Benchmarking predictive models, nutrients and irrigation for management of downy and powdery mildews and white blister

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Final report for VG07070

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Purpose of the project: This project details the outcomes of a 3 year study on evaluating the efficacy, development and economics of disease predictive models (decision support tools), nutrient management, irrigation timing regimes, a limited number of alternative chemistries and resistant cultivars against conventional diseae control methods.

Final report completed: November 2011

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Benchmarking predictive models, nutrients and irrigation for management of downy and powdery mildew and white blister: VG07070

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Media summary

This project focused on white blister on brassicas, powdery mildew on cucurbits and downy mildew and anthracnose on lettuce. The project determined the efficacy and economics which could be achieved with weekly fungicide sprays, disease predictive models, irrigation timing and growing a resistant variety, but the latter was the most superior IPM tool. It evaluated the benefits of nutrient management; developed a disease predictive model for powdery mildew of cucurbits and a detection kit for airborne spores of white blister.

Major outcomes of the research were:

White Blister of brassicas:

The world's first white blister spore detection kit was developed for use with the white blister disease predictive model (Brassica$_{spot}$™). This detection kit is a major addition to the current disease control tools. Scientific and economic analysis of field trials demonstrated that the number of fungicide spray applications based on predictions of the Brassica$_{spot}$™ model could be reduced by 12-13, which corresponded to a 77% reduction in disease on broccoli heads and an increase in profits of 13%, during dry conditions. Irrigating broccoli crops in the morning rather than the evening reduced disease by 58% and increased profit by 3%. Growing a broccoli variety with resistance to white blister reduced disease by 99% and increased profit 11%.

Downy mildew of lettuce:

A disease predictive model for downy mildew on lettuce (BREMCAST™) showed timing fungicide sprays based on model predictions could reduce spray programs by 1-3, reduce disease by 50-70% and increase profits by 20%. High rates of calcium nitrate applied to lettuce seedlings reduced susceptibility to downy mildew and anthracnose.

Powdery mildew:

The world's first powdery mildew disease predictive model (PMRI) for powdery mildew of cucurbits grown under Australian conditions was developed and a preliminary trial indicated a reduction of one spray could be achieved.

This project was part of the HAL Plant Pathology Program and the commissioned research was undertaken with the support of HAL; the Federal Government; the Department of Primary Industries, Victoria; and The University of Queensland.

Recommendations for future research and development:

- ***Downy mildew of lettuce:*** Format the BREMCAST™ model into user-friendly software and validate it in the field.
- ***White blister:*** Under Australian conditions test the white blister spore detection kit alone and incombination with the Brassica$_{spot}$™ model; investigate modifications to the models spray threshold and determine the physiological stage when broccoli heads are susceptible to white blister infection.
- ***Powdery mildew of cucurbits***: Further refine the cucurbit powdery mildew disease predictive model and test it on commercial crops.

Recommendations to industry:

To maximize the production of broccoli, lettuce and cucurbits and meet the high aesthetic standards of the marketplace:

- Where possible grow resistant varieties, although they still need to be sprayed.
- Irrigate broccoli crops at 4 am instead of 8 pm to reduce the incidence of white blister.
- Spray Chinese cabbage with fungicides 14 days before harvest to control white blister.
- Use calcium nitrate based fertilizers instead of ammonium or potassium based fertilisers.
- Use disease predictive models as decision support tools, especially during dry conditions.

Technical summary

The objective of this project was to benchmark disease predictive models, irrigation and nutrient management as IPM tools for white blister on brassicas and downy mildew on lettuce and to develop a disease predictive model for powdery mildew on cucurbits.

Irrigation

- Irrigation of broccoli in the morning rather than the evening reduced incidence of white blister by 58% and improved farm profit by 3%. Growing a broccoli variety resistant to white blister compared to one with little resistance reduced white blister incidence by 99% and increased farm profit by 11%.

Nutrients

- Application of high rates of calcium nitrate to lettuce seedlings reduced their susceptibility to downy mildew and anthracnose. In contrast, the application of ammonium nitrate increased disease susceptibility. The application of a commercially available calcium product did not improve the control of downy mildew in the field although further research on the timing of applications is required.

Disease predictive models (decision support tools)

- The first disease predictive model for powdery mildew on cucurbits was developed for Australian conditions. An initial field trial to evaluate use of the model reduced fungicide spray programs by one application without affecting yield of zucchini. A subsequent trial, yet to be harvested has reduced 4 fungicide sprays.
- Under dry conditions, use of the Brassica$_{spot}$™ models and the weekly spray program reduced white blister incidence on heads by 77%, but none of these programs differed significantly in their disease control. The models reduced spray programs by 12-13 sprays and were 13% more profitable than the weekly spray program under dry conditions.
- Under wetter conditions, however, either the weekly sprays of protectant fungicides or the grower spray program of systemic and protectant fungicides was 75% more economical and reduced white blister incidence by 63-98%, compared with the use of current versions of the Brassica$_{spot}$™ model.
- Good control of white blister on the wrap leaves of Chinese cabbage was achieved with a single application of a registered systemic fungicide 14 days before harvest. The disease rating was by 20% and profit was increased by 60%.
- An in-field test kit was developed to detect the availability of airborne inoculum of the white blister pathogen (*Albugo candida).* The kit was based on "lateral flow" technology (immunochromatographic test strips) and developed from monoclonal antibodies generated from recognition sites on *A. candida* Race 9 zoosporangia. The test kit was quantified in the field by comparing it with spore trap data. Low detection of zoosporangia corresponded with no visible symptoms of white blister on Brussels sprout crops in the UK.
- None of the methods for timing fungicide sprays to control downy mildew on lettuce was consistently superior. Use of the BREMCAST™ model, compared with various versions of the DownCast model, provided better control of downy mildew when systemic sprays were used late in the spray program. BREMCAST™ reduced downy mildew incidence by 50-70% and increased farm profit by 20% with a similar number or 1-3 sprays less than the grower spray program.

