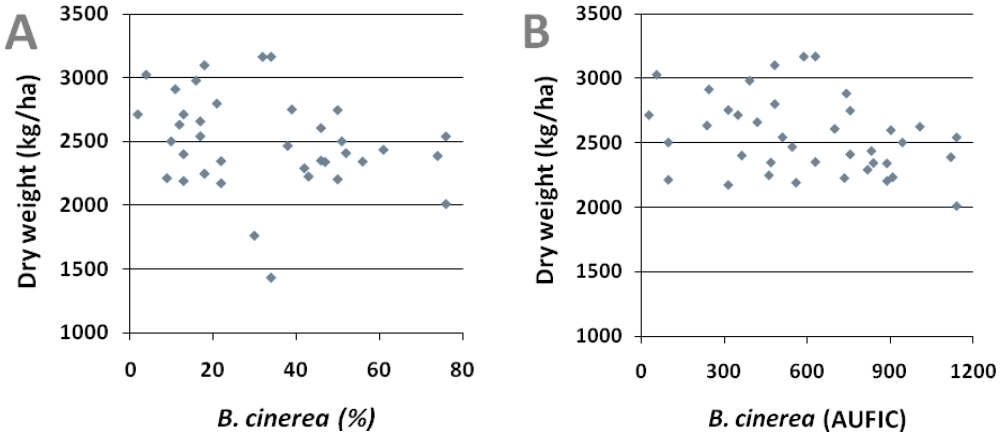


Figure 3.3.4.3 - Relationship between **(A)** fungal incidence of *B. cinerea* on December 2 and yield of dried flowers (kg/ha), and **(B)** AUFIC of *B. cinerea* between December 2-16 and yield of dried flowers (kg/ha, 2009-10 season).

Year/s	Pathogen Yield attribute	Fungal incidence of flowers:		AUFIC
		Dec. 2[3], 4[2] or 11[1]	**Dec-22**	
2007-08[1]	***S. sclerotiorum****			
	Pyrethrin assay (%)	-	-	r = -0.4, 0.05 < P < 0.1
2008-09[2]	***B. cinerea****	-	-	-
	Fresh weight of flowers (kg/ha)	r = -0.44, 0.02 < P < 0.05	-	-
	Dry weight of flowers (kg/ha)	r = -0.52, 0.01 < P < 0.02	-	r = -0.42, 0.01 < P < 0.05
	Pyrethrin yield (kg/ha)	r = -0.43, 0.02 < P < 0.05	-	r = -0.37, 0.05 < P < 0.1
	Total pyrethrin assay (%)	-	r = -0.56, P < 0.01	-
	Ratio (PI/PII)	r = -0.38, 0.05 < P < 0.1	-	-
	S. sclerotiorum			
	Dry weight of flowers (kg/ha)	r = -0.39, 0.05 < P < 0.1	-	-
2009-10[3]	***B. cinerea******			
	Dry weight of flowers (kg/ha)	r = -0.27, 0.05 < P < 0.1	-	r = -0.32, 0.02 < P < 0.05
2007-09[1,2]	***B. cinerea***	**Dec. 4-11**	**Dec-22**	**AUFIC**
	Pyrethrin PI assay (%)	r = -0.85, P < 0.01	-	-
	Pyrethrin ratio (PI:PII)	r = -0.77, P < 0.01	-	-
	Total pyrethrin assay (%)	r = -0.76, P < 0.01	-	-
	Pyrethrin yield (kg/ha)	r = -0.63, P < 0.01	-	-
	OD fraction	r = -0.47, P < 0.01	-	-
	Dry weight of flowers (kg/ha)	r = -0.36, 0.05 < P < 0.02	-	-
	Fresh weight of flowers (kg/ha)	-	r = -0.54, P < 0.01	-
	Pyrethrin PII assay (%)	-	r = -0.40, 0.02 < P < 0.01	-
	S. sclerotiorum			
	Total pyrethrin assay (%)	r = -0.45, P < 0.01	-	-
	Pyrethrin yield (kg/ha)	r = -0.44, P < 0.01	-	-
	Pyrethrin PI assay (%)	r = -0.41, P < 0.01	-	-
	Dry weight of flowers (kg/ha)	r = -0.35, 0.05 < P < 0.02	-	-
	Fresh weight of flowers (kg/ha)	-	r = -0.56, P < 0.01	r = -0.33, 0.05 < P < 0.02

* AUFIC (4-22 December).
** AUFIC (4-22 December).
***AUFIC (2-16 December).
[1] Trial year in parentheses

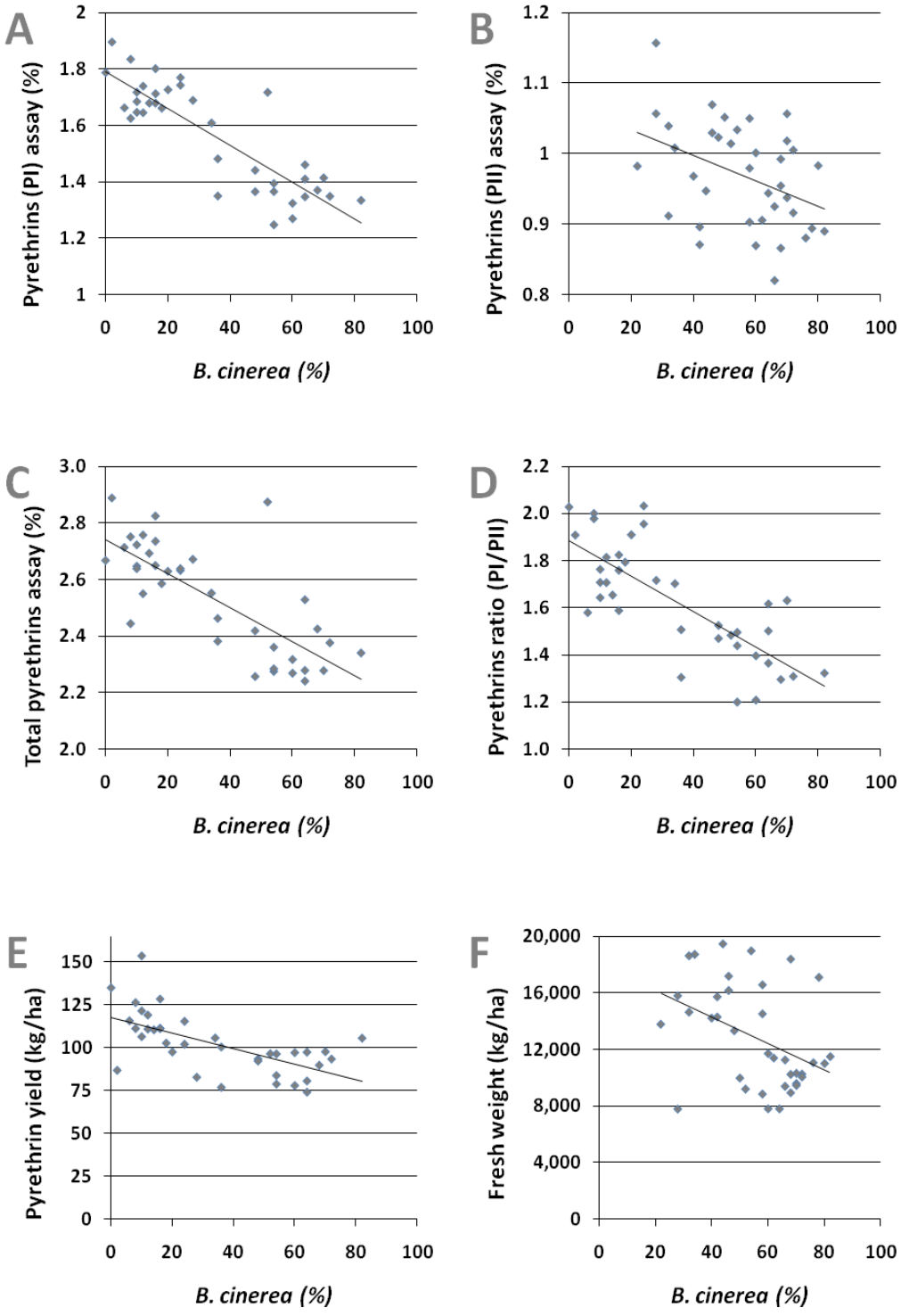
Table 3.3.4 - Borderline and significant negative correlations between AUFIC/fungal incidence of pyrethrum flowers and yield quality attributes from field trials (2007-08, 2008-09, 2009-10).

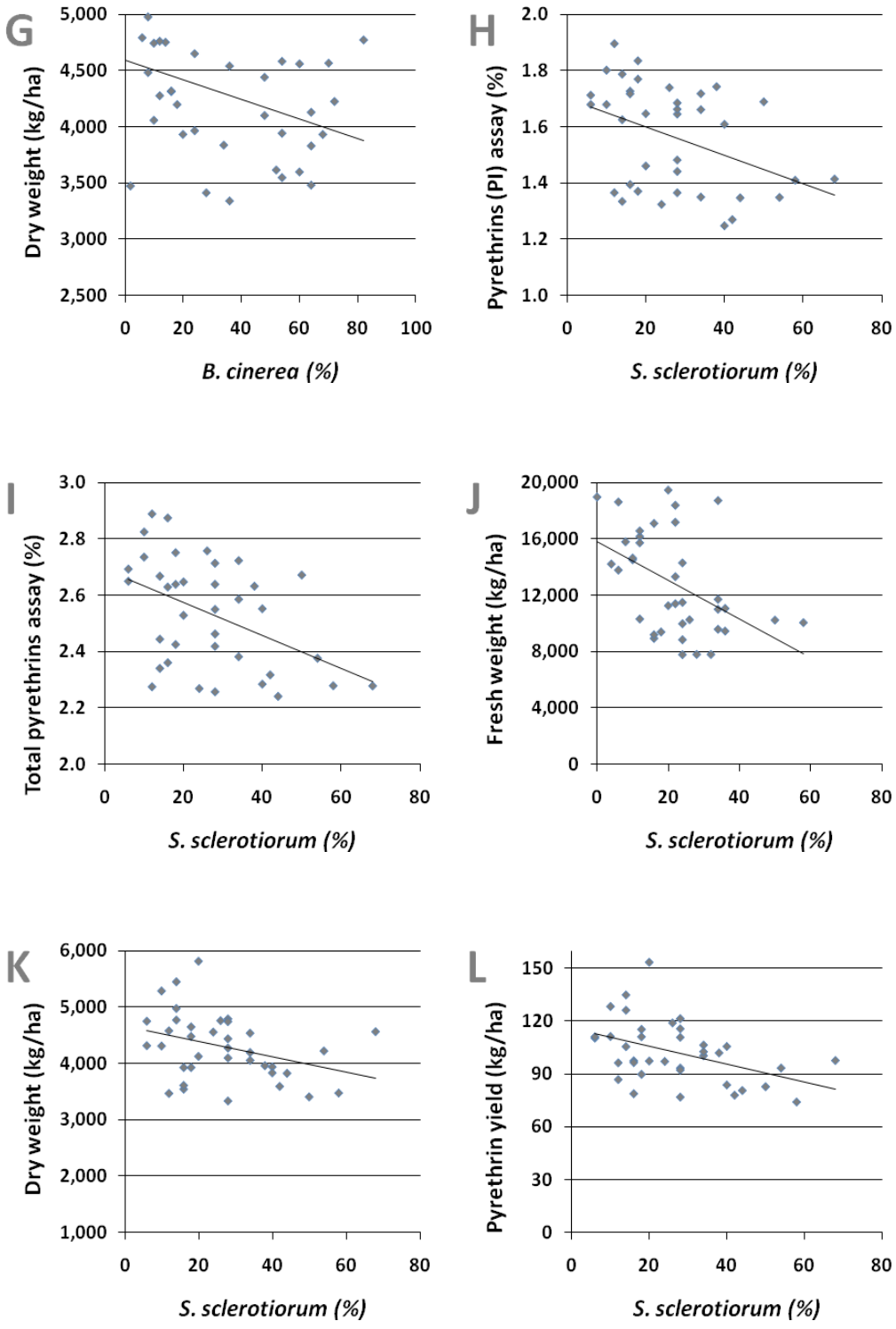
Further negative correlations between AUFIC/fungal incidence and yield qualities were pooling data from replicated plot trials on Werrin Farm over consecutive flower harvests (Table 3.3.4). Correlations between these variables within each season of trials and across multiple years were further investigated using linear regression analysis, with numerous significant relationships

discovered. Regression data including R^2, intercept, slope, *P,* the standard error for the estimate of Y (SEEy) and coefficient of variation (CV%) are shown (Appendix 3.3.3 G).

Similarly to increasing strength of negative correlations, regressing fungal incidence and yield data from consecutive years of replicated plot trials on Werrin Farm (2007-08, site 07-3; 2008-09, site 08-4) revealed stronger relationships between yield properties and fungal incidence of flowers or AUFIC (Appendix 3.3.3 G). Fungal incidence of *B. cinerea* in flowers between 4-11 December demonstrated significant relationships with pyrethrin (PI) assay fraction (Figure 3.3.4.4 A; R^2 = 0.73, *P* = <.001), total pyrethrin assay (Figure 3.3.4.4 C; R^2 = 0.58, *P* = <.001), ratio (PI/PII) of pyrethrin assay constituents (Fig. 3.3.4.4D; R^2 = 0.59, *P* = <.001), kilograms of pyrethrins yielded per hectare (Figure 3.3.4.4 E; R^2 = 0.4, *P* = <.001), dry flower weight (Figure 3.3.4.4 G; R^2 = 0.13, *P* = 0.028) and the optical density (OD) fraction (R^2 = 0.23, *P* = 0.003; Appendix 3.3.3 G). Incidence of *B. cinerea* at Werrin Farm field trials on 22 December during 2007 and 2008 provided a significant relationship when regressed against pyrethrin (PII) assay fraction (Figure 3.3.4.4 B; R^2 = 0.16, *P* = 0.014). Incidence of *S. sclerotiorum* in flowers between 4-11 December over consecutive harvests showed significant relationships with dry flower weight (Figure 3.3.4.4 K; R^2 = 0.13, *P* = 0.032), kilograms of pyrethrins yielded per hectare (Figure 3.3.4.4 L; R^2 = 0.19, *P* = 0.007), pyrethrin (PI) assay component (Figure 3.3.4.4 H; R^2 = 0.17, *P* = 0.012) and total pyrethrin assay (Figure 3.3.4.4 I; R^2 = 0.21, *P* = 0.004). On 22 December the fungal incidence of *S. sclerotiorum* and fresh flower weight (kg/ha) also produced a significant regression (Figure 3.3.4.4 J; R^2 = 0.22, *P* = 0.003), while AUFIC between 4-22 December and fresh weight showed a similar but weaker relationship (R^2 = 0.11, *P* = 0.04; Appendix 3.3.3 G).

Figure 3.3.4.4 - Plotted linear regressions between fungal incidence/AUFIC and yield data from consecutive replicated plot fungicide trials at Werrin Farm (2007-08, 2008-09); **(A)** incidence of *B. cinerea* 4-11 December/pyrethrins (PI) assay fraction (*P* = <.0001, R^2 = 0.73), **(B)** incidence of *B. cinerea* 22 December/pyrethrins (PII) assay component (*P* = 0.013, R^2 = 0.16), **(C)** incidence of *B. cinerea* 4-11 December/total pyrethrin assay (*P* = <.0001, R^2 = 0.58), **(D)** incidence of *B. cinerea* 4-11 December/pyrethrin (PI/PII) ratio (*P* = <.0001, R^2 = 0.59), **(E)** incidence of *B. cinerea* 4-11 December/pyrethrin yield (*P* = <.0001, R^2 = 0.4), **(F)** incidence of *B. cinerea* 22 December/fresh flower weight (*P* = 0.011, R^2 = 0.17), **(G)** incidence of *B. cinerea* 4-11 December/dry weight (*P* = 0.028, R^2 = 0.13), **(H)** incidence of *S. sclerotiorum* 4-11 December/pyrethrins (PI) assay (*P* = 0.0123, R^2 = 0.17), **(I)** incidence of *S. sclerotiorum* 4-11 December/total pyrethrin assay (*P* = 0.005, R^2 = 0.21), **(J)** incidence of *S. sclerotiorum* 22 December/fresh flower weight (*P* = 0.003, R^2 = 0.22), **(K)** incidence *S. sclerotiorum* 4-11 December/dry flower weight (*P* = 0.032, R^2 = 0.13), and **(L)** incidence of *S. sclerotiorum* 4-11 December/pyrethrin yield (*P* = 0.007, R^2 = 0.19).


A
Pyrethrins (PI) assay (%)
2
1.8
1.6
1.4
1.2
1
0
20
40
60
80
100
B. cinerea (%)
B
Pyrethrins (PII) assay (%)
1.2
1.1
1
0.9
0.8
0
20
40
60
80
100
B. cinerea (%)
C
Total pyrethrins assay (%)
3.0
2.8
2.6
2.4
2.2
2.0
0
20
40
60
80
100
B. cinerea (%)
D
Pyrethrins ratio (PI/PII)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0
20
40
60
80
100
B. cinerea (%)
E
Pyrethrin yield (kg/ha)
150
125
100
75
50
25
0
0
20
40
60
80
100
B. cinerea (%)
F
Fresh weight (kg/ha)
20,000
16,000
12,000
8,000
4,000
0
0
20
40
60
80
100
B. cinerea (%)


G
Dry weight (kg/ha)
5,000
4,500
4,000
3,500
3,000
2,500
0
20
40
60
80
100
B. cinerea (%)
H
Pyrethrins (PI) assay (%)
2.0
1.8
1.6
1.4
1.2
1.0
0
20
40
60
80
S. sclerotiorum (%)
I
Total pyrethrins assay (%)
3.0
2.8
2.6
2.4
2.2
2.0
0
20
40
60
80
S. sclerotiorum (%)
J
Fresh weight (kg/ha)
20,000
16,000
12,000
8,000
4,000
0
0
20
40
60
80
S. sclerotiorum (%)
K
Dry weight (kg/ha)
6,000
5,000
4,000
3,000
2,000
1,000
0
20
40
60
80
S. sclerotiorum (%)
L
Pyrethrin yield (kg/ha)
150
120
90
60
30
0
0
20
40
60
80
S. sclerotiorum (%)

3.3.5 *In vitro* fungicide sensitivity screening

EC_{50} values of *B. cinerea* isolates to iprodione ranged from 0.13-8.48 µg a.i./ml with an average of 1.62 µg a.i./ml. There was a suggestion of a bimodal frequency distribution of sensitivities (Fig. 3.3.5A), although a larger number of isolates would need to be tested to investigate this further. Isolates of *S. sclerotiorum* reported EC_{50} values ranging between 0.02-0.61 µg/ml, with a mean of 0.18 µg a.i./ml, with a unimodal frequency distribution of sensitivities. Estimated concentrations (EC_{50}) of iprodione required to inhibit 50% of mycelial growth of isolates of *B. cinerea* and *S. sclerotiorum* evaluated are listed (Appendix 3.3.4), with sensitivity profiles of each pathogen to fungicides shown (Figure 3.3.5 A; 3.3.5 B).

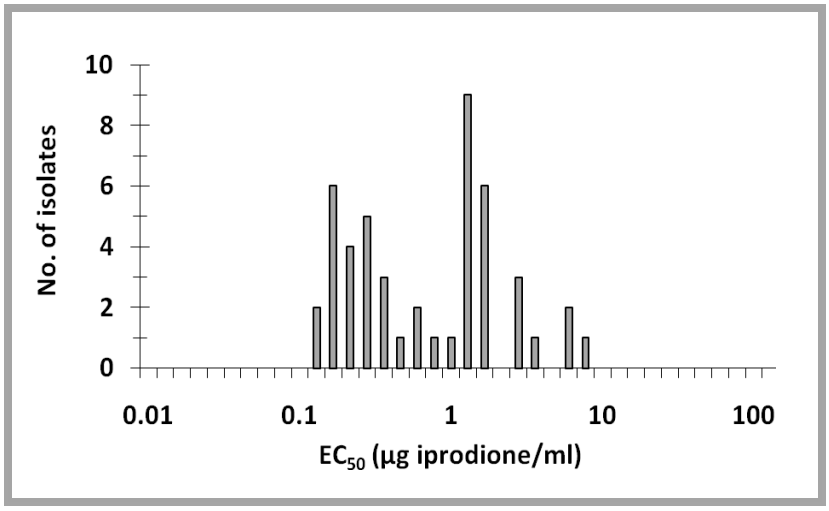


Figure 3.3.5 A - Sensitivity profile of *B. cinerea* isolates to iprodione.

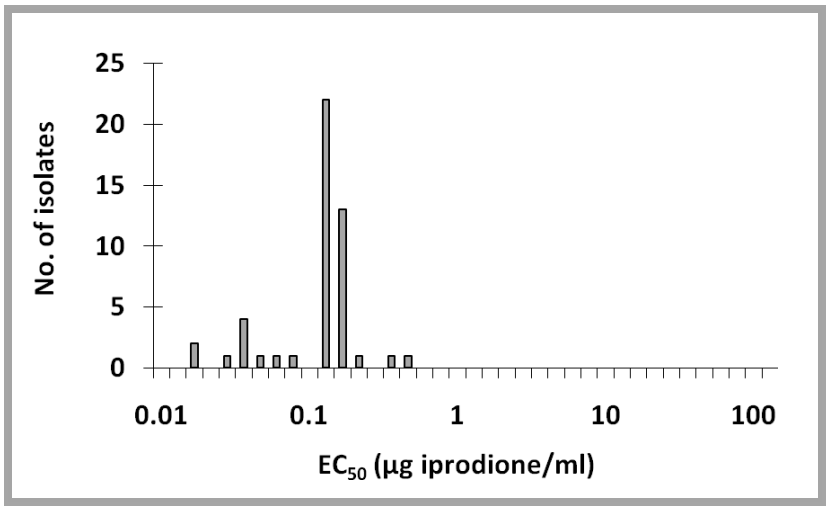


Figure 3.3.5 B - Sensitivity profile of isolates of *S. sclerotiorum* to iprodione.

3.4 Discussion

Year one, 2007-08

The occurrence of *B. cinerea* prior to *S. sclerotiorum* during the flowering period would suggest the initial fungicide application should be targeted primarily against the former pathogen. Prior to cutting the crop into windrows, incidence of *B. cinerea* peaked at a mean of 87.5% and incidence of *S. sclerotiorum* reached 48.7% in nontreated. In agreement with results from chapter 1, this indicates flower infection by *B. cinerea* is initiated prior to that of *S. sclerotiorum,* and higher incidences of *Botrytis* can be anticipated sooner than *Sclerotinia*. Between the third and penultimate flower evaluations incidence of *B. cinerea* and *S. sclerotiorum* declined in both treatments, suggesting a factor other than fungicide application could have affected disease development. Climate data for this site indicates 44.8 mm of rain in the week preceding *Sclerotinia* flower blight decline. Most of this precipitation fell in two rainfall events, with 26.1 mm on 3 December and 15.5 mm on 7 December. Data from all trial sites weather stations were operating during three years of field trials for the evaluation of alternative fungicides for flower disease control are summarised (Appendix 2.5.1 - 2.5.4). Despite fungicide treatments providing reduced incidences of *B. cinerea* and *S. sclerotiorum,* no significant increases in dry weight of flowers harvested or pyrethrin yields were detected. The recommended commercial applications of Folicur^{R}/Bavistin^{R} showed significantly higher pyrethrin assay than plots receiving no treatment. Commercial treatment plots also yielded a borderline significant increase in average pyrethrin (PII) assay fraction. A negative correlation of borderline statistical significance between the AUFIC of *S. sclerotiorum* during December and pyrethrin assay was noted.

Year two, 2008-09

Similarly to the previous year, the mean incidence of *B. cinerea* in nontreated plots rose sharply between 4-22 December, increasing from a mean of 30.0 to 76.5%. After cutting, mean incidence in nontreated continued to rise to 89.5% by 14 January, before declining to 20.5% one week later and immediately prior to harvest of windrows. A proportion of diseased flowers appeared to have completely abscised by this time, potentially leaving a greater percentage of healthy or recently infected flowers. The mean incidence of *S. sclerotiorum* in nontreated was 26.5% during

early December, and failed to increase further throughout the remainder of the study. Environmental conditions this year may not have been as conducive for infection by *S. sclerotiorum* as during 2007-08. Mean December rainfall across all trial sites during 2007 and 2008 averaged 49.4 mm and 18.5 mm respectively, while mean consecutive hours of leaf wetness per day in December declined from 6.4 to 3.7 hours in successive years. Across all trial sites on average, incidence of *S. sclerotiorum* from nontreated strips during 2008-09 was significantly lower than 2007-08, while incidence of *B. cinerea* showed no significant variation among years. All fungicides except the commercial spray regime resulted in significantly lower frequency of *B. cinerea* than no treatment on 4 December; while Switch^{R}, Filan^{R}, Rovral^{R} and Scala^{R} had lower incidences than commercial treatments at this time. Commercial and Filan^{R} treatments significantly lowered disease on 22 December compared to nontreated plots, while Switch^{R} and Scala^{R} applications provided significantly lower incidence of *B. cinerea* after air drying of crop material. Flowers sampled on 4 December from all treatments except the commercial showed significantly lower incidences of *S. sclerotiorum* than no treatment. Conversely, on 22 December the commercial treatment provided the only significant reduction in *Sclerotinia* flower disease frequency compared to control plots. During drying in windrows on 14 January mean incidence of this pathogen was significantly lower in Filan^{R}, Rovral^{R}, Prosaro^{R} and Scala^{R} than nontreated and commercial. In the absence of fungicides, the incidence of *B. cinerea* in flowers may continue to increase after crop has been cut into windrows for drying, even during a relatively dry December period. Presumably the windrow affords a humid and conducive environment for continued fungal development for some time after cutting. Numerous significant negative correlations and linear relationships were demonstrated between measured fungal incidence of flowers and yield attributes (Table 3.3.3 C; App. 3.3.3 H)

Year three, 2009-10

Within individual pyrethrum fields, modified treatment provided significantly lower *Botrytis* flower blight incidence than no treatment at more sites than commercial recommendations at initial assessment, significantly exceeding disease reductions of commercial sprays at four sites. Similarly at the final disease evaluation, incidence of *B. cinerea* of flowers from modified was significantly lower than nontreated strips at more sites than commercial treatments, notably outperforming commercial sprays in seven fields. Incidence of *S. sclerotiorum* across all sites was generally low throughout sampling, however both fungicide treatments significantly reduced incidence at both assessment times. Modified treatment in turn provided a significantly lower mean incidence of *S.*

sclerotiorum than commercial treatments at the final evaluation. Within trial sites, the incidence of *Sclerotinia* flower blight was significantly reduced by both fungicide treatments at a similar number of sites on 2 December, however by the final assessment modified treatments resulted in notably lower incidences of this pathogen at almost twice as many sites as commercial applications. Yield measurements varied considerably among trial sites, with few notable yield increases attributable to fungicide treatment within individual pyrethrum fields. Across all sites however both commercial and modified treatments significantly increased fresh weight of all cut material, dry weight of flowers and pyrethrins per hectare in comparison to nontreated plots. Fresh weight of flowers per hectare was also notably higher with modified treatment. Borderline and significant negative correlations between disease incidence and AUFIC of *B. cinerea* and dry weight of flowers (kg/ha) were demonstrated. This provides limited evidence fungicides able to significantly reduce flower disease incidence during December are associated with increased yields. The low incidence of *S. sclerotiorum* through the sampling period led to no significant correlations against yield measurements.

Variability of flower disease incidence between years

Over the duration of three consecutive years of fungicide trials in commercial pyrethrum crops during the flowering period and mean disease incidence of *B. cinerea* and *S. sclerotiorum* in nontreated plots varied significantly among years. Across all field trial sites each year and in the absence of fungicides, incidence of *B. cinerea* (81.4%) during the first year (2007-08) was significantly higher ($P = 0.05$) than incidence reported in the final year of field trials (62.9%). Mean incidences of *S. sclerotiorum* in nontreated of 24.9% 22.7% in 2008-09 and 2009-10 respectively were significantly lower ($P < .001$) than 2007-2008 which had a mean incidence of 62.8% (Appendix 2.3.3 H).

There was no measured improvement in flower yield from fungicide treatments when data was analysed by analysis of variance. This may have been due to a variable effect of fungicides among replicates on fungal incidence, or suggest that fungicides had been unable to manipulate incidence consistently. Correlation and regression analyses were therefore used to investigate relationships between AUFIC or fungal incidence of flowers and measured yield properties. There were numerous significant negative correlations and linear regressions between disease intensity (AUFIC) or fungal incidence of both flower disease agents and yield and pyrethrin assay. Incidence of

B. cinerea was significantly negatively correlated with fresh and dry weight of flowers harvested, pyrethrins yielded per hectare and the pyrethrin (PII) assay component. The incidence of *S. sclerotiorum* in flowers bordered on being significantly negatively correlated with dry flower yields. The few points of high incidence or AUFIC in Figures 3.3.4.2 A-G represent three nontreated plots amongst eighteen fungicide treated plots comprising the trial.

Numerous examples showing significant relationships between fungal incidence of flowers or AUFIC (particularly *B. cinerea*) and yield measurements have been demonstrated. The strongest of these relationships were between the incidence of *B. cinerea* in replicated plot trials over consecutive years (between 4-11 December) and decreases in; pyrethrin (PII) assay concentration (P <.0001, R^2 = 0.73), total pyrethrin assay (P = <.0001, R^2 = 0.58) and kilograms of pyrethrins yielded per hectare (P <.0001, R^2 = 0.4), allowing for some quantitative estimation of how yield attributes might be affected by changes in fungal incidence of flowers. Several fungicide treatments proved equally, or more, effective than the current commercial recommendations at providing lower diseases incidences during early and mid December, a time when lower frequency of *B. cinerea* has been shown a factor in higher flower yields. Although analysis of variance reported no yield increase from fungicides, comparing fungicide effect on yield of dried flowers (kg/ha) using Tukey's adjusted pair wise probabilities showed Filan^R and Rovral^R treatments provided statistical increases in dry flower yields compared to nontreated. Other differences among fungicide treatments may have been overwhelmed by few data points per treatment as well as sample variability. While not strictly assessing the effect of fungicide treatment on fungal incidence or yield qualities of flowers, linear regression analysis did however allow some estimations of yield and assay to be made based on disease incidence measurements.

In vitro fungicide sensitivity screening

Of the forty six isolates of *B. cinerea* evaluated, 58.7% reported EC_{50} values exceeding the baseline sensitivity of 0.56 µg a.i./ml. reported by Lennox and Spotts (2003), and 98% of isolates of *B. cinerea* were less sensitive to iprodione than the intrinsic EC_{50} toxicity of 0.15 µg a.i./ml reported by Leroux (2004). Resistance of *B. cinerea* to iprodione is associated with enhanced biological ability of the fungus to produce protective enzymes such as catalase (Steel, 1996), and has been documented in strawberry (Pappas *et al,* 1979) and ornamental greenhouse production in the United States and

Greece (LaMondia and Douglas, 1997; Myresiotis *et al,* 2007). Cross resistance of *B. cinerea* to multiple dicarboximide fungicides has been reported in vineyards (Beever *et al,* 1989), glasshouse cucumber (Elad *et al,* 1992) and strawberry (Pappas *et al,* 1979); while resistance of *B. cinerea* to both benzimidizole and dicarboximide fungicide classes has been documented in greenhouse cropping (LaMondia and Douglas, 1997; Yourman and Jeffers, 1999). Additionally, cultures of *B. cinerea* resistant to benzimidazole fungicides such as carbendazim were shown more likely to have reduced sensitivity to dicarboximide fungicides including iprodione (Beever *et al,* 1989; Yourman and Jeffers, 1999). The previous use in pyrethrum fields of benzimidazole fungicides such as carbendazim (Bavistin^{R}, Spinflo^{R}) and dicarboximide fungicides such as procymidone (Fortress^{R}, Sumiclex^{R}) may therefore have allowed some level of reduced sensitivity to develop in the population of *B. cinerea* to iprodione and other dicarboximide fungicides. This may partially explain a higher mean EC_{50} value than the reported baseline value, as iprodione has yet to be used for flower disease control in Tasmanian crops.

These findings suggest a low level of reduced sensitivity exists in the population of *B. cinerea* tested in this study, with the mean EC_{50} of 1.62 µg a.i./ml to iprodione calculated being consistent with the EC_{50} range of mildly resistant isolates (1-1.5 µg a.i./ml) reported recently (Myresiotis *et al,* 2007), and significantly less than the mean EC_{50} value (7.1 µg a.i./ml) of resistant isolates (LaMondia and Douglas, 1997). The mean EC_{50} values of carbendazim and iprodione for inhibiting mycelial growth of *B. cinerea* measured in this study were 318073 and 1.62 µg a.i./ml respectively (App. 2.3.4 B; 3.3.4). This provides indication that iprodione is more effective at inhibiting mycelial growth and may provide better control of *Botrytis* flower disease than carbendazim, with most fungal isolates evaluated previously in this study (Chapter 2) demonstrating resistance to this fungicide.

Isolates of *S. sclerotiorum* reported EC_{50} values ranging between 0.02-0.61 µg/ml, with a mean of 0.18 µg a.i./ml. This indicates no evidence of reduced sensitivity in comparison to wild-type isolate EC_{50} values, which varied between 0.163-0.734 with a mean of 0.428 µg a.i./ml (Liu *et al,* 2009). Compared to the mean EC_{50} value of *S. sclerotiorum* reported to carbendazim of 606.42 µg a.i./ml (Chapter 2, App. 2.3.4 A), iprodione (applied as Rovral^{R} Aquaflo) could be expected to achieve better control of *S. sclerotiorum* flower disease through superior inhibition of mycelial growth, and be a replacement option for the deregistered fungicide carbendazim. Iprodione provided better control of stem rot of oilseed rape caused by *S. sclerotiorum* than carbendazim in recent studies, and

cross resistance with benzimidizole and dicarboximide fungicides observed with *B. cinerea* has not been reported from crop diseases caused by *S. sclerotiorum* after three decades of extensively using both fungicide classes (Ma *et al,* 2009).

3.5 Conclusions

2007-08 - Cumulative disease incidence (AUFIC) of both *B. cinerea* and *S. sclerotiorum* was significantly lowered by all fungicide treatments prior to cutting, aside from commercial, which provided no significant decrease in *B. cinerea*. Alternating fungicide applications of Folicur^{R}/Filan^{R} and Folicur^{R}/Switch^{R} provided comparable or improved reductions in fungal incidence of flower disease pathogens compared to commercial applications of Folicur^{R}/Bavistin^{R}, while no fungicide treatments elicited significant yield responses.

2008-09 - All fungicides except the commercial spray regime resulted in significantly lower incidences in flowers of *B. cinerea* and *S. sclerotiorum* than no treatment on 4 December; while Switch^{R}, Filan^{R}, Rovral^{R} and Scala^{R} applications provided notably lower incidences of *B. cinerea* than commercial treatments. During drying on 14 January, incidence of *S. sclerotiorum* was significantly lower in the windrow in Filan^{R}, Rovral^{R}, Prosaro^{R} and Scala^{R} than nontreated and commercial. The most significant relationships between fungal incidence of flowers and yield qualities resulted from incidence of *B. cinerea* on 4 December and dry weight and pyrethrins yielded per hectare, while declining pyrethrin PII assay fraction was associated with increasing incidence of *B. cinerea* from material sampled on 22 December. Stronger relationships again were shown using data from consecutive years of fungicide trials at Werrin Farm during 2007-08 and 2008-09. Tukey's adjusted pair wise probabilities showed Filan^{R} and Rovral^{R} treatments provided statistical increases in dry matter compared to no treatment, making these fungicides the most effective products tested for flower disease control in this trial, certainly worthy of *in vitro* testing to determine the relative sensitivity of field populations of flower disease pathogens to potentially validate inclusion into the flowering fungicide program in future if required.

2009-10 - Modified fungicide treatment provided significantly lower *Botrytis* flower blight incidences than no treatment at more sites than commercial recommendations at initial assessment, notably exceeding disease reductions of commercial sprays at four sites. Similarly at the final disease evaluation, incidence of *B. cinerea* from modified plots was significantly lower than nontreated at more sites than commercial treatments, also outperforming commercial treatment in seven fields.

Averaged across all sites, modified treatment provided significantly greater fresh and dry weight of material harvested than no treatment, while commercial spray applications significantly increased dry matter compared to nontreated plots. Further evidence of associations between higher incidences of *B. cinerea* in the first week of December or AUFIC through December and lower dry flower yields were demonstrated with correlation analysis.