Economics

- Economic analyses of all trials indicated that no individual treatment (unsprayed, disease predictive models, weekly spray programs, growers spray programs and alternative chemistries) was consistently superior. In some cases the unsprayed control was more

economical than all other treatments. In practice even resistant varieties should be sprayed as there is a risk that the pathogen will overcome varietal resistance.

- Of the limited number of fungicide alternatives benchmarked against the disease predictive models and weekly or grower spray programs, only Bion (half rate) had some efficacy and favourable economics, when applied before the disease appeared in lettuce crops.

Recommendations for future research of substantial benefit to industry (refer to Chapter 13 for details)

- Improve the Brassica$_{spot}$™ model by modifying the spray threshold "Disease Index".
- Produce an Australian model to avoid the access and cost issues of the Brassica$_{spot}$™ model.
- Determine the physiological stage when broccoli heads are most susceptible to white blister.
- Validate the white blister spore detection kit under Australian conditions, alone and in conjunction with the Brassica$_{spot}$™ disease predictive model.
- Determine the role of oospores in the epidemiology of white blister on broccoli.
- Reformat the BREMCAST™ model into more user-friendly software and validate it in the field.
- Evaluate and improve the cucurbit powdery mildew disease predictive model.
- Link with private providers to deliver a website for access to disease predictive models.
- Investigate the use of Vapour Pressure Deficit (VPD), Fuzzy Logic models and CART models to replace the use of leaf wetness data.
- Develop a rapid molecular test to identify pathogen resistance to the major systemic fungicides.
- Determine if nutrient excesses or deficiencies influence disease incidence and severity in the field.
- Investigate alternative fungicide chemistries for use in situations where disease pressure is low.

Chapter 1

Introduction

1.1 Project aim and background

The aim of the project was to benchmark disease predictive models, irrigation timing and nutrient management as IPM tools for white blister on brassicas, downy mildew on lettuce and powdery mildew on cucurbits. All of these crops contribute significantly to the Australian vegetable industry (Table 1.1). This project arose from the IPM Gap Analysis Project HAL VG06092 (Porter *et al.* 2007) which enlisted growers, allied industry representatives and scientists, and identified six areas of interest for future research commissioned under the HAL Plant Pathology Program. This report describes the research undertaken for one sub-section of that program, the HAL 3.2 Foliage Diseases Plant Pathology Program.

Table 1.1 The production and value of brassicas, lettuce and cucurbits in Australia.

Commodity [A]	Area (ha)	Production (tonnes)	Gross value ($M)
Broccoli and cauliflower	10,666	133,487	121.7
Cucurbits (cucumber, pumpkin & zucchini)	3,693	125,952	88.6
Lettuce	9,530	135,015	113.4

[A]AHSH 2004.

1.1.2 Crop loss estimates

In the IPM Gap Analysis, the vegetable industry estimated that crop losses caused by downy mildew on field-grown lettuce were 10-30% amounting to an average loss of $4,000/ha with the cost of control considered to be high, at over $750/ha. Crop losses on cucurbits caused by powdery mildew were estimated to be $1,000-2,500/ha with the cost of control regarded as moderate at $250-750/ha. No data was available for white blister.

1.2 Nutrients

The acceptable range for nutrient levels in brassicas, cucurbits and lettuce is well documented. Growers believe their nutrient management is generally appropriate, but are concerned that high levels of nitrogen could be increasing the susceptibility of crops to disease. High nitrogen levels increase the susceptibility of lettuce seedlings and cabbages to downy mildew and render canola more susceptible to white blister (Minchinton *et al.* 2005; Huber 1981). Generally high nitrogen increases the susceptibility of plants to powdery mildew infections (Spencer 1978). The form of nitrogen, either NH_4-N or NO_3-N, can influence the severity of many diseases. For example, powdery mildew of wheat, *Erysiphe*, increases with increased NO_3-N (Huber and Watson 1974). There are reports of nutrients such as phosphorous and potassium influencing disease pressure, especially for white blister, but these reports may not be applicable to Australian vegetable cropping areas because fertilisers are already widely used (Minchinton *et al.* 2005; Huber 1981).

1.3 Irrigation

Overhead irrigation can affect the duration of leaf wetness depending on its time of application. Leaf wetness is a key factor in predicting the behaviour of fungal pathogens in most crop disease-prediction models (Pitblado 1988; Huber and Gillespie 1992; Kushalappa 2001; Kennedy and Giles 2003). Most fungal pathogens require the presence of water on the plant surface for germination and infection (Lacy 1986; Rotem *et al.* 1978), but powdery mildews appear to prefer high relative humidity, dew or mist (Butt 1978).

Irrigation management can be used to reduce disease pressure from downy mildew and white blister (Minchinton *et al.* 2005; Wu *et al*. 2001), while strawberry growers overhead irrigate crops in the afternoon to degrade powdery mildew conidia, even though these crops rely on trickle irrigation for moisture. This approach may not be feasible for cucurbits because of expense, lack of water and the observation by Chorin and Palti (1962) that *S. fuliginea* on cucurbits can tolerate a broad range of moisture.

Systematic surveys showed that overhead irrigation of radish and spring onion crops in the evening compared with dawn increased the incidence of white blister and downy mildew, respectively (Minchinton *et al.* 2005). The downy mildew disease-predictive model forecasted fewer sprays on spring onions when an irrigation of 2 mm was applied during the period from midnight to 04:00 h. However, efficacy of this approach to control downy mildew was not confirmed due to lack of disease (Minchinton *et al.* 2005). Trickle-irrigated lettuce crops required fewer fungicide sprays to control downy mildew because of lower disease pressure compared with overhead irrigated crops (Wu *et al.* 2001). Cucurbits grown in Queensland are very susceptible to powdery mildew and most production is trickle irrigated because of low water availability (G. Mac Mannus, pers comm). There is no evidence that manipulating timing of trickle irrigations will have benefits in disease reduction over and above that achieved by moving from overhead to trickle irrigation. Experience has shown that growers move from overhead and furrow irrigation to trickle irrigation because of water shortages and not because of reduced disease pressure.

1.4 White blister

1.4.1 The causal pathogen

White blister is caused by the oomycete *Albugo candida* (Pers.) Kuntze [= *Cystopus candidus* (Pers.) Lev., *A. cruciferarum* (DC.) Gray], an obligate parasite. The disease is polycyclic in Brassica vegetable crops.

1.4.2 Symptoms

Symptoms of white blister take two forms. White raised lesions, up to 5 mm in diameter on the abaxial leaf surface which penetrate to the adaxial leaf surface and contain zoosporangia. Symptoms of hypertrophy and hyperplasia, contain internally formed oospores and are considered to result from seed infections or from infection of the apical meristem (Fig 1.1).

Fig 1.1 Symptoms of white blister.
Left, Broccoli head with both primary and secondary symptoms of white blister. Middle, lesion of the abaxial leaf surface. Right, hypertrophy and hyperplasia on an axillary broccoli shoot.

1.4.3 Reproduction

The life cycle of *A. candida* is shown in Fig 1.2 (Rimmer 2007).

1.4.3.1 Asexual reproduction and infection

Zoosporangia form on sporangiophores that develop from fungal mycelium underneath the host epidermis (Fig 1.3). Theses structures build up, causing the host epidermis to rupture and release the zoosporangia, which are dispersed by wind and rain. Zoosporangia absorb water, swell and release four to twelve zoospores which possess one long and one short flagellum that allows the zoospore to swim through water. When a zoospore comes to rest on a susceptible host plant, the flagella are absorbed; the zoospore encysts and germinates by a germ tube. The germ tube penetrates the host tissue through a stoma to form an intercellular mycelium which penetrates host cells and produces globose to knob-like, nutrient absorbing, haustoria. *Albugo* continues to spread throughout the intercellular spaces (Verma *et al*. 1975).

1.4.3.2 Sexual reproduction and infection

Sexual reproduction usually occurs in systemically infected tissues causing symptoms of hypertrophy and hyperplasia of the host organ, such as a "staghead" of the inflorescence. Oogonia and antheridia are formed from the mycelium in intercellular spaces. The antheridium attaches to the side of the oogonium and a nucleus flows through a penetration tube from the antheridium to the oogonium. Nuclei combine and the outer periplasm of the oogonium wall becomes thick and dark, and a chocolate brown oospore is formed (Fig 1.4). Oospores may entirely fill hypertrophied plant parts. Weathering and decay of the host plant subsequently releases them. Oospores can germinate directly on the host by a germ tube or release 40-60 zoospores that infect the plant in the same way as the zoospores liberated from sporangia (Verma and Petrie 1975). Infection from zoospores produced by oospores has been difficult to demonstrate (Petkowski 2008), but they are reported to infect plants after passing through the gut of a snail, due to exposure to enzymes in the snail gut (Liu and Rimmer 1993). In contrast, Kumar *et al.* (1995) working with rapeseed-mustard obtained infection from 1g/pot of oosporic material.

Fig 1.3 Sporangia produced in leaf lesions.

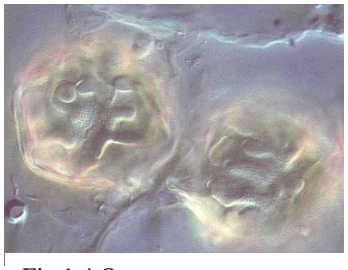

Fig 1.4 Oospores.