Iprodione (Rovral^{R} Aquaflo) demonstrated efficacy at inhibiting mycelial growth of *B. cinerea* and *S. sclerotiorum* in agar plate testing, with no significant levels of reduced sensitivity in either pathogen reported. Maximum EC_{50} for *B. cinerea* and *S. sclerotiorum* were 8.5 µg a.i./ml and 0.6 µg a.i./ml respectively.

4. Carpogenic germination, morphological and genetic studies of *S. minor:* A previously unreported agent of flower disease in Tasmanian pyrethrum fields.

4.1 Introduction

Presently two flower diseases are recognised as being significant in Tasmanian pyrethrum fields - *Botrytis* blight caused by *B. cinerea* and *Sclerotinia* blight caused by *S. sclerotiorum* (Pethybridge *et al,* 2008a). Ray blight caused by the fungus *P. ligulicola* is also common, causing a severe dieback of pyrethrum in spring, but does not currently cause significant damage to flowers (Sarah Pethybridge, Botanical Resources Australia, *personal communication*). In addition to flower blight, *S. sclerotiorum* causes periodic crown rot of pyrethrum via myceliogenic germination of sclerotia (Pethybridge *et al,* 2008a). As pyrethrum flowers are produced at a height of approximately 0.8 to 1.5 m above soil level, it is assumed that *S. sclerotiorum* infects flowers following carpogenic germination of sclerotia and subsequent release of windblown ascospores. In support of this, Pethybridge *et al.* (2010) reported a significant spatial association between the presence of apothecia of *S. sclerotiorum* and flower blight in pyrethrum crops. Pyrethrum is also a host of *S. minor* (Melzer *et al,* 1997), which also causes crown rot disease in Tasmania following myceliogenic germination (Pethybridge *et al,* 2008a). It has been assumed that *S. minor* does not contribute to flower blights of pyrethrum in Tasmania as the teleomorph stage required for ascospore release and flower infection has not previously been reported under Tasmanian conditions. However, as described previously (Chapter 1), fungi morphologically similar to *S. minor* have been isolated from surface sterilised pyrethrum flowers obtained from the field.

Both *S. sclerotiorum* and *S. minor* are common in pyrethrum fields (Sarah Pethybridge, Botanical Resources Australia, *personal communication*), with numerous vegetables grown in rotation with pyrethrum also prone to *Sclerotinia* diseases including carrots, lettuce, peas and beans. For these crops, myceliogenic germination of sclerotia has been demonstrated as the means infection and reproduction of *S. minor* in Tasmania, with only *S. sclerotiorum* considered capable of infecting aerial plant tissues with windblown ascospores (Pung and Cross, 2004; DPIWE, 2010c). Few reports of carpogenic germination in field cropping of *S. minor* exist, with *in vitro* ascospore production and confirmation of pathogenicity toward sunflower the sole record of the teleomorph of this fungus in Australia (Ekins *et al,* 2002). Despite no reports of sexual reproduction by *S. minor* in Tasmania, predictive climate modelling suggests ascospore development could occur with temperatures of 11-17°C required for carpogenic germination (Ekins *et al,* 2002). The cool, temperate climate and isolation of fungi tentatively identified as *S. minor* from diseased pyrethrum flowers (described in chapter 1) may suggest favourable conditions allow the teleomorph stage to

develop in Tasmanian crops, infecting flowers with windblown ascospores liberated from germinating sclerotia. More thorough examination of the isolates identified as *S. minor* on a preliminarily basis and confirmation of fungal identity, evidence of carpogenic germination infield or culture and the ability to cause flower disease from artificial infection would however be required to substantiate this hypothesis.

A number of methods are reported to differentiate between *S. sclerotiorum* and *S. minor*. Growth habit and size of sclerotia in plate culture as well as dimensions of asci and ascospores are noted as sufficiently descriptive to separate phytopathogenic *Sclerotinia* isolates at species level (Purdy, 1955; Willets and Wong, 1980; Ekins *et al,* 2002). No characteristic morphological differences were noted among species during stages of stipe and apothecial development from a scanning electron microscopy study (Jayachandran *et al,* 1987). Mean ascospore length and width of *S. minor* are reported to range between 12.7-16.1 µm and 6.3-7.4 µm respectively, while mean asci length of 141.0 µm and width of 8.9 µm are additionally noted (Purdy, 1955). More recently Ekins *et al* (2002) reported ascospore length and width of Australian isolates of *S. minor* varied between 10.32-13.46 µm and 4.70-5.86 µm respectively, with asci length and breadth ranging between 133.81-138.67 µm and 8.77-9.23 µm respectively. The study also reported ascus length and width of *S. sclerotiorum* to vary between 123.83-126.09 µm and 8.17-8.55 µm respectively, while ascospore dimensions fell into ranges of 9.03-11.57 µm and 3.98-5.08 µm for length and width respectively; showing asci and ascospores of *S. sclerotiorum* are generally smaller than *S. minor* (Ekins *et al,* 2002). *S. minor* in plate cultures produces irregularly dispersed sclerotia across plate surface, while *S. sclerotiorum* on culture plates often produces sclerotia in one or more concentric ring formations (Willets and Wong, 1980). Sclerotia of *S. minor* reportedly vary between 0.5-2 mm in size, and are generally much smaller than those of *S. sclerotiorum* which from 3-10+ mm in size (Purdy, 1955; Ekins *et al,* 2005). Morphology in plate culture, in addition to sclerotia, asci and ascospore dimensions could therefore be used in combination to differentiate between *S. sclerotiorum* and *S. minor* isolated from plant material symptomatic of crown rot and flower blight infections.

Objectives of this study were therefore:

- To further investigate fungal identity of isolates from diseased pyrethrum flowers tentatively identified as *S. minor*, using morphological and genetic techniques (described in chapter 1).
- To evaluate the biological ability of tentatively identified isolates of *S. minor* to produce ascosporic inoculum and contribute to flower disease epidemics in Tasmania.
- To evaluate on a cautionary basis the relative sensitivity of fungal isolates tentatively identified as *S. minor* to fungicides including iprodione, a new fungicide for flower disease control in Australian pyrethrum crops.

4.2 Materials and methods

Collection of isolates

During the mycofloral survey of diseased pyrethrum flowers described previously (Chapter 1), fungal isolates tentatively identified as *S. minor* were collected from commercial pyrethrum fields in Tasmania and stored for further study as described in chapter 1 (1.3.2 - Survey of mycoflora associated with diseased pyrethrum flowers; App. 1.3.2 C). These isolates had notably smaller sclerotia than *S. sclerotiorum* when isolated from material including diseased flowers after incubation in humid boxes (Plate 1.3.2). In total, 46 isolates tentatively identified as *S. minor* by sclerotial size and morphological characteristics in plate culture were collected. Seven isolates were obtained from diseased foliage and 38 isolates from diseased flower samples from commercial pyrethrum fields.

A further isolate was collected from field site 08-1 (Tollymores Road, Table Cape) during a field investigation, where several sclerotia with apothecia were observed and collected. Sclerotia were approximately 1.0-2.5 mm in length and 1.0-2.0 mm in width. Apothecia were dipped into autoclaved, sterile water to collect ascospores. Spore concentration was then determined with an improved Neubauer haemocytometer (Paul Marienfield GMbH & Co., Germany). To obtain single spore isolates, an aliquot (100 µl) of suspension was pipetted onto PDA in petri plates and spread with a sterile glass rod. Plates were incubated in the dark (20°C/24 h) and observed under the

microscope. Mycelium from individual colonies was removed and placed onto PDA in petri plates and incubated in the dark to obtain single spore isolates for storage.

A subset of ten isolates was selected from all isolates obtained from pyrethrum fields and tentatively identified as *S. minor,* for further investigation to confirm identity (Table 4.1.3 H). Nine isolates of the 38 collected from flowers during three years of sampling were randomly selected. Isolate 08-1 was included on the basis of its origin, a single spore isolate from a mature apothecial structure attached to sclerotia consistent in size with that of *S. minor.* As described previously, these apothecia were found in a pyrethrum field at Table Cape late during the flowering period. These ten isolates were representative of all isolates identified as *S. minor* on the basis of growth habit in culture and sclerotial dimensions, and were collected across a range of pyrethrum cultivation districts in Tasmania (Table 4.2.1).

Isolate	District	Isolated from:	Year collected
08-1	Table Cape	apothecia	2008
08-5 A	Forth	flower	2009
08-7 B	Penguin	flower	2009
09-8 E	Don	flower	2010
09-8 A	Don	flower	2010
09-8 Y	Don	flower	2010
09-1 A	North Motton	flower	2010
09-3 B	North Motton	flower	2010
09-3 D	North Motton	flower	2010
09-5 A	North Motton	flower	2010

Table 4.2.1 - Fungal isolates selected for further studies.

Mycelial plugs of isolates were removed from cryostorage and incubated at 20°C on PDA plates. Five PDA plates of each isolate were cultured until sclerotia formation was complete, totalling 50 plates. Selected isolates were also subcultured from PDA plates to wholemeal agar plates (WMA) as described by Ekins *et al* (2002) for successful induction of carpogenic germination of *S. minor in vitro*. This growth media was made by combining 20 g wholemeal flour, 8 g agar and 400 ml distilled water, mixing thoroughly before autoclaving for 30 minutes at 121°C and pouring into 90 × 10 mm

plastic petri plates once sufficiently cooled. Three replicate plates of each isolate were incubated, totalling 30 plates.

Morphological identification of fungal isolates (sclerotia)

The length and width of sclerotia from PDA culture plates of each isolate were measured with a Zeiss Stemi DRC stereo microscope (10 ×) and incremented microspore eyepiece. Twenty five sclerotia from four separate PDA plates were measured per isolate, totalling one hundred sclerotia per isolate.

Conditioning sclerotia for carpogenic germination of fungi tentatively identified as S. minor

Using methodology adapted from Ekins *et al* (2002), sclerotia were produced *in vitro* and conditioned to encourage carpogenic germination. Sclerotia from hyphal tipped cultures of the ten selected isolates were plated onto V8 vegetable juice agar, incubated in darkness at room temperature and refreshed onto new plates at seven day intervals. V8 agar was made in 400 ml Schott bottles from 64 ml V8 vegetable juice, 8 g bacteriological agar and 336 ml of distilled water mixed together prior to reducing acidity to approximately pH 6.3 by addition of NaOH. After autoclaving for 30 minutes at 121°C, media was transferred to a water bath set at 60°C prior to pouring V8 agar into 90 × 10 mm plastic petri plates. Third generation cultures from V8 media plates were then transferred to WMA plates and incubated at 20°C in darkness for four weeks. Mature sclerotia were then scraped onto sterile filter paper and dried in a laminar flow cabinet for two days.

Once thoroughly dry, sclerotia of each isolate were placed into 25 ml deep petri plates containing 10ml of autoclaved, distilled water. Two replicate petri plates each containing approximately 200 sclerotia were incubated per isolate. Petri plates were sealed with parafilm and incubated in a Contherm Phytotron Climate Simulator Growth Cabinet (Contherm Scientific, Upper Hutt, New Zealand) in darkness with a temperature pattern of 15°C for 8 hours followed by 10°C for 16 hours for 1-2 months duration or until the emergence of apothecial stipes. After 12 weeks incubation and no formation of stipes, Growth Cabinet maintenance required moving plates containing sclerotia and sterile water to the closest environmental substitute available, a 10°C cool

room. After two weeks darkness at 10°C, apothecial stipes or stipe initials were evident on sclerotia in both replicate plates of all isolates except isolate 08-7 (Plate 4.2.2).

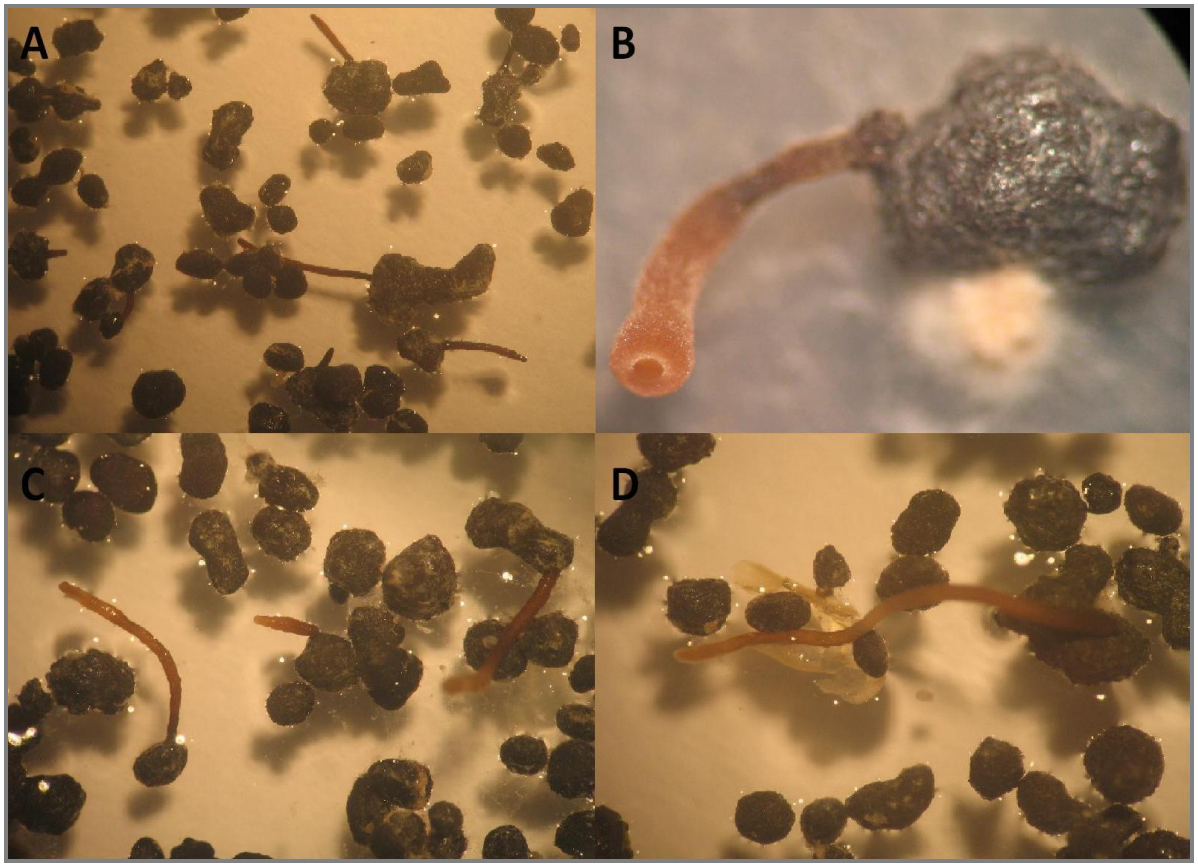


Plate 4.2.2 - Apothecial stipes on sclerotia of isolates **(A)** 09-3B, **(B)** 08-5A, **(C)** 09-8Y and **(D)** 08-1 (3 × magnification).

One plate of each ten isolates was then placed in a light box under 36 W fluorescent daylight tubes (Osram Luminex daylight 860, Germany) illuminated for 8 hours per day at 10°C while the second replicate was incubated at room temperature (20°C) under the same light conditions. Following initiation of apothecial disc development after one week at 10°C, plates at room temperature were relocated to the refrigerated light box (10°C). Mature apothecia then developed from multiple sclerotia from both replicate plates from 8 of the ten isolates conditioned (Plate 4.2.3 A-D, E-H).

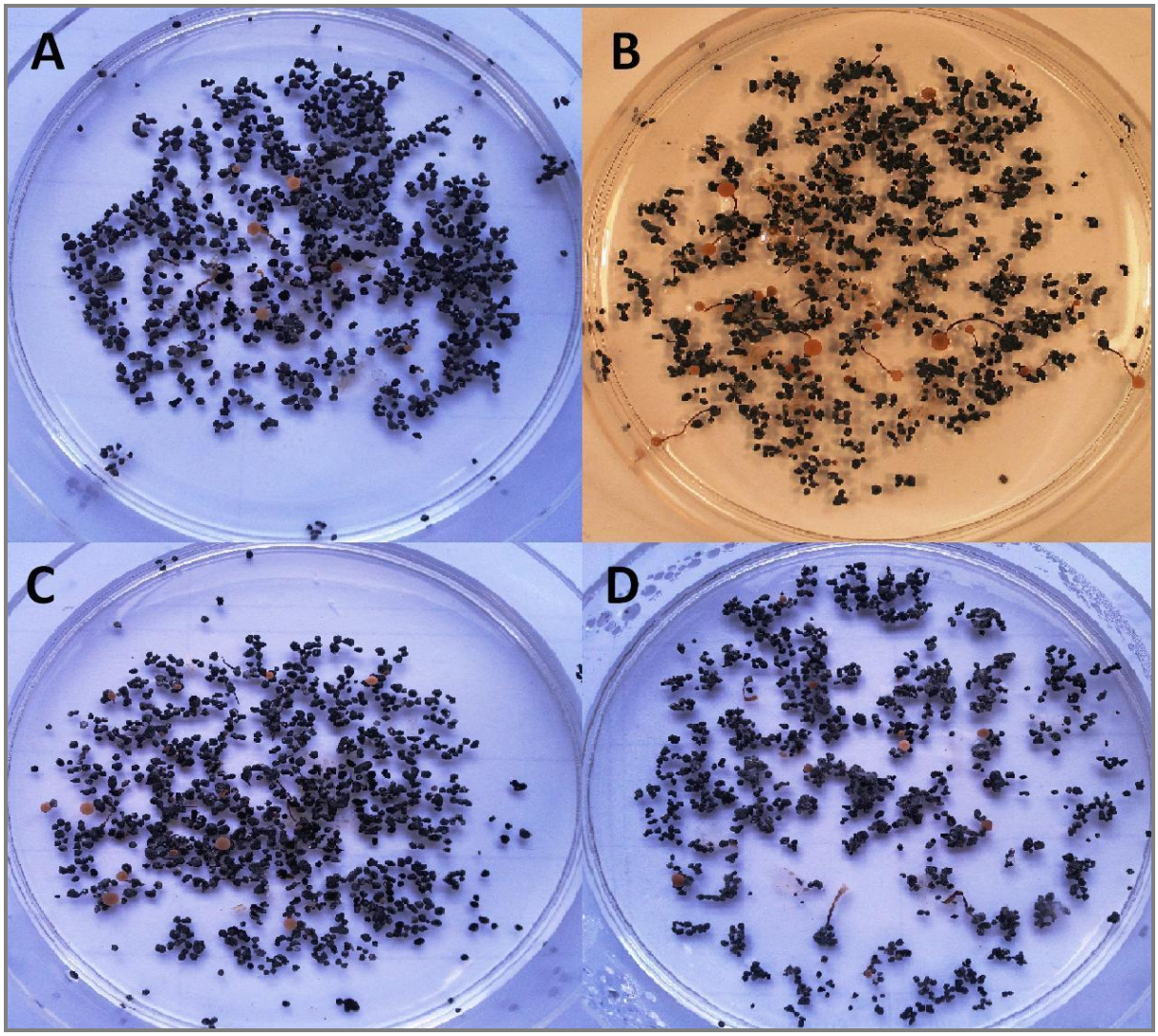


Plate 4.2.3 (A-D) - Apothecia developing on numerous sclerotia of isolates **(A)** 09-8Y, **(B)** 09-3B, **(C)** 09-3D and **(D)** 09-5A.

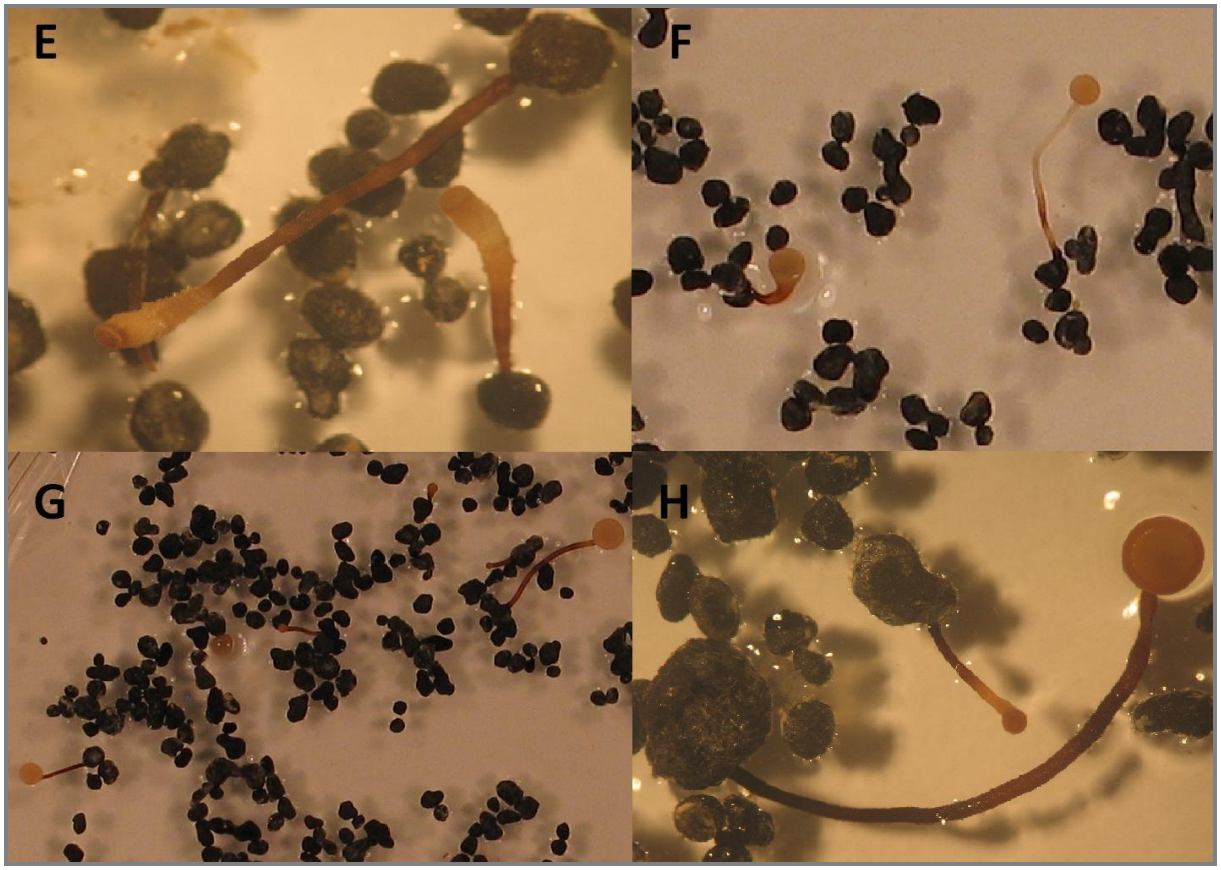


Plate 4.2.3 (E-H) - Mature and developing apothecia of isolates **(E)** 09-8E, **(F)** 08-5A, **(G)** 09-3D and **(H)** 09-3B (3-6 × magnification)

Asci and ascospore morphology of isolates

Ten mature asci and one hundred ascospores from all isolates undergoing complete carpogenic germination were measured. Individual, mature apothecia were cut from sclerotia with sterile tweezers and scalpel, before being gently squashed under a microscope slide coverslip in several drops of sterile water. A Zeiss Axiolab compound microscope and incremented microscope eyepiece (Switzerland) were used to measure length and width of structures at 400 × magnification (Plate 4.2.4 A-E). Spore germination rates were evaluated for all isolates carpogenically germinating, using ten replicate PDA plates per isolate. An aliquot (50 µl) of spore suspension of known volume was pipetted and spread with a glass rod under aseptic conditions onto each plate, before incubation at 20°C overnight. Agar sections were cut from plates, covered with coverslips and assessed with a Zeiss Axiolab compound microscope (100-200×) for ascospore germination rate after 24 hours.

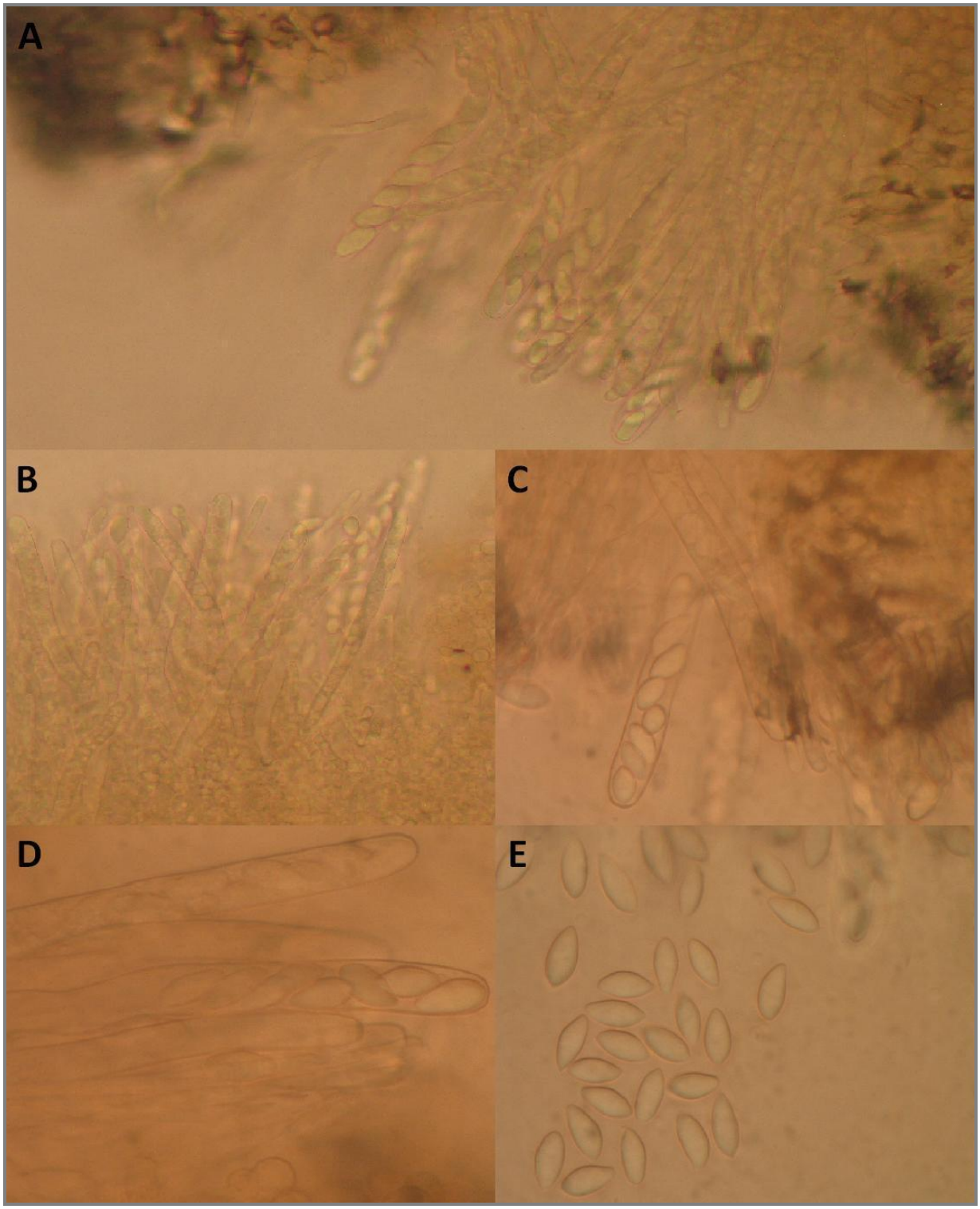


Plate 4.2.4 - Isolate 09-3B; **(A, B, C and D)** Asci attached to apothecia with ascospores visible within and **(E)** discharged ascospores floating in solution (400-1000 × magnification).

Genetic (sequencing) identification of fungal isolates

Cultures on PDA of each of the 10 isolates were sent to fungal taxonomist Dr. Roger Shivas (Department of Employment, Economic Development and Innovation) for further confirmation of identity by sequencing of the ITS region. One potato dextrose agar (PDA) culture plate of each of the ten selected isolates was used for genomic sequencing analysis. This was to determine if genetic material obtained was consistent with current records existing in genomic databases of the internal transcribed spacer (ITS) region of *S. minor,* comprising ITS1, 5.8S rDNA, and ITS2.

Sclerotia of the ten isolates were removed from agar plates and placed in 2.0 ml safe-lock tubes (Eppendorf) before being lysed with a Tissue Lyser (Qiagen) for 1 min at 30 hz/s in the presence of the equivalent of 0.5 ml of 0.5 mm glass beads (Daintree Scientific). A Gentra Puregene kit (Qiagen) was used to extract fungal DNA using the manufacturer's instructions.

A Phusion High-Fidelity PCR Master Mix (Finnzymes, Thermo Scientific) incorporating 25 µl of 2 × Master Mix with HF Buffer, 1 µl each of 10 mM of primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*. 1990) and 1 µl of DNA template was used for PCR amplification of the internal transcribed spacer (ITS) region. A Bio-Rad C1000 thermal cycler was used for the amplification of PCR products under conditions of 98°C for 30 seconds, 35 cycles at 98°C for 10 seconds, 55°C for 30 seconds, 72°C for 30 seconds, followed by a final extension duration of 5 min at 72°C. A QIAquick PCR Purification Kit (Qiagen) was utilized to purify PCR products before sequencing using an AB 3730xl DNA Analyser (Applied Biosystems) by Macrogen Incorporated (Seoul, Korea).

A phylogenic tree which compared ITS sequences from pyrethrum isolates with published sequences on GeneBank was produced by Dr. Jason Scott (Tasmanian Institute of Agricultural Research, University of Tasmania) as described (Figure 4.3.3).

In vitro fungicide sensitivity screening of S. minor to fungicides

Fungicide infused agar plates were prepared as described previously (Chapter 2, materials and methods), with seven concentrations of iprodione (0, 0.01, 0.05, 0.5, 5, 50 and 100 µg a.i./ml of agar) and three replicate plates per concentration used for each fungal isolate evaluated. Seven isolates of *S. minor* were screened against carbendazim and tebuconazole, while thirty seven isolates were evaluated against iprodione. Actively growing colonies were maintained on PDA plates at 20°C, before 5 mm cork borings from colony edge were removed, inverted and placed centrally on plates of differing fungicide concentrations. Plates were sealed and incubated at 20°C until mycelial growth approached the perimeter of 0 µg a.i./ml concentration plates, at which point all plates were measured. EC_{50} values were calculated with PROBIT analysis using SAS (V9.2) as previously described (Chapter 2 materials and methods).

4.3 Results

4.3.1 Evidence of carpogenic germination of *S. minor* in a pyrethrum field

While visiting a field trial in the Table Cape area on 27 November during 2008, (Site 08-1, Tollymores road, 40.95° S 145.69° E) scattered plants throughout the crop of wilted appearance were noticed (Plate 4.3.1 A). The majority of flowers on numerous wilted plants were shrivelled and severely reduced in size and number, having already ceased development (Plate 4.3.1 B). Upon closer inspection of such plants, foliage was found to be severely wilted, and colonised by profuse white mycelium, obvious also on basal stems (Plate 4.3.1 C). After a closer inspection of another plant rotted back almost to its growing points beneath the flower canopy, an abundance of small sclerotia with apothecia of differing growth stages were observed (Plates 4.3.1 C-E; apothecia indicated by superimposed arrows).

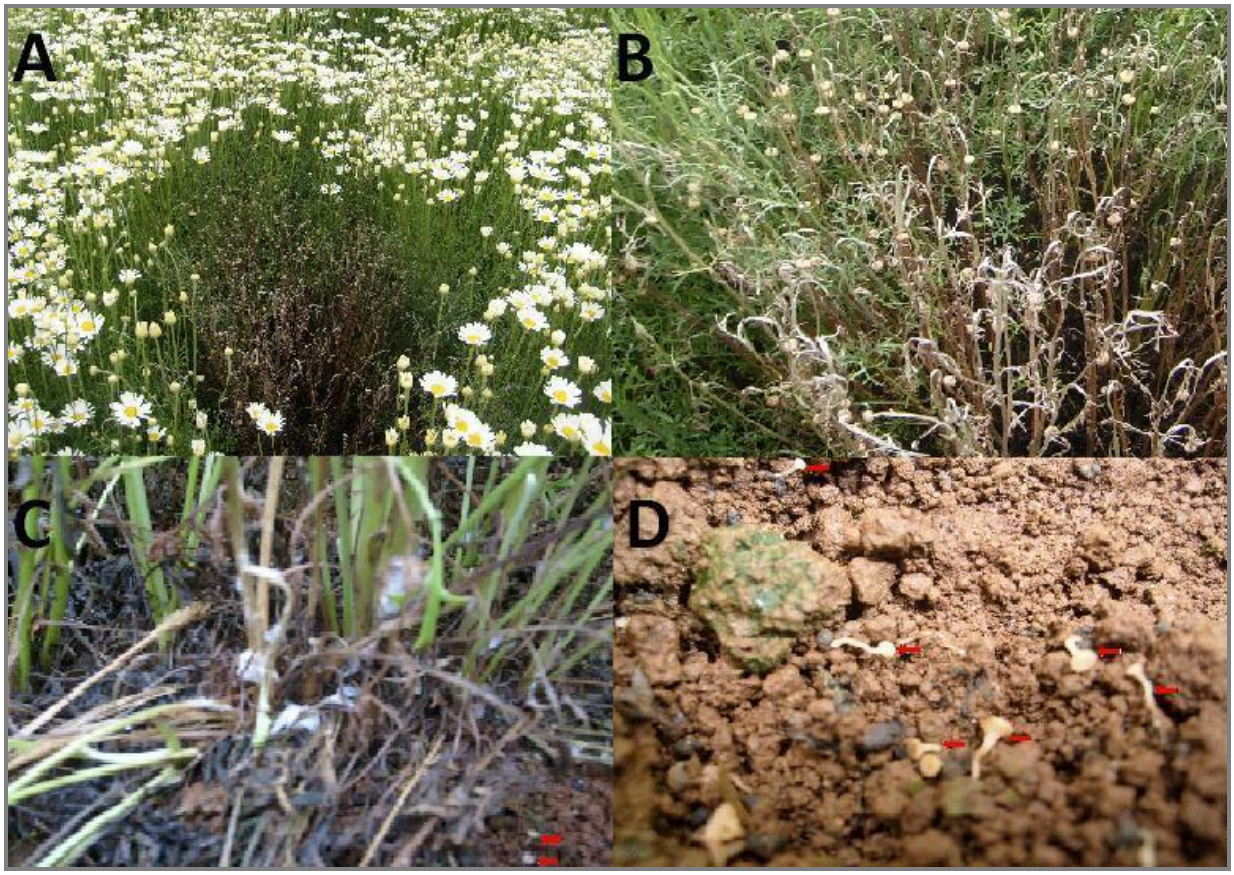


Plate 4.3.1 - (A) Diseased pyrethrum plant at site 08-1, **(B)** diseased flowers, **(C)** rotted out growing points and necrotic foliage colonised with mycelium with soil bound apothecia, and **(D)** numerous apothecia observed at ground level.

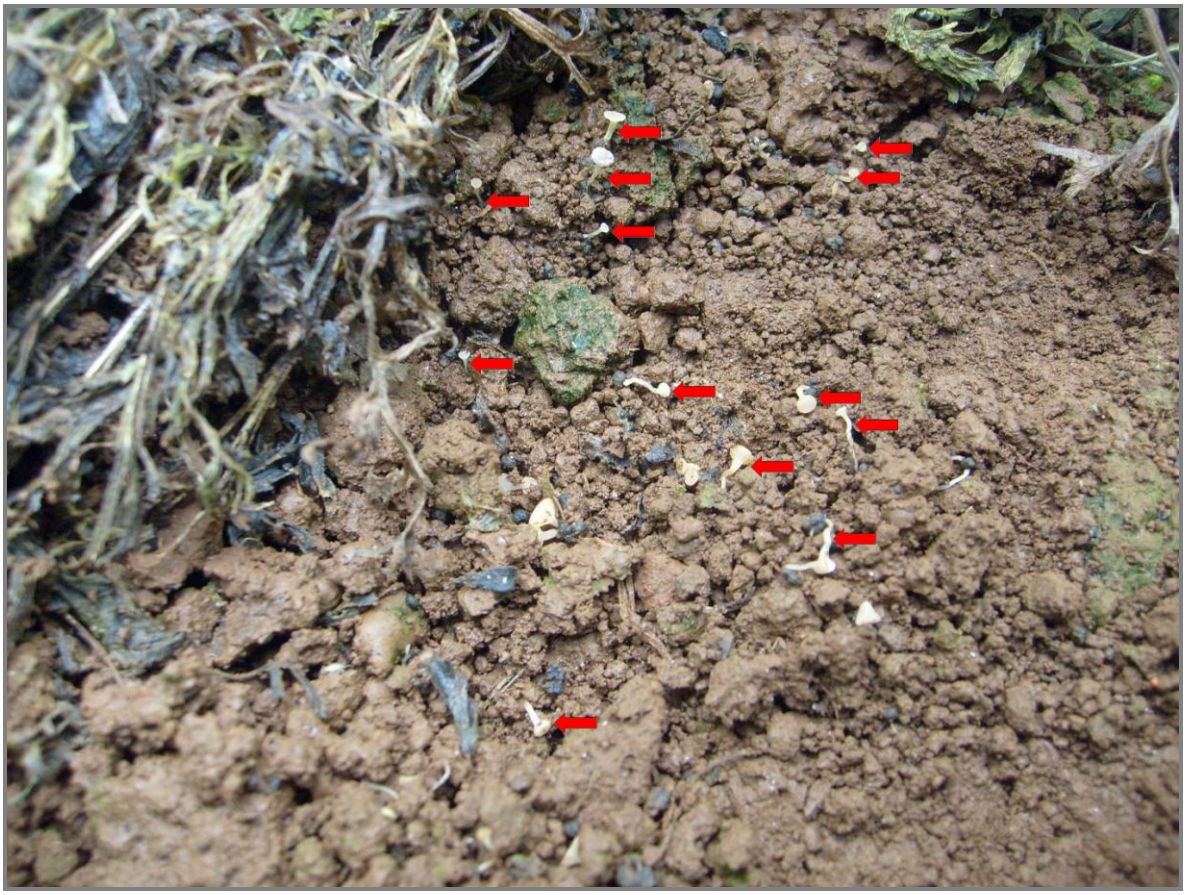


Plate 4.3.1 E - Apothecia near soil surface with apothecia, shown with superimposed arrows.