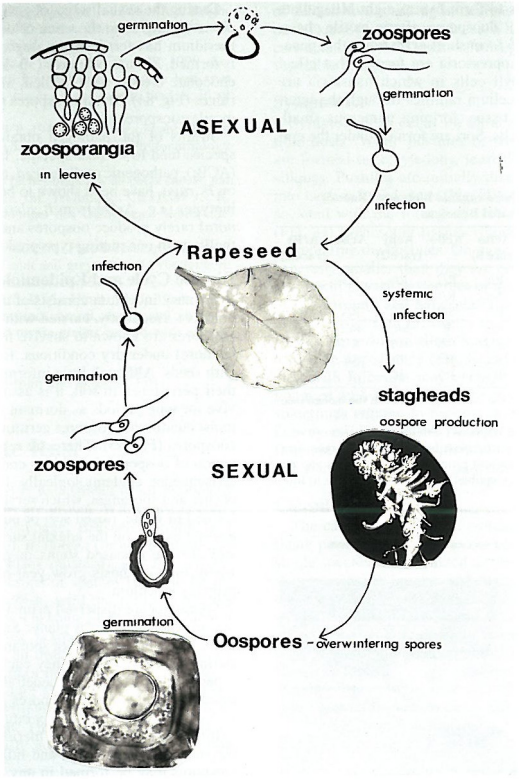

Fig 1.2 Life cycle of *Albugo candida* (Rimmer 2007).

1.4.4 Epidemiology

There are two sources of oospore-contaminated seed and plant debris (Verma *et al.* 1988). Petrie (1975) suggested oospore contaminated seed was a major mode of survival and spread of white blister. Oospores in soil and plant debris are considered to be the primary source of inoculum (Saharan and Verma 1992), but Verma *et al*. (1988) did not detect oospores in hypertrophied plant material which had been buried for 6 months. Additionally Petkowski (2008) could not produce infected seedlings from germinated oospores collected from white blister lesions on broccoli. In contrast oospores from *B. rapa* germinated at temperatures from 10-20°C, with an optimum of 13°C (Verma and Petrie 1975).

Sporangia release zoospores at any time of the day or night, but mainly from 04:00 h to 16:00 h (Kennedy pers comm.). A film of free water is essential for zoospore release (Lakra *et al*. 1989). They are dispersed by wind, rain splash, and on farm implements and clothing. Only young tissue is susceptible to infection from zoospores (Kennedy pers. comm.). The optimal temperature for zoospore release from sporangia is 13°C with a range from 2-20°C. At 20°C it takes only one h for most zoospores to reach a host stoma, and by 3 h of leaf wetness, infection has occurred. Infection of Brussels sprouts occurs over a temperature range from 5-25°C and the latency period is from 6-37 days (Gilijamse *et al*. 1998). White blister is a polycyclic disease (Saharan and Verma 1992). Re-infection may occur several times during the life of the crop following initial disease expression. Growers report that white blister and downy mildews are worst in autumn when fogs are prevalent and leaf wetness durations are longer (Minchinton *et al.* 2005).

1.4.5 Races

There are 17 named races of *A. candida*. The first 10 are generally host specific to genera or species on Brassicaceae, whilst the remaining races are generally associated with oilseed rape and mustards such as canola and Indian mustards and are often variety specific. Races are not considered to cross from one host to the other under field conditions (Table 1.2).

Table 1.2 Races of *A. candida.*

Race	Scientific name	Common name	Reference
1	*Raphanus sativus*	radish	Pound and Williams (1963)
2	*Brassica juncea*	Indian mustard	Pound and Williams (1963)
2V	*B. napus*	fodder rape, oil rape	Petrie (1994)
3	*Armoracia rusticana*	horse radish	Pound and Williams (1963)
4	*Capsella bursa-pastoris*	shepherd's purse	Pound and Williams (1963)
5	*Sisymbrium officinale*	hedge/tumble mustard	Pound and Williams (1963)
6	*Rorippa islandica*	marsh/yellow watercress	Pound and Williams (1963)
7	*B. rapa (campestris)*	mustard, rape, rapeseed, field mustard, pak choi, Chinese cabbage, spinach Mustard, turnip	Verma *et al* (1975)
7A	*B. rapa* var. *oleifera*	turnip rape cv. Torch	Pidskalny and Rimmer (1985); Petrie (1988)
7V	*B. rapa*	turnip rape cv. Reward	Petrie (1994)
8	*B. nigra*	Black mustard	Delwiche and Williams (1977)
9	*B. oleracea*	broccoli, Brussels sprouts, cauliflower, kale, kohlrabi	Hill *et al.* (1988)
10	*B. kaber* (*Sinapis arvensis*)	charlock	Williams (1985); Hill *et al* (1988)
11	*B. carinata*	Ethiopian mustard	Williams (1985)
12	*B. juncea*	Indian mustard	Verma *et al.* (1999)
13	*B. rapa*	turnip rape cv. Toria	Verma *et al.* (1999)
14	*B. juncea*	Indian mustard cv. RL 1359	Gupta and Saharan (2002)
15	*B. juncea*	Indian mustard cv. Kranti	Gupta and Saharan (2002)
16	*B. juncea*	Indian mustard cv. Kranti	Gupta and Saharan (2002)
17	*B. juncea*	Indian mustard cv. RH 30	Gupta and Saharan (2002)