Sclerotia collected were all approximately 1.0-2.5 mm in length and 1.0-2.0 mm in width (Plate 4.3.1 F). Single ascospore isolates grown on PDA in petri plates, produced mycelium and sclerotia morphologically consistent with that reported of *S. minor* (Plate 4.3.1 G).

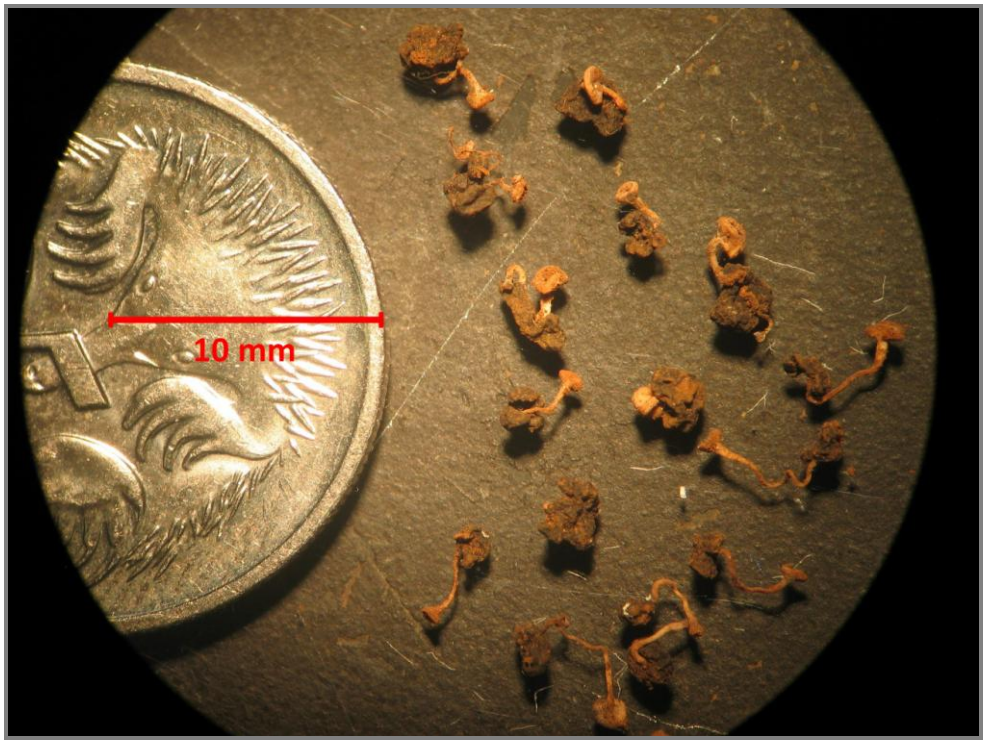


Plate 4.3.1 F - Sclerotia with apothecia collected from Table Cape pyrethrum field, November 2008.

Plate 4.3.1 G - Culture plate (90 mm diameter) of single spore isolate 08-1 on PDA growth media.

4.3.2 Morphological studies

Morphological identification of fungal isolates (sclerotia)

Sclerotial diameters of Australian isolates of *S. minor* from sunflower were reported to range from 0.5-2 mm, varying significantly from the size of resting structures of *S. sclerotiorum* which range from 3-10 mm or more in diameter (Ekins *et al,* 2002).

The average dimensions of one hundred sclerotia measured for each of the ten isolates varied between 1.60-2.24 mm in length and 1.08-1.36 mm in width. Across all isolates mean length and width was 1.78 mm and 1.20 mm respectively (Table 4.3.2 A). Potato dextrose agar (PDA) plate cultures of isolates produced growth consistent with that documented for *S. minor,* forming sclerotia irregularly scattered across the entire media plate surface (Plate 4.3.2.1). All measurements of sclerotia fell within ranges described for *S. minor* described within the literature by, and well below the 4-13 mm size range of sclerotia reported for Australia isolates of *S. sclerotiorum* (Purdy, 1955; Willets and Wong, 1980; Ekins *et al,* 2002)

Isolate	**Sclerotia**	
	Length (mm)	**Width (mm)**
08-5 A	2.24	1.36
09-8 A	1.72	1.16
09-8 E	1.72	1.08
09-8 Y	1.88	1.25
08-7 B	1.87	1.35
09-1 A	1.74	1.11
09-3 B	1.74	1.22
09-3 D	1.6	1.16
09-5 A	1.54	1.08
08-1	1.75	1.19
Minimum	0.3	0.2
Maximum	3.6	3.2
Mean (± σ)	**1.78** ± **0.71** × **1.19** ± **0.38**	

Table 4.3.2 A - Average size of sclerotia per isolate (σ = Standard deviation around the mean).

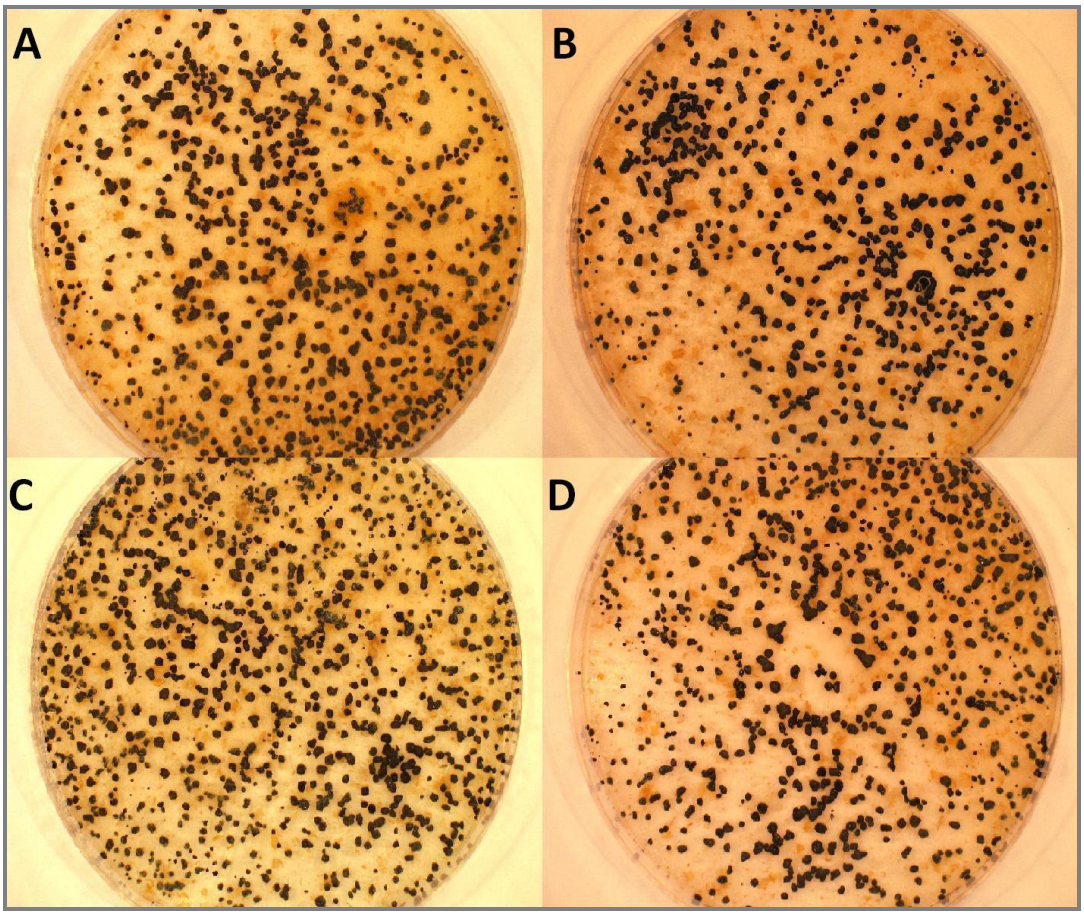


Plate 4.3.2.1 - Fungal isolates 09-8A **(A)**, 09-8Y **(B)**, 09-3D **(C)** and 08-1 **(D)** on potato dextrose agar (PDA) media plates (90 mm diameter).

Asci and ascospore morphology of isolates

Mean asci and ascospore dimensions for each isolate are shown where carpogenic germination occurred (Table 4.3.2 B). Across sporulating isolates mean asci length varied between 126.5-138.83 µm while width ranged between 8.25-11.00 µm. Seven isolates reported asci length and width measurements within limits described for *S. minor* by Purdy (1955), Willets and Wong (1980) and more recently Ekins *et al* (2002). Except for a marginally lower mean asci length of 126.5 µm, the remaining isolate (09-3D) displayed asci dimensions within the documented range. Mean ascospore length ranged between 11.88-15.75 µm amongst isolates, with average widths varying from 5.83-8.85 µm. Across all asci measured mean length was 134.21 µm and width 9.21 µm, while mean ascospore dimensions of 13.51 µm and 7.57 µm were reported for length and width respectively (Table 4.3.2 B). Averaged across the eight isolates which underwent carpogenic

germination of the ten evaluated, all mean asci and ascospore measurements were within or marginally greater than those reported of Australian isolates of *S. minor*, and all well above the generally smaller dimensions of asci and ascospores documented for *S. sclerotiorum* (Ekins *et al,* 2002). Furthermore, this demonstrates 80% of isolates tested were able to carpogenically germinate and produce viable ascospores in culture (Plate 4.3.2.2), these isolates being representative of all isolates collected from diseased pyrethrum flowers and in-field apothecia tentatively identified as *S. minor*. This may suggest conditioning of sclerotia during this experiment provided a suitable environment for most, but not all isolates to carpogenically germinate, or that only a majority of isolates of *S. minor* obtained from Tasmanian pyrethrum fields and evaluated in this study were able to germinate carpogenically even under favourable conditions.

Isolate	**Asci**		**Ascospore**	
	Length (µm)	**Width (µm)**	**Length (µm)**	**Width (µm)**
08-5 A	134.25	8.25	13.13	7.75
09-8 A	138.25	9.23	14.38	5.75
09-8 E	137.75	8.75	15.75	8.75
09-8 Y	138.33	9.23	11.88	5.83
09-3 B	132.25	8.88	14.79	8.85
09-3 D	126.5	8.83	13.04	7.88
09-5 A	131.5	9.5	13.18	7.63
08-1	134.88	11	11.98	8.13
Minimum	105	5	7.75	3.75
Maximum	155	12.5	22.5	12.5
Mean (± σ)	**134.21 ± 12.33 × 9.21 ± 2.04**		**13.51 ± 2.45 × 7.57 ± 1.7**	

Table 4.3.2 B - Mean asci and ascospore size of isolates undergoing sexual reproduction (σ = Standard deviation around the mean).

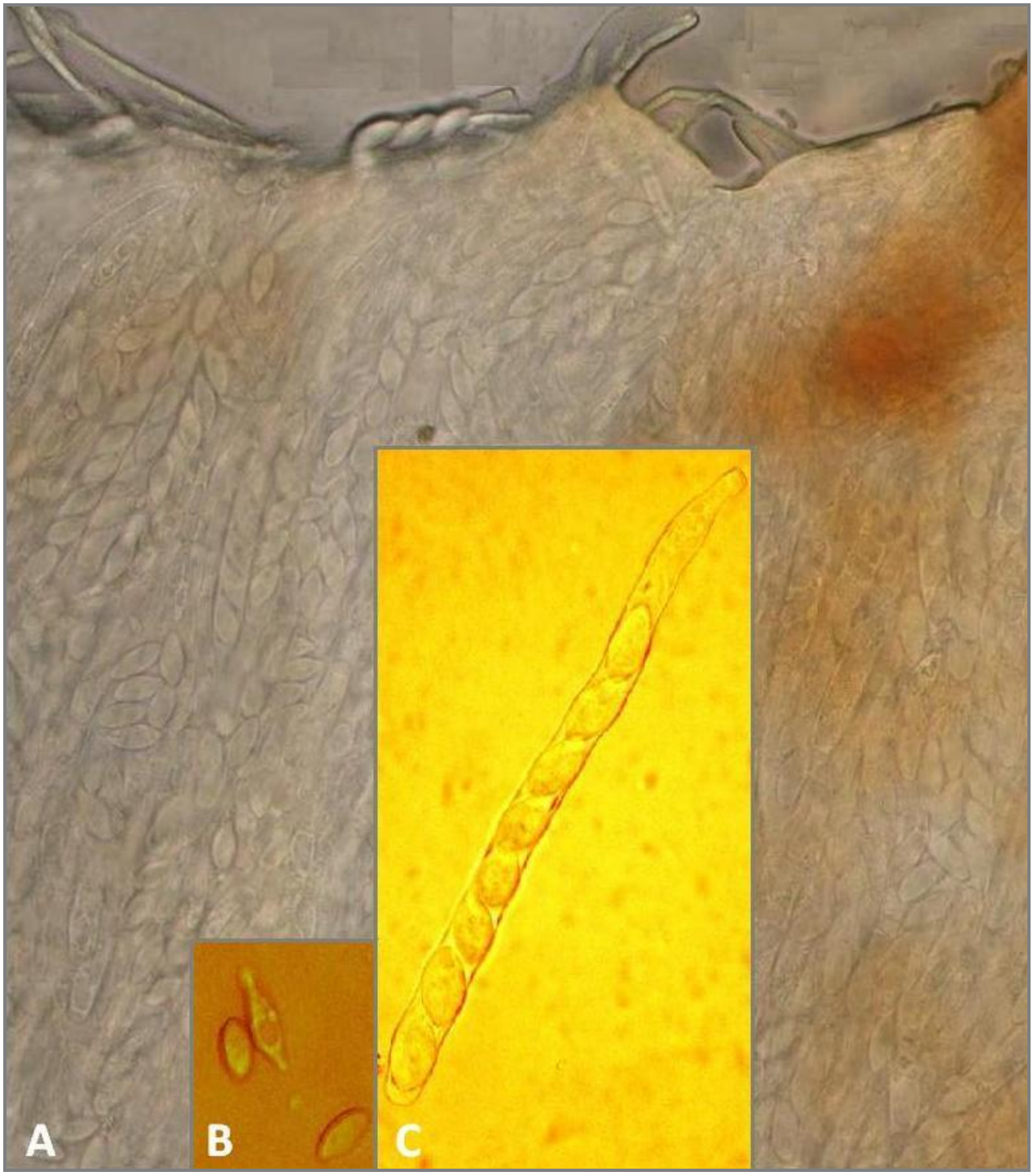


Plate 4.3.2.2 - (A) Panorama of section of apothethial disc with asci and ascospores visible with **(B)** ascospore with developing germ tube and **(C)** individual asci containing eight ascospores inset (All at 1000 × magnification)

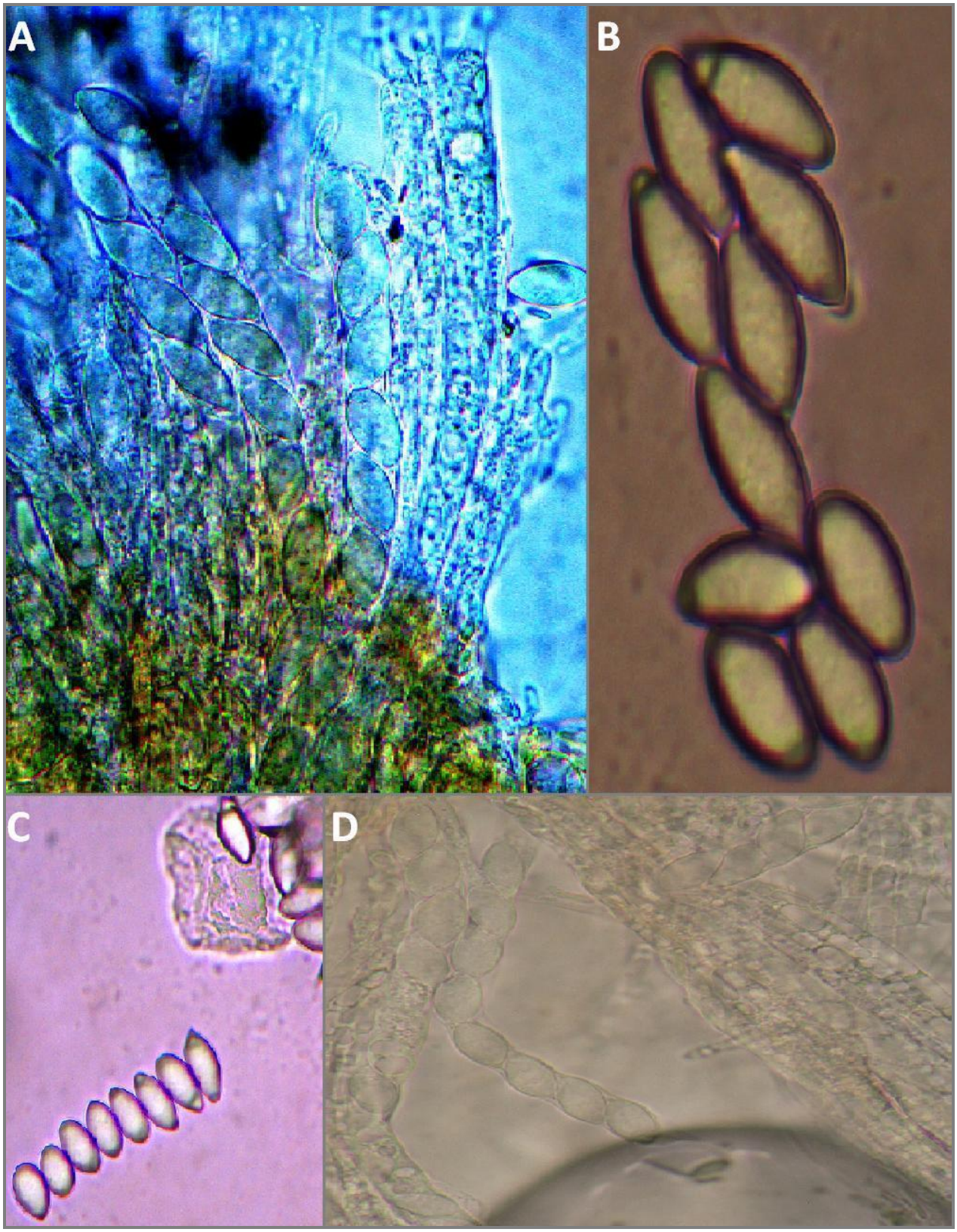


Plate 4.3.2.3 - Isolate 08-1; (A) Numerous asci containing ascospores attached to apothecial disc**, (B)** cluster of ascospores in solution, **(C)** eight ascospores floating free in solution, **(D)** asci with ascospores visible (All at 1000 × magnification).

Ascospore viability

Germination rates varied between 88-100% across all isolates, while mean ascospore viability of isolates ranged between 93.3-99.3% (Table 4.3.2 C). Spore germination rates of all carpogenically germinating isolates were consistently high after 1 day incubation at 20°C, averaging 96.3% across all isolates.

Replicate	Isolate							
	08-5A	**09-8A**	**09-8E**	**09-8Y**	**09-3B**	**09-3D**	**09-5A**	**08-1**
1	98	90	90	92	99	97	99	100
2	97	94	91	95	99	100	96	100
3	100	97	90	97	100	96	99	99
4	100	95	99	95	99	93	95	99
5	99	94	95	94	100	88	96	100
6	94	98	92	91	98	96	98	97
7	98	99	100	93	99	90	98	99
8	100	96	92	96	100	92	99	99
9	99	94	89	99	100	93	95	100
10	97	97	95	95	94	89	98	100
Mean (per isolate)	98.2	95.4	93.3	94.7	98.8	93.4	97.3	99.3
Minimum = 88								
Maximum = 100								
Mean (± σ) = 96.3 ± 3.33								

Table 4.3.2 C - Mean ascospore germination rates of sporulating isolates after 24 hours incubation on PDA media plates and minimum, maximum and mean germination across all plates (σ = Standard deviation around the mean).

4.3.3 Genetic (sequencing) identification of fungal isolates

Sequencing results of described ITS regions (Appendix 4) of the ten selected isolates were compared to the nucleotide sequence database (GeneBank) reference data for representative sequences of *S. minor*, *S. sclerotiorum* and *S. trifoliorum* (Accessed on 20/05/2011 via the National Centre for Biotechnology Information website, http://www.ncbi.nlm.nih.gov/).

Phylogenetic analysis of the ITS region indicated that sequences of *S. trifoliorum* and *S. minor* formed two separate groups (Figure 4.3.3). Sequences of *S. sclerotiorum* were more variable, but grouped separately from S*. trifoliorum* and *S. minor* (Figure 4.3.3). Sequences from all isolates obtained from pyrethrum and identified morphologically as *S. minor* grouped closely with other *S. minor* sequences on Genebank. This provided further evidence that these isolates were *S. minor*.

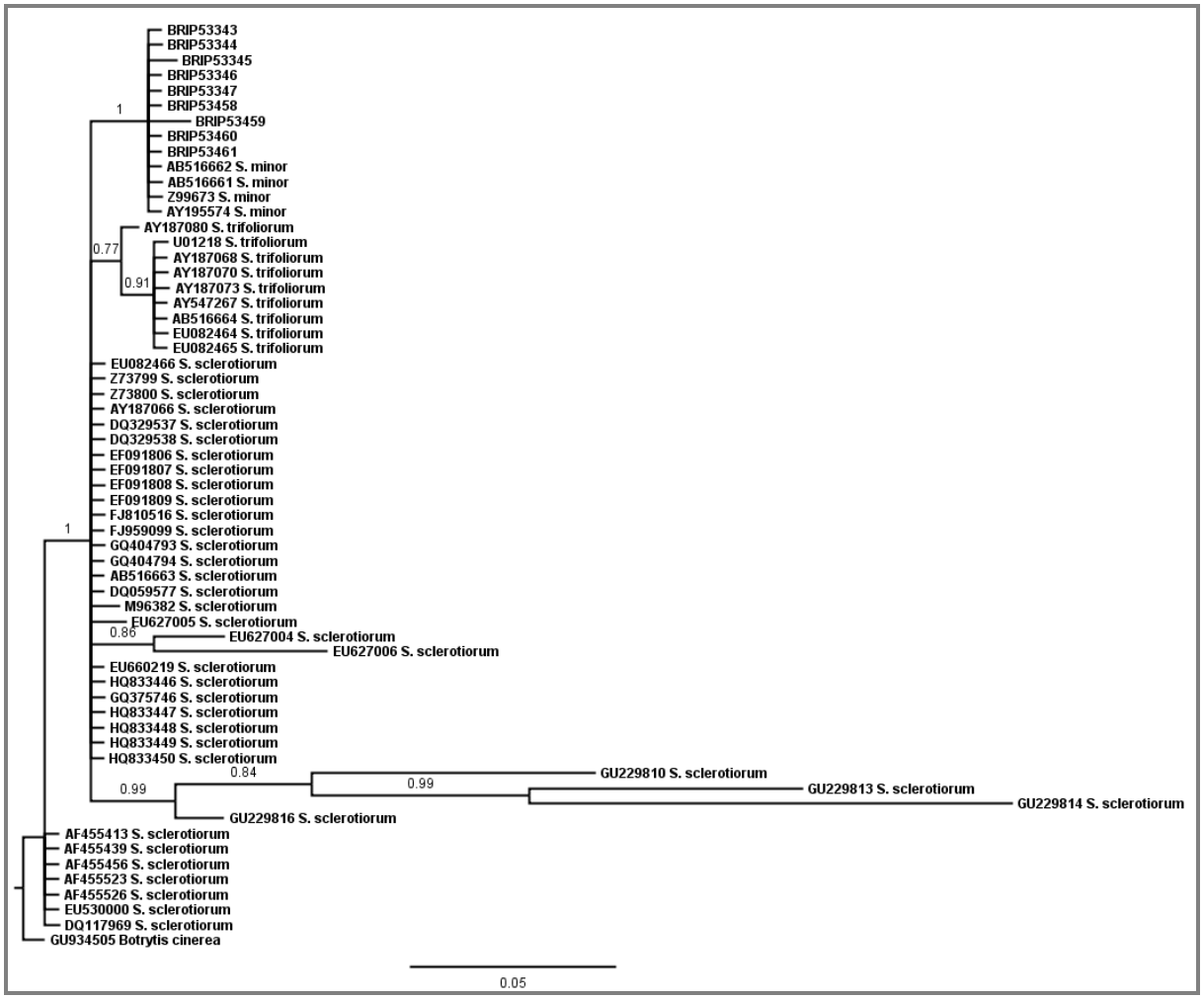


Figure 4.3.3 - Phylogenetic tree based Bayesian inference (BI) of ITS1, 5.8s and ITS2 genetic region of *Sclerotinia* spp. Sequences labelled with a species designation were obtained from the GeneBank database, with the preceding code indicating the GeneBank accession number. Sequences without species designation were sequenced in this study, i.e. those preceded by 'BRIP'. Sequences were aligned using Clustal W v1.4 (Thompson *et al*, 1994), and manually cropped to the limits of the smallest available sequence. BI was conducted on this alignment with MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003), using the predefined Felsenstein model with invariant sites (F81+I; Felsenstein, 1981), selected as the model of best fit by MrModelTest v2 (Nylander, 2004). BI was undertaken using two concurrent sets of four chains (one cold, three heated) for 1,000,000 generations with sampling every 100 generations. At completion, log-likelihood values for each set were compared visually to select the proportion of values to be burnt in, thus ensuring that stationarity of the BI was achieved; burn-in was determined to be 10% of samples (1000 samples). Branch values indicate posterior probabilities for each clade, with a value of 0.95 or greater considered significant (Leache & Reeder, 2002). Tree is rooted using a single isolate of *B. cinerea*. Scale bar indicates expected rate of substitutions per site.

4.3.4 *In vitro* sensitivity of *S. minor* to fungicides

For the seven isolates screened against tebuconazole and carbendazim, means EC_{50} values of 0.009 and 1.92 µg a.i./ml were recorded respectively (Appendix 4.3.4). EC_{50} values of isolates of *S. minor* to iprodione ranged between 0.08-1.22 µg a.i./ml. with a mean of 0.3 µg a.i./ml. (Appendix 4.3.4), with 97% of isolates reported EC_{50} values of <1 µg a.i./ml. The frequency distribution of sensitivities suggested a unimodal distribution (Figure 4.3.4).

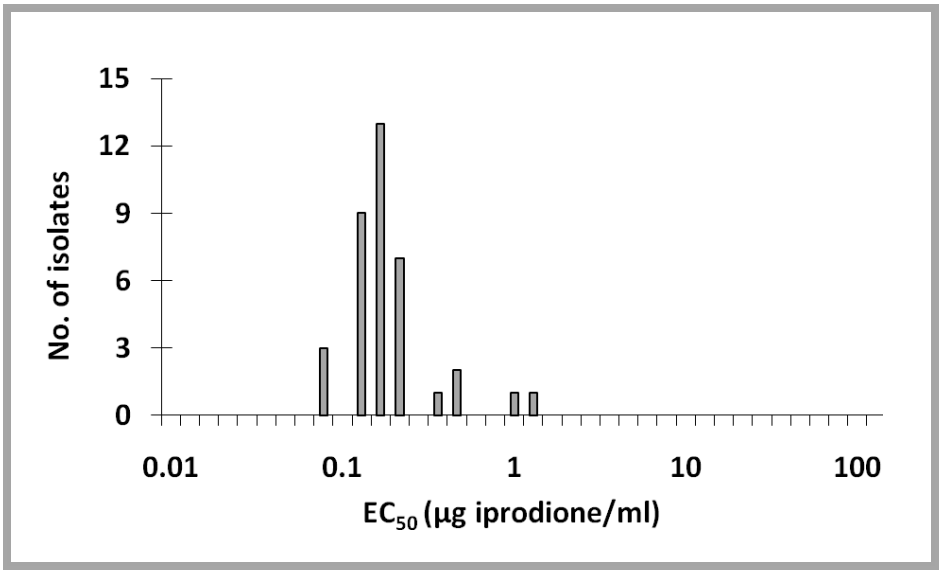


Figure 4.3.4 - Frequency distribution of iprodione EC_{50} values for *S. minor* isolates.

4.4 Discussion

Over three successive pyrethrum harvests, 46 fungal isolates displaying sclerotial size and growth habit in plate culture consistent with that reported within literature for *S. minor* were collected from Tasmanian pyrethrum fields. Thirty eight of these were isolated from diseased pyrethrum flowers, seven from diseased foliage and one from apothecial ascospores. Isolates were obtained from numerous cultivation districts in North-west Tasmania (Table 4.2.1 A). Isolates were obtained from several districts in North-west Tasmania. This indicates the presence of *S. minor* in flowers of Tasmanian pyrethrum crops and suggests the potential involvement of this fungus in

flower disease, with inoculum known present in pyrethrum fields causing annual crown rot disease epidemics (MacDonald, 1995) as well as white mould of green bean crops in Tasmania (DPIWE, 2010b).

Fungal growth in plate culture and measured dimensions of sclerotia, asci and ascospores during *in vitro* studies provided considerable evidence supporting the identity of isolates from pyrethrum flowers as *S. minor.* Sequencing of the ITS region of isolates provided further evidence for identification as *S. minor*. The ITS region of *S. sclerotiorum*, *S. minor*, *Sclerotinia trifoliorum* and *Botrytis cinerea* has a high degree of sequence similarity (e.g. Qin *et al*. 2010). However, phylogenetic analysis of the ITS region indicated that sequences of *S. trifoliorum* and *S. minor* formed two separate groups, and that while sequences of *S. sclerotiorum* were more variable, they grouped separately from S*. trifoliorum* and *S. minor*. Sequences from all isolates obtained from pyrethrum identified morphologically as *S. minor*, grouped with other sequences of *S. minor* on Genebank and separate to *S. trifoliorum*, *S. sclerotiorum* and *B. cinerea*. This provided further evidence that these isolates were *S. minor*.

Given that pyrethrum flowers are generally produced some 0.8-1.5 m above the soil level, the presence of *S. minor* in flowers most likely requires carpogenic germination of sclerotia and production and release of wind-borne ascospores. Reports of carpogenic germination of *S. minor* and the role of ascospores in plant disease are rare, although Ekins *et al* (2002) predicted the potential for carpogenic germination of *S. minor* in Southern Australia by CLIMEX modeling. In my study, small sclerotia were observed to carpogenically germinate and produce ascospores in one Tasmanian pyrethrum field late during the flowering period of 2008. Subsequent isolation of fungal cultures from ascospores confirmed the identity of the fungus as *S. minor*. This therefore demonstrated the occurrence of the perfect stage of *S. minor* in the field, with production of airborne inoculum during flowering required to achieve flower infection.

The potential for carpogenic germination was not uncommon in isolates of *S. minor* from pyrethrum, as of the ten isolates selected for more detailed evaluation during these studies; eight successfully underwent all stages of carpogenic germination in the laboratory. A further isolate

produced only stipe initials and the remaining isolate showed no sign of the teleomorph stage developing. Ascospores of high mean germination rates were obtained from apothecia harvested from all sexually reproducing isolates. This demonstrates the ability of most isolates of *S. minor* from pyrethrum fields tested to undergo carpogenic germination *in vitro*, and produce ascospores of high viability. Conditioning of sclerotia during these studies provided an environment sufficient for the majority of isolates to produce airborne inoculum potentially able to infect pyrethrum flowers and other above ground plant tissue. Temperature ranges used during sclerotial conditioning were within optimum limits (11-17°C) reported for apothecial ontogeny of *S. minor* (Ekins *et al,* 2002). These temperature ranges are also observed within mean maximum and minimum monthly temperatures recorded at Forthside research station during spring and early summer over forty five years data collection (Appendix 4.2), located almost centrally amongst Tasmanian pyrethrum cultivation districts. On the basis of these studies, this indicates most isolates of *S. minor* from flowers in Tasmanian pyrethrum fields are capable of carpogenic germination under local conditions often observed during flower anthesis. However, even given optimum environmental conditions not all isolates of *S. minor* from Tasmanian pyrethrum fields will undergo carpogenic germination for reasons undetermined.

Despite a high proportion of isolates of *S. minor* being capable of carpogenic germination in the laboratory with subsequent production of large number of viable ascospores, *S. minor* was isolated from flowers in the field at relatively low frequency in comparison to *S. sclerotiorum* (Table 4.4). Carpogenic morphology and phylogeny confirmed fungal identity for a subset of isolates, suggesting tentative identification as *S. minor* was valid and the estimated incidence in flowers accurate (Table 4.4). *S. sclerotiorum* was present in all (25) fields sampled during the mycofloral survey, with a mean incidence near harvest of 25.8% across all sites. *S. minor* was identified on the basis of sclerotial size on flowers in humid boxes from 15 of 25 (66.7%) pyrethrum fields from which flowers were sampled, with lower mean incidence of 4.36% (Table 4.4). Subsequently, the 39 isolates of *S. minor* obtained from diseased flowers identified on the basis of cultural morphology and from which a subset were further characterized was from seven of twenty five fields (28%) surveyed in total. This suggests that carpogenic germination of *S. minor* in the field was quite prevalent, but within fields may not be common, even though environmental conditions appear favourable and *S. minor* is a common cause of crown rot in pyrethrum fields in Tasmania suggesting sclerotial inoculum would be present. The apparent rareness of apothecia of *S. minor* compared to *S. sclerotiorum* could be partially due to a more stringent temperature range required for *S. minor*.

Sclerotinia sclerotiorum produced apothecia over a temperature range from 4°C (Smith and Boland 1989) to 30°C (Huang and Kozub 1993). The temperature range for apothecia development for *S. minor* was from 11 to 17°C (optimum 15°C) and a few hours at 20°C or higher was sufficient to inhibit stipe initiation (Hawthorne 1976). Hence, this would suggest apothecia production for *S. minor* during summer in Tasmania is likely to occur only in cooler seasons.

The isolation and identification of *S. minor* from surface sterilised pyrethrum flowers suggest *S. minor* as a possible cause of flower disease. *In vitro* fungicide sensitivity testing of isolates from diseased flowers identified as *S. minor* reported EC_{50} values which were more sensitive to tebuconazole than the baseline sensitivity reported for *Sclerotinia* sp. of 0.013 µg a.i./ml. (Hsiang *et al,* 1997). A mean EC_{50} value of 1.92 µg a.i./ml was measured for carbendazim (Appendix 4.3.4), and at more than ten times the reported baseline of 0.16 µg a.i./ml (Qian and Fox, 1994) may indicate a level of resistance present to this fungicide. Compared to the baseline sensitivity value of iprodione reported from isolates causing *S. minor* peanut blight in Virginia (USA) of 0.5 µg a.i./ml (Smith *et al,* 1995), isolates from pyrethrum flowers were highly sensitive to this fungicide suggesting the flowering fungicide program may provide some level of control for potential flower disease caused by this fungus.

Year	Site	Incidence 2-4 Dec.		Incidence 14-22 Dec.	
		S. minor	***S. sclerotiorum***	***S. minor***	***S. sclerotiorum***
2008-09*	08-1	5	17	1	9
	08-2	4	8	0	2
	08-3	1	15	1	8
	08-4	1	14	0	14
	08-5	5	18	13	23
	08-6	1	8	0	36
	08-7	7	20	4	50
	08-8	2	27	1	21
	08-9	5	20	0	27
	08-10	7	11	14	59
2009-10**	09-9	0	24	0	6
	09-10	0	58	0	25
	09-8	34	69	60	60
	09-1	0	29	1	33
	09-2	1	12	1	20
	09-3	0	23	10	9
	09-4	0	36	0	57
	09-5	2	13	3	30
	09-15	0	17	0	35
	09-16	0	11	0	48
	09-6	0	6	0	18
	09-7	0	11	0	8
	09-13	0	8	0	28
	09-11	0	22	0	10
	09-12	0	8	0	10
Mean		**3**	**20.2**	**4.36**	**25.84**
Minimum		**0**	**6**	**0**	**2**
Maximum		**34**	**69**	**60**	**60**
Standard deviation (σ)		**6.87**	**15.10**	**12.28**	**17.69**

*Flowers sampled on 4 and 22 December.

**Flowers sampled on 2 and between 14-18 December.

Table 4.4 - Comparative incidence of *S. minor* and *S. sclerotiorum* from nontreated plots during 2008-09 and 2009-10.

Conclusions

Fungal cultures tentatively identified as *S. minor* were isolated from diseased pyrethrum flowers, with fungal identity later confirmed using morphological, reproductive, genetic and phylogenic analysis.

Carpogenic germination of sclerotia of *S. minor* was observed in one pyrethrum field during flowering, constituting the first such report of sexual reproduction and contribution toward airborne ascospore inoculum of *S. minor* in Australian agriculture. Furthermore, 8 of 10 isolates of *S. minor* selected for further study completed carpogenic germination *in vitro* and produced ascospores of high viability

Isolates of *S. minor* were relatively insensitive to inhibition of mycelial growth by the fungicide carbendazim, while tebuconazole and iprodione effectively inhibited growth in agar plate tests.

5. Greenhouse flower inoculation studies: Effect of *B. cinerea, S. sclerotiorum* and *S. minor* spore inoculation on disease symptoms, flower development, and yield and assay.

5.1 Introduction

Flower diseases of pyrethrum in Tasmania caused by the fungal pathogens *B. cinerea* and *S. sclerotiorum* have been documented as affecting flower yields adversely for some years (MacDonald, 1995). Despite this, little specific information exists detailing the effect infection by flower disease pathogens may have on flower yields and pyrethrin content, either separately or as a combined flower disease epidemic. No quantitative data presently exists either regarding effect of flower infection on yield aspects such as dry flower weight and pyrethrin assay in comparison to healthy flowers harbouring no inoculum. Whether the effect of flower inoculation on measured yield qualities and flower development varies among commercial pyrethrum cultivars grown in Tasmania is also unknown.

Field investigations into the effects of fungicide applications on floral disease incidence *(B. cinerea* and *S. sclerotiorum)* as well as yield attributes was described in chapters 2 and 3 of this study. This provided some comparison of yield between areas of higher disease incidence receiving no fungicide spray applications, and fungicide treated plots with significantly lower disease incidences. However, flower infection and disease development was uncontrolled in commercial crops, relying on seasonal disease resulting from inoculum levels and environmental conditions. Results from these studies were variable, although some significant linear relationships between incidence of *B. cinerea* and *S. sclerotiorum* and yield, and yield components were obtained. However, as demonstrated in chapter 1, several other fungi are present in pyrethrum flowers and may also be affecting yield or quality. The variability of the field studies highlighted the necessity to assess the pathogenicity of individual fungi suspected as pathogens of pyrethrum flowers under more controlled conditions.

The confirmation of *S. minor* being isolated from surface-sterilised flowers in Tasmanian pyrethrum fields (Chapters 1 and 4) and the demonstration of carpogenic germination and production of viable ascospores *in vitro* suggest this fungus may also be a pathogen of pyrethrum flowers. However, confirmation of pathogenicity through Koch's postulates is required.

More recently it has been noted that *S. sclerotiorum* flower blight in Tasmania causes infected flowers to fragment upon harvest, resulting in decreased flower yields as a portion of material remains infield (Pethybridge *et al,* 2010). Further information on whether flower infection by *S. sclerotiorum* or *B. cinerea* effects flower development or reduces moisture content leading to drier, potentially more fragile flowers would therefore be of interest.

Grape infection by *B. cinerea* often results in development of 'noble rot' with favourable conditions, a disease able to cause significant desiccation of grapes (Elad *et al,* 2004). Infection by *B. cinerea* was reportedly the foremost cause of premature flower senescence of blackcurrant flowers (Sharon *et al,* 2004), also resulting in apricot flower desiccation and abscission (Faes and Staehelin, 1923). Premature development and ripening of plant reproductive structures is often observed during *S. sclerotiorum* plant diseases (Willets and Wong, 1980), while disintegration of sunflower heads from head rot caused by *S. sclerotiorum* and can reduce yields of seed harvested (Gregiore *et al,* 2000). Pethybridge *et al* (2010) suggests drier pyrethrum flower heads may be more likely to disintegrate prior to or during cutting and harvest, failing to be removed. Whether infections of *B. cinerea, S. sclerotiorum* and *S. minor* of pyrethrum flowers affect flower development or moisture content, and the potential effect on harvested flower yields in Australian crops from disintegration of flowers is unknown.

Objectives of these studies were therefore to evaluate:

- The effect of flower inoculation with conidia of *B. cinerea* on yield, assay and flower development during anthesis.
- The effect on yield, pyrethrin concentration and flower ontogeny of flowers inoculated with ascospores of *S. sclerotiorum.*
- If the effect of flower inoculation varied significantly among plant cultivars.
- Whether flower inoculation with ascospores of *S. minor* resulted in visible symptoms consistent with flower disease in comparison to nontreated flowers.
- If pure cultures of *S. minor* could be reisolated from pyrethrum flowers displaying symptoms of flower blight after inoculation with ascospores of *S. minor*, demonstrating the completion of Koch's postulates and pathogenicity towards pyrethrum flowers.

5.2 Materials and methods

Pyrethrum plants of four cultivars and approximately twelve months in age were obtained from commercial pyrethrum fields for inoculation with spore suspensions of *B. cinerea* and *S. sclerotiorum*. Plants of one variety were additionally obtained for inoculation with ascospores of *S. minor* (Table 5.2.1). Plants of each variety were transplanted into 8 litre pots and maintained in a greenhouse at day and night time temperature regimes of between 21-24°C and 12-18°C respectively. A relative humidity of between 75-85% was maintained in the greenhouse.

Production of inoculum

Five isolates were randomly selected from all isolates of *B. cinerea* obtained from diseased pyrethrum flower samples across numerous cultivation districts in North West Tasmania (Table 5.2.2). Mycelial plugs of *B. cinerea* in storage were revived on PDA plates and incubated in darkness at 20°C for approximately four weeks or until profuse conidial growth covered the entire plate surface. Plates were then flooded with sterile water and gently agitated, before transferring inoculum suspensions into 100 ml Schott bottles through a 10 μm sieve to remove mycelial detritus.

Mycelial plugs of *S. sclerotiorum* were removed from cryostorage and incubated on PDA plates at room temperature. After twelve weeks stipe initials formed on mature sclerotia, further developing into mature apothecial discs. Apothecia were cut from sclerotia with sterile implements and placed in sterile glass McCartney bottles containing distilled, autoclaved water. Bottles were then agitated to thoroughly disperse ascospores from apothecia. Mature apothecia from selected isolates of *S. minor* sexually reproducing *in vitro* were cut from sclerotia with sterile implements, and placed in sterile 20 ml McCartney bottles containing between 1-4 ml of sterile water based on number of apothecia per isolate. Bottles were then agitated to disperse ascospores in suspension.

All bottles containing inoculum were agitated again immediately prior to estimating conidia or ascospore numbers. Spore suspensions of each isolate were pipetted into a Marienfeld haemocytometer and concentrations estimated with a Zeiss Axiolab compound microscope at 400 × magnification. A 100 μl aliquot of suspension was pipetted into each of ten replicate PDA plates per isolate and spread with a sterile glass rod to test viability of spores. Plates were taped shut with

cling film and incubated on the bench at room temperature for 24 h. Spores were assessed using a Zeiss compound microscope at 100 × magnification and 100 spores per plate observed for germination. Spores were considered germinated if the germ tube was greater than half the length of the spore after 24 h incubation.

Spore suspensions used in all flower inoculation experiments were diluted to concentrations ranging between 3.75×10^4 - 6.04×10^4 per millilitre, falling within limits reported in to achieve successful infection (Bautista-Banos *et al,* 2001; Harikrishana and del Rio, 2006). Spore suspensions of *B. cinerea* and *S. sclerotiorum* used in inoculations were mixed preparations of inoculum, each containing five isolates (Table 5.2.1). *S. minor* isolate 09-3B produced mature apothecia and viable ascospores before other isolates during *in vitro* studies described in chapter 4, and was used to inoculate a number of small pyrethrum plants available on hand in a preliminary experiment. Fourteen flowers were inoculated with ascospores while eleven were dipped in sterile water. Following preliminary inoculation with one isolate of *S. minor*, eight isolates of *S. minor* underwent carpogenic germination concurrently producing mature apothecial discs. These sporulating isolates were separated into two groups of four, each containing similar ascospore numbers. Isolates of each group were combined in separate 100 ml Schott bottles labelled 'Inoculum A' and 'Inoculum B', and diluted to 30 ml total volume. Estimated number of spores per millilitre and average germination rates for each isolate and inoculum preparation are shown (Tables 5.2.2 and 5.2.3).

Flower inoculation

All flowers were of similar early maturity when treated, having between 0-30% of disc florets open at time of inoculation. Strung jewellers tags (Esselte, Australia) measuring 21 × 13 mm were used to identify flowers individually on replicate plants throughout the duration of greenhouse trials. Inoculum of *S. sclerotiorum* was applied to flowers by pipetting and gently rubbing 0.15 µl of ascospore suspension onto the flower surface. Inoculum was limited in quantity and applied only to disc florets. Flower inoculation with inoculum of *B. cinerea* and *S. minor* proceeded via individually dipping flowers into spore suspension contained in a 25 ml beaker. Each flower received between 0.6-0.7 ml of inoculum across both disc and ray florets in the process, the beaker rinsed with distilled water and inoculum refreshed between each replicate plant.

Flower and plant numbers per treatment and variety in addition to estimated spore numbers applied to individual flowers on replicate plants are summarised (Table 5.2.2) Nontreated flowers (control treatments) for each greenhouse inoculation experiment underwent the same process with identical volumes of sterile, distilled water. After inoculation, plants were incubated under high humidity for two days by covering pots with plastic bags with stakes positioned to prevent flower contact with plastic. Thereafter, overhead misting irrigation equivalent to 25 mm of precipitation was applied twice weekly after inoculation to encourage disease development (Plate 5.2).

Plate 5.2 - Variety trial of flower inoculation with ascospores of *S. sclerotiorum*, overhead misting irrigation in operation.

Assessment

Two weeks after flower inoculation, five flowers per plant were harvested to determine efficacy of spore suspension inoculations. Flowers were surface sterilised and incubated in humid boxes as described in chapter 2 materials and methods. After 15-20 days incubation, flowers were microscopically assessed for presence of mycelium, conidiophores or sclerotia consistent with that of *B. cinerea, S. sclerotiorum* and *S. minor*. For assessment of flower yield and assay from treatments

of *B. cinerea* and *S. sclerotiorum*, ten flowers from each replicate plant per variety were harvested. Each replicate of ten flowers was weighed fresh, oven dried at 60°C for 2 days and reweighed to obtain dry weight. Flowers were then ground into a fine powder with pestle and mortar in preparation for laboratory pyrethrin assay evaluation as described previously (Chapter 2.2). Flower maturity was assessed at weekly intervals after treatment with inoculum or sterile water. Flowers were categorised for maturity (FMS), with flowers from treatments harvested once 50% or more flowers reached an FMS value of seven (Plate 1.2.2). Flowers of this developmental stage closely reflect the maturity of pyrethrum flowers observed immediately prior to harvest in commercial fields, and therefore a suitable indicator at which to conclude greenhouse experiments. Only fresh flower weights were recorded during inoculation studies with *S. minor*, with flowers incubated to determine success of inoculation.

At fourteen days following treatment, flowers inoculated with spores of *B. cinerea* and *S. sclerotiorum* were observed for visible symptoms of flower disease. Flowers treated with ascospore suspensions of *S. minor* were photographed and assessed for visual differences among treatments and flower disease symptoms at seven day intervals for two weeks after inoculation. Immediately prior to final assessment of inoculation experiment B with *S. minor*, all flowers on plants in excess of five treated or five control flowers were removed to allow a visual comparison between all plants of both treatments as shown (Plate 5.3.1.8).

Flower pathogen	Greenhouse experiment	Plant variety	No. plants per treatment	Flowers per plant inoculated	Est. number spores applied per flower	Mean (%) spore viability
Botrytis cinerea	Variety trial	Pyoneer Pyper Pyrate RS7	* * * 10	* * * 15	404,680	98.2
Sclerotinia sclerotiorum	Variety trial	Pyoneer Pyper Pyrite RS5	10 10 10 10	15 15 15 15	6,150	95.4
Sclerotinia minor	Preliminary inoculation	mixed	10	1-3	262,500	95.9
	Inoculation A Inoculation B	BRA 1 BRA 1	10 10	5 5	284,000 292,000	97.4 95.2

*high plant mortality from apparent crown rot disease.

Table 5.2.1 - Summary of cultivar, plant and flower number per treatment as well as estimated number of conidia or ascospores applied to individual flowers.

Pathogen	Trial	Isolate	No. of apothecia	Distilled H_2O (ml)	Conc. per ml	Estimated total no.	Spore suspension (ml)		
							Total volume	Approx. conc.	per flower
Botrytis cinerea	***Botrytis***	07-1C 07-5A 07-3B 07-10D 07-7J	na na na na na	20 20 20 20 20	8.5×10^4 4.5×10^4 5.8×10^4 7.6×10^4 3.8×10^4	1.7×10^6 9×10^5 1.16×10^6 1.52×10^6 7.6×10^5	100	604,000	0.67
Sclerotinia sclerotiorum	**Variety trial**	07-6F 07-10F 07-2A 07-3D 07-7C	4 2 2 3 3	2.2 2.2 2.2 2.2 2.2	7.5×10^5 2.5×10^5 3.5×10^5 4×10^5 3×10^5	1.65×10^6 5.5×10^5 7.7×10^5 8.8×10^5 6.6×10^5	11	410,000	0.015
Sclerotinia minor	**Preliminary Inoculation**	09-3B*	10	10	3.75×10^4	3.75×10^5	10	375,000	0.7
	Inoculation A	08-5 09-8Y 09-5A 08-1	9 5 6 2	4 2 3 1	2.15×10^4 8.5×10^3 1.15×10^4 4.5×10^3	8.6×10^4 1.7×10^4 3.45×10^4 4.5×10^3	30	473,333	0.6
	Inoculation B	09-8A 09-8E 09-3B 09-3D	4 1 10 6	2 1 4 3	8.5×10^3 6.5×10^3 2.35×10^4 9.5×10^3	1.7×10^4 6.5×10^3 9.4×10^4 2.85×10^4	30	486,667	0.6

*preliminary inoculation experiment**.**

Table 5.2.2 - Concentrations of ascospore and conidial suspensions used for flower inoculation.

Pathogen	Isolate	Replicate										mean
		1	2	3	4	5	6	7	8	9	10	
Botrytis cinerea	07-1C	100	99	99	99	100	100	100	99	99	100	99.5
	07-5A	99	99	98	99	100	100	97	98	98	99	98.7
	07-3B	100	100	100	100	100	100	100	100	100	100	100
	07-10D	96	97	95	91	93	95	94	94	90	93	93.8
	07-7J	98	98	99	100	100	99	98	99	99	100	99
Sclerotinia sclerotiorum	07-6F	88	89	88	83	90	81	89	88	91	92	87.9
	07-6F	92	89	88	87	90	95	89	88	89	92	89.9
	07-10F	99	100	100	98	96	95	99	99	100	98	98.4
	07-2A	89	94	96	100	99	93	94	96	99	98	95.8
	07-3D	97	97	99	100	98	99	98	100	100	100	98.8
Sclerotinia minor	07-7C	93	91	90	96	90	99	97	95	98	92	94.1
	08-5	98	97	100	100	99	94	98	100	99	97	98.2
	09-8A	90	94	97	95	94	98	99	96	94	97	95.4
	09-8E	90	91	90	99	95	92	100	92	89	95	93.3
	09-8Y	92	95	97	95	94	91	93	96	99	95	94.7
	09-3B	99	99	100	99	100	98	99	100	100	94	98.8
	09-3B*	97	91	94	99	100	94	92	96	98	98	95.9
	09-3D	97	100	96	93	88	96	90	92	93	89	93.4
	09-5A	99	96	99	95	96	98	98	99	95	98	97.3
	08-1	100	100	99	99	100	97	99	99	100	100	99.3

*preliminary inoculation experiment

Table 5.2.3 - Germination viability of spore suspensions.

5.3 Results

5.3.1 Inoculation and disease symptoms

Flower inoculation

Artificial flower infection with conidial and ascospore suspensions was successful, with high numbers of inoculated flowers having conidiophores, mycelium or sclerotia consistent with *B. cinerea, S. sclerotiorum* or *S. minor* after incubation (Table 5.3.1). Flower infection with *B. cinerea* resulted in conidiophores morphologically consistent with *B. cinerea* on 94% of inoculated flowers, with nontreated or control plants showing only 5% incidence of this pathogen. The incidence of *S. sclerotiorum* identified from ascospore inoculated flowers after incubation averaged 95% across

cultivars, with nontreated plants having low incidences averaging ≤1.5% among varieties. *S. sclerotiorum* was identified by having conspicuously larger sclerotia than *S. minor* (Plate 1.3.2), while the smaller size and different colour of sclerotia of *B. cinerea* described previously allowed differentiation between fungal pathogens (Chapter 1.2, plate 1.2.1).

Flower inoculation with *S. minor* was also successful in each trial. Preliminary inoculation of a small number of flowers led to incidences of 78.6% and 18.9% for ascospore suspension and distilled water treatments respectively. Flowers inoculated with ascospores of *S. minor* in Inoculation Experiments 'A' and 'B' resulted in incidences of *S. minor* of 92% and 98% respectively, in comparison to control treatments which averaged 8% incidence in flowers after incubation (Table 5.3.1). Fifty flowers per treatment (five per plant) in Inoculation Experiments A and B were used to determine the incidence of *S. minor* in flowers after incubation. This strongly suggests ascospores of *S. minor* are able to infect developing flowers under environmental conditions similar to those observed in Tasmanian pyrethrum fields.

Fungal pathogen	**Greenhouse trial**	**Plant variety**	**Treatment (%)**	
			Inoculated	**Nontreated**
Botrytis cinerea	Variety trial	Pyoneer	*	*
		Pyper	*	*
		Pyrate	*	*
		RS7	94	5
Sclerotinia sclerotiorum	Variety trial	Pyoneer	98	4
		Pyper	94	0
		Pyrite	94	0
		RS5	94	2
Sclerotinia minor	Preliminary inoculation	mixed	78.6	18.9
	Inoculation A	BRA 1	92	10
	Inoculation B	BRA 1	98	6

*high plant mortality from apparent crown rot infection

Table 5.3.1 - Incidence of flower disease pathogens identified from treated, surface sterilised and incubated flowers collected fourteen days after inoculation.

Observed flower disease symptoms

Symptoms of flower disease indicated pipette and particularly dip inoculations with spore suspensions had been successful. Two weeks after inoculation with conidia of *B. cinerea*, most ray florets on numerous flowers had perished with the remaining few being necrotic and shrivelled in appearance. Many capitula had perimeters blackened with necrosis, these regions of dead tissue covering sizeable areas of the flower surface in some instances (Plate 5.3.1.2). As well as displaying these obvious signs of necroses, flowers inoculated with conidia had ≤30% of disc florets open. Flowers treated with sterile water also had approximately ≤30% disc florets open, but showed no signs of necroses and had most or all ray florets intact (Plate 5.3.1.1).

Plate 5.3.1.1 - (A) Flowers of pyrethrum variety RS7 treated with sterile water and **(B)** conidial suspension of *B. cinerea* of two weeks after treatment, cut for incubation.

Two weeks after inoculation with ascospores of *S. sclerotiorum,* many artificially infected flowers had necrotic areas of disc florets ranging from small to large in size while most or all ray florets were intact (Plate 5.3.1.2). Necrotic regions of tissue were light to dark brown in colour, appearing limited to where inoculum was applied to the flower surface by pipette. Nontreated flowers had most or all ray florets intact also, but showed no symptoms of necrosis or disease on any disc florets of all varieties (Plate 5.3.1.2).

Plate 5.3.1.2 - (A) Flowers 1-5 on plant 3 (control) and **(B)** flowers 1-5 on plant 5 eight days after pipette inoculation with ascospore suspension of *S. sclerotiorum*.

Fourteen days after preliminary inoculation of flowers with ascospore suspensions of *S. minor*, symptoms of flower disease were evident (Plate 5.3.1.3). Many, or all, ray florets on most artificially infected flowers were desiccated and stunted in appearance, while areas of brown to black necrotic disc florets covered significant areas of flower surfaces. Despite necrosis, most disc florets on some inoculated flowers remained unopened while on others necrosis appeared to have halted development of many flowers entirely. Some flowers treated with sterile water did show a minor loss of ray florets from possible disease, disc florets of all such nontreated flowers developing with no signs of pathogenic activity.

Plate 5.3.1.3 - After 14 days **(A)** nontreated flowers and **(B)** flowers inoculated with *S. minor.*

One week after flower treatment with ascospores of *S. minor* during Inoculation Experiment A, symptoms of disease began to develop. At this stage many ray florets on numerous flowers to which ascosporic inoculum was applied starting to shrivel or were partially desiccated. Small patches of light to dark brown necrosis were apparent on the surface of some artificially infected flowers, while nontreated flowers all had ray and disc florets of normal and healthy appearance (Plate 5.3.1.4). Two weeks after treatment the desiccation of most or all ray florets on the majority of inoculated flowers was far more pronounced, with some darkened in colour. Necrosis of disc florets covered small to large areas of flower surfaces ranging in colour from light brown to black (Plate 5.3.1.5). Most or all ray florets of flowers treated with sterile water remained intact at the conclusion of the experiment, with small areas of necrotic tissue observed in a few flowers.

Plate 5.3.1.4 - (A) Nontreated and **(B)** flowers inoculated with *S. minor* seven days after treatment.

Plate 5.3.1.5 - (A) Nontreated and **(B)** flowers inoculated with *S. minor* two weeks after treatment.

Inoculation Experiment B resulted in symptoms similar or more pronounced than experiment A from ascospores of *S. minor*. Seven days after treatment with sterile water or ascospore suspension, numerous ray florets of many flowers inoculated with *S. minor* ascospores were shrivelled and reduced in size with some having a noticeable brown tinge (Plate 5.3.1.6). Patches of disc florets on many artificially infected flowers had darkened to a light brown colour, these apparent early stages of necrosis often observed around the outer ring of pistilate florets. Ray and disc florets of flowers dip treated with water were generally healthy in appearance and intact at this time, with very few showing any symptom of disease (Plate 5.3.1.6). Two weeks after dip treatment the majority of ray florets of most flowers inoculated with *S. minor* were severely withered, with some tinged brown in colour or missing. Regions of disc florets displaying necrosis on many artificially infected flowers had darkened to a deep brown, were larger, and covered most carpelate florets on many flower heads (Plate 5.3.1.7). Some flowers had necrotic patches of disc florets similar to those observed from pipette inoculation of flowers with ascospores of *S. sclerotiorum*, however symptoms were far more pronounced during inoculation experiments using *S. minor*, likely due to significantly higher amounts of inoculum used. Fissures between severely desiccated and darkened disc floret regions of some inoculated flowers were also observed to have opened, with flower development all but ceased two weeks after infection. Most nontreated flowers were of generally healthy appearance two weeks post treatment, with most or all ray florets intact and approximately two thirds of disc florets open (Plate 5.3.1.7).

Plate 5.3.1.6 - After 7 days **(A)** nontreated flowers and **(B)** flowers inoculated with *S. minor*.

Plate 5.3.1.7 - After 14 days **(A)** nontreated flowers and **(B)** flowers inoculated with *S. minor*.

Visual comparison of nontreated and inoculated flowers before and after cutting showed obvious differences among treatments. Flowers on replicate plants not treated with inoculum or sterile water as well as all jewellers tags denoting flower and treatment were removed prior to comparison, leaving five flowers per plant. Plants were lined up in two rows of five, with noticeably fewer disc florets evident on flowers inoculated with *S. minor* than plants treated with distilled water observed from above (Plate 5.3.1.8). After cutting, flowers of each replicate plant were arranged sequentially across a sheet of A3 paper in ten rows, plant one in background and plant ten being in the foreground (Plate 5.3.1.8). In comparison to nontreated flowers, ray florets of many flowers inoculated with *S. minor* were again reduced or absent. Disc florets of most flowers treated with distilled water appeared healthy and were yellow to light brown in colour, with carpelate florets of several flowers brown to dark brown. Ascospore infection resulted in flower colour ranging from yellow through to dark brown or black, in addition to a higher number of flowers having disc florets of darker appearance than nontreated flowers, with (Plate 5.3.1.8).

Flower inoculation with ascospores of *S. minor* cultured *in vitro* demonstrated pathogenicity of *S. minor* towards pyrethrum flowers, giving rise to symptoms consistent with flower disease. Symptoms of *S. minor* flower disease observed during this study appear very similar to those reported within the literature and observed during these studies of *S. sclerotiorum* flower blight, making differentiating between *Sclerotinia* species flower blights from only in-field disease symptoms a difficult task.

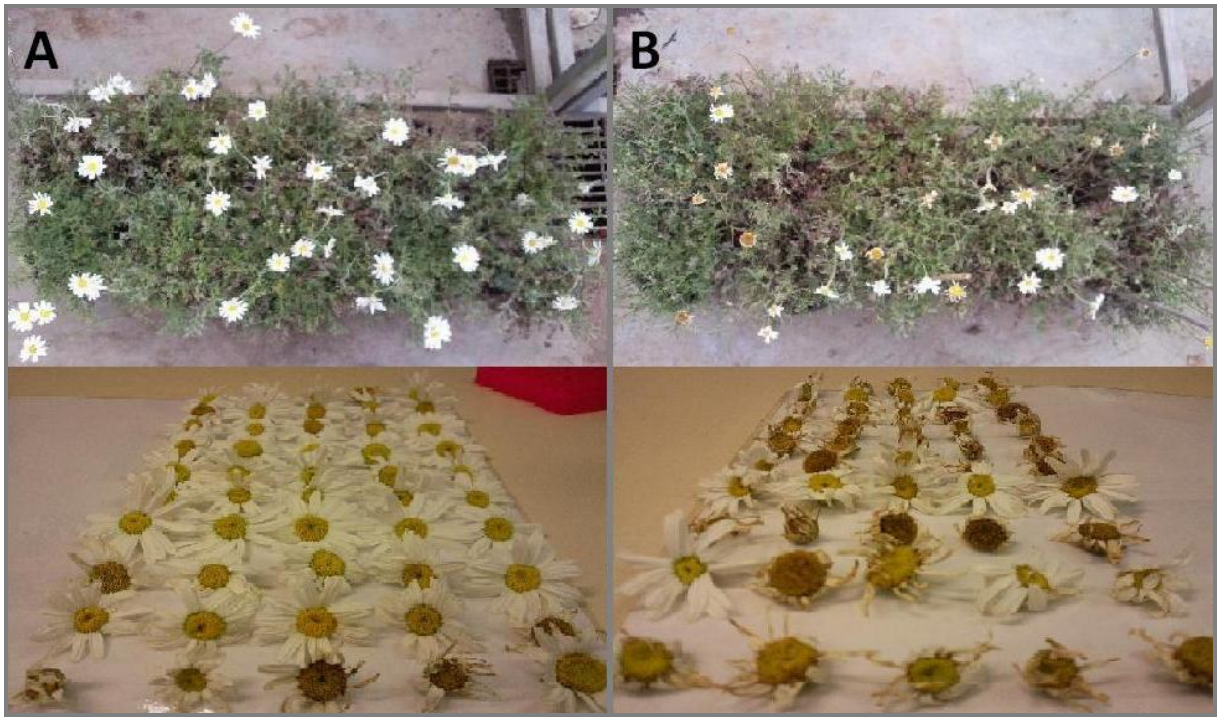


Plate 5.3.1.8 - Plants two weeks after treatment and harvested flowers before surface sterilisation of inoculation experiment B from **(A)** nontreated and **(B)** ascospore inoculated treatments.

5.3.2 Effect on flower development

Flower development

Following inoculation with *B. cinerea*, maturity of nontreated and inoculated flowers was measured after 23 days. Flowers of plant varieties utilised for inoculation studies with *S. sclerotiorum* were assessed at weekly intervals until an FMS of 7 (Plate 1.2.2) or higher was reached by most flowers, while a comparison of mean FMS values among treatments was conducted three weeks after inoculation. All flowers treated during preliminary inoculation studies with *S. minor* as well as experiments A and B were assessed two weeks after treatment, with flowers then harvested for incubation to confirm inoculation efficacy (Table 5.3.1).

Flower inoculations with spore suspensions of *B. cinerea, S. minor* and *S. sclerotiorum* all resulted in significant differences between measured aspects of flower development compared to nontreated flowers. Flowers of variety RS7 artificially infected with conidia of *B. cinerea* were observed to have reached an FMS value bordering on being significantly higher than nontreated

flowers 23 days after treatment (P = 0.062, Table 5.3.2). The dry matter percentage of flowers inoculated with *B. cinerea* also bordered on being significantly greater than nontreated flowers (P = 0.083). This may indicate flower infection of cultivar RS7 with conidia can lead to flower development and senescence occurring more rapidly, effectively reducing the lifespan of infected flowers and resulting in them drying prior to healthy, uninfected flowers.

Flower maturity was measured 14 days after dip inoculation with ascospores of *S. minor* in all inoculation experiments (Preliminary and Inoculation Experiments A and B). Highly significant increases in mean FMS values of artificially infected flowers were noted in both inoculation experiments (P < 0.001, Table 5.3.2), indicating an infection period of only two weeks may significantly hasten flower senescence and mortality.

Three weeks after pipette inoculation with ascospores of *S. sclerotiorum*, mean FMS values from flowers of cultivar Pyper bordered on being significantly greater than nontreated flowers. Inoculated flowers of varieties RS5 and Pyoneer reported significant and highly significant increases in FMS values compared to flowers treated with sterile water during this period (Table 5.3.2). The number of days after treatment when the majority of flowers from each cultivar attained FMS values of seven or higher also showed variation among treatments. Inoculated flowers of varieties Pyper and Pyoneer reached a final or harvestable FMS in significantly fewer days than nontreated flowers, while in variety RS5 this difference bordered on statistical significance. The mean dry matter percentage of inoculated flowers from cultivar Pyper was significantly greater than flowers on replicate plants treated with sterile water. A similar difference was noted in variety Pyrite; however the dry matter percentage of treated flowers bordered on being significantly higher than nontreated flowers in this instance.

Fungal pathogen	Flower maturity/DM%	Plant variety	Treatment		F pr.	LSD
			Inoculated	Nontreated		
Botrytis cinerea	Mean FMS (after 23 days)	RS7	7	6.84	0.062	0.169
	Dry matter (DM) %		49.15	37.18	0.083	13.68
Sclerotinia minor	Mean FMS (after 14 days)	BRA I [A]	5.26	4.36	<.001	0.48
		BRA I [B]	5.96	4.56	<.001	0.28
Sclerotinia sclerotiorum	Mean FMS (after 21 days)	Pyper	6.73	6.43	0.055	0.307
		RS5	6.7	6.31	0.021	0.325
		Pyrite	6.53	6.35	-	-
		Pyoneer	6.77	5.93	<.001	0.203
	Days ≥50% flowers = FMS ≥7	Pyper	27.6	37	0.001	5.27
		RS5	23.4	27.8	0.074	4.87
		Pyrite	24.2	26.6	-	-
		Pyoneer	15.3	21.2	0.008	4.13
	Dry matter (DM) %	Pyper	43.2	33.1	0.021	9.01
		RS5	29.8	33.4	-	-
		Pyrite	31.48	28.71	0.059	3.84
		Pyoneer	33	35.4	-	-

[A] Inoculation experiment A
[B] Inoculation experiment B

Table 5.3.2 - Summary of flower development assessments for greenhouse flower inoculation studies.

5.3.3 Effect on flower yield and pyrethrin assay

Flower inoculation with conidia of *B. cinerea* resulted in highly significant reductions in fresh and dry flower weights of flowers compared to treatment with sterile water, this decline also reported in milligrams yielded of pyrethrins per replicate of 10 flowers. Neither pyrethrins I or pyrethrins II assay fractions (% of dry weight) were reduced in concentration by artificial flower infection with *B. cinerea*, however total pyrethrin assay bordered on being statistically significantly lower in inoculated flowers in comparison to non-inoculated (Table 5.3.3). Overall, differences in dry weight of flowers and pyrethrins assay (% of dry weight) led to a significant reduction in the weight of pyrethrins within 10 flowers, with inoculated flowers containing almost two times less pyrethrins than non-inoculated flowers (Table 5.3.3). Statistically significant differences among treatments provide limited data defining the potential impact on yield qualities from a known incidence of *B. cinerea* flower disease (Table 5.3.3) Flowers from plants inoculated with *B. cinerea* had a mean incidence of 94%, and showed reductions in fresh and dry flower weights as well as total weight of pyrethrins yielded of 48.6%, 40.6% and 48.8% respectively compared to nontreated flowers with 5%

disease incidence. This frequency of flower infection also gave rise to a 15.2% decline in total pyrethrins assayed which bordered on statistical significance (Table 5.3.3).

Dip inoculation of flowers with inoculum of *S. minor* resulted in significant reductions of fresh flower weights in Inoculation Experiments A ($P < 0.001$) and B ($P = 0.032$) respectively (Table 5.3.3). Flowers from all inoculated plants had a mean incidence of 95% *S. minor*, and inoculated flowers had reductions in fresh flower weight of 27.3**%** and 18.0**%** for Inoculation Experiments A and B respectively. As flowers in these experiments were retained for assessment of incidence, the dry weight of flowers was not measured.

Pipette inoculation with ascospores of *S. sclerotiorum* resulted in fresh flower weights of one cultivar (Pyper) bordering on being significantly lower ($0.05 < P < 0.1$) than nontreated (Table 5.3.3). Inoculated flowers had a mean incidence of *S. sclerotiorum* of 94%, with fresh flower weights 24.6% lower than nontreated flowers which had 0% incidence of *S. sclerotiorum*. Flowers of this variety also reported the most prominent statistical effects on maturity, development and senescence from inoculation. Within the remaining cultivars and across all plants of each treatment no other significant effect on yield or assay was detected.

Pathogen	Plant variety	Yield/assay component per replicate (10 flowers)	Treatment		F pr.	LSD
			Inoculated	Nontreated		
Botrytis cinerea	RS7	Fresh weight (g)	1.48	2.88	<.001	0.55
		Dry weight (g)	0.63	1.06	<.001	0.15
		Pyrethrins (mg)	7.32	14.3	<.001	3.03
		Pyrethrins (PI)	0.71	0.84	-	-
		Pyrethrins (PII)	0.52	0.60	-	-
		Total pyrethrins (%)	1.23	1.45	0.092	0.26
Sclerotinia sclerotiorum	Pyoneer	Fresh weight (g)	3.94	4.04	-	-
		Dry weight (g)	1.30	1.44	-	-
		Pyrethrins (mg)	14.20	13.30	-	-
		Total pyrethrins (%)	1.19	1.16	-	-
	Pyper	Fresh weight (g)	2.85	3.78	0.095	1.03
		Dry weight (g)	1.11	1.23	-	-
		Pyrethrins (mg)	14.70	14.40	-	-
		Total pyrethrins (%)	15.13	14.12	-	-
	Pyrite	Fresh weight (g)	4.44	4.50	-	-
		Dry weight (g)	1.40	1.46	-	-
		Pyrethrins (mg)	22.4	20	-	-
		Total pyrethrins (%)	1.77	1.58	-	-
	RS5	Fresh weight (g)	3.92	4.06	-	-
		Dry weight (g)	1.15	1.25	-	-
		Pyrethrins (mg)	16.03	16.49	-	-
		Total pyrethrins (%)	1.61	1.68	-	-
Sclerotinia minor	BRA 1 [A]	Fresh weight (g)	1.92	2.64	<.001	0.33
	BRA 1 [B]	Fresh weight (g)	2.10	2.56	0.032	0.42

[A] Inoculation experiment A
[B] Inoculation experiment B

Table 5.3.3 - Summary of yield and assay results for all greenhouse flower inoculation studies, with each measured attribute the mean value of 10 replicate plants.