1.4.6 Disease control

1.4.6.1 Chemical control

Early control of white blister was based the use of copper fungicides, then moved to the use of other protectant fungicides, such as the dithiocarbamates and inorganics. Later systemic fungicides such as acylalanines (e.g. metalaxyl), dimethomorph and more recently the strobilurins (e.g. azoxystrobin), have shown efficacy against white blister on broccoli (Minchinton *et al.* 2004, 2007). Additionally a number of biological control agents, surfactants, and activators of plant defence systems have been evaluated. Whilst some are promising, none are likely to supersede the systemic fungicides in the field in the immediate future (Akem *et al.* 2011; Minchinton *et al*. 2004, 2007). Unfortunately *A. candida* reportedly has a predisposition to rapidly develop resistance to systemic fungicides (Rimmer 2007). It is therefore imperative to implement fungicide resistance management strategies, especially as there is field evidence of metalaxyl resistance in Werribee South (Len Tesseriero, pers. comm.).

1.4.6.2 Resistance

Resistance to *A. candia* in broccoli is recessive and there are reportedly 12 genes for resistance to *Albugo* (Clive Ockenden, pers. comm.). Resistance to *A. candida* in radish is considered to be associated with a single dominant gene (Williams and Pound 1963; Bonnet 1981; Kole *et al*. 1996), while resistance in *B. napus* appears to be controlled by several dominant genes (Fan *et al*. 1983; Verma and Bhowmik 1989). Increasing numbers of resistant broccoli varieties are being released commercially but some, such as Booster, have already lost their resistance because of the lack of application of preventative sprays.

1.4.5.3 Cultural management

Cultural practices to reduce *A. candida* are: (i) Prevention of free water on leaves during the morning and night; (ii) avoiding overhead irrigation; (iii) improving air movement by increasing plant spacing; and (iv) crop rotation.

1.5 Downy mildew

1.5.1 The causal pathogen

Downy mildew of lettuce is caused by *Bremia lactucae* Regal, which is an obligate parasite of the Peronosporaceae. A number of pathotypes can be distinguished based on their virulence response when they are inoculated onto a differential set of lettuce genotypes with different resistance genes (DM genes).

1.5.2 Symptoms

Symptoms of downy mildew on lettuce, *Latucae sativa* L. are initially an angular shaped light green to yellowing of leaves delineated by major veins (Fig 1.5). In this area “downy” white spores are produced on the abaxial leaf surface and the area turns chlorotic and later necrotic (Fig 1.6). Systemic infections appear as a dark brown discolouration of the vascular stem tissue. The fungus can infect all growth stages of lettuce crops.

Fig 1.5 Early symptoms of downy mildew on lettuce.

Fig 1.6 Older symptoms of downy mildew on lettuce.

1.5.3 Reproduction

The life cycle of downy mildew is shown in Fig 1.7.

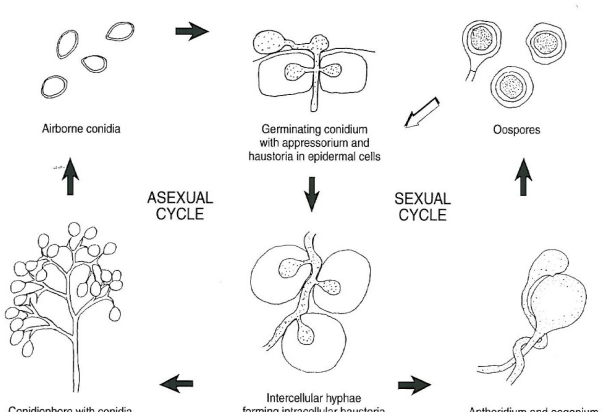

Fig 1.7 Life cycle of downy mildew (Lucas *et al*. 1995).

1.5.3.1 Asexual reproduction and infection

Up to three sporangiophores can arise from each stomata on the abaxial leaf surface. In free water, sporangiospores germinate via a germtube, produce an appressorium and an infection peg directly penetrates the host epidermal cell wall, but not the plasmalema, forming vesicles. Hypha ramify intercellularly producing haustoria. The infection process can be complete within three h under an optimum temperature of 10-22ºC with a range of -5 to 30ºC. The latent period is 5-14 days under optimal conditions of 20-22ºC, but longer under lower temperatures.

1.5.3.2 Sexual reproduction and infection

The fungus is heterothallic and two mating types (B1 and B2) are required for oospore production, although homothallic types have been reported. Oospore formation in field crops is variable, but they have been observed in the stems of systemically infect plants (Crute and Dixon 1981; van Bruggen and Scherm 1997). Ontogeny of *B. lactucae* oospores has been described by Crute and Dixon (1981) with oogonia and antheridia production commencing about four days after inoculation and completing about 15 days later with mature oospores. Germination of oospores is rare and they appear to require incubation in water and crop debris (van Bruggen and Scherm 1997), but oospores in crop debris are considered to be the primary source of infection (Crute and Dixon 1981).