5.3.4 *Sclerotinia minor* - completion of Koch's postulates

Mycelium and sclerotia with characteristics and dimensions consistent with *S. minor* were identified from 95% of artificially infected flowers across Inoculation Experiments A and B (Table 5.3.4, Plate 5.3.4). Nontreated flowers averaged 8% pathogen incidence after treatment with sterile water and incubation. Sclerotia from ten arbitrarily selected flowers inoculated with ascospores of *S. minor* from both experiments were removed and sterilised, with resultant colonies on PDA plates hyphal tipped after two days to obtain pure cultures. All ten cultures from both Inoculation Experiments A and B produced cultures on potato dextrose agar with mycelial and sclerotial growth, development and morphology consistent with that reported within literature and observed of *S. minor* (Plate 5.3.4 C-D). Inoculation Experiment A produced sclerotia with length and width ranging between 0.4-3.6 and 0.3-2.6 mm respectively. Length of sclerotia averaged 2.01 mm and width 1.28 mm across the ten cultures obtained. Length and width of sclerotia from the ten resultant cultures

of Inoculation Experiment B varied between 0.3-4.6 and 0.3-3.4 mm respectively, with mean values of 1.83 and 1.25 mm (Table 5.3.4, data shown in Appendix 5.3.4 A-B)

Inoculum of *S. minor* used in these experiments was obtained from diseased pyrethrum flowers sampled from areas receiving no fungicide applications, purified through culture on nutrient media, and resulted in flower disease symptoms consistent with those associated with *S. sclerotiorum* flower blight of pyrethrum in Tasmania. The successful re-isolation onto nutrient media of pure cultures from incubated flowers completes Koch's postulates, providing strong evidence *S. minor* is a pathogenic agent able to elicit flower disease in Tasmanian pyrethrum fields.

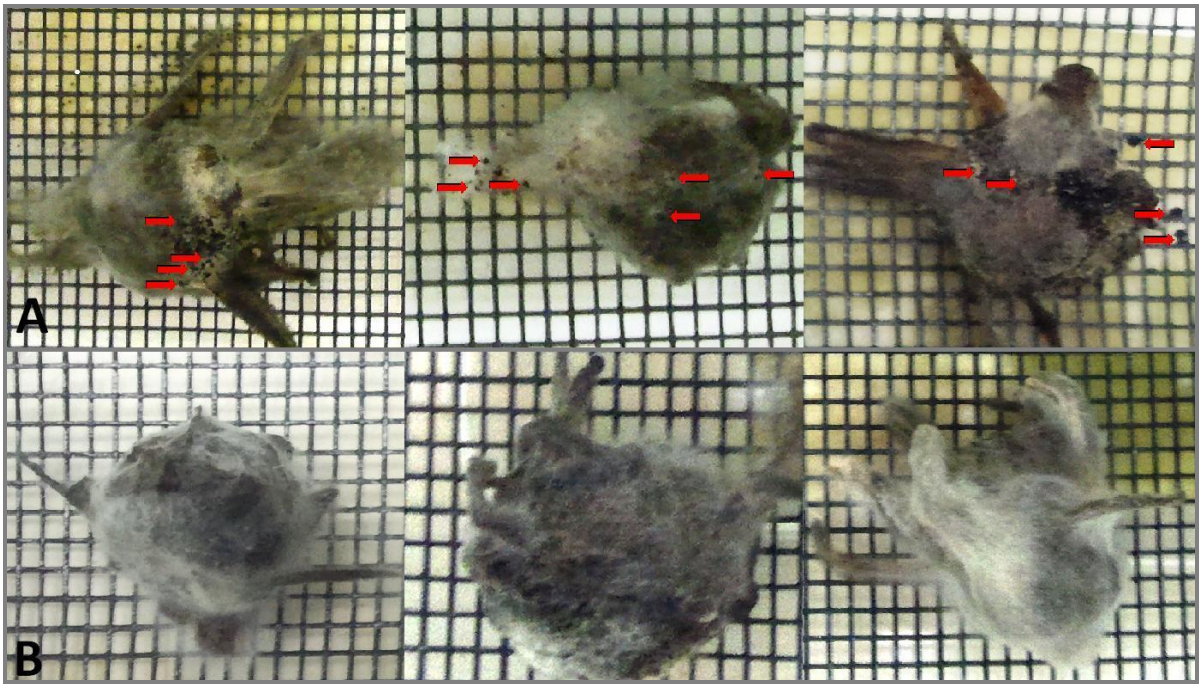


Plate 5.3.4 - (A) Developing and mature sclerotia (denoted by arrows) on and around mycelium and flowers and **(B)** mycelial growth consistent with *S. minor* on inoculated flowers after 20 days incubation.

Culture	Inoculation A		Inoculation B	
	Length (mm)	Width (mm)	Length (mm)	Width (mm)
1	1.85	1.30	1.95	1.27
2	1.95	1.21	1.88	1.26
3	1.82	1.18	1.80	1.12
4	1.72	1.08	2.21	1.43
5	2.20	1.38	1.99	1.40
6	2.23	1.41	1.76	1.19
7	1.91	1.18	1.60	1.14
8	2.16	1.34	1.53	1.06
9	2.12	1.38	1.86	1.29
10	2.16	1.41	1.73	1.30
Minimum	0.4	0.3	0.3	0.3
Maximum	3.6	2.1	4.7	3.4
Mean (± σ)	**2.01 ± 0.53 × 1.28 ± 0.31**		**1.83 ± 0.66 × 1.25 ± 0.37**	

Table 5.3.4 - Mean dimensions (mm) of one hundred sclerotia cultured from sterile, bisected sclerotia obtained from flowers treated with inoculum of *S. minor* during Inoculation Experiments A and B (σ = Standard deviation around the mean).

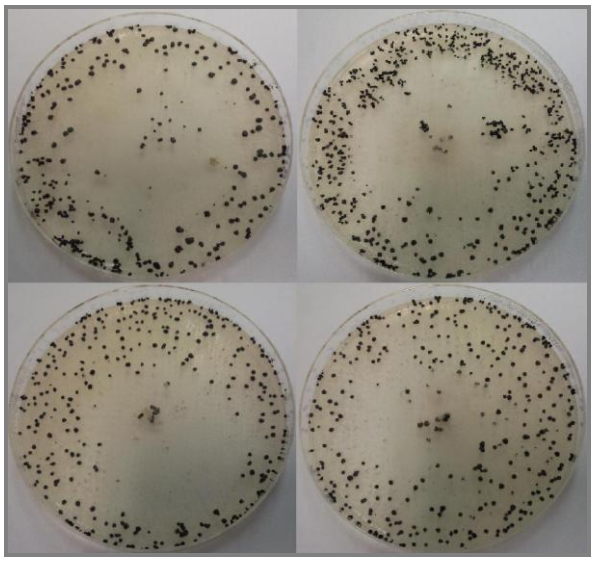


Plate 5.3.4 C - Pure cultures obtained from inoculation experiment A.

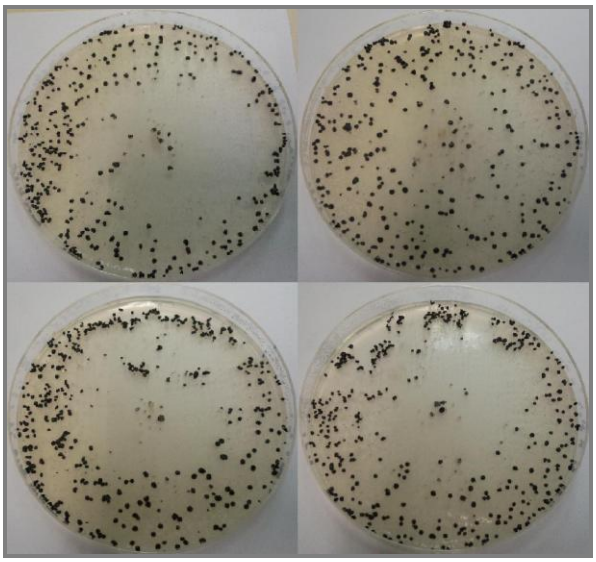


Plate 5.3.4 D - Hyphal tipped isolates from inoculation experiment B.

5.4 Discussion

Despite being applied at a similar rate to that reported to successfully achieve infection in bean (Harikrishnan and del Rio, 2006), it is possible inoculum of *S. sclerotiorum* may have been applied to a limited flower surface area and spread too thinly across four plant varieties in this experiment to promote development of more obvious flower disease symptoms and to adversely affect flower yield qualities. Compared to dip inoculation of flowers with spores of *B. cinerea* and *S. minor* the area of flower surface covered was significantly smaller. Inoculum of *S. sclerotiorum* used to promote flower infection in these studies appeared sufficient to show differences in flower development and dry matter content across numerous commercial varieties of pyrethrum. A notable decline in yield (fresh flower weight) was noticed in only one of four varieties compared to nontreated flowers, suggesting inoculation could have been optimised by diluting spore suspension to allow dip inoculation and greater coverage of flower surfaces.

Individually, each flower disease causing fungus displayed the capability to adversely affect aspects of flower development and/or attributes of yield measured. *B. cinerea* alone caused significant reductions in dry flower weight and pyrethrins yielded, while high incidences of flower infection by *S. sclerotiorum* and *S. minor* demonstrated borderline or significant reductions in fresh flower weights respectively. Infection of *B. cinerea* resulted in symptoms of ray floret desiccation and flower abscission (Plate 5.3.1.1), this pathogen also reported as the preeminent cause of premature blackcurrant flower senescence (Sharon *et al,* 2004). Premature development and abscission of floral organs is often reported from plant diseases caused by *S. sclerotiorum* (Willets and Wong, 1980), with flowers treated with *S. minor* inoculum following this trend in terms of developmental stage and pronounced symptoms of disease compared to nontreated flowers.

Statistical differences among treatments effecting flower development and dry matter content provide limited evidence supporting the suggestion by Pethybridge *et al* (2010) that infected flowers are dryer and may fragment upon harvesting, reducing the number of flowers harvested as demonstrated to occur in sunflower cultivation (Gregiore *et al,* 2000).

Ascosporic inoculum of *S. minor* was shown to successfully infect, elicit symptoms consistent with flower disease and significantly reduce fresh flower weights when applied to developing pyrethrum flowers. The successful reisolation, culture, and identification of fungi isolated from living, inoculated flowers as *S. minor* completed Koch's postulates as described (Agrios, 1995) and demonstrated the complete disease cycle potentially involved in *S. minor* flower blight of pyrethrum in Tasmania. The effect of flower infection of *S. minor* on yield attributes, and the potential of decaying flowers to contribute inoculum towards crown rot epidemics in Tasmanian pyrethrum crops is however still unknown. Successful flower inoculation with ascospores of *S. minor* demonstrated infection ability, while symptoms of flower disease observed post-inoculation additionally established the pathogenicity of this fungus toward pyrethrum flower tissues. *S. minor* has been demonstrated as pathogenic toward sunflower in Australia, with isolates obtained also capable of carpogenic germination *in vitro* (Ekins *et al,* 2002).

Symptoms of flower disease induced by inoculation with *B. cinerea, S. minor* and *S. sclerotiorum* were documented. Discerning from observable disease symptoms alone whether flower infection is caused by *S. sclerotiorum* or *S. minor* is unlikely to be successful.

Inoculum of each fungi resulted in borderline or significant differences in measured flower maturity. Twenty three days after infection with *B. cinerea,* inoculated flowers bordered on being significantly more mature than control replicates, while two weeks post-treatment this difference from artificial infection with *S. minor* was highly significant. Of the four varieties inoculated with ascospores of *S. sclerotiorum*, three had borderline or significantly higher flower maturity three weeks after treatment than flowers with between 0-4% disease incidences. Inoculated flowers of two cultivars additionally reached a harvestable flower maturity in significantly fewer days than flowers treated with sterile water.

The dry matter content of flowers inoculated with *B. cinerea* bordered on being significantly greater than nontreated flowers at 23 days after infection. One of four cultivars treated with inoculum of *S. sclerotiorum* had a significantly higher dry matter percentage than control flowers, while in one additional variety this difference among treatments bordered on being significant.

Flowers with 94% incidence of *B. cinerea* had significantly lower fresh weights (-48.6%), dry weights (-40.6%), and weight of pyrethrins yielded (-48.8%) compared to flowers with 5% incidence of this pathogen. A reduction in total pyrethrin assay bordering on statistical significance was also reported. Flowers with 92% and 98% incidence of *S. minor* had significantly lower fresh weights (-27.3% and -18%) than flowers with 10% and 16% frequency of this pathogen respectively. Incidence of *S. sclerotiorum* of 94% resulted in a reduction in fresh flower weights which bordered on statistical significance compared 0% incidence of this pathogen.

The successful re-isolation from artificially infected flowers of fungal cultures consistent with morphology and sclerotial dimensions of *S. minor* completes the final requirement of Koch's postulates, providing evidence that *S. minor* is indeed an agent of flower disease in Tasmanian pyrethrum fields.

5.5 Conclusions

Individually, known agents of flower disease *B. cinerea* and *S. sclerotiorum* resulted in significantly lower flower weight, pyrethrum assay, moisture content as well as more rapid flower senescence from flower inoculation with conidia *(Botrytis)* or ascospores *(Sclerotinia)* than nontreated flowers.

Pathogenicity of *S. minor* toward pyrethrum flowers was demonstrated, with completion of Koch's postulates and reisolation of pure culture from flowers of artificially infected pyrethrum plants.

Significantly lower fresh flower weights resulted from inoculation with ascospores of *S. minor*, while observed symptoms of *S. minor* flower blight were indistinguishable from observed and reported symptoms of *S. sclerotiorum* flower blight of pyrethrum.

Summary of findings

Epidemiological studies showed flower infection by *B. cinerea* occurred earlier than *S. sclerotiorum*, and that polycyclic development of generations of conidia of *B. cinerea* during flowering leads to a faster rise in the incidence of diseased flowers.

S. minor was regularly isolated from diseased pyrethrum flowers in north western Tasmania, and carpogenic germination of *S. minor* was documented in one pyrethrum field. Eight of 10 isolates of *S. minor* collected from pyrethrum flowers underwent carpogenic germination *in vitro,* producing mature apothecia and viable ascospores.

Completion of Koch's postulates demonstrated *S. minor* to be pathogenic toward pyrethrum flowers in Tasmanian fields, while inoculation with ascospores of *S. minor* resulted in symptoms of flower disease and significantly lower fresh flower weights after only two weeks.

Evaluation of the flowering fungicide program for flower disease control in Tasmanian pyrethrum crops showed numerous reductions in disease incidence (more often *S. sclerotiorum*) and modest increases in some yield attributes, likely contributed to by widespread resistance of local isolates of *B. cinerea* to carbendazim identified in this study.

The dicarboximide fungicide iprodione (Rovral^{R} Aquaflo) demonstrated efficacy in reducing incidence of *B. cinerea* and *S. sclerotiorum* in flowers during field trials and at inhibiting mycelial growth in laboratory tests, with no evidence of significant resistance in isolates during sensitivity screening. This fungicide presents as the best available option to replace carbendazim in the flowering fungicide program in Tasmanian pyrethrum fields, given the reduced sensitivity and recent removal from registration of the latter. Iprodione and tebuconazole demonstrated effective inhibition of mycelial growth of *S. minor,* while reduced sensitivity of this fungus was reported to carbendazim.

Individually, inoculation of pyrethrum flowers with *B. cinerea* and *S. sclerotiorum* significantly reduced flower weights or pyrethrins concentration, hastened flower senescence and/or reduced moisture content of flowers prior to harvest compared to nontreated flowers.

Summary of findings and further research:

Recommendations for further research

Further investigate the role of *S. minor* in flower disease epidemiology of pyrethrum, as well as a potential source of primary inoculum for crown rot disease caused by *S. minor* in Tasmania.

Evaluate the effect of flower inoculation with ascospores of *S. minor* on pyrethrin yield and assay attributes.

Evaluate the performance of iprodione as a fungicide for control of flower diseases in Australian pyrethrum crops, following inclusion to replace carbendazim.

Monitor sensitivity to fungicides used for flower disease control of isolates collected from diseased flowers annually of *S. sclerotiorum, S. minor* and *B. cinerea*.

Evaluate potential soil treatments for the reduction of sclerotial inoculum of *Sclerotinia* species, including biocontrol agents such as *Coniothyrium minitans.*

Investigate other, potentially more accurate methods (than hand and hedge trimmer harvesting of flowers) of evaluating effect of flower disease and fungicide applications on yield attributes from field trials, including non-destructive methods such as computer analysis of flower numbers from photographic images. Furthermore, it would be interesting to investigate the quality of aerial image that might be obtained with a high quality digital camera mounted to multi rotor radio-controlled helicopter (Quadcopter) should computer analysis of flowers per unit area prove promising. Having a high degree of stability and requiring limited technical experience to operate, devices such as these may in future provide a means to non-destructively estimate yield (or other aspects of crop production) of large areas or even whole paddocks.

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Appendices

Appendix 1.3.2 A - Isolates of *S. sclerotiorum* collected for further study.

1	07-3 A	**16**	07-6 H	**31**	07-10 F	**46**	07-2 C	**61**	07-9 B
2	07-3 B	**17**	07-6 J	**32**	07-10 L	**47**	07-2 D	**62**	07-9 C
3	07-3 C	**18**	07-5 D	**33**	07-10 H	**48**	07-2 E	**63**	07-9 E
4	07-3 I	**19**	07-5 B	**34**	07-10 I	**49**	07-2 G	**64**	07-9 F
5	07-3 E	**20**	07-5 C	**35**	07-10 J	**50**	07-2 H	**65**	07-9 H
6	07-3 G	**21**	07-5 E	**36**	07-10 K	**51**	07-2 K	**66**	07-9 J
7	07-3 F	**22**	07-5 F	**37**	07-10 M	**52**	07-2 L	**67**	07-9 K
8	07-3 M	**23**	07-5 H	**38**	07-4 A	**53**	07-8 B	**68**	07-7 A
9	07-6 A	**24**	07-5 I	**39**	07-4 B	**54**	07-8 C	**69**	07-7 B
10	07-6 B	**25**	07-5 J	**40**	07-4 C	**55**	07-8 D	**70**	07-9 I
11	07-6 C	**26**	07-1 A	**41**	07-4 D	**56**	07-8 I	**71**	PFT A
12	07-6 E	**27**	07-1 B	**42**	07-4 E	**57**	07-8 G	**72**	07-1 F
13	07-6 F	**28**	07-1 D	**43**	07-10 B	**58**	07-8 H	**73**	07-8 A
14	07-6 I	**29**	07-1 J	**44**	07-2 A	**59**	07-8 K		
15	07-6 G	**30**	07-10 G	**45**	07-2 B	**60**	07-9 A		

Appendix 1.3.2 B - Isolates of *B. cinerea* collected and stored for further study.

1	07-3 A	**11**	07-6 D	**21**	07-1 A	**31**	07-4 D	**41**	07-8 E
2	07-3 B	**12**	07-6 E	**22**	07-1 B	**32**	07-4 E	**42**	07-9 B
3	07-3 D	**13**	07-6 I	**23**	07-1 C	**33**	07-4 G	**43**	07-9 D
4	07-3 E	**14**	07-6 J	**24**	07-1 D	**34**	07-4 H	**44**	07-9 G
5	07-3 G	**15**	07-5 A	**25**	07-1 H	**35**	07-2 B	**45**	07-9 H
6	07-3 I	**16**	07-5 B	**26**	07-1 I	**36**	07-2 E	**46**	07-7 C
7	07-3 J	**17**	07-5 C	**27**	07-10 D	**37**	07-2 F	**47**	07-7 E
8	07-3 L	**18**	07-5 D	**28**	07-4 A	**38**	07-2 G	**48**	07-7 J
9	07-6 B	**19**	07-5 I	**29**	07-4 B	**39**	07-2 H	**49**	07-10 D
10	07-6 C	**20**	07-5 K	**30**	07-4 C	**40**	07-2 I		

Appendix 1.3.2 C - Fungal isolates collected and preliminarily identified as *S. minor* stored for further studies.

Isolate	Host plant	Isolated from:	Year collected
07-13 A	Pyrethrum	foliage	2008
07-13 D	Pyrethrum	foliage	2008
07-12 C	Pyrethrum	foliage	2008
07-12 F	Pyrethrum	foliage	2008
07-12 G	Pyrethrum	foliage	2008
07-11 B	Pyrethrum	foliage	2008
07-11 F	Pyrethrum	foliage	2008
08-1	na	apothecia	2008
08-5 A	Pyrethrum	flower	2009
08-5 B	Pyrethrum	flower	2009
08-5 C	Pyrethrum	flower	2009
08-5 D	Pyrethrum	flower	2009
08-5 E	Pyrethrum	flower	2009
08-7 A	Pyrethrum	flower	2009
08-7 B	Pyrethrum	flower	2009
09-8 B	Pyrethrum	flower	2010
09-8 C	Pyrethrum	flower	2010
09-8 E	Pyrethrum	flower	2010
09-8 F	Pyrethrum	flower	2010
09-8 G	Pyrethrum	flower	2010
09-8 H	Pyrethrum	flower	2010
09-8 K	Pyrethrum	flower	2010
09-8 L	Pyrethrum	flower	2010
09-8 O	Pyrethrum	flower	2010
09-8 Q	Pyrethrum	flower	2010
09-8 A	Pyrethrum	flower	2010
09-8 J	Pyrethrum	flower	2010
09-8 O	Pyrethrum	flower	2010
09-8 V	Pyrethrum	flower	2010
09-8 W	Pyrethrum	flower	2010
09-8 X	Pyrethrum	flower	2010
09-8 Y	Pyrethrum	flower	2010
09-8 A	Pyrethrum	flower	2010
09-8 B	Pyrethrum	flower	2010
09-8 C	Pyrethrum	flower	2010
09-8 D	Pyrethrum	flower	2010
09-8 E	Pyrethrum	flower	2010
09-8 F	Pyrethrum	flower	2010
09-1 A	Pyrethrum	flower	2010
09-3 A	Pyrethrum	flower	2010
09-3 B	Pyrethrum	flower	2010
09-3 C	Pyrethrum	flower	2010
09-3 D	Pyrethrum	flower	2010
09-3 E	Pyrethrum	flower	2010
09-3 G	Pyrethrum	flower	2010
09-5 A	Pyrethrum	flower	2010

Appendices:

Appendix 1.3.2 D - Incidence (%) of mycoflora indentified on diseased pyrethrum flowers.

Date sampled	Site	*Alternaria*	*Penicillium*	*Stemphyllium*
17-Nov	08-1	9	46	28
	08-2	11	54	31
	08-3	15	62	19
	08-4	39	7	34
	08-5	42	13	21
	08-6	8	62	20
	08-7	9	57	27
	08-8	13	41	18
	08-9	1	71	11
	08-10	5	59	4
4-Dec	08-1	64	7	0
	08-2	69	28	0
	08-3	73	3	0
	08-4	41	10	0
	08-5	68	9	0
	08-6	67	6	0
	08-7	51	7	2
	08-8	85	7	0
	08-9	68	5	0
	08-10	60	16	0
22-Dec	08-1	52	2	64
	08-2	37	2	76
	08-3	75	3	89
	08-4	27	4	92
	08-5	49	2	83
	08-6	40	5	79
	08-7	40	1	50
	08-8	63	0	23
	08-9	63	6	52
	08-10	66	4	29

Appendices:

Appendix 2.2.1 - Flowering fungicide application details for trial sites; 2007-08, 2008-09 and 2009-10.

Year	Site	Date	Fungicide	Date	Fungicide	Date	Fungicide	Date	Fungicide
2007-08	07-1	4/10/2007	Bavistin	8/12/2007	Folicur	17/12/2007	Bavistin		
	07-2	23/11/2007	Folicur	1/12/2007	Bavistin	7/12/2007	Folicur	14/12/2007	Bavistin
	07-3	21/11/2007	Folicur	29/11/2007	Bavistin	10/12/2007	Folicur	14/12/2007	Bavistin
	07-4	26/11/2007	Folicur	3/12/2007	Bavistin	10/12/2007	Folicur	17/12/2007	Bavistin
	07-5	24/11/2007	Folicur	2/12/2008	Bavistin	15/12/2007	Folicur		
	07-6	5/12/2007	Folicur	14/12/2007	Bavistin	21/12/2007	Folicur		
	07-7	27/11/2007	Folicur	7/12/2007	Bavistin	21/12/2007	Folicur		
	07-8	25/11/2007	Folicur	8/12/2007	Bavistin				
	07-9	23/11/2007	Folicur	5/12/2007	Bavistin	12/12/2007	Folicur		
	07-10	19/11/2007	Folicur	26/11/2007	Bavistin	4/12/07	Folicur	11/12/2007	Bavistin
2008-09	08-1	26/11/2008	Folicur	15/12/2008	Bavistin				
	08-2	24/11/2008	Folicur	9/12/2008	Bavistin	19/12/2008	Folicur		
	08-3	25/11/2008	Folicur	4/12/2008	Bavistin	10/12/2008	Folicur	22/12/2008	Bavistin
	08-4	17/11/2008	Folicur	4/12/2008	Bavistin	17/12/2008	Folicur	23/12/2008	Bavistin
	08-5	18/11/2008	Folicur	3/12/2008	Bavistin	15/12/2008	Folicur	22/12/2008	Bavistin
	08-6	12/11/2008	Folicur	24/11/2008	Bavistin	1/12/2008	Folicur	11/12/2208	Bavistin
	08-7	12/11/2008	Folicur	24/11/2008	Bavistin	1/12/2008	Folicur	10/12/2008	Bavistin
	08-8	14/11/2008	Folicur	24/11/2008	Bavistin	3/12/2008	Folicur	13/12/2008	Bavistin
	08-9	21/11/2008	Folicur	28/11/2008	Bavistin	12/03/2008	Folicur	12/11/2008	Bavistin
	08-10	21/11/2008	Folicur	26/11/2008	Bavistin	3/12/2008	Folicur	10/12/2008	Bavistin
2009-10	09-1	19/11/2009	Bavistin	30/11/2009	Folicur	7/12/2009	Bavistin	18/12/2009	Folicur
	09-2	19/11/2009	Bavistin	30/11/2009	Folicur	7/12/2009	Bavistin	18/12/2009	Folicur
	09-3	19/11/2009	Bavistin	30/11/2009	Folicur	7/12/2009	Bavistin	18/12/2009	Folicur
	09-4	19/11/2009	Bavistin	30/11/2009	Folicur	7/12/2009	Bavistin	18/12/2009	Folicur
	09-5	19/11/2009	Bavistin	30/11/2009	Folicur	7/12/2009	Bavistin	18/12/2009	Folicur
	09-6	19/11/2009	Bavistin	29/11/2009	Folicur	2/12/2009	Bavistin	16/12/2009	Folicur
	09-7	19/11/2009	Bavistin	29/11/2009	Folicur	8/12/2009	Bavistin	16/12/2009	Folicur
	09-8	21/11/2009	Folicur	1/12/2009	Bavistin	8/12/2009	Folicur	15/12/2009	Bavistin
	09-9	19/11/2009	Folicur	1/12/2009	Bavistin	8/12/2009	Folicur	15/12/2009	Bavistin
	09-10	20/11/2009	Folicur	1/12/2009	Bavistin	8/12/2009	Folicur	15/12/2009	Bavistin
	09-11	20/11/2009	Folicur	1/12/2009	Bavistin	8/12/2009	Folicur	15/12/2009	Bavistin
	09-12	20/11/2009	Folicur	1/12/2009	Bavistin	8/12/2009	Folicur	15/12/2009	Bavistin
	09-13	20/11/2009	Folicur	30/11/2009	Bavistin	8/12/2009	Folicur	15/12/2009	Bavistin
	09-14	20/11/2009	Folicur	1/12/2009	Bavistin	9/12/2009	Folicur	15/12/2009	Bavistin
	09-15	20/11/2009	Folicur	1/12/2009	Bavistin	9/12/2009	Folicur	16/12/2009	Bavistin
	09-16	19/11/2009	Folicur	1/12/2009	Bavistin	9/12/2009	Folicur	16/12/2009	Bavistin
	09-17	20/11/2009	Folicur	1/12/2009	Bavistin	8/12/2009	Folicur	16/12/2009	Bavistin

Appendix 2.2.2 - Flower sampling dates each season.

Year	Site	Flower sampling dates				
		1st	2nd	3rd	4th	5th
2007	07-1	15-Nov	30-Nov	11-Dec	16-Dec	21-Dec
	07-2	15-Nov	30-Nov	11-Dec	16-Dec	21-Dec
	07-3	16-Nov	4-Dec	11-Dec	16-Dec	21-Dec
	07-4	15-Nov	29-Nov	11-Dec	16-Dec	21-Dec
	07-5	15-Nov	4-Dec	11-Dec	16-Dec	21-Dec
	07-6	15-Nov	4-Dec	11-Dec	18-Dec	21-Dec
	07-7	13-Nov	30-Nov	10-Dec	18-Dec	21-Dec
	07-8	13-Nov	30-Nov	10-Dec	18-Dec	21-Dec
	07-9	13-Nov	30-Nov	10-Dec	18-Dec	21-Dec
	07-10	15-Nov	29-Nov	11-Dec	16-Dec	21-Dec
2008	08-1	17-Nov	4-Dec	22-Dec	-	-
	08-2	17-Nov	4-Dec	22-Dec	-	-
	08-3	17-Nov	4-Dec	22-Dec	-	-
	08-4	17-Nov	4-Dec	22-Dec	-	-
	08-5	17-Nov	4-Dec	22-Dec	-	-
	08-6	17-Nov	4-Dec	22-Dec	-	-
	08-7	17-Nov	4-Dec	22-Dec	-	-
	08-8	17-Nov	4-Dec	22-Dec	-	-
	08-9	17-Nov	4-Dec	22-Dec	-	-
	08-10	17-Nov	4-Dec	22-Dec	-	-
2009	09-1	2-Dec	15-Dec	-	-	-
	09-2	2-Dec	15-Dec	-	-	-
	09-3	2-Dec	15-Dec	-	-	-
	09-4	2-Dec	15-Dec	-	-	-
	09-5	2-Dec	16-Dec	-	-	-
	09-6	2-Dec	16-Dec	-	-	-
	09-7	2-Dec	16-Dec	-	-	-
	09-8	2-Dec	14-Dec	-	-	-
	09-9	2-Dec	14-Dec	-	-	-
	09-10	2-Dec	18-Dec	-	-	-
	09-11	2-Dec	14-Dec	-	-	-
	09-12	2-Dec	14-Dec	-	-	-
	09-13	2-Dec	14-Dec	-	-	-
	09-14*	na	na	-	-	-
	09-15	2-Dec	18-Dec	-	-	-
	09-16	2-Dec	16-Dec	-	-	-
	09-17*	na	na	-	-	-

* Site abandoned due to low plant density.

Appendix 2.2.3 - Yield sampling details at field trial sites over three years.

Year	**2007-08**	**2008-09**	**2009-10**
Method	Hand harvesting	Hand harvesting	Hedge trimmer
Quadrat size (m^2)	0.7 × 0.7 m (0.49 m^2)	0.7 × 0.7 m (0.49 m^2)	1 × 2.4 m (2.4 m^2)
No. trial sites	10	10	17
No. replicates (yield)	4	6	4
No. replicates (assay)	1	2	4
Area sampled per treatment (plot/site)	1.96 m (1.4^2 m)	2.94 m (1.72^2 m)	9.6m (3.1^2 m)
Area sampled per treatment (total)	19.6 m (4.43^2 m)	29.4 m (5.42^2 m)	144m (12^2 m)

Appendices:

Appendix 2.3.1 A - Incidence (%) of *B. cinerea* in treatments plots at field sites during 2007-08.

Site	Assessment	Harvest	Commercial	Nontreated	F pr.	LSD	CV%
07-5	1	14-Nov	0	6	0.04	5.65	129.1
	2	3-Dec	60	40	0.1	-	33.5
	3	10-Dec	86	85	0.86	-	10.3
	4	17-Dec	100	98	0.14	-	2
	5	21-Dec	96	99	0.29	-	4.3
07-1	1	14-Nov	0	0	-	-	-
	2	29-Nov	4	7	0.49	-	120.3
	3	10-Dec	14	28	0.01	10.31	33.7
	4	17-Dec	24	48	0.01	15.89	30.3
	5	21-Dec	24	52	0.02	22.18	40
07-2	1	14-Nov	3	0	0.35	-	316.2
	2	29-Nov	6	7	0.83	-	108.8
	3	10-Dec	30	43	0.02	10.69	20.1
	4	17-Dec	49	70	0.04	20.1	23.2
	5	21-Dec	25	87	<.001	14.86	18.2
07-3	1	15-Nov	2	1	0.55	-	166.7
	2	3-Dec	10	18	0.12	-	52.4
	3	10-Dec	55	75	0.05	20.3	21.4
	4	17-Dec	52	88	<.001	15.12	14.8
	5	21-Dec	73	96	0.004	13.15	10.7
07-4	1	14-Nov	2	3	0.73	-	178.9
	2	28-Nov	10	14	0.46	-	67.2
	3	10-Dec	56	64	0.39	-	23.1
	4	17-Dec	57	89	<.001	12.09	11.4
	5	21-Dec	76	96	0.01	12.52	10
07-6	1	14-Nov	0	4	0.35	-	316.2
	2	3-Dec	2	4	0.49	-	144.3
	3	10-Dec	16	47	<.001	10.31	22.4
	4	17-Dec	12	61	<.001	12.09	22.7
	5	21-Dec	81	81	1	-	11
07-7	1	12-Nov	0	2	0.35	-	316.2
	2	29-Nov	1	0	0.35	-	316.2
	3	9-Dec	60	66	0.35	-	15.1
	4	17-Dec	51	85	<.001	14.31	14.4
	5	21-Dec	81	75	0.17	-	8.1
07-8	1	12-Nov	0	4	0.35	-	316.2
	2	29-Nov	0	1	0.35	-	316.2
	3	9-Dec	21	37	0.03	13.64	32.3
	4	17-Dec	32	65	<.001	17.71	25
	5	21-Dec	38	81	<.001	16.31	18.8
07-9	1	12-Nov	5	13	0.3	-	126.1
	2	29-Nov	0	2	0.35	-	316.2
	3	9-Dec	64	87	0.01	14.58	13.2
	4	17-Dec	88	95	0.1	-	6.5
	5	21-Dec	87	87	1	-	9.6
07-10	1	14-Nov	1	2	0.55	-	166.7
	2	28-Nov	7	2	0.27	-	149.1
	3	10-Dec	3	28.4	0.02	19.23	84
	4	17-Dec	9	58	<.001	15.03	30.8
	5	21-Dec	24	60	<.001	13.84	22.6

Appendix 2.3.1 B - Incidence (%) of *S. sclerotiorum* in treatments plots at field sites during 2007-08.

Site	Assessment	Harvest	Commercial	Nontreated	F pr.	LSD	CV%
07-5	1	14-Nov	5	6	0.822	-	123.6
	2	3-Dec	3	5	0.455	-	100.8
	3	10-Dec	10	27	0.03	14.86	55.1
	4	17-Dec	10	27	0.084	-	73.5
	5	21-Dec	21	57	0.002	18.59	32.7
07-1	1	14-Nov	0	0	-	-	-
	2	29-Nov	0	0	-	-	-
	3	10-Dec	8	15	0.211	-	70.8
	4	17-Dec	41	69	0.003	15.38	19.2
	5	21-Dec	41	79	<.001	15.38	17.6
07-2	1	14-Nov	2	0	0.141	-	193.6
	2	29-Nov	1	7	0.06	-	108.3
	3	10-Dec	35	55	0.05	19.97	30.4
	4	17-Dec	44	36	0.265	-	26.4
	5	21-Dec	28	82	<.001	12.74	15.9
07-3	1	15-Nov	8	1	0.178	-	166.7
	2	3-Dec	0	3	0.172	-	210.8
	3	10-Dec	14	8	0.16	-	55.7
	4	17-Dec	16	46	0.021	24.13	53.4
	5	21-Dec	20	43	0.039	21.45	46.7
07-4	1	14-Nov	5	0	0.153	-	200
	2	28-Nov	3	0	0.172	-	210.8
	3	10-Dec	12	16	0.424	-	53.6
	4	17-Dec	28	34	0.242	-	24.2
	5	21-Dec	36	76	<.001	11.41	14
07-6	1	14-Nov	3	1	0.397	-	176.8
	2	3-Dec	0	0	-	-	-
	3	10-Dec	11	52	<.001	18.23	39.7
	4	17-Dec	42	72	0.005	17.94	21.6
	5	21-Dec	22	53	0.001	14.22	26
07-7	1	12-Nov	1	4	0.195	-	134.2
	2	29-Nov	0	0	-	-	-
	3	9-Dec	20	22	0.524	-	22.6
	4	17-Dec	27	61	0.029	29.62	46.2
	5	21-Dec	22	23	0.883	-	46.1
07-8	1	12-Nov	3	1	0.397	-	176.8
	2	29-Nov	0	0	-	-	-
	3	9-Dec	29	51	0.019	17.41	29.8
	4	17-Dec	38	62	0.004	13.74	18.8
	5	21-Dec	46	80	<.001	5.65	6.1
07-9	1	12-Nov	2	10	0.029	6.92	79.1
	2	29-Nov	1	0	0.347	-	316.2
	3	9-Dec	12	32	0.002	10.44	32.5
	4	17-Dec	70	69	0.833	-	10.4
	5	21-Dec	12	53	<.001	12.2	25.7
07-10	1	14-Nov	2	1	0.667	-	235.7
	2	28-Nov	18	6	0.051	-	69.1
	3	10-Dec	5	16	0.017	8.47	55.3
	4	17-Dec	35	42	0.479	-	38.7
	5	21-Dec	44	82	0.003	20.63	22.4

Appendices:

Appendix 2.3.2 A - Fungal incidence (%) of flowers of *B. cinerea* from treatment plots during field trials 2008-09.