1.5.4 Epidemiology

Sources of inoculum are oospores on seed and in debris, inoculum from wild lettuce and sporangia from infected crops. Sporangiophores are produced after 6-7 h of darkness under high relative humidity conditions. When the RH drops to 50-90% as the morning progresses, sporangiophores twist and sporangiospores are flung into the air and dispersed by wind. Maximun spore release occurs about 10:00 h. The optimum temperature for sporulation is 20 ºC with a range of 5-24ºC. Sporangia can survive only a few days and their longiviety is influenced by solar radiation, RH and temperature. Released sporangia require 3-4 h of leaf wetness for infection and usually infect on the same day as their release. Downy mildew is more prevalent under sprinkler irrigation than furrow irrigation and is even less in sub-surface drip irrigation (Scherm and van Bruggen 1995b).

1.5.5 Disease control

1.5.5.1 Chemical control

Early control of downy mildew on lettuce was based on the use of multisite copper compounds and the dithiocarbamates, fentins, chloronitrines and phthalimides. Since the mid 1970's systemic fungicides such as cymoxanil, fosetyl-Al, phenylamides, propamocarb, dimethomorph, fluazinam have been widely used. The 1990's saw the introduction of new groups of systemic fungicides such as the stobilurins, amino acid amide carbamates and benzothiadiazoles. Some of these systemic fungicides are single site inhibitors and resistance has rapidly emerged in downy mildew to these chemicals (Gisi 2002). Development of fungicide resistance has led to the implementation of fungicide resistance strategies.

1.5.5.2 Resistant cultivars

Resistant cultivars contain either single dominant genes or multiple genes. *B. lactucae* has proved very resilient in overcoming resistance. Consequently even resistant cultivars require fungicide protectants to maintain the resistance. In Australia there are up to 27 DMR (downy mildew resistant) genes recognised for lettuce varieties and varieties are sold with their DMR gene numbers listed.

1.5.5.3 Cultural management

Cultural practices to reduce *B. lactucae* are: (i) Prevention of free water on leaves during the morning and night; (ii) avoiding overhead irrigation; (iii) improving air movement by increasing plant spacing; (iv) deep ploughing crop residues to reduce carry-over of inoculum; (v) crop rotation; and (vi) maintaining weed management.

1.6 Powdery mildew

Prepared by Zaiton Sapak, Victer Galea and Daryl Joyce.

1.6.1 Causal pathogen

Powdery mildew of cucurbits can be caused by two types of fungal pathogens: *Podosphaera fusca* (syn. *Sphaerotheca fuliginea*) and *Golovinomyces cichoracearum* (syn. *Erysiphe cichoracearum*). In Australia, *P. fusca* is considered to be the primary causal pathogen of the disease (O'Brien *et al*. 1988; Letham and Priest 1989). *P. fusca* is an obligate fungal pathogen in the family *Erysiphaceae* and sub-family *Cystotheceae*. This pathogen can be identified either through cleistothecium (sexual) or conidium and mycelium (asexual) characteristics. Studies with *P. fusca* must rely on use of conidia and identification must rely on conidium and mycelium characteristics because of the rarity of cleistothecia in the cucurbit field (McGrath *et al.* 1999) and the difficulty in producing fertile ascospores in a laboratory (Nicot *et al*. 2002).

1.6.2 Symptoms of the disease

Powdery mildew is the most widespread and most serious foliar fungal disease of cucurbits (Zitter *et al*.1996). In the early stage of development, the disease appears as small patches of white fungal colonies on adaxial (Fig 1.8 left) and abaxial leaf surfaces. Under conducive conditions, these white patches grow into large lesions and may cover the whole leaf surface, petioles, and stems (Fig 1.8 middle). The disease affects all commercially grown cucurbit species by causing premature leaf senescence, which exposes the fruit to sunscald. Normally, fruit produced from severely infected plants are of low quality in taste, texture and size and are regarded as low quality products by the market (Sitterly 1978). Cucurbit leaves with severe infection lose their green colour and become brown and withered (Fig 1.8 right). Due to the premature loss of leaves, cucurbit plants easily become stunted and die. Dik (1999), who studied the relationship between disease severity and the yield of the glasshouse cucumbers infected by powdery mildew, showed that the disease greatly reduces yield and market quality.

Fig 1.8 Symptoms of powdery mildew on zucchini plants in the glasshouse at the Gatton Campus of The University of Queensland. Evident are small white fungal colony patches on upper and lower leaf surfaces (left), on stems (middle). Severe infection caused brown and shrivelled leaves and later defoliation of plants (right).

1.6.3 Reproduction

The life cycle of *P. fusca* on cucurbits is shown in Fig 1.9. The life cycle of powdery mildew is initiated by either conidia or ascospores and comprises several important biological processes such as germination, infection, colonisation, sporulation, dispersal and perennation (survival).

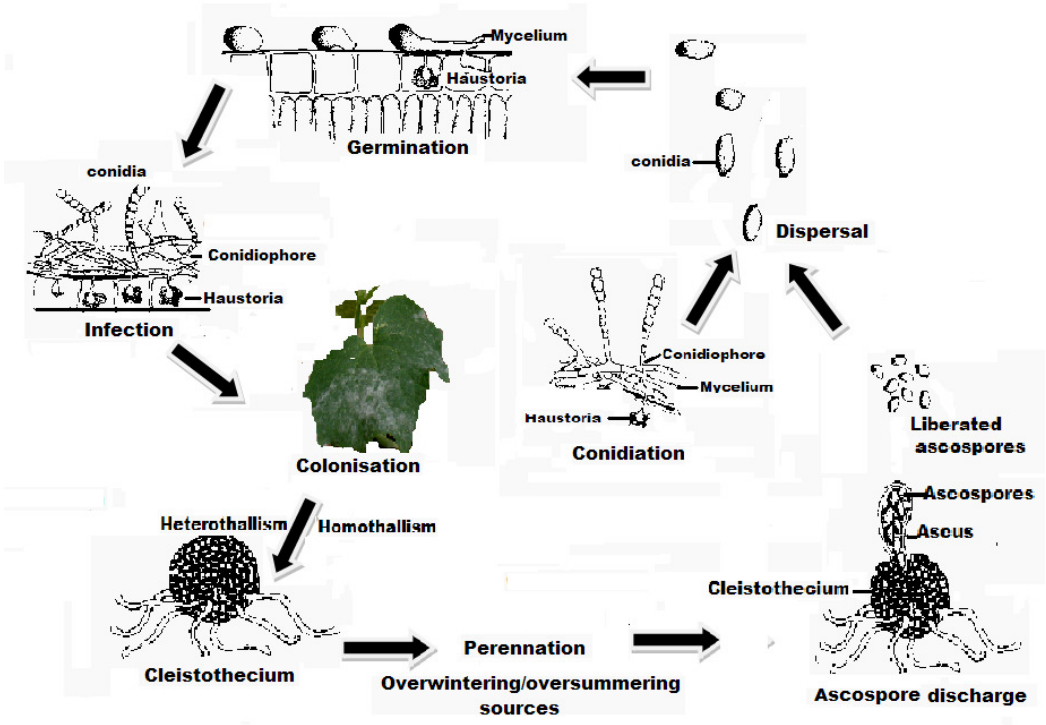

Fig 1.9 Life cycle of *Podosphaera fusca* (Image source: Agrios 1997).

1.6.3.1 Asexual reproduction and infection

Most of the studies on powdery mildew of cucurbits suggested that conidia are the main inoculum source for the infection and spread of the disease (Hashioka 1936; Reuveni and Rotem 1974; Cheah *et al.* 1996). Conidia develop as a chain, with each new conidium forming at the base of the previous conidium (Hammett and Manners 1973). Yarwood and

Gardner (1964) reported that a single conidium of each conidiophore of *P. fusca* matured daily in the middle of the day, were dispersed by wind and could remain viable for up to 8 days (McGrath and Thomas 1996).

On a susceptible host, conidia germinate to produce a forked germ tube without obvious appressoria after 12 h incubation (Kuzuya *et al*. 2006). Between 12 h to 24 h the single tube then elongates to form long hyphae and appressoria on the underside of the hyphae. In the following 24 h, a penetration peg emerges from the underside of the appressorium, penetrates the epidermal cell by an enzymatic digestion activity and turgid mechanical force (Green *et al*. 2002). The penetration peg enters the epidermal cell and forms a haustorium, which absorbs nutrients from the host plant tissues. When the first haustorium is established, additional germ tubes are formed from other points on the same conidium. These tubes extend and branch to form a mass of mycelia, more appressoria and pegs and colonise the leaf. The incubation period was reported to be 3-7 days (McGrath and Thomas 1996).

1.6.3.2 Sexual reproduction

Cleistothecia are considered to be the over-wintering or over-summering source of infection (Butt 1978), but they are very rare and have not been observed in some studies (McGrath and Thomas 1996). Attempts to produce fertile ascospores have often failed (Nicot *et al*. 2002). No detailed study of powdery mildew infection by ascospores is available so far and in Australia there is no record of the occurrence of fertile ascospores in cucurbit fields.

1.6.4 Epidemiology

1.6.4.1 Disease development

Under field conditions *P. fusca* is classified as a summer pathogen as temperatures for disease development range from 20-30ºC (Reuveni and Rotem 1974; Cheah *et al*. 1996). Germination of conidia occurs over a range of temperatures from 15 to 30ºC with an optimum of 25ºC. Germination declined sharply at temperatures of 15ºC or lower and above 30ºC as conidia and germ tubes shrivel at high temperatures (Hashioka 1936; Reuveni and Rotem 1974; Cheah *et al.* 1996). Colonisation, sporulation and dispersal of *P. fusca* on squash in Israel were favoured by dry conditions of 45%-50% RH, while infection and conidial survival were encouraged by high humidity of 90%-95% RH (Reuveni and Rotem 1974). Temperature and relative humidity are considered the dominant factors for powdery mildew development (Yarwood 1957, Butt 1978). However, Anderson (1936), Schnathorst (1965) and Jarvis *et al*. (2002) suggested that vapour pressure deficit (VPD) was a more meaningful description of the effect of atmospheric moisture than relative humidity on the biological processes of powdery mildews. VPD gives a more accurate indicator of evaporation rate, without depending on temperature. The ability of powdery mildew to germinate and sporulate under dry conditions may be associated with the higher than average water content of spores (Yarwood 1950). Conversely, excessive water or rainfall is detrimental as it can (i) wash conidia off leaves, (ii) physically damage mycelium and conidiophores and (iii) cause abnormal germination (Sivapalan 1993). Powdery mildews are more prevalent in the shade than in full sunlight (Yarwood 1957), but this is more related to host physiology than direct effects of light on the pathogen (Jarvis *et al.* 2002).