Sample time	Site	Commercial	Nontreated	F pr.	LSD	CV%
1	08-1	0	0	-	-	-
	08-2	5	0	0.153	7.29	200
	08-3	0	0	-	-	-
	08-4	1	0	0.347	-	316.2
	08-5	2	1	0.545	-	166.7
	08-6	1	4	0.29	-	167.3
	08-7	0	3	0.04	2.824	129.1
	08-8	0	1	0.347	-	316.2
	08-9	0	1	0.347	-	316.2
	08-10	0	1	0.347	-	316.2
2	08-1	10	4	0.313	-	125.8
	08-2	21	25	0.648	-	57.9
	08-3	3	8	0.272	-	122
	08-4	14	9	0.463	-	89.1
	08-5	22	7	0.018	11.64	55.1
	08-6	7	9	0.7	-	98.8
	08-7	4	26	0.014	16.22	74.2
	08-8	0	6	0.04	5.65	129.1
	08-9	3	12	0.113	-	106.5
	08-10	0	3	0.04	2.824	129.1
3	08-1	53	79	0.013	18.95	19.7
	08-2	85	91	0.201	-	7.7
	08-3	25	71	<.001	16.06	22.9
	08-4	54	64	0.357	-	27.4
	08-5	71	90	0.001	9.22	7.9
	08-6	71	79	0.31	-	15.6
	08-7	74	83	0.153	-	11.5
	08-8	62	97	<.001	10.44	9
	08-9	28	61	<.001	13.64	21
	08-10	10	35	0.001	11.53	35.1

Appendix 2.3.2 B - Pathogen incidence (%) of *S. sclerotiorum* in flowers throughout sampling period during 2008-09.

Sample time	Site	Commercial	Nontreated	F pr.	LSD	CV%
1	08-1	30	25	0.423	-	34
	08-2	27	32	0.409	-	30.8
	08-3	15	29	0.025	11.8	36.6
	08-4	18	28	0.166	-	45.1
	08-5	12	29	0.039	15.9	53.2
	08-6	21	17	0.587	-	58.8
	08-7	17	25	0.141	-	36.9
	08-8	8	36	<.001	12.1	37.7
	08-9	14	28	0.108	-	58.3
	08-10	21	17	0.545	-	52.6
2	08-1	5	17	0.165	-	112.7
	08-2	6	8	0.587	-	79.9
	08-3	12	15	0.511	-	51.1
	08-4	5	14	0.055	-	66.6
	08-5	9	18	0.094	-	55.6
	08-6	3	8	0.02	3.99	49.8
	08-7	1	20	0.016	14.3	93.4
	08-8	23	27	0.57	-	42.7
	08-9	7	20	0.056	-	68.3
	08-10	12	11	0.817	-	57.5
3	08-1	0	9	0.07	-	151.1
	08-2	1	2	0.667	-	235.7
	08-3	0	8	<.001	2.82	48.4
	08-4	15	14	0.883	-	71.9
	08-5	0	23	0.005	13.9	83.1
	08-6	13	36	0.015	17.1	47.9
	08-7	20	50	<.001	11.5	22.6
	08-8	19	21	0.733	-	44.7
	08-9	16	27	0.14	-	49.3
	08-10	12	59	<.001	17.9	34.5

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Appendix 2.3.3 A - Flower maturity stage (FMS) at first flower sampling during 2009-10.

Site	Commercial	Nontreated	F pr.	LSD	CV%
09-1	3.17	3.37	0.023	0.173	3.9
09-2	3.43	3.79	0.088	-	6.4
09-3	3.02	3.14	0.02	0.087	2.1
09-4	3.26	3.26	0.143	-	6.4
09-5	3.25	3.59	0.029	0.339	7.4
09-6	3.42	3.78	<.001	0.21	4.4
09-7	3.51	3.81	0.087	-	8.6
09-8	3.32	3.3	0.792	-	5
09-9	3.19	3.26	0.633	-	4.8
09-10	3.29	3.72	0.002	0.207	4.3
09-11	3.09	3.11	0.097	-	2
09-12	3.12	3.31	0.179	-	5.5
09-13	3.1	3.59	0.004	0.251	5.5
09-14	3.41	3.65	0.089	-	8.4
09-15	3.64	4.14	0.025	0.343	6.4
09-16	3.44	3.72	<.001	0.207	4.4
09-17	3.38	3.7	0.015	0.284	6

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Appendix 2.3.3 B - Pathogen incidence (%) of *B. cinerea* in flowers from trial plots at both sampling occasions.

Sample time	Site	Commercial	Nontreated	F pr.	LSD	CV%
1	09-1	18	52	0.001	15.98	31.3
	09-2	38	61	0.021	18.59	25.8
	09-3	13	43	<.001	9.78	24
	09-4	17	46	0.02	23.06	50.2
	09-5	34	50	0.084	-	30.6
	09-6	9	46	<.001	10.82	27
	09-7	10	22	0.116	-	67.4
	09-8	42	74	0.003	17.86	21.1
	09-9	26	34	0.252	-	34.2
	09-10	56	76	0.132	-	28.5
	09-11	21	51	<.001	8.78	16.7
	09-12	32	47	0.073	-	29.1
	09-13	24	30	0.446	-	43.8
	09-14	63	79	0.073	-	17.2
	09-15	60	50	0.214	-	21.3
	09-16	60	76	0.029	13.84	14
	09-17	21	66	<.001	13.54	21.3
2	09-1	51	56	0.508	-	21.3
	09-2	40	58	0.018	13.93	19.5
	09-3	67	62	0.48	-	16.5
	09-4	56	54	0.71	-	14.9
	09-5	56	58	0.831	-	25.2
	09-6	5	44	<.001	7.65	21.4
	09-7	4	23	0.003	10.31	52.4
	09-8	75	86	0.043	10.57	9
	09-9	20	50	0.003	16.71	32.7
	09-10	64	87	0.002	11.53	10.5
	09-11	48	84	<.001	15.89	16.5
	09-12	52	80	0.003	15.72	16.3
	09-13	42	37	0.491	-	27.7
	09-15	70	77	0.322	-	14.3
	09-16	84	87	0.583	-	9.7

Appendix 2.3.3 C - Pathogen incidence (%) of *S. sclerotiorum* from flower samples.

Sample time	Site	Commercial	Nontreated	F pr.	LSD	CV%
1	09-1	29	29	1	-	42.9
	09-2	4	12	0.035	7.29	62.5
	09-3	11	23	0.021	9.65	38.9
	09-4	4	36	<.001	14.49	49.7
	09-5	9	13	0.397	-	64.3
	09-6	4	6	0.58	-	109.5
	09-7	3	11	0.053	-	79.9
	09-8	8	69	<.001	10.94	19.5
	09-9	10	24	0.042	13.35	53.8
	09-10	13	58	<.001	14.68	28.3
	09-11	12	22	0.108	-	51.4
	09-12	10	8	0.524	-	52.7
	09-13	7	8	0.82	-	89.4
	09-14	6	23	0.009	11.53	54.5
	09-15	2	17	0.008	9.78	70.6
	09-16	15	11	0.561	-	80.2
	09-17	8	24	0.035	14.58	62.5
2	09-1	24	24	1	-	40.1
	09-2	1	8	0.083	-	124.2
	09-3	18	8	0.12	-	69.9
	09-4	3	53	<.001	15.12	37
	09-5	15	25	0.105	-	43.3
	09-6	16	18	0.752	-	57
	09-7	25	8	0.019	13.45	55.9
	09-8	2	59	<.001	7.29	16.4
	09-9	7	5	0.572	-	89.4
	09-10	0	11	0.063	-	146.6
	09-11	0	3	0.04	2.824	129.1
	09-12	11	8	0.521	-	74.4
	09-13	12	28	0.005	9.78	33.5
	09-15	17	35	<.001	5.88	15.5
	09-16	27	48	0.011	14.68	26.8

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Appendix 2.3.3 D - Dry matter (kg/ha) and pyrethrins (kg/ha) from treatment plots at trial sites.

Yield	Site	Commercial	Nontreated	F pr.	LSD	CV%
Dry matter (kg/ha)	09-1	3100	2410	0.008	437.9	9.2
	09-2	2467	2436	0.84	-	8.4
	09-3	2191	2226	0.86	-	12.4
	09-4	2542	2607	0.74	-	10.2
	09-5	3167	2748	0.26	-	16
	09-6	2213	2351	0.67	-	19.3
	09-7	2502	2173	0.38	-	21.2
	09-8	2291	2388	0.61	-	10.8
	09-9	1688	1431	0.3	-	20.6
	09-10	2343	2010	0.09	-	11
	09-11	2799	2502	0.11	-	8.5
	09-12	3166	2340	0.15	-	25.6
	09-13	2036	1761	0.23	-	499.4
	09-15	2233	2204	0.9	-	14.1
	09-16	2624	2541	0.78	-	15.8
Pyrethrins (kg/ha)	09-1	73.9	53.8	0.014	14.26	12.9
	09-2	50.6	53.5	0.7	-	19.8
	09-3	48.1	51	0.49	-	11.1
	09-4	56.9	62.1	0.27	-	10.4
	09-5	68.1	59	0.32	-	18.7
	09-6	39.5	43.3	0.54	-	20
	09-7	39.9	34.7	0.48	-	26.2
	09-8	52.4	53.8	0.76	-	11.7
	09-9	31.8	28.8	0.49	-	18.7
	09-10	46.3	41.3	0.13	-	9.4
	09-11	62	54.2	0.08	-	9
	09-12	68.9	48.7	0.13	-	28
	09-13	42	36.3	0.26	-	16.5
	09-15	47.7	51	0.56	-	14.9
	09-16	58.9	59.8	0.87	-	12

Appendix 2.3.3 E - Comparison of pyrethrins (PII) assay (%) and total pyrethrin assay (%) from treatments plots within sites.

Assay quality	Site	Commercial	Nontreated	F pr.	LSD	CV%
Pyrethrins (PII)	09-1	1.19	1.08	0.366	-	13.5
	09-2	0.99	1.13	0.178	-	12.6
	09-3	1.11	1.19	0.176	-	6.4
	09-4	1.11	1.18	0.43	-	9.3
	09-5	1.12	1.13	0.854	-	10.4
	09-6	0.92	0.89	0.411	-	6.1
	09-7	0.81	0.83	0.811	-	12.4
	09-8	1.23	1.08	0.023	0.125	6.3
	09-9	0.97	1.11	0.204	-	14.2
	09-10	0.94	1.13	0.008	0.122	6.8
	09-11	1.22	1.09	0.003	0.063	3.1
	09-12	1.11	1.04	0.443	-	12.6
	09-13	1.06	1.07	0.869	-	10.8
	09-15	1.1	1.16	0.347	-	8.5
	09-16	1.13	1.11	0.904	-	12.5
Pyrethrins total (%)	09-1	2.57	2.41	0.224	-	6.7
	09-2	2.18	2.35	0.495	-	14.9
	09-3	2.37	2.48	0.048	0.11	2.6
	09-4	2.41	2.57	0.007	0.098	2.3
	09-5	2.32	2.34	0.83	-	5.3
	09-6	1.96	2	0.421	-	3.8
	09-7	1.71	1.73	0.85	-	8.4
	09-8	2.47	2.43	0.637	-	4.6
	09-9	2.04	2.17	0.274	-	7.3
	09-10	2.15	2.23	0.315	-	4.9
	09-11	2.38	2.34	0.059	-	1.2
	09-12	2.34	2.23	0.426	-	8.6
	09-13	2.25	2.22	0.73	-	3.9
	09-15	2.31	2.52	0.037	0.19	4.5
	09-16	2.47	2.56	0.531	-	7.8

Appendix 2.3.3 F - Pyrethrin (PI) assay values (%) and pyrethrin ratio (PI %/PII %) from treatment plots at field trial sites 2009-10.

Assay quality	Site	Commercial	Nontreated	F pr.	LSD	CV%
Pyrethrins (PI)	09-1	1.38	1.33	0.293	-	4.8
	09-2	1.2	1.23	0.854	-	17.9
	09-3	1.25	1.29	0.589	-	6.1
	09-4	1.3	1.39	0.252	-	8
	09-5	1.2	1.21	0.943	-	5.8
	09-6	1.03	1.11	0.156	-	6.4
	09-7	0.91	0.91	0.976	-	11
	09-8	1.24	1.36	0.246	-	9.6
	09-9	1.08	1.06	0.707	-	5.8
	09-10	1.21	1.09	0.211	-	9.8
	09-11	1.16	1.25	0.104	-	4.9
	09-12	1.23	1.19	0.559	-	7.6
	09-13	1.19	1.15	0.679	-	10.1
	09-15	1.21	1.35	0.13	-	8.6
	09-16	1.34	1.44	0.24	-	8.1
Pyrethrins ratio (PI/PII)	09-1	1.19	1.23	0.774	-	15.5
	09-2	1.21	1.09	0.234	-	11.1
	09-3	1.13	1.08	0.623	-	11.3
	09-4	1.18	1.2	0.921	-	17.4
	09-5	1.09	1.07	0.887	-	12.7
	09-6	1.12	1.26	0.165	-	10.4
	09-7	1.12	1.12	1	-	15.1
	09-8	1.02	1.27	0.072	-	14.6
	09-9	1.12	0.97	0.245	-	15.5
	09-10	1.3	0.97	0.036	0.299	15.3
	09-11	0.96	1.14	0.015	0.132	7.3
	09-12	1.12	1.15	0.748	-	11
	09-13	1.14	1.11	0.849	-	20.2
	09-15	1.11	1.17	0.612	-	14.3
	09-16	1.2	1.32	0.382	-	13.8

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Appendix 2.3.3 G - Mean LSD and coefficient of variation values separating treatments each year by yield (kilograms of dry weight of flowers and pyrethrins yielded).

Year	LSD (mean)		Coefficient of variation (mean)	
	DW flowers (kg/ha)	Pyrethrins (kg/ha)	DW flowers (kg/ha)	Pyrethrins (kg/ha)
2007-08	1195.16	25.55	68.55	14.30
2008-09	775.14	18.52	13.48	14.20
2009-10	594.85	13.70	14.55	15.95

Appendix 2.3.3 H - Variation between different years of disease incidence (%) and yield qualities, averaged across all sites.

Measured attribute	2007	2008	2009	F pr.	LSD
FMS (nontreated)	4.93a	4.34a	4.7a	0.13	-
FMS (commercial)	5.11b	4.15a	4.80b	0.001	0.49
B. cinerea (% nontreated)	81.40b	75a	62.87a	0.05	16.70
B. cinerea (% commercial)	60.50a	53.30a	48.90a	0.55	-
S. sclerotiorum (% nontreated)	62.80b	24.90a	22.70a	<.001	17.33
S. sclerotiorum (% commercial)	29.20b	9.60a	11.90a	<.001	8.99
DW flowers (kg/ha, nontreated)	3653	4392	2275	<.001	534.90
DW flowers (kg/ha, commercial)	3746b	4550c	2491a	<.001	476.20
Pyrethrins (kg/ha, nontreated)	76.70b	98.80c	48.80a	<.001	14.31
Pyrethrins (kg/ha, commercial)	80.77b	104.51c	52.47a	<.001	13.92
PI (nontreated, %)	1.20a	1.39b	1.22a	0.044	0.16
PI (commercial, %)	1.29ab	1.39b	1.20a	0.023	0.15
PII (nontreated, %)	0.97a	0.99b	1.08b	0.041	0.10
PII (commercial, %)	1.00a	1.02a	1.07a	0.373	-
P total (nontreated, %)	2.22a	2.38a	2.31a	0.305	-
P total (commercial, %)	2.29a	2.41a	2.26a	0.236	-
Ratio (nontreated, PI %/PII %)	1.25ab	1.41b	1.14a	0.01	0.18
Ratio (commercial, PI %/PII %)	1.29ab	1.38b	1.13a	0.018	0.19

Appendix 2.3.4 A - EC_{50} (µg a.i./ml) values of carbendazim and tebuconazole to *S. sclerotiorum* isolates.

No.	Isolate	Host plant	Place of origin	EC_{50} (µg a.i./ml) carbendazim	EC_{50} (µg a.i./ml) tebuconazole
1	07-3 A	Pyrethrum	Forth	0.501	1.057
2	07-3 B	Pyrethrum	Forth	0.344	0.363
3	07-3 C	Pyrethrum	Forth	1343*	0.106
4	07-3 E	Pyrethrum	Forth	0.175	0.768
5	07-3 F	Pyrethrum	Forth	0.208	0.199
6	07-3 G	Pyrethrum	Forth	0.201	0.242
7	07-3 I	Pyrethrum	Forth	0.947	0.143
8	07-3 M	Pyrethrum	Forth	0.138	0.128
9	07-6 A	Pyrethrum	Penguin	0.139	0.086
10	07-6 B	Pyrethrum	Penguin	0.301	0.116
11	07-6 C	Pyrethrum	Penguin	1.057	0.133
12	07-6 E	Pyrethrum	Penguin	1.046	0.172
13	07-6 F	Pyrethrum	Penguin	0.395	0.194
14	07-6 G	Pyrethrum	Penguin	0.805	0.127
15	07-6 H	Pyrethrum	Penguin	0.334	0.029
16	07-6 I	Pyrethrum	Penguin	0.110	0.276
17	07-6 J	Pyrethrum	Penguin	1.099	-
18	07-5 B	Pyrethrum	Kindred	55658*	0.228
19	07-5 C	Pyrethrum	Kindred	0.189	0.203
20	07-5 D	Pyrethrum	Kindred	0.192	0.742
21	07-5 E	Pyrethrum	Kindred	0.592	0.067
22	07-5 F	Pyrethrum	Kindred	0.336	0.226
23	07-5 H	Pyrethrum	Kindred	0.277	0.163
24	07-5 I	Pyrethrum	Kindred	0.259	0.094
25	07-5 J	Pyrethrum	Kindred	0.297	0.985
26	07-1 A	Pyrethrum	Barrington	0.296	0.071
27	07-1 B	Pyrethrum	Barrington	0.155	0.743
28	07-1 D	Pyrethrum	Barrington	0.151	0.093
29	07-1 J	Pyrethrum	Barrington	0.156	0.036
30	07-10 B	Pyrethrum	Wesley Vale	0.140	0.222
31	07-10 F	Pyrethrum	Wesley Vale	1.658	0.130
32	07-10 G	Pyrethrum	Wesley Vale	0.142	0.227
33	07-10 H	Pyrethrum	Wesley Vale	0.356	0.094
34	07-10 I	Pyrethrum	Wesley Vale	0.444	0.092
35	07-10 J	Pyrethrum	Wesley Vale	0.708	0.397
36	07-10 K	Pyrethrum	Wesley Vale	0.435	0.137
37	07-10 L	Pyrethrum	Wesley Vale	0.567	0.289
38	07-10 M	Pyrethrum	Wesley Vale	0.535	0.106
39	07-4 A	Pyrethrum	Forth	0.288	0.123
40	07-4 B	Pyrethrum	Forth	0.619	0.226
41	07-4 C	Pyrethrum	Forth	1178*	-
42	07-4 D	Pyrethrum	Forth	0.185	-
43	07-4 E	Pyrethrum	Forth	0.602	0.321
44	07-2 A	Pyrethrum	Sassafras	1.166	0.129
45	07-2 B	Pyrethrum	Sassafras	0.357	0.106
46	07-2 C	Pyrethrum	Sassafras	0.910	0.121
47	07-2 D	Pyrethrum	Sassafras	1.607	0.113
48	07-2 E	Pyrethrum	Sassafras	0.866	0.109
49	07-2 G	Pyrethrum	Sassafras	0.590	0.137
50	07-2 H	Pyrethrum	Sassafras	1.032	0.113
51	07-2 K	Pyrethrum	Sassafras	0.390	0.143

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52	07-2 L	Pyrethrum	Sassafras	0.592	0.109
53	07-8 B	Pyrethrum	Table Cape	0.301	0.241
54	07-8 C	Pyrethrum	Table Cape	0.343	0.117
55	07-8 D	Pyrethrum	Table Cape	0.319	0.030
56	07-8 G	Pyrethrum	Table Cape	0.433	0.165
57	07-8 H	Pyrethrum	Table Cape	0.925	0.124
58	07-8 I	Pyrethrum	Table Cape	0.517	0.151
59	07-8 K	Pyrethrum	Table Cape	0.855	0.128
60	07-9 A	Pyrethrum	Table Cape	0.742	0.160
61	07-9 B	Pyrethrum	Table Cape	2.316	0.241
62	07-9 C	Pyrethrum	Table Cape	0.842	0.252
63	07-9 E	Pyrethrum	Table Cape	0.398	0.418
64	07-9 F	Pyrethrum	Table Cape	0.276	0.137
65	07-9 H	Pyrethrum	Table Cape	0.320	0.270
66	07-9 I	Pyrethrum	Table Cape	0.194	0.154
67	07-9 J	Pyrethrum	Table Cape	0.907	0.180
68	07-9 K	Pyrethrum	Table Cape	1.131	-
69	07-7 A	Pyrethrum	Table Cape	1.376	0.194
70	07-7 B	Pyrethrum	Table Cape	0.461	0.120
71	AWI - 1108	Bean	N/NW Tas.	0.498	0.136
72	BGO - 4A08	Bean	N/NW Tas.	0.185	0.074
73	BGO - 5A08	Bean	N/NW Tas.	0.099	0.080
74	BGO - 6A08	Bean	N/NW Tas.	0.263	0.155
75	BGO - 8A08	Bean	N/NW Tas.	0.555	0.088
76	CRA - 108	Bean	N/NW Tas.	0.319	0.016
77	DOB - 108	Bean	N/NW Tas.	0.350	0.086
78	DOB - 708	Bean	N/NW Tas.	0.251	0.025
79	FAS - MBI	Bean	N/NW Tas.	0.185	0.077
80	FGR - 108	Bean	N/NW Tas.	0.226	0.155
81	GMA - 508	Bean	N/NW Tas.	0.660	0.111
82	GRE - 708	Bean	N/NW Tas.	0.567	0.113
83	IVE - 3A08	Bean	N/NW Tas.	0.348	0.194
84	IVE - 508	Bean	N/NW Tas.	0.301	0.013
85	LOA08 - 3	Bean	N/NW Tas.	0.247	0.117
86	LOC - 1008	Bean	N/NW Tas.	0.060	0.071
87	MED - 4A08	Bean	N/NW Tas.	0.589	0.160
88	MED - 5A08	Bean	N/NW Tas.	0.493	0.159
89	MEU - 908	Bean	N/NW Tas.	0.228	0.038
90	PFT A	Pyrethrum	Penguin	0.771	0.094
91	PLA - 4A08	Bean	N/NW Tas.	0.096	0.063
92	PLA - 508	Bean	N/NW Tas.	0.113	0.137
93	PLA - 6A08	Bean	N/NW Tas.	0.259	0.183
94	PLA - 7A08	Bean	N/NW Tas.	0.255	0.067
95	SUM - 408	Bean	N/NW Tas.	0.240	0.058
96	WOL - 108	Bean	N/NW Tas.	0.836	0.038
Minimum, maximum				**0.006, 55658**	**0.013, 1.057**
Mean ± σ (Standard deviation)				**0.5 ± 0.392**	**0.183 ± 0.183**

*Highly insensitive EC_{50} values to carbendazim not used to calculate mean EC_{50} and standard deviation.

Appendix 2.3.4 B - EC_{50} (µg a.i./ml) values of carbendazim and tebuconazole to *B. cinerea* isolates.

No.	Isolate	Host plant	Place of origin	EC_{50} (µg a.i./ml) carbendazim	EC_{50} (µg a.i./ml) tebuconazole
1	07-3 A	Pyrethrum	Forth	256.312	0.165
2	07-3 B	Pyrethrum	Forth	>1000	0.180
3	07-3 D	Pyrethrum	Forth	274.101	0.214
4	07-3 E	Pyrethrum	Forth	269.180	0.186
5	07-3 G	Pyrethrum	Forth	429.511	0.222
6	07-3 I	Pyrethrum	Forth	>1000	0.270
7	07-3 J	Pyrethrum	Forth	>1000	0.192
8	07-3 L	Pyrethrum	Forth	1.048	0.930
9	07-6 B	Pyrethrum	Penguin	0.710	0.131
10	07-6 C	Pyrethrum	Penguin	154.696	0.222
11	07-6 D	Pyrethrum	Penguin	>1000	0.657
12	07-6 E	Pyrethrum	Penguin	147.517	0.385
13	07-6 I	Pyrethrum	Penguin	0.670	0.264
14	07-6 J	Pyrethrum	Penguin	0.943	0.486
15	07-5 A	Pyrethrum	Kindred	>1000	0.642
16	07-5 B	Pyrethrum	Kindred	>1000	0.390
17	07-5 C	Pyrethrum	Kindred	0.338	0.303
18	07-5 D	Pyrethrum	Kindred	>1000	0.291
19	07-5 I	Pyrethrum	Kindred	>1000	0.192
20	07-5 K	Pyrethrum	Kindred	>1000	0.272
21	07-1 A	Pyrethrum	Barrington	0.460	1.148
22	07-1 B	Pyrethrum	Barrington	0.181	0.516
23	07-1 C	Pyrethrum	Barrington	0.301	0.397
24	07-1 D	Pyrethrum	Barrington	0.707	0.787
25	07-1 H	Pyrethrum	Barrington	>1000	0.312
26	07-1 I	Pyrethrum	Barrington	>1000	0.610
27	07-10 A	Pyrethrum	Wesley Vale	0.426	10.745
28	07-10 D	Pyrethrum	Wesley Vale	>1000	0.428
29	07-4 A	Pyrethrum	Forth	0.350	0.290
30	07-4 B	Pyrethrum	Forth	>1000	0.541
31	07-4 C	Pyrethrum	Forth	>1000	0.458
32	07-4 D	Pyrethrum	Forth	>1000	0.387
33	07-4 E	Pyrethrum	Forth	>1000	0.564
34	07-4 G	Pyrethrum	Forth	0.366	0.516
35	07-4 H	Pyrethrum	Forth	0.038	0.291
36	07-2 B	Pyrethrum	Sassafras	0.124	0.522
37	07-2 E	Pyrethrum	Sassafras	>1000	0.242
38	07-2 F	Pyrethrum	Sassafras	0.240	0.591
39	07-2 G	Pyrethrum	Sassafras	>1000	0.230
40	07-2 H	Pyrethrum	Sassafras	0.168	0.849
41	07-2 I	Pyrethrum	Sassafras	0.107	0.704
42	07-8 E	Pyrethrum	Table Cape	>1000	0.333
43	07-9 B	Pyrethrum	Table Cape	>1000	0.363
44	07-9 D	Pyrethrum	Table Cape	0.116	0.509
45	07-9 G	Pyrethrum	Table Cape	>1000	0.364
46	07-9 H	Pyrethrum	Table Cape	>1000	0.823
47	07-7 C	Pyrethrum	Table Cape	>1000	0.570
48	07-7 E	Pyrethrum	Table Cape	>1000	0.326
49	07-7 J	Pyrethrum	Table Cape	>1000	0.224
Minimum, maximum				**0.038, 5.203E+35**	**0.131, 10.475**
Mean ± σ (Standard deviation)				**1.062E+34 ± 7.43E+34**	**0.637 ± 1.49**

Appendices:

Appendix 2.5.1 A - Weekly rainfall totals, average temperature and relative humidity, 2007-08.

Site Code	Date	Total rainfall (mm)	Consecutive hours LW per day	Consecutive hours LW >7 per day	Relative humidity (%)			Temperature (°C)		
					Mean	Max.	Min.	Max.	Min.	Mean
07-5	22-28 Oct	55.09	11.71	9.43	89.92	96.66	78.14	18.39	7.37	13.08
	29 Oct - 4 Nov	17	12	9.14	93.63	100	79.89	18.54	6.09	11.44
	5-11 Nov	60.7	13	9.86	93.71	100	80.13	20.14	7.01	12.68
	12-18 Nov	57.38	13.14	9.29	92.88	100	77.74	24.33	8.96	15.67
	19-25 Nov	2.53	14.86	8.71	90.61	100	72.06	24.79	9.07	16.26
	26 Nov - 2 Dec	30.73	15.14	10.14	94.57	100	81.81	23.04	9.51	15.44
	3-9 Dec	33.77	13.43	10	95.83	100	84.54	22.4	7.64	14.33
	10-16 Dec	2.78	15	10.71	91.18	100	73.41	24	7.84	14.71
	17-21 Dec	17.53	13.4	6	92.53	99.74	81.14	24.24	11.62	16.37
07-10	22-28 Oct	51.52	10.43	8.14	82.85	94.96	65.01	19.37	7.2	13.26
	29 Oct - 4 Nov	14.21	12	4.14	84.04	99.6	63.07	17.79	7.21	11.86
	5-11 Nov	15.74	10.29	7.29	83.74	99.06	61.96	19.16	7.34	12.85
	12-18 Nov	1.76	11.43	7.86	82.90	99.67	57.39	22.84	9.99	15.85
	19-25 Nov	27.18	10.71	7	79.13	99.2	54.23	24.04	9.09	16.21
	26 Nov - 2 Dec	0	13.14	8.71	78.28	98.44	54.19	23	9.36	15.98
	3-9 Dec	22.59	11	5	83.31	99.63	59.03	22	9.21	15.37
	10-16 Dec	2.54	10.71	5.86	72.75	98.01	41	24.06	8.5	15.88
	17-20 Dec	0	0.5	0.25	53.07	66.725	39.3	27.78	17.3	21.13
07-8	22-28 Oct	11.4	1.57	1.14	70.95	83.99	53.8	23.59	7.7	15.02
	29 Oct - 4 Nov	26.37	0.71	0.57	83.71	99.86	59.77	17.01	5.8	11.26
	5-11 Nov	4.31	1.29	0.57	82.68	99.81	57.06	18.93	6.83	12.46
	12-18 Nov	26.13	4.29	4.29	82.17	99.86	55.93	22.24	8.89	15.49
	19-25 Nov	3.55	4.29	2.14	76.78	95.83	51.86	23.41	10.51	16.36
	26 Nov - 2 Dec	2.29	0.29	0	78.42	96.06	58.76	20.56	11.94	15.84
	3-9 Dec	29.68	7.86	0	84.82	99.39	65.03	19.21	9.49	14.59
	10-16 Dec	1.77	0.86	0	74.46	95.61	50.46	20.47	9.77	15.02
	17-20 Dec	0	0	0	65.19	79.9	50.88	23.08	14.63	18.32
07-3	26 Nov - 2 Dec	20.32	9	5.5	41.74	27.86	35.32	20.49	10.64	15.26
	3-9 Dec	44.68	12.43	7.57	42.34	27.26	35.48	19.36	9.44	14.79
	10-16 Dec	2.79	11.86	5.33	42.17	25.11	33.28	19.91	9.71	15.11
	17-20 Dec	17	15.25	5	45.5	25.45	35.5	20.58	13.38	16.26
07-6	24-30 Oct	24.05	14.14	9.43	87.08	99.86	62.96	18.39	7.67	12.87
	31 Oct - 6 Nov	9.38	7.57	2.86	79.31	99.37	51.8	18.54	6.07	11.65
	7-13 Nov	15.99	10.43	6.14	81.82	99.74	56.87	20.14	7.97	13.9
	14-20 Nov	25.38	11.43	5.71	79.17	99.44	51.7	24.33	8.67	16.25
	21-27 Nov	0.76	11.71	4.57	77.46	99.59	45.34	24.79	6.13	14.65
	28 Nov - 4 Dec	86.61	12.43	8.71	86.77	99.81	63.89	23.04	8.77	15.69
	5-11 Dec	12.68	12.29	5.86	85.5	99.93	56.8	22.4	5.24	13.55
	12-18 Dec	4.82	8.14	5.57	74.51	96.2	48.09	24	9.24	16.15

Appendices:

Appendix 2.5.1 B - Weather data 2007-08, summary.

Site	Average daily RH (%)			Mean daily Temperature (°C)		
	October	November	December	October	November	December
07-5	91.59	92.64	93.52	12.25	14.61	15.06
07-10	84.08	81.21	73.36	12.81	14.64	16.80
07-8	75.81	79.89	78.18	13.63	14.53	15.34
07-4	*	*	*	*	15.29	15.24
07-6	86.13	79.82	82.09	12.56	14.44	14.96
Average	**84.40**	**83.39**	**81.79**	**12.82**	**14.70**	**15.48**

Appendix 2.5.1 C - Weather data 2007-08, summary.

Site	Average rainfall (mm/day)			Average longest consecutive period LW per day (hours)			Average longest consecutive period LW >7 per day (hours)		
	October	November	December	October	November	December	October	November	December
07-5	6.6	5.25	2.58	13.2	13.33	13.95	10.1	9.07	9.43
07-10	6.82	1.58	1.26	11.67	11.13	9	7.11	6.87	5.1
07-8	2.94	1.41	1.78	1.4	2.67	3.26	1	1.7	0
07-4	*	4.06	3.22	*	7.8	12.75	*	3.5	6.53
07-6	3.01	2.88	3.85	13.75	10.13	11.28	8.25	5.2	6.67
Average	**4.84**	**3.04**	**2.54**	**10.00**	**9.01**	**10.05**	**6.62**	**5.27**	**5.54**

Appendices:

Appendix 2.5.2 A - Weekly rainfall totals, average temperature and relative humidity, 2008-09.

Site Code	Date	Total rainfall (mm)	Consecutive hours LW per day	Consecutive hours LW >7 per day	Relative humidity (%)			Temperature (°C)		
					Mean	Max.	Min.	Max.	Min.	Mean
08-8	11-17 Nov	-	7.29	4.43	-	-	-	23.36	5.39	14
	18-24 Nov	-	7.86	5.57	-	-	-	21.24	7	13.19
	25 Nov - 1 Dec	-	6.43	4	-	-	-	19.93	7.97	13.05
	2-8 Dec	-	7.86	5.14	-	-	-	18.56	5.34	11.79
	9-15 Dec	-	7.43	4.29	-	-	-	19.93	6.46	12.91
	16-22 Dec	-	7.86	2.57	-	-	-	23.29	6.09	14.06
	23-30 Dec	-	0.14	0	-	-	-	23.74	20.74	22.31
08-4	11-17 Nov	4.05	5.57	3.71	79.62	99.37	53	20.59	6.71	13.69
	18-24 Nov	43.4	7.29	5	83.91	99.01	62.43	20.09	6.63	13.09
	25 Nov - 1 Dec	14.2	4.71	3.86	85.32	98.79	60.39	20.76	8.61	13.35
	2-8 Dec	16.47	7.29	5	84.93	99.59	62	18.19	5.26	11.34
	9-15 Dec	27.66	7.29	5.57	82.88	99.81	56	20.77	7	13
	16-22 Dec	7.86	4.86	3.86	77.53	96.27	48.66	23.61	7.61	14.59
	23-29 Dec	0	0	0	43.86	50.44	36.83	24.10	21.69	22.87
08-2	11-17 Nov	0.5	4.29	3.43	68.79	85.70	48.56	22.97	10.37	16.03
	18-24 Nov	0.75	7.67	6.67	88.94	99.92	70.5	19.9	5.75	12.06
08-1	10-16 Nov	6.31	11.43	7.29	92.67	100.00	78.14	19.14	6.53	12.78
	17-23 Nov	19.78	6.29	5.57	93.16	99.93	79.67	19.17	5.4	12.56
	24-30 Nov	12.18	7.43	4.14	92.47	99.63	78.43	19.4	9.5	13.7
	1-7 Dec	39.27	15	8	94.33	100	79.57	17.9	4.63	11.27
	8-14 Dec	30.44	13.43	6.57	93.53	100	80.4	19.13	6.46	12.85
	15-21 Dec	1.77	9	3.14	89.59	100	71.79	20.96	5.97	13.46
	22-28 Dec	0	1.71	0.43	66.02	72.63	57.49	24.31	20.59	22.3

Appendices:

Appendix 2.5.2 B - Weather data 2008-09, summary.