1.6.4.2 Dispersal

Release of *P. fusca* conidia into the air is generally increased with wind speed and surface dryness, but surface wetness, low temperatures and high RH inhibit sporulation (Reuveni and Rotem 1974). Generally for powdery mildews, dispersal of conidia is positively associated with wind velocity, temperature, and solar radiation but negatively associated with relative humidity and leaf wetness (Hammett and Manner 1971; Sutton and Jones 1979). Electrostatic charges generated from fluctuations of humidity or from exposure to infrared radiation reportedly also disperse conidia (Leach 1976; Jarvis *et al.* 2002). Dispersal of conidia is often correlated with the onset of rain which may be linked with high velocity wind and this energy

could help to dislodge the conidia (Bainbridge and Legg 1976; Jarvis *et al*. 2002). In contrast, heavy rain is not conducive to dispersal and such rain can cause damage to conidia and conidiophore structures (Yarwood 1978a; Sivapalan 1993). In addition to the environmental factors, the conidia of powdery mildews can also be transported from one location to another by human activities (Schepers 1984).

1.6.5 Disease control

Management of powdery mildew has largely relied on fungicides, but with the development of fungicide resistance and the consumer pull to "softer" or less harmful products, biofungicides, biorational and non-harmful chemicals are constantly being evaluated. Additional methods for managing powdery mildews are the use of genetic resistance, cultural practices and integrated pest management (IPM).

1.6.5.1 Chemical control

"Softer products" often require frequent application and can be expensive, but may have merit in glasshouse hydroponic production where the application of registered fungicides is limited. Biofungicides are (i) products or microorganisms that can induce host defence mechanisms such as Bion™, or (ii) microorganisms that compete either through parasitism or antibiosis for space or nutrients (Bélanger and Labbé 2002). Biological products registered for control of powdery mildews are Serenade® (*Bacillus subtilis*), AQ10® (*Ampelomyces quisqualis* Ces) and Sporodex® (*Sporothrix flocculosa*). Biorationals include natural and mineral oils, peroxides, cow's milk, extracts of neem, jojoba and cinnamon, and salts of monovalent cations such as potassium, sodium and ammonium bicarbonate (Nunez-Plaenius *et al.* 2008; Bettiol 1999). Soft products were extensively evaluated by Akem *et al.* (2011) for control of *P. fusca* on cucurbits in Queensland.

1.6.5.2 Resistance

Genetic resistance to powdery mildew, caused by *P. fusca*, has been introduced in commercial varieties of melon, cucumber and squash. To date seven races of *P. fusca* have been reported on melon (Jahn *et al.* 2002) and four of these races have been reported in Australia (McGrath and Thomas 1996). An extensive evaluation of zucchini, squash and cucumber varieties for resistance to powdery mildew was recently undertaken by Akem *et al.* (2011). Introduction of varieties with resistance to specific races, however, increases the risk of development of new races. Additionally, resistant varieties also require preventative spray programs to reduce the risk of the resistance failing.

1.6.5.3 Cultural control

Use of crop rotation, removal of debris and alternative hosts breaks the disease cycle. Increasing ventilation reduces humidity, especially in glasshouses, and has given good control for powdery mildew on glasshouse grown cucurbits (Jhooty and McKeen 1965; Reuveni and Rotem 1974; Butt 1978).

1.7.1 Disease predictive models

Prepared by Victor Galea

The influence of weather on disease is well known (Jones 1986). Disease predictive models are a mathematical description of an attempt to forecast the future development or appearance of a disease in a crop, based on climatic measurements made within the crop (Madden and Ellis 1988; Parry 1990; Galea and Minchinton 2005). Models can be based on climatic variables such as temperature, relative humidity, leaf wetness and on an understanding of how the fungus reproduces and infects under field conditions (Fritt *et al.* 1989).

There are several motivations for use of disease predictive models (Fry and Fohner 1985). They can increase income by reallocating disease management resources to other areas of

production. The risk of large unexpected crop losses is reduced. They provide the means to lower pesticide application to crops, which alleviates concerns for human health and pollution of the environment. Disease predictive models may assist in the management of fungicide resistance strategies by assisting the grower to identify the most appropriate timing for the application of systemic (curative) compounds. Consequently they are an ideal tool for integrated pest management (IPM).

Factors that contribute to growers' adoption of predictive models (Kable 1991; Maloy 1993; Polley 1983) are as follows.
1. Significant economic losses are associated with the crop disease;
2. Economically viable control measures must be available;
3. Seasonal variability may make appearance of the disease difficult