Site	Average daily RH (%)		Mean daily Temperature (°C)	
	November	December	November	December
08-8	*	*	13.47	15.54
08-4	82.93	70.50	13.52	15.78
08-2	78.09	*	14.20	*
08-5	76.21	76.28	15.47	16.34
Average	**79.07**	**73.39**	**14.17**	**15.89**

Appendix 2.5.2 C - Weather data 2008-09, summary.

Site	Average rainfall (mm/day)		Average longest consecutive period LW per day (hours)		Average longest consecutive period LW >7 per day (hours)	
	November	December	November	December	November	December
08-8	*	*	7.25	5.45	4.75	2.81
08-4	2.94	1.77	6.05	4.45	4.3	3.32
08-2	0.10	*	5.85	*	4.92	*
08-5	2.92	1.75	1.89	3.8	1	2.85
Average	**1.99**	**1.76**	**5.26**	**4.57**	**3.74**	**2.99**

Appendix 2.5.2 D - Comparative pathogen incidence (%) of flowers at sampling times during 2008-09 of *B. cinerea* and *S. sclerotiorum* from nontreated plots.

Sampling date	Pathogen incidence		F pr.	LSD	CV%
	B. cinerea	*S. sclerotiorum*			
17-Nov	1.1	26.6	<0.001	4.079	31.3
4-Dec	10.9	15.8	0.14	6.67	53.2
22-Dec	75	24.9	<0.001	17.32	36.9

Appendices:

Appendix 2.5.3 A - Weekly rainfall totals, average temperature and relative humidity, 2009-10.

Site Code	Date	Total rainfall (mm)	Consecutive hours LW per day	Consecutive hours LW >7 per day	Relative humidity (%)			Temperature ºC		
					Mean	Max.	Min.	Max.	Min.	Mean
09-7	1-7 Oct	1.7	0.86	0.50	77.09	-	-	16.27	2.97	9.28
	8-14 Oct	35.4	6.64	3.44	86.83	-	-	15.81	5.44	9.91
	15-21 Oct	6	1.36	0.85	82.90	-	-	20.09	5.11	11.66
	22-28 Oct	0.5	0.79	0.86	79.20	-	-	20.47	6.04	12.07
	29 Oct - 4 Nov	23.2	4.64	2.40	84.59	-	-	22.81	8.89	14.58
	5-11 Nov	0.6	5.50	2.75	82.07	-	-	26.19	8.93	16.62
	12-18 Nov	1.1	2.57	1.87	83.17	-	-	27.86	9.96	16.70
	19-25 Nov	18	5.07	2.72	84.68	-	-	24.73	9.53	15.42
	26 Nov - 2 Dec	34.8	5.93	2.99	85.07	-	-	21.83	9.71	14.74
	3-9 Dec	5.7	3.36	1.81	78.46	-	-	22.21	7.24	13.63
	10-16 Dec	3.4	5.67	2.82	77.17	-	-	23.78	8.38	15.11
09-15	1-6 Oct	1.9	2.14	0.43	72.35	95.16	45.61	16.27	9.16	2.53
	7-13 Oct	31.3	8.71	4.57	79.51	97.5	55.94	15.81	9.79	4.04
	14-20 Oct	9.7	5.29	2.14	78.41	96.69	48.73	19.97	11.26	4.44
	21-27 Oct	0.5	6.57	2.29	74.46	97.99	42.74	22.49	12.15	4.2
	28 Oct - 4 Nov	23.4	4.86	4.14	81.68	98.8	47.84	23.77	14.63	9.1
09-5	28 Sep - 4 Oct	4.1	6.43	3.57	93.46	-	-	18.67	4	9.89
	5-11 Oct	1.2	9	4.91	92.43	-	-	19.31	2.06	8.7
	12-18 Oct	15.7	10.93	5.74	96.72	-	-	18.93	4.14	10.11
	19-25 Oct	0	6.93	3.58	91.72	-	-	22.61	4.5	11.42
	26 Oct - 1 Nov	0.9	6.86	3.76	94.95	-	-	23.59	9.43	14.38
	2 - 8 Nov	18.6	7.5	5	92.24	-	-	24.67	6.01	13.57
	9 - 15 Nov	19.7	6.07	4.45	94.03	-	-	28.2	9.69	16.49
	16-22 Nov	19.3	5.5	3.95	94.13	-	-	25.13	8.99	14.99
	23-29 Nov	17.6	11.64	5.3	94.98	-	-	22.69	9.53	14.73
	30 Nov - 6 Dec	4.9	2.93	1.65	89.66	-	-	23.63	8.99	14.2
	7 - 13 Dec	6.8	6.5	3.27	88.77	-	-	22.36	8.03	13.63
	14 - 20 Dec	10.3	1.25	2.62	92.25	-	-	24.58	8.1	14.8
09-12	22-28 Nov	26.27	1.86	1	98.986	46.3	74.98	24.66	6.74	15.38
	29 Nov - 5 Dec	13.86	4.29	3.14	99.929	54.73	82.78	23.8	9.34	15.77
	6-12 Dec	16.3	5.14	3.71	100	57.51	84.96	22.11	6.76	14.06
	13-19 Dec	4.83	2	1.57	86.229	40.5	64.30	25.2	12.34	18.31

Appendices:

Appendix 2.5.3 B - Weather data 2009-10, summary.

Site	Average daily RH (%)			Mean daily Temperature (°C)		
	October	November	December	October	November	December
09-7	81.64	84.02	78.67	11.35	15.51	14.38
09-5	93.69	93.65	90.01	10.77	14.98	14.09
09-12	*	76.21	76.28	*	15.47	16.34
Average	**87.66**	**84.63**	**81.65**	**11.06**	**15.32**	**14.94**

Appendix 2.5.3 C - Weather data 2009-10, summary.

Site	Average rainfall (mm/day)			Average longest consecutive period LW per day (hours)			Average longest consecutive period LW >7 per day (hours)		
	October	November	December	October	November	December	October	November	December
09-7	1.43	2.56	0.63	2.60	5.03	3.97	1.48	2.72	2.05
09-5	0.61	2.51	1.29	8.21	7.17	4.18	4.39	4.36	2.64
09-12	*	2.92	1.75	*	1.89	3.80	*	1.00	2.85
Average	**1.02**	**2.66**	**1.22**	**5.40**	**4.70**	**3.98**	**2.94**	**2.69**	**2.51**

Appendix 2.5.4 A - Monthly relative humidity (%) and temperature (°C) values.

Year	Site	Average daily RH (%)			Mean daily Temperature °C		
		October	November	December	October	November	December
2007-08	07-5	91.59	92.64	89.24	12.25	14.61	15.98
	07-10	84.13	81.09	73.36	12.66	14.76	16.80
	07-8	75.81	79.89	77.21	13.63	14.53	15.66
	07-4	*	*	*	*	15.29	15.24
	07-6	86.13	79.82	79.78	12.56	14.44	15.54
	Average	**84.41**	**83.36**	**79.90**	**12.78**	**14.73**	**15.84**
2008-09	08-8	*	*	*	*	13.47	15.54
	08-4	*	82.93	70.5	*	13.52	15.78
	08-2	*	78.09	*	*	14.2	*
	08-1	*	93.2	83.12	*	12.89	15.61
	Average		**84.74**	**76.8**		**13.52**	**15.64**
2009-10	09-7	81.64	84.02	78.67	12.18	16.96	15.50
	09-15	*	76.52	83.19	*	11.16	13.22
	09-5	79.88	73.31	72.56	10.52	14.98	14.09
	09-12	*	76.21	77.01	*	15.47	16.07
	Average	**80.76**	**77.51**	**77.86**	**11.35**	**14.64**	**14.72**

Appendices:

Appendix 2.5.4 B - Mean monthly rainfall totals (mm.) and mean hours leaf wetness (LW) per day across sites.

Year	Rainfall (Total)			Mean consecutive hours LW per day			Mean consecutive hours LW >7 per day		
	October	November	December	October	November	December	October	November	December
2007-08	36.17	71.08	49.35	2.33	7.79	6.41	1.55	4.71	4.21
2008-09	25.09	33.31	18.51	0.58	4.48	3.65	0.26	2.98	2.24
2009-10	26.75	50.27	16.60	4.06	3.4	1.67	2.06	2.02	1.07

Appendix 3.2.1 - Yield sampling details at field trial sites over three years.

Year	**2007-08**	**2008-09**	**2009-10**
Trial type	Replicated field trial	Replicated field trial	Multiple trial sites
Method	Hand harvesting	Hand harvesting	Hedge trimmer
Quadrat size (m^2)	0.7 × 0.7 m (0.49 m^2)	0.7 × 0.7 m (0.49 m^2)	1 × 2.4 m (2.4 m^2)
No. treatments	4	7	3
No. treatment plots per site	4	4	1
No. trial sites	1	1	15
No. replicates (yield)	4	6	4
No. replicates (assay)	1	2	4
Area sampled per treatment (plot/site)	1.96 m (1.4^2 m)	2.94 m (1.72^2 m)	9.6 m (3.1^2 m)
Area sampled per treatment (total)	7.84 m (2.8^2 m)	11.76 m (3.43^2 m)	144 m (12^2 m)

Appendix 3.3.1 A - The effect of fungicide treatment on mean flower developmental stage (FMS), replicated trial Werrin farm (2007-08).

Treatment	**Sampling date/Flower developmental stage**					
	19-Nov	**4-Dec**	**11-Dec**	**18-Dec**	**22-Dec**	**10-Jan**
Nontreated	1.57	3.08	3.54	5.12	5.34	6.24
Folicur^R/Filan^R	1.42	3.14	3.61	4.93	5.07	6.14
Folicur^R/Bavistin^R	1.34	3.06	3.67	5.02	5.48	6
Folicur^R/Switch^R	1.38	2.93	3.58	5.28	5.51	6.03
F pr.	0.056	0.754	0.829	0.546	0.475	0.579
LSD	0.1695	-	-	-	-	-

Appendices:

Appendix 3.3.2 A - Effect of fungicide on flower developmental stage (FMS), Werrin Farm (2008-09).

Treatment	Sampling date	
	4-Dec	22-Dec
Nontreated	2.95	4.04
Switch^{R}	2.77	4.16
Filan^{R}	2.81	4.20
Rovral^{R}	2.86	3.97
Prosaro^{R}	2.78	4.08
Scala^{R}	2.93	4.24
Commercial	2.86	4.01
F pr.	0.257	0.084
LSD	**-**	**-**

Appendix 3.3.2 B - Tukey's adjusted pair wise probabilities comparing fungicide effect on yield of dried flowers 2008-09 (kg/ha).

	Commercial	Filan^{R}	Nontreated	Prosaro^{R}	Rovral^{R}	Scala^{R}	Switch^{R}
Commercial		**0.039**	0.998	0.383	**0.063**	0.350	1
Filan^{R}	0.039		0.015	0.785	1.000	0.818	0.043
Nontreated	0.998	**0.015**		0.179	**0.025**	0.161	0.996
Prosaro^{R}	0.383	0.785	0.179		0.905	1	0.414
Rovral^{R}	0.063	1.000	0.025	0.905		0.926	0.071
Scala^{R}	0.350	0.818	0.161	1	0.926		0.380
Switch^{R}	1	**0.043**	0.996	0.414	**0.071**	0.380	

Appendices:

Appendix 3.3.3 A - Flower maturity stage (FMS) of flower samples from field sites sampled December 2 (2009-10).

Site	Commercial	Modified	Nontreated	F pr.	LSD
09-1	3.17	3.13	3.37	0.023	0.172
09-2	3.43	3.61	3.79	0.088	-
09-3	3.02	3.03	3.14	0.02	0.087
09-4	3.26	3.02	3.26	0.143	-
09-5	3.25	3.12	3.59	0.029	0.339
09-6	3.42	3.27	3.78	<.001	0.212
09-7	3.51	3.26	3.81	0.044	0.418
09-8	3.32	3.25	3.3	0.792	-
09-9	3.19	3.17	3.26	0.633	-
09-10	3.29	3.4	3.72	0.002	0.206
09-11	3.09	3.18	3.11	0.097	-
09-12	3.12	3.32	3.31	0.179	-
09-13	3.1	3.29	3.59	0.004	0.251
09-14	3.41	3.88	3.65	0.089	-
09-15	3.64	3.87	4.14	0.025	0.342
09-16	3.44	3.15	3.72	<.001	0.206
09-17	3.38	3.26	3.7	0.015	0.284

Appendix 3.3.3 B - Flower developmental stage (FMS) near harvest time 2009-10 (14-18 December).

Site	Commercial	Modified	Nontreated	F pr.	LSD
09-1	4.75	5.13	4.92	0.429	-
09-2	4.58	4.44	4.56	0.736	-
09-3	4.96	5.15	4.69	0.226	-
09-4	5.08	4.57	4.57	0.056	0.468
09-5	5.32	5.55	5.77	0.663	-
09-6	5.34	5.19	4.62	0.213	-
09-7	5.21	5.93	5.82	0.109	-
09-8	4.03	3.81	3.92	0.497	-
09-9	3.81	4.41	4.21	0.178	-
09-10	5.37	5.36	4.98	0.32	-
09-11	3.86	4.17	4.18	0.291	-
09-12	3.84	3.99	4.13	0.304	-
09-13	4.14	4.17	4.01	0.814	-
09-15	5.5	5.04	5.2	0.028	0.32
09-16	5.14	5.05	4.9	0.245	-

Appendix 3.3.3 C - Fungal incidence (%) in flowers from treatment plots at trial sites of *B. cinerea* and *S. sclerotiorum* on December 2 (2009-10).

Fungal incidence *B. cinerea* in flowers (%)					
Site	**Commercial**	**Modified**	**Nontreated**	**F pr.**	**LSD**
09-1	18b	12b	52a	<.001	13.13
09-2	38b	39b	61a	0.026	17.88
09-3	13b	13b	43a	<.001	9.66
09-4	17b	11b	46a	0.003	18.1
09-5	34ab	16b	50a	0.005	18.19
09-6	9b	4b	46a	<.001	8.98
09-7	10ab	2b	22a	0.013	12.33
09-8	42b	13c	74a	<.001	16.06
09-9	26ab	13b	34a	0.007	11.73
09-10	56ab	22b	76a	<.001	22.4
09-11	21b	17b	51a	<.001	8.15
09-12	32b	18c	47a	0.002	13.72
09-13	24	21	30	0.391	-
09-14	63ab	30c	79a	<.001	18.44
09-15	60	57	50	0.417	-
09-16	60b	29c	76a	<.001	13.72
09-17	21b	13b	66a	<.001	11.39
Mean	**32b**	**19.4c**	**51.4a**	**<.001**	**11**
Fungal incidence *S. sclerotiorum cinerea* in flowers (%)					
Site	**Commercial**	**Modified**	**Nontreated**	**F pr.**	**LSD**
09-1	29	13	29	0.064	-
09-2	4	8	12	0.066	-
09-3	11b	7b	23a	0.005	8.72
09-4	4b	8b	36a	<.001	12.39
09-5	9	7	13	0.378	-
09-6	4	3	6	0.656	-
09-7	3	4	11	0.061	-
09-8	8b	6b	69a	<.001	11.04
09-9	10b	7b	24a	0.014	11.25
09-10	13b	5b	58a	<.001	12.33
09-11	12a	5b	22a	0.011	10.22
09-12	10	7	8	0.647	-
09-13	7	4	8	0.624	-
09-14	6b	2b	23a	0.001	9.58
09-15	2b	7b	17a	0.009	8.81
09-16	15	6	11	0.309	-
09-17	8b	6b	24a	0.015	12.39
Mean	**9.1b**	**6.2b**	**23.17a**	**<.001**	**6.63**

Appendix 3.3.3 D - Estimated dry weight of flowers and pyrethrins yielded (kg/ha) from treatment plots at field trial sites 2009-10 season.

Dry weight flowers yielded (kg/ha)					
Site	**Commercial**	**Modified**	**Nontreated**	**F pr.**	**LSD**
09-1	3100 a	2633 ab	2410 b	0.022	458
09-2	2467	2753	2436	0.162	-
09-3	2191	2401	2226	0.562	-
09-4	2542	2912	2607	0.215	-
09-5	3167	2980	2748	0.492	-
09-6	2213	3026	2351	0.315	-
09-7	2502	2714	2173	0.365	-
09-8	2291	2714	2388	0.118	-
09-9	1688	1350	1431	0.348	-
09-10	2343	2347	2010	0.164	-
09-11	2799	2659	2502	0.359	-
09-12	3166	2248	2340	0.102	-
09-13	2036	1632	1761	0.124	-
09-15	2233	2597	2204	0.246	-
09-16	2624	2881	2541	0.518	-
Pyrethrins yielded (kg/ha)					
Site	**Commercial**	**Modified**	**Nontreated**	**F pr.**	**LSD**
09-1	73.9 a	64 ab	53.8 b	0.018	12.59
09-2	50.6	59.3	53.5	0.403	-
09-3	48.1	50.3	51	0.768	-
09-4	56.9	66.5	62.1	0.304	-
09-5	68.1	66	59	0.505	-
09-6	39.5	57.4	43.3	0.241	-
09-7	39.9	49.5	34.7	0.141	-
09-8	52.4	59.9	53.8	0.196	-
09-9	31.8	25.3	28.8	0.348	-
09-10	46.3	50	41.3	0.1	-
09-11	62	57.5	54.2	0.274	-
09-12	68.9	45.5	48.7	0.075	-
09-13	42	32.9	36.3	0.129	-
09-15	47.7	54.1	51	0.508	-
09-16	58.9	68.7	59.8	0.224	-

Appendix 3.3.3 E - Effect of fungicide treatments at different sites on pyrethrins I (PI) and pyrethrins II (PII) content 2009-10 (% content by weight).

Pyrethrins I (PI) content (% content by weight)					
Site	**Commercial**	**Modified**	**Nontreated**	**F pr.**	**LSD**
09-1	1.38	1.4	1.33	0.225	-
09-2	1.2	1.24	1.23	0.938	-
09-3	1.25	1.17	1.29	0.211	-
09-4	1.29	1.38	1.39	0.464	-
09-5	1.2	1.25	1.21	0.761	-
09-6	1.03	1.07	1.11	0.311	-
09-7	0.91	0.99	0.91	0.273	-
09-8	1.24	1.35	1.36	0.389	-
09-9	1.08	1.06	1.06	0.973	-
09-10	1.21	1.05	1.09	0.114	-
09-11	1.16	1.23	1.25	0.411	-
09-12	1.23	1.17	1.19	0.792	-
09-13	1.19	1.11	1.15	0.536	-
09-15	1.21	1.3	1.35	0.309	-
09-16	1.34	1.45	1.44	0.642	-
Pyrethrins II (PII) content (% content by weight)					
Site	**Commercial**	**Modified**	**Nontreated**	**F pr.**	**LSD**
09-1	1.19	1.23	1.08	0.362	-
09-2	0.98	1.09	1.13	0.245	-
09-3	1.11	1.09	1.19	0.399	-
09-4	1.11	1.09	1.18	0.55	-
09-5	1.12	1.15	1.13	0.86	-
09-6	0.92	0.98	0.89	0.411	-
09-7	0.81	0.97	0.83	0.065	-
09-8	1.23 a	1.05 b	1.07 b	0.013	0.113
09-9	0.96	0.96	1.11	0.272	-
09-10	0.94 b	1.27 a	1.13 ab	0.013	0.195
09-11	1.22	1.09	1.09	0.147	-
09-12	1.11	1	1.03	0.417	-
09-13	1.05	1.06	1.07	0.982	-
09-15	1.09	0.98	1.16	0.124	-
09-16	1.12	1.16	1.11	0.85	-

Appendix 3.3.3 F - Effect of fungicide treatments at different sites on total pyrethrins content 2009-10 (% content by weight).

Site	Commercial	Modified	Nontreated	F pr.	LSD
09-1	2.57	2.62	2.41	0.155	-
09-2	2.18	2.33	2.35	0.644	-
09-3	2.37 b	2.25 c	2.48 a	0.003	0.103
09-4	2.41	2.46	2.57	0.103	-
09-5	2.32	2.4	2.34	0.671	-
09-6	1.96	2.05	2.01	0.258	-
09-7	1.71 b	1.97 a	1.73 b	0.029	0.197
09-8	2.47	2.41	2.43	0.758	-
09-9	2.04	2.02	2.17	0.313	-
09-10	2.15	2.32	2.23	0.288	-
09-11	2.38	2.32	2.34	0.393	-
09-12	2.34	2.17	2.22	0.398	-
09-13	2.25	2.17	2.22	0.409	-
09-15	2.31 b	2.27 b	2.51 a	0.021	0.169
09-16	2.47	2.61	2.56	0.611	-

Appendix 3.3.3 G - Coefficients of determination (R^2), intercepts, slopes, standard error of the estimate for y (SEEy), and coefficients of variation (CV) for borderline ($0.05 < P < 0.1$) and significant ($P < 0.05$) linear relationships between flower pathogen incidences or AUFIC during December, and measured yield attributes of flowers harvested.

	Fungal incidence/AUFIC	Yield attribute	R^2	Intercept	Slope ($y = mx + c$)	SEEy	CV (%)	P =
2008-09 (21xy pairs)	***B. cinerea* Dec-4**	Dry weight (kg/ha)	0.268	4892.40	y = -28.3x + 4892.4	212.55	12.66	0.016
		Fresh weight (kg/ha)	0.194	1088.97	y = -5.4x + 1088.9	49.84	13.13	0.046
		Pyrethrin yield (kg/ha)	0.185	121.26	y = -0.588x + 121.26	5.61	13.24	0.052
	B. cinerea* AUFIC	Dry weight (kg/ha)	0.179	6142.93	y = -2.37x + 6142.93	848.65	13.40	0.056
		Pyrethrin yield (kg/ha)	0.137	149.11	y = -0.052x + 149.11	21.75	13.63	0.098
	***B. cinerea* 22nd Dec-22**	Pyrethrin PII assay (%)	0.317	1.19	y = -0.0036x + 1.1936	0.079	6.83	0.008
	***S. sclerotiorum* Dec-4**	Dry weight (kg/ha)	0.155	4897.01	y = -20.85x + 4897.01	280.721	13.60	0.078
2007-09 (37 xy pairs)	***B. cinerea* Dec. 4-11**	Dry weight (kg/ha)	0.131	4588.72	y = -8.69x + 4588.73	158.99	12.88	0.028
		Fresh weight (kg/ha)	0.17	17992	y = -92.56 + 17992	2007.55	26.24	0.011
		Pyrethrin yield (kg/ha)	0.40	117.83	y = -0.456x + 117.83	3.95	13.44	<.0001
		Pyrethrin PI assay (%)	0.73	1.79	y = -0.00655x + 1.79	0.028	6.27	<.0001
		Pyrethrin ratio (PI:PII)	0.592	1.89	y = -0.00757x + 1.88	0.045	9.53	<.0001
		OD fraction	0.23	0.944	y = -0.000171x + 0.94	0.002	0.83	0.003
		Total pyrethrin assay (%)	0.578	2.74	y = -0.00606x + 2.74	0.037	5.02	<.0001
	***B. cinerea* Dec-22**	Pyrethrin PII assay (%)	0.162	1.07	y = -0.0018x + 1.071	0.041	7.05	0.0136
	***S. sclerotiorum* Dec. 4-11**	Dry weight (kg/ha)	0.125	4657.44	y = -13.69x + 4657.44	188.39	12.92	0.0322
		Pyrethrin yield (kg/ha)	0.189	115.73	y = -0.51x + 115.73	5.43	15.63	0.0071
		Pyrethrin PI assay (%)	0.166	1.70	y = -0.00504x + 1.7	0.059	11.01	0.0123
		Total pyrethrin assay (%)	0.21	2.69	y = -0.00584x + 2.69	0.059	6.89	0.0047
	***S. sclerotiorum* Dec-22**	Fresh weight (kg/ha)	0.22	15839	y = -138.08x + 15839	1094.98	25.41	0.0033
	S. sclerotiorum* AUFIC	Fresh weight (kg/ha)	0.11	14031	y = -15.64x + 14031	3291.54	97.92	0.0454

***AUFIC 4-22 December**

Appendix 3.3.4 - EC_{50} values *B. cinerea* and *S. sclerotiorum* to iprodione (µg/ml. of active ingredient).

No.	*B. cinerea*		*S. sclerotiorum*	
	Isolate	**EC_{50} (µg/ml)**	**Isolate**	**EC_{50} (µg/ml)**
1	07-3 A	0.130	07-3 A	0.231
2	07-3 B	2.274	07-3 J	0.235
3	07-3 C	0.239	07-3 B	0.193
4	07-3 D	0.792	07-3 H	0.074
5	07-3 E	0.849	07-3 E	0.245
6	07-3 J	1.514	07-3 F	0.141
7	07-6 C	0.490	07-3 I	0.193
8	07-6 D	0.977	07-3 G	0.180
9	07-6 E	0.406	07-6 B	0.067
10	07-6 G	0.591	07-6 D	0.607
11	07-6 H	0.413	07-6 A	0.054
12	07-6 I	0.194	07-6 E	0.161
13	07-6 J	0.237	07-6 F	0.347
14	07-5 A	1.600	07-6 G	0.291
15	07-5 C	1.292	07-6 H	0.297
16	07-5 D	2.848	07-5 A	0.170
17	07-5 F	2.573	07-5 B	0.155
18	07-5 G	2.486	07-5 C	0.252
19	07-5 I	4.263	07-5 D	0.169
20	07-5 K	1.872	07-5 E	0.199
21	07-1 A	0.306	07-5 F	0.205
22	07-1 C	0.706	07-5 H	0.223
23	07-1 D	0.330	07-5 S	0.140
24	07-1 F	1.055	07-1 B	0.060
25	07-1 H	0.503	07-1 C	0.022
26	07-1 I	1.467	07-1 E	0.198
27	07-1 J	0.374	07-1 I	0.183
28	07-10 A	0.335	07-10 A	0.057
29	07-10 D	1.385	07-10 B	0.129
30	07-10 F	0.611	07-10 D	0.050
31	07-4 B	1.122	07-10 E	0.189
32	07-4 C	4.573	07-10 F	0.169
33	07-4 G	0.212	07-10 I	0.183
34	07-2 B	0.294	07-4 A	0.025
35	07-2 E	7.894	07-4 B	0.221
36	07-2 F	0.438	07-2 A	0.084
37	07-2 G	2.790	07-2 D	0.134
38	07-2 I	0.478	07-2 E	0.192
39	07-8 C	4.265	07-2 H	0.214
40	07-9 B	1.455	07-8 C	0.546
41	07-9 D	0.264	07-9 A	0.103
42	07-9 G	0.517	07-9 C	0.157
43	07-9 H	0.203	07-9 D	0.224
44	07-7 C	8.476	07-9 J	0.205
45	07-7 E	5.480	07-9 Z	0.189
46	07-7 J	2.774	07-7 B	0.214
Minimum		**0.13**		**0.022**
Maximum		**8.476**		**0.607**
Mean ± σ		**1.616 ± 1.93**		**0.186 ± 0.11**

Appendices:

Appendix 4.1 - Base pair sequences of internal transcribed spacer (ITS) regions of ten selected isolates; A = adenosine, G = guanine, C = cytosine and T = thymine.

BRIP 53343: *S. minor* 100%

Collector's number: 08-5

TGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTGCTT
TGGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTCTGAGTA
CTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGG
CATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAGGCTCTA
AAATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTCTGCAAA
AAACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAA

BRIP 53344: *S. minor* 100%

Collector's number: 09-8 A

ACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTG
CTTTGGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTCTGA
GTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGG
GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAGGCT
CTAAAATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTCTGC
AAAAAACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAT

BRIP 53345: *S. minor* 99% (1bp difference)

Collector's number: 09-1 A

ACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTACTATTACTTTGTTG
CTTTGGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTCTGA
GTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCG
ATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGG
GGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAGGCT
CTAAAATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTCTGC
AAAAAACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATA

Appendices:

BRIP 53346: *S. minor* 100%

Collector's number: 09-3 D

GCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTGCTTT
GGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTCTGAGTAC
TATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAA
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAGGCTCTAA
AATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTCTGCAAAA
AACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAA

BRIP 53347: *S. minor* 100%

Collector's number: 09-5 A

GAACCTGCGGAAGGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTG
TTGCTTTGGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTC
TGAGTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCC
GGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAG
GCTCTAAAATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTC
TGCAAAAAACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAATAA

BRIP 53458: *S. minor* 100%

Collector's number: 08-1

TGAACCTGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTG
TTGCTTTGGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTC
TGAGTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAAT
GCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCC
GGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAG
GCTCTAAAATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTC
TGCAAAAAACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAT

Appendices:

BRIP 53459: *S. minor* 99% (2bp diff)

Collector's number: 08-7

TTCCGTAGGTGAACCTGCGGAAGGATCATTACAGAGAACATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATT
ATTACTTTGTTGCTTTGGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATT
AATGTCGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACG
CAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCT
TGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAG
TAATGGCAGGCTCTAAAATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCG
GTGTGCTTCTGCAAAAAACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCAT
ATCAAT

BRIP 53460: *S. minor* 100%

Collector's number: 09-8 E

GCGGAAGGATCTAATCAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTGCTTT
GGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTCTGAGTAC
TATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAA
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGGC
ATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAGGCTCTAA
AATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTCTGCAAAA
AACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATCAAT

BRIP 53461: *S. minor* 100%

Collector's number: 09-8 Y

TGCGGAAGGATCATTACAGAGTTCATGCCCGAAAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTGCTT
TGGCGAGCTGCTCTTCGGGGCCTTGTATGCTCGCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTCTGAGTA
CTATATAATAGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATA
AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGG
CATGCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAGGCTCTA
AAATCAGTGGCGGCGCCGCTGGGTCCTGAACGTAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTCTGCAAA
AAACCCAATTTTCTATGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATAT

Appendices:

BRIP 53462: *S. minor* 100%

Collector's number: 09-3 B

AAGGGTAGACCTCCCACCCTTGTGTATTATTACTTTGTTGCTTTGGCGAGCTGCTCTTCGGGGCCTTGTATGCTC
GCCAGAGAATATCAAAACTCTTTTTATTAATGTCGTCTGAGTACTATATAATAGTTAAAACTTTCAACAACGGAT
CTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCAT
CGAATCTTTGAACGCACATTGCGCCCCTTGGTATTCCGGGGGGCATGCCTGTTCGAGCGTCATTTCAACCCTCAA
GCTCAGCTTGGTATTGAGTCCATGTCAGTAATGGCAGGCTCTAAAATCAGTGGCGGCGCCGCTGGGTCCTGAACG
TAGTAATATCTCTCGTTACAGGTTCTCGGTGTGCTTCTGCAAAAAACCCAATTTTCTATGGTTGACCTCGGATCA
GGTAGGGATACCCGCTGAACTTAAGCATA

Appendix 4.2 - Maximum, minimum and mean monthly temperature (Degrees Celcius) at Forthside Vegetable Research Station.

	Highest max. daily temp. (°C)	Lowest min. daily temp.(°C)	Mean daily max. temp. (°C)	Mean daily min. temp. (°C)
January	30.2	0	20.6	11
February	30.8	0.9	21.1	11.6
March	29	1.2	19.8	10.4
April	24	-2.3	16.9	8.2
May	19.9	-2.6	14.4	6.2
June	17.6	-2.6	12.2	4.1
July	15.8	-3.2	11.5	3.6
August	16.9	-2.9	12.1	4.2
September	20.3	-2.6	13.3	4.9
October	24.1	-2.8	15.4	6.2
November	28.1	-0.6	17.1	8.1
December	28.6	0.2	18.9	9.6

Source: DPIWE (2010) Climate averages for Forthside Research Station (1966-2010) 41.21°S, 146.26°E. http://www.bom.gov.au/climate/averages/tables/cw_091186.shtml

Appendix 5.3 A - Dimensions (mm.) of sclerotia cultured from surface sterilised and bisected sclerotia, Inoculation Experiment A of *S. minor* (L=length; W=width).

Sclerotia	Culture 1:		Culture 2:		Culture 3:		Culture 4:		Culture 5:		Culture 6:		Culture 7:		Culture 8:		Culture 9:		Culture 10:	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	2	1.1	1.5	1.1	2.2	1.2	1.7	1	2.6	1.4	2.1	1.3	2.4	1.2	1.2	1.1	2.1	1.3	1.7	1.3
2	1.4	1.2	1.9	1.1	1.3	1.1	1.3	1	1.8	1.4	1.7	1.3	1.9	1.1	1.7	1.3	2	1.4	2.1	1.2
3	1.9	1.8	1.9	1	2.7	1.2	1.6	1.1	2.1	1.3	2.4	1.6	0.6	0.4	1.8	1.7	2.3	1.3	0.8	0.6
4	1.7	1.6	2.9	1.3	2.7	1.3	1.4	1.2	2	1.5	1.9	1.3	2.1	1.1	1.8	1.4	2.2	1	2.5	1.3
5	1.7	1.6	0.6	0.4	2.1	1.9	1.9	1	3.1	1.4	2.5	1.3	2	0.8	1.8	1.5	2.3	1.3	2.1	1.2
6	3	1.2	2.4	0.5	1.7	1.1	2.2	1.1	1.7	1.3	2.3	1.3	1.5	1.1	2.1	1.3	1.8	1.4	2.1	1.1
7	1	0.8	2.3	1.1	2.2	1.2	1.8	0.7	2.6	1.4	2.1	0.9	1.9	1.1	2.4	1.6	1.8	1.7	2.6	1.4
8	0.9	0.7	2.1	1.1	1.7	1	1.7	1	2.1	0.9	2.1	1.3	2.1	1.1	2.6	1.3	1.8	1.4	2.3	1.3
9	1.7	1.6	1.9	1	2.1	1.1	1.9	1	2.6	1.4	2.1	1.2	2.2	1.4	3.1	1.3	2.6	1.4	2.1	1.3
10	1.1	0.8	2.7	1.6	1.4	1.3	0.4	0.3	2.1	1.6	1.7	1.5	2.9	1.3	0.8	0.6	1.7	1.5	2.6	2
11	1.8	1.3	1.9	0.9	1.4	1.2	1.4	1.1	1.8	1.4	2.5	1.4	1.8	1.3	1.4	1.3	1.8	1.4	1.8	1.7
12	1.7	1.6	1.6	1.3	1.9	1.1	1.4	1.1	2.1	1.5	1.7	1.3	2.1	0.8	1.8	1.1	2.6	1.4	1.8	1.4
13	1.6	1.5	2.4	1.2	0.7	0.9	1.4	1.4	1.7	1.3	2.3	1.3	1.9	0.9	1.9	1.3	1.9	1.3	2.2	1
14	2.6	1	2.2	1.4	1.3	1.1	1.4	1.2	3.1	1.5	2.3	1.3	1.5	1.1	1.9	1.5	1.7	1.3	1.7	1.3
15	2.7	1.5	2.1	1.1	1.6	1.2	1.4	1.1	2.3	1.3	2.1	1.3	1.7	1.1	2	1.5	2.3	1.3	2.1	1.3
16	1.4	1	2.3	1.9	1.9	1.1	1.9	1	2.5	1.4	2.6	1.4	2.1	1.1	2.9	1.4	3.1	1.5	2	1.5
17	1.9	1.3	1.9	1.1	1.7	1.1	2.2	1.1	2.3	1.3	1.8	1.7	1.9	1.1	3.1	1.4	2.6	2	3	1.6
18	1.4	1	2.3	1.9	1.7	0.7	1.3	1	1.7	1.3	3.1	1.5	2.3	1.9	3.1	1.5	2.1	1.3	0.8	0.6
19	3.6	1.5	2.4	1.8	2.7	1.2	1.3	1.2	2.1	1.3	1.5	1.3	2.3	1.1	2.3	1.1	1.7	1.3	2.1	1.2
20	1.7	1.3	1.6	1.2	2.1	1.2	2.1	1	2.1	0.9	2.6	1.4	2.3	1.2	2.3	1.3	2.3	1	1.8	1.5
21	1.7	1.3	1.6	1.5	1.7	1.1	1.4	1.1	2.1	1.6	2.3	1.3	1.9	1.1	2.3	1	2.3	1.3	2.3	1.3
22	2.9	2.1	2.1	0.8	1.6	1.3	1.6	1.2	1.5	1.3	2.1	1.3	2.1	1.1	2.5	1.5	0.8	0.6	2.5	1.6
23	2.2	1.8	1.6	1.2	0.7	0.9	2.2	1.1	1.8	1.4	3.1	1.5	0.6	0.4	2.6	0.7	2.3	1.3	2.3	1.3
24	1.9	1.5	2.1	1.1	2.2	1.2	2	1.3	1.8	1.4	1.7	1.3	1.6	1.2	2.6	1.4	1.8	1.4	2.6	1.4
25	1.6	1	2	0.8	1.7	1	1.5	1	1.8	1.5	2.3	1.3	2.4	0.5	2.7	1.6	2.3	1.3	2.9	1.4
26	1	0.8	2.3	1.2	1.4	1.2	2.1	1.1	3.1	1.4	2.5	1.4	2.1	1.1	2.7	0.8	2.3	1.3	1.8	1.4
27	0.8	0.6	1.5	1.1	1.9	1.1	1.3	1	1.1	1.1	1.8	1.4	1.8	1.2	2.9	1.8	1.7	1.3	2.1	1.6
28	1.6	1.5	1.6	1.3	2.7	1.2	1.7	0.9	2.1	0.9	2	1.4	1.5	1.1	3.1	1.4	2.3	1.3	3	1.6
29	2.1	1.7	1.9	1.1	2.1	1.2	1.9	1	1.7	1.3	1.8	1.4	1.6	1.2	2.8	1.5	3.1	1.5	1.8	1.4
30	1.8	1.4	1.5	1.1	2.2	1.2	2.2	1.1	2.5	2.1	2.5	1.6	1.6	1.5	2.6	0.7	0.8	0.6	3.1	1.5
31	2.4	1.3	1.8	1.3	0.7	0.9	2.7	1.2	2.6	1.4	2.1	1.3	1.6	1.2	2.6	1.3	2.6	1.4	2.5	1.3
32	2	1.1	1.8	1.2	1.3	1.1	2.1	1.8	2.4	1.6	2	1.5	1.6	1.2	1.8	1.1	1.8	1.7	2.6	2
33	1.8	1.4	2.8	1.4	2.7	1.3	2.2	1.7	2.1	1.2	2.5	2.1	1.9	0.7	2.5	1.4	1.7	1.5	2.5	2.1
34	1	0.7	1.6	1.5	1.3	1.1	1.9	1	2.9	1.4	2.6	1.4	2.3	1.4	1.1	1.1	2.5	1.6	2.3	1.3
35	0.9	0.7	0.6	0.4	2.7	1.2	1.7	1	2.3	1	3.1	1.5	1.5	1.1	0.8	0.6	2.6	1.4	2.5	2.1
36	1.5	1.4	1.5	1.3	1.9	1.1	1.4	1.1	1.5	1.3	0.8	0.6	2.7	1.2	1.7	1.3	2.3	1.3	1.5	1.3
37	1.6	1.5	1.9	1	2.1	1.9	1.3	1	1.9	1.3	2.5	2.1	0.6	0.4	1.1	1.1	2.6	1.4	2.3	1.3
38	2.5	0.9	2.1	1.1	2.2	1.2	1.7	0.8	2.1	1.2	2.5	1.3	1.9	1.1	2.5	1.4	2.1	1.5	3.1	1.5
39	1.6	1	1.6	1.3	1.3	1.1	1.9	0.7	2	1.4	1.7	1.3	1.6	1.2	3.1	1.4	1.8	1.4	1.1	1.1
40	1.6	1.4	2.3	1.1	2.1	1.2	2.5	1.5	2.1	1.3	2.9	1.8	1.9	1	2.9	1.8	2.1	1.5	2.5	1.6
41	2.5	1.2	2.1	1.1	1.7	1.1	1.9	1	2.1	1.2	2	1.5	2.1	1.1	1.7	1.3	2.5	1.6	1.9	1.2
42	1.3	1.2	1.8	1.2	2.7	1.2	1.5	1	3.1	1.4	2.2	1	1.9	1.3	3.1	1.4	0.8	0.6	2.1	1.5
43	1.7	1.2	2.3	1.4	2.2	0.5	0.4	0.3	3.1	1.4	2.3	1	1.5	1.1	2.1	1.3	2.3	1	2.4	1.6
44	1.5	0.9	1.9	1.3	1.9	1.1	2.7	1.2	3.1	1.4	2.5	2.1	1.9	1.4	1.1	1.1	1.7	1.3	2.5	1.3
45	1.9	1.3	2.1	1.1	2.5	1.6	1.9	1	2.2	1	2.3	1.3	2.3	1.4	2.6	1.4	3.1	1.5	1.8	1.7
46	1.7	1.3	1.6	1.2	2.7	1.2	1.8	0.7	3.1	1.5	1.7	1.3	2.1	1.1	3.1	1.5	2.5	2.1	2.1	1.1
47	2.1	1.4	2.4	1.2	1.9	0.8	1.4	1.1	2.6	1.4	1.8	1.7	1.5	1.3	1.1	1.1	2.1	1.5	2.6	2
48	1.6	1.3	2.1	0.9	1.9	1.1	2.1	1.1	2.1	1.2	1.8	1.4	2.3	1.4	2.5	1.6	2	1.4	3.1	1.5
49	0.9	0.7	2.7	1.2	2.2	1.2	1.9	1	3.1	1.5	2.3	1.3	1.9	1.1	3.1	1.4	1.8	1.4	2	1.5
50	1.5	1.4	2.3	1.4	1.4	1.2	1.6	1.1	2.5	2.1	1.7	1.3	2.3	1.2	2	1.5	0.8	0.6	2.2	1
51	1.7	1.2	1.6	1.2	2.1	1.2	1.9	1	2.5	2.1	2.3	1.3	2.4	1.8	2.1	1.3	1.7	1.3	2.1	1.3
52	2.5	0.7	2.1	1.1	2	1.4	2.1	1.8	1.8	1.5	2.5	1.4	1.8	1.3	1.8	1.4	1.8	1.5	3.1	1.5
53	1.5	1	1.9	1.4	1.7	1.1	1.3	1	2.5	2.1	1.7	1.3	0.9	0.9	2.5	1.4	2.6	1.4	2.6	0.7
54	2.9	1.2	2.4	1.8	1.6	1.2	1.4	1.1	2.5	1.3	2.3	1.3	1.5	1.1	2.1	1.5	2.1	1.3	2.3	1.3
55	1.6	1.3	2.8	1.4	1.7	1.1	1.9	1	2.3	1.3	1.7	1.3	2.3	1.9	0.8	0.6	2.3	1.3	2.1	1.2
56	1.5	1	1.7	1.1	2.1	1.2	1.7	1	1.1	1.1	2.5	1.4	2.3	1.9	2.1	1.2	0.8	0.6	2.9	1.8
57	1.9	1.1	1.6	1.2	1.8	0.8	2.7	1.2	2.1	0.9	2.6	1.4	2.1	1.1	1.8	1.4	1.8	1.4	1.8	1.5
58	2	1.2	1.5	1.1	1.7	0.7	2.1	1.8	2.5	1.4	2.6	2	1.9	1.3	2.6	1.4	1.8	1.5	2.4	1.6
59	1.9	1.5	2.9	1.3	2.2	1.2	0.4	0.3	2.5	1.4	3.1	1.5	1.5	1.1	1.7	1.3	2.3	1.3	2.5	2.1
60	1.4	1.5	0.6	0.4	1.4	1.5	1.3	1	1.7	1.3	1.8	1.4	2.4	1.2	2.1	1.5	1.7	1.3	2.5	2.1
61	2.8	1.2	2.3	1.1	1.7	1.3	2.2	1.1	2.1	1.3	1.8	1.4	1.6	1.2	1.7	1.3	2.5	1.3	1.8	1.4
62	1.5	1.5	1.5	1.1	2.6	1.4	1.7	0.9	2.3	1.3	2.5	1.3	1.9	1	2.5	1.4	2	1.5	2.3	1
63	1.6	1.6	2.4	1.8	1.9	1.1	1.4	1.4	2	1.4	2.5	2.1	2.9	1.3	2.1	1.3	2.4	1.6	2.3	1.3
64	1.6	1.3	1.9	1.3	2.1	1.2	1.6	1.2	2.1	1.1	0.8	0.6	1.5	1.1	2.1	1.2	2.5	1.4	2.5	1.4
65	1.6	1.4	2.3	1.9	2.7	1.3	1.3	1.2	1.8	1.4	1.7	1.3	2.1	1.1	1.7	1.3	2.1	1.2	1.8	1.5
66	1.3	1.3	1.6	1.2	1.7	0.9	1.7	1.2	1.8	1.4	3.1	1.5	0.6	0.4	1.8	1.4	2.6	1.4	1.7	1.3
67	2.9	0.9	2.1	1.1	2.2	1.2	2.1	1.3	1.8	1.7	1.9	1.3	2.4	1.2	3.1	1.5	2.5	2.1	2	1.4
68	2.6	1.2	1.9	0.7	1.7	1	2.2	0.4	1.8	1.4	2.5	1.4	2.3	1.2	2.3	1	2.3	1.3	1.8	1.7
69	1.6	1.4	2.3	1.9	1.4	1.2	2.2	1.1	1.8	1.4	1.8	1.4	1.9	1.1	2.4	1.6	1.8	1.4	1.7	1.5
70	1.6	1.1	1.9	0.9	1.7	1.3	1.3	1	1.8	1.5	2.5	1.6	1.9	1.3	2.5	1.6	2.1	1.1	2.3	1.3
71	2.6	1	1.3	1.1	0.4	0.4	1.9	1	1.7	1.3	2.9	1.4	2.1	1.1	2.1	1.5	2.9	1.8	2.5	1.3
72	1.2	0.9	1.9	1.1	1.7	1	1.1	1	2.6	1.4	2.5	1.6	2.4	1.2	2.6	2	1.9	1.3	2	1.4
73	1.8	1.2	2.1	1.1	2.2	1.2	2.2	1.1	1.7	1.3	1.8	1.4	1.5	1.1	1.8	1.4	3.1	1.5	2.1	1.5

74	1.6	1.4	1.3	1.1	2.1	1.9	1.7	1.3	2.1	1.3	2.5	1.3	1.6	1.3	2.1	1.2	2.2	1	1.8	1.4
75	1.9	1.9	2.9	1.3	1.3	1.1	1.7	1.2	2.3	1.3	3.1	1.5	2.4	1.8	2.5	1.3	2.5	1.4	2.3	1.1
76	2.2	1.7	1.5	1.3	1.7	1.4	2.5	1.1	2.3	1.3	2.5	2.1	2.3	1.9	2.5	2.1	2	1.4	2.5	1.6
77	1.6	1.2	0.9	0.9	1.1	1.1	1.4	1.1	2.5	1.4	2.6	1.4	1.8	1.3	1.7	1.3	2.5	2.1	2.3	1.3
78	1.7	1.7	2.8	1.4	2.5	1.2	1.4	1.1	2.1	1.3	3.1	1.5	2.3	1.1	1.8	1.7	1.7	1.3	2.1	1.6
79	1.9	1.8	2.3	1.4	1.4	1.2	1.7	1.2	1.8	1.7	2.3	1.3	1.6	1.5	1.7	1.5	1.8	1.4	2.6	2
80	1.8	1.3	2.9	1.3	2.7	1.3	1.1	1	2.3	1.3	2.1	1.3	1.9	1.3	2.5	2.1	2.1	1.3	1.9	1.3
81	1.9	1.8	1.7	1	1.9	0.8	2.1	1.3	2.5	2.1	2.4	1.6	2.9	1.3	3	1.6	2.5	2.1	1.7	1.3
82	2.4	1.6	1.6	1.5	2.1	1.1	2.7	1.2	2.1	1.6	2	1.5	1.7	1.1	1.9	1.3	1.7	1.3	0.8	0.6
83	1.9	1.8	1.9	1.3	1.4	1.5	0.4	0.3	1.8	1.4	2.1	1.1	0.9	0.9	2.5	1.4	2.1	1.2	1.7	1.3
84	2	1.5	1.6	1.2	1.7	1.3	2.1	1.8	2.1	1.5	2.1	1.3	1.6	1.2	2.5	1.4	2	1.5	2.1	1.5
85	1.6	1.2	2.2	1.4	1.4	1.2	1.9	0.7	1.7	1.3	2.5	1.3	1.3	1.1	1.5	1.3	2.1	1.5	1.8	1.4
86	1.8	1.7	2.3	1.9	0.4	0.4	1.7	1.2	2.5	1.3	2.1	1.3	2.9	1.3	1.7	1.3	2.6	0.7	2.1	0.9
87	1.9	1.4	2.3	1.1	2.2	1.8	1.3	1	2.3	1.3	2.6	0.7	1.9	1.3	2.6	0.7	1.7	1.3	2.1	1.1
88	2	1.5	0.6	0.4	1.4	1.2	1.4	1.2	2.1	0.9	1.7	1.3	2.3	1.9	1.8	1.4	1.5	1.3	2.1	1.3
89	1.9	1.5	1.6	1.5	2.1	1.9	2.7	1.2	2.6	1.4	1.8	1.7	1.3	1.1	1.7	1.3	2.1	1.6	1.5	1.3
90	2.9	1.2	2.3	1.9	1.3	1.3	1.1	1	2.3	1.3	2.1	1.2	2.4	1.2	3.1	1.5	2.9	1.4	1.7	1.5
91	2.2	1.4	1.9	0.9	1.1	1.1	2.1	1.8	1.7	1.3	1.7	1.3	1.9	0.7	2.6	1.4	1.8	1.4	3	1.6
92	1.9	1.6	1.6	1.2	2.1	1.9	2.6	1.3	1.7	1.3	2.3	1.3	1.5	1.1	2.1	1.5	1.5	1.3	3.1	1.5
93	1.8	1.3	2.4	1.8	1.7	0.9	1.7	1.2	2.6	1.4	2.5	1.4	1.6	1.2	1.5	1.3	3.1	1.5	1.8	1.7
94	1.9	1.6	2.1	0.8	1.5	1.1	2	1.3	2.1	1.3	1.7	1.3	1.9	1.3	0.8	0.6	2.5	2.1	1.8	1.4
95	2.2	1.4	2.9	1.3	1.4	1.2	1.6	1.1	2.9	1.8	2.5	2.1	0.6	0.4	2.2	1	2.1	1.5	2.5	2.1
96	1.1	1	1.5	1.1	1.6	1.2	1.9	0.7	3.1	1.4	2.1	1.5	2.3	1.1	2.5	1.4	1.8	1.5	0.8	0.6
97	1.7	1.2	1.8	1.3	1.7	1.3	1.4	1.1	2.5	1.4	2.5	1.6	2.7	1.6	2.1	1.5	1.5	1.3	2.5	2.1
98	2.4	1.4	1.6	1.3	1.4	1.3	1.3	1	2.6	1.4	2.3	1.3	2.9	1.3	1.7	1.3	3	1.6	1.8	1.4
99	2.5	1.5	2	0.8	2.7	1.3	0.4	0.3	1.8	1.4	2.5	1.6	2.3	1.9	1.5	1.3	2.4	1.6	2.3	1
100	2.5	1.4	1.8	1.2	1.3	1.1	2.1	1	2.1	1.2	2.6	2	1.8	1.3	2.6	1.4	2.3	1	1.7	1.3

Appendix 5.3 B - Dimensions (mm.) of sclerotia cultured from surface sterilised and bisected sclerotia, Inoculation Experiment B of *S. minor*:

Sclerotia	Culture 1:		Culture 2:		Culture 3:		Culture 4:		Culture 5:		Culture 6:		Culture 7:		Culture 8:		Culture 9:		Culture 10:	
	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W	L	W
1	2.5	1.4	0.6	0.4	1.4	0.9	1.7	1.5	2.8	1.6	1.9	1	2	1.7	1.8	1.3	1.9	1.7	1.8	1.8
2	4.4	1.3	0.6	0.4	2.5	1.4	2.9	1.7	2.8	1.7	1.6	1.5	2.8	1.1	0.7	0.5	1.2	1.1	2.2	1.3
3	2.7	1.7	4.3	1.3	0.4	0.3	1.8	1.4	2.7	1.3	2.1	0.8	1.8	1	1.5	1.4	1.9	1.7	2.1	1.6
4	0.7	0.4	2.5	1.5	1.9	0.9	3	1.6	2.7	1.6	1.3	1.1	1.5	1.5	1.9	1	2.2	1.2	1.9	1.8
5	2.7	1.4	2.6	1.7	4.3	1.2	2.9	1.7	1.8	1.6	3.5	1.4	1.5	1.2	2	0.7	2.3	1.3	2.1	1.2
6	2.6	1.5	1	1.1	1.4	1.1	2.8	1.3	0.7	0.6	2	1.1	2.3	1.3	1.5	1.4	2.4	1.9	1.8	1.7
7	2.6	1.5	1.3	1.2	2.6	1.6	4.7	1.5	1.7	1.4	1.8	1.7	1.5	1.5	1.8	0.9	1.9	1.7	1.5	1.1
8	1.5	1.2	2.6	1.4	0.4	0.3	2.8	1.6	2.9	1.6	1	0.7	1.4	0.9	1.2	0.8	2.4	1	2.3	1
9	1.1	1.1	3	1.1	2.6	1.3	3	1.9	4.6	1.5	1.6	1.5	1.5	1.1	2	1.6	1.6	1.1	2.1	1.6
10	2.5	1.4	2.5	1.5	2.4	1	1.9	1.6	1.6	1.4	1.6	1.6	1.8	1	1.6	1.1	2.7	1.5	1	0.8
11	2.6	1.5	0.4	0.4	1	1	1.7	1.3	2.2	1.2	1.6	1.5	1.5	1.5	1.5	1.5	2.2	1.2	2	1.9
12	2.6	1.5	2.5	1.5	1.4	1.1	3.4	1.3	2.8	1.7	2.5	1	1.9	1.1	1.7	1.3	1.9	1.7	1.6	1.6
13	0.7	0.4	2.4	1.1	4.3	1.2	3	1.6	2.9	1.9	0.9	0.7	1.5	1.5	1.4	0.8	3.8	1.6	3	1.3
14	2	1	2.4	1.4	2.5	1.4	2.9	1.7	0.7	0.6	1.6	1.5	2	0.8	1.8	1.3	2.3	1.3	1.1	0.9
15	0.5	0.4	1.5	1.4	2.4	1.3	1.7	1.4	1.6	1.3	2.1	0.8	2	1.7	2.3	1.2	2	1.4	1.8	1.7
16	2.6	1.5	2.5	1.5	2.5	1.4	2.9	1.7	2.3	1.3	1.7	1.2	1.9	1.1	1.5	1.4	1.1	0.8	2.6	1.5
17	2.5	1.1	1.3	1.1	2.6	1.3	1.7	1.3	2.8	1.7	2.9	1.1	1.7	1.7	0.7	0.5	1.1	0.8	1.9	1.4
18	2.5	1.4	1.9	1	1.3	1.1	1.9	1.6	1.8	1.6	2	1.1	1.4	1.4	1.9	1	1.9	1.7	1.7	1.2
19	2.5	1.6	2.1	1	1.5	1.3	2.8	1.8	2.3	1.5	1.5	1.4	1.6	1.2	1.2	0.8	1.9	1.4	1.1	1.1
20	1.4	1.1	1.3	1.2	2.4	1.6	2.6	2	2.8	1.7	0.9	0.7	1.6	1.3	0.8	0.8	1.6	1.3	1.8	1.7
21	2.4	1.5	2.4	1.6	2.4	1.5	2.4	1.3	1.6	1.3	1.1	0.9	1.2	1.1	1.5	1.4	1.9	1.7	1.1	0.9
22	1.6	1.4	2.6	1.4	2.5	1.4	0.8	0.6	2.4	2	0.8	0.6	0.5	0.6	1.7	1.3	1.8	1.1	2.3	1.4
23	3.3	1.4	2.4	1.4	2.2	1.7	1.7	1.3	2.5	2	1.6	1.3	1.8	1.3	1	1.5	1.9	1.3	2.3	1.5
24	2.3	1.8	3	1.1	3	1	3.6	1.6	2.4	1.8	1.9	1.4	1.5	1.5	1.8	1.2	1.3	1.1	1.7	1.1
25	0.7	0.4	2.1	1.2	2.5	1.4	2.3	1.2	0.9	0.6	1	0.7	1.8	1.4	0.9	0.6	1.3	0.9	2.1	1.6
26	2.2	1	1.7	0.9	1.7	1.3	2.5	2	1.3	1.2	0.9	0.7	0.9	0.9	1.6	1.1	1.8	1.6	1	0.8
27	1.8	1.4	1.5	1.3	1.9	0.9	2.9	1.7	1.3	1.3	1.7	1.3	0.9	0.7	0.8	0.6	3.1	1.3	1.7	1.6
28	1.9	1.3	2.2	1.8	0.6	0.3	3.4	1.3	2.2	1.2	0.9	0.7	1.3	1.4	2	1.6	1.6	1.1	1.8	1.7
29	1.4	1.2	1.9	1	1.3	1	1.9	1.6	2.5	2	1.6	1.3	1.9	1.1	1.9	1	2	1.4	1.7	1.2
30	2	1	2.5	1.5	1.3	1.1	1.4	1.2	1.6	1.4	1.6	1.5	0.7	0.6	1.9	1	1.2	1.1	1.7	1.6
31	2.5	1.4	1.5	1.4	2.2	1.7	1.7	1.4	2.8	1.7	2.1	1.7	1.5	1.5	1.5	1.4	1.2	0.9	1.1	0.9
32	1.4	1.2	2.3	1.5	2	1	2.8	1.9	2.8	1.7	2	1.1	3.4	1.4	1.6	1.1	1.9	1.7	1.9	1.4
33	2.3	1.8	2.5	1.5	2.1	0.9	2.6	2	2.7	1.6	1	0.7	0.9	0.9	1.4	0.9	2	1.4	2.3	1.4
34	2.5	1.7	1.4	1.2	2.5	1.4	0.8	0.6	2.6	1.5	1.5	1.4	0.9	0.7	1.2	0.8	1.6	1.1	1	0.8
35	1.8	1.3	1.7	1.4	2.4	1.3	2.4	1.5	2.4	1.1	1.6	1.5	1.5	1.5	1.4	1.3	1.8	1.2	1.1	1.1
36	2.6	1.5	3.2	1.4	2.4	1.3	2.4	1.3	2.3	1.3	1.7	1.2	2	1.7	1.6	1.1	1.4	1.1	2.3	1.5

Appendices:

37	1.8	1.4	2.2	1.8	1	1	1.7	1.4	1.8	1.6	2.6	1.1	2.7	1.1	2	1.1	1.6	1.3	2	1.9
38	2.1	1.1	2.4	1.4	1.5	1.3	2.5	1.2	3.5	1.6	0.8	0.6	1.6	1.3	1.5	1.4	1.8	1.6	1.8	1.7
39	0.7	0.4	2	1.3	3	1	1.7	1.3	2.1	1.6	1.6	1.5	2.5	1.1	1.9	1	2.4	1.9	2.2	1.3
40	1.9	1.4	1.8	1.3	2.2	1.7	2.1	1.6	2	1.1	2.1	1.7	1.4	1.4	2	1.6	2.3	1.3	2.2	1.3
41	2.5	1.6	1.3	1.2	2.1	1.1	2.6	2	1.9	1.6	2	1.1	0.7	0.6	1.6	1.1	2.7	1.5	1.7	1.2
42	2.3	1.8	1.5	1.4	2	1.3	2.8	1.8	1.9	1.2	3.5	1.4	1.5	1.3	1.6	1.2	1.9	1.8	2.3	1.9
43	1.9	1.4	1.8	1.4	1.7	1.3	2.7	1.7	0.7	0.6	1.7	1.2	1.5	1.5	2.4	0.8	2.1	1.6	1.5	1.1
44	1.8	1.3	2.4	1.6	1.8	1.2	2.5	1.8	2.5	2	1.3	1.1	1.4	0.9	0.8	0.6	2.3	1.3	1.7	1.6
45	2.1	1.1	1.4	1.2	1.9	1.3	2.4	1.5	1.3	1.2	1.5	1.4	1.4	1.4	0.3	0.3	1.8	1.1	1.7	1.6
46	3.1	1.6	2.2	1.8	2.3	1.4	2.3	1.2	2.4	1.2	3.6	3.4	3.5	3.4	2.4	1.1	1.6	1.1	1.1	0.9
47	1.1	1.1	1.7	1.3	0.4	0.3	3.4	1.3	2	1.6	1	0.7	1.2	0.9	1.5	0.9	2.1	1.4	1.9	1.4
48	2.5	1.7	1.9	1	2.4	1.5	1.7	1.4	1.8	1.2	2.5	0.9	0.8	0.7	1.4	0.9	1.6	1.1	1.3	1.1
49	1.8	1.3	1.5	1	2.2	1.7	2.9	1.7	2.7	1.8	1.7	0.8	1.2	0.9	0.8	0.6	1.9	1.5	1.5	1.3
50	2.3	1.8	0.9	1	2	1	2.6	1.1	1.8	1.6	0.8	0.6	1.5	1.2	1.7	1.6	1.2	1.1	1.5	1.1
51	1.5	1.2	2.4	1.4	1.3	1.1	2.5	2	1.2	1.2	2.6	1.4	1.4	1	1.5	1.2	1.8	1.6	2.3	1.5
52	2.5	1.6	1.7	1.3	1.5	0.9	2.4	1.3	2.8	1.7	2.5	1.2	0.5	0.6	1.5	1.2	2.3	1.3	2.3	1.9
53	2.2	1.8	1.7	1.4	0.4	0.3	2	1.4	1.6	1.4	1.8	1.5	2	1.2	0.7	0.5	1.8	1.2	1.8	1.8
54	1.8	0.9	2.4	1.6	2.5	1.4	3.4	1.3	2	1.5	1.3	0.9	1.5	1.3	1.5	1.3	2.4	1.4	2	1.6
55	1.7	1.4	2	1.3	0.6	0.3	2.1	1.5	2.7	1.6	1.6	1.6	0.8	0.7	2.5	1.3	1.1	0.8	1.8	1.3
56	1.7	1	3	1.1	1.3	1.1	2.3	1.2	2.3	1.3	2	1.1	0.6	0.6	2.4	1.1	2.8	1.4	1.2	1.1
57	2.5	1.4	1.7	1.3	1.8	1.3	2.4	1.3	1.3	1.2	1.8	1.7	1.5	1.3	1.2	1.1	1.6	1.4	2.2	1.3
58	2	1	2.4	1.4	1.7	1.3	2.1	1.6	2	1.5	1	0.7	1.7	1.1	2	1.1	1.8	1.2	1.9	1.4
59	0.7	0.4	2	1.1	3	1	2.5	2	2.2	1.2	2.8	1.1	1.1	1	1.9	1	0.9	0.8	1	0.8
60	1.6	1	2.5	1.5	1.3	1.1	2.8	1.6	2.7	1.9	2.6	1.1	2.5	1.4	1.4	0.9	3.1	2.2	2	1.9
61	2.7	1.4	1.7	1.3	1.3	1	2.2	1.6	2.9	1.9	2.5	1	1.2	1.2	0.8	0.8	1	0.8	1.1	0.9
62	1.9	1.3	1.9	1	0.6	0.3	1	0.6	1.8	1.6	1.5	1.4	0.3	0.3	1.4	1.3	1	0.9	1.8	1.5
63	2.6	1.5	2.4	1.7	2.4	1.5	2.9	1.7	1.6	1.3	3.6	3.4	0.6	0.6	2	1.2	2.8	0.9	1.5	1.1
64	1.8	1.4	0.6	0.4	1	1	2.9	1.7	2.7	1.8	1.8	1.2	2	1.3	0.7	0.5	1.9	1.5	1.7	1.2
65	1.4	1.2	1.5	1.4	1.5	1.3	1	0.6	1.3	1.3	1.6	1.3	2.8	0.8	1.6	1.1	2.4	1.5	1.1	1.1
66	1.5	1.2	1.7	1.4	2.5	1.4	2.4	1.5	2.6	1.7	1.8	1.1	2.9	0.9	1.2	1	1.2	0.9	1.8	1.4
67	2.1	1.3	2.4	1.6	0.4	0.3	1.9	1.6	0.9	0.6	1.2	1	1.1	0.8	1.5	1.4	2	1.4	2.3	1.4
68	2.1	1.3	1	1.1	1.4	1.1	2.3	1.2	1.7	1.4	1.3	0.9	1.5	1	1.4	0.8	1.6	1.1	2.8	1.1
69	2.2	1.8	2	1.1	1.3	1.1	2.1	1.5	1.6	1.3	1.6	1.2	1.6	1.2	1.8	1.3	1.8	1.2	2.3	1.4
70	1.9	1.4	1.7	1.3	2.6	1.3	1.4	1.2	1.6	1.4	1.3	0.9	3.4	1.4	1.8	1.2	2.1	1.9	1.9	1.4
71	1.8	1.4	1.7	1.3	1.7	1.3	1.7	1.4	2.3	1.5	3.5	1.4	1.9	1.1	0.9	0.6	1.9	1.5	1.5	1.1
72	2.1	1.1	2.4	1.4	2.1	1.7	1.7	1.3	2.1	1.5	1.3	1.2	1.5	1.1	1.6	1.2	1.1	0.8	2	1.7
73	1.6	1.4	1.3	1.2	1.9	0.9	2.1	1.5	1.7	1.4	1.6	1.2	0.9	0.9	1.2	1	2.3	1.3	1.8	1.4
74	2.5	1.7	1.8	1.4	2.5	1.4	1	0.6	2.1	1.6	1.6	1.3	0.9	0.7	2.4	0.9	1.2	1.1	2.3	1.6
75	2.6	1.5	2.4	1.6	1.7	1.2	1.3	1.2	0.9	0.6	2.1	1.1	2.5	1.1	3.5	3.3	2.4	1.5	1.1	0.9
76	0.7	0.4	2.4	1.4	1.4	1.1	1	0.6	1.7	1.7	2.1	1.3	2.4	1	1.5	1.3	1.8	1.6	0.8	0.8
77	2.5	1.6	1.9	1	1.8	1.3	2.2	1.6	2.2	1.2	0.9	0.7	1.5	1.3	1.7	1	1.9	1.5	0.9	0.9
78	1.4	1.1	2.5	1.5	2	1	1.7	1.3	1.8	1.6	1.6	1	2	1.8	1.1	0.9	1	0.8	1.1	0.9
79	1.8	1.3	1.7	1.4	0.6	0.3	2.8	1.8	2.3	1.5	1.3	0.9	0.3	0.3	1.5	1.1	2.4	1.4	2.1	1.3
80	2.1	1.1	0.6	0.4	2.4	1.5	1.9	1.6	2.7	1.8	1.6	1.2	2.3	1.1	2	1.3	2	1.4	1.7	1.2
81	1.8	1.4	1.4	1.2	2	1	2.4	1.5	2.3	1.5	1.6	1	1.5	1	1.5	1.2	2.9	1.1	1.8	1.5
82	1.5	1.2	1.3	1.1	1.3	1	1.4	1.2	1.3	1.2	2.1	1.4	2.5	0.9	1.5	1.2	1.1	0.8	2.3	1.4
83	1.1	1	2	1.3	0.4	0.3	1.4	1.3	1.6	1.3	2.8	2	1.5	1.3	2	1.2	2.4	1.3	1.7	1.6
84	1	1	1.8	1.3	1.3	1	2.3	1.2	1.3	1.2	2.1	1.1	1.2	1.2	0.8	0.8	2.9	2	2.8	1.1
85	2.2	1.8	1.5	1.4	1.8	1.2	1.9	1.6	1.3	1.2	2	1.1	0.3	0.3	1.4	0.9	2.4	1.5	0.9	0.8
86	1.1	1	0.9	1	1	0.9	1.9	1.2	2.2	1.2	2.1	1.7	1.5	1.3	2	1.2	1.2	0.9	2.2	1.3
87	1.4	1.1	1	1	1.3	1	1.4	1.2	0.9	0.6	1.6	1.6	2	1.1	0.8	0.6	1	0.8	1.1	0.9
88	1.9	1.4	1	1	1.7	1.2	2.1	1.5	1.6	1.4	1.8	1.4	2	1.3	0.7	0.5	1	0.8	1.7	1.2
89	1.8	1.3	1.8	1.4	1	0.9	1	0.6	1.6	1.3	1.5	0.9	1.1	0.8	1.9	1	1.2	0.9	0.4	0.3
90	2.5	1.6	1.4	1.2	2	1.2	1.8	1.4	1.8	1.6	1	0.7	2	1.7	1.4	1.3	1.1	0.8	0.9	0.8
91	2.6	1.5	1.3	1.1	1.5	1.3	2.4	1.5	1.6	1.3	1.5	1.4	1.7	1.2	2.5	1.7	1.2	1.1	0.9	0.9
92	1.1	1.1	1.3	1.1	2.4	1.5	1.4	1.2	2.3	1.5	0.9	0.7	0.8	0.7	2	1.2	2.3	1.3	1.8	1.5
93	1.4	1.2	0.6	0.4	1.4	1.1	1.7	1.3	1.6	1.3	1.8	1.2	1.7	1.1	0.6	0.5	1.2	0.9	2.3	1.5
94	1.9	1.3	2.4	1.6	1.9	0.9	2.4	1.5	1.3	1.2	1.8	1.1	2.5	1.4	0.6	0.5	1.8	1.6	1.1	0.9
95	1.9	1.4	1.8	1.4	1.7	1.2	1.7	1.3	1.6	1.3	2.1	1.1	2.3	1.1	1.6	1.1	1.8	1.2	0.9	0.8
96	2.5	1.7	3	1.6	1	1	2.3	1.2	0.9	0.6	2.1	1.3	1.5	1.2	0.8	0.6	2.4	1.5	2.1	1.3
97	2.1	1.1	1.9	1	1.3	1.1	2.3	1.2	2.1	1.6	0.8	0.6	2	1.4	0.7	0.5	1.9	1.5	2.2	1.3
98	0.7	0.4	1.5	1	2	1	1.7	1.4	1.6	1.4	1.6	1.3	2	1.1	2.4	0.6	2.4	1.5	1.1	1.1
99	2	1	1.7	1.3	0.9	0.9	1.9	1.6	1.3	1.3	0.9	0.7	1.5	1.3	1.5	1.2	2	1.4	1	0.8
100	1.6	1	1.5	1.4	1.8	1.3	2.1	1.5	0.7	0.6	2	1.1	0.6	0.6	1.4	0.9	1.2	0.9	2.3	1.5

Appendix 5.3.4 - EC_{50} values of isolates of *S. minor* to fungicides (µg/ml. of active ingredient).

No.	Fungicide	Active ingredient (a.i.)	Isolate	Place of Origin	EC_{50} (µg/ml)	Min.	Max	Mean ± σ (SD)
1	**Folicur**[R]	**tebuconazole**	76702 B	Kindred	0.132			
2			76702 F	Kindred	0.110			
3			72704 C	Kindred	0.072			
4			72704 F	Kindred	0.126	**0.055**	**0.132**	**0.098 ± 0.03**
5			72704 G	Kindred	0.079			
6			75003 A	Kindred	0.116			
7			75003 D	Kindred	0.055			
1	**Bavistin**[R]	**carbendazim**	75003 D	Kindred	0.946			
2			76702 B	Kindred	2.624			
3			76702 F	Kindred	1.242			
4			75003 A	Kindred	2.598	**0.95**	**2.62**	**1.92 ± 0.68**
5			72704 G	Kindred	2.542			
6			72704 F	Kindred	1.730			
7			72704 C	Kindred	1.758			
1	**Rovral**[R]	**iprodione**	76702 F	Kindred	0.323			
2			76702 B	Kindred	0.123			
3			75003 A	Kindred	0.250			
4			72704 G	Kindred	0.128			
5			72704 F	Kindred	0.086			
6			72704 C	Kindred	0.089			
7			08-1	Table Cape	0.195			
8			09-5 A	North Motton	0.246			
9			09-3 E	North Motton	0.616			
10			09-3 D	North Motton	0.229			
11			09-3 A	North Motton	0.300			
12			08-7 B	Penguin	0.225			
13			08-7 A	Penguin	0.657			
14			09-8 H	Don	0.136			
15			09-8 E	Don	0.275			
16			09-8 D	Don	0.237			
17			09-8 C	Don	0.318			
18			09-8 B	Don	0.274			
19			09-8 A	Don	0.960	**0.086**	**1.225**	**0.302 ± 0.23**
20			09-8 F	Don	0.241			
21			09-8 Y	Don	0.157			
22			09-8 V	Don	0.337			
23			09-8 S	Don	0.567			
24			09-8 Q	Don	0.173			
25			09-8 O	Don	0.300			
26			09-8 P	Don	0.209			
27			09-8 L	Don	0.204			
28			09-8 K	Don	0.392			
29			09-8 J	Don	0.216			
30			09-8 G	Don	1.225			
31			09-8 F	Don	0.086			
32			09-8 C	Don	0.320			
33			09-8 B	Don	0.191			
34			08-5 E	Forth	0.147			
35			08-5 D	Forth	0.153			
36			08-5 C	Forth	0.326			
37			08-5 B	Forth	0.247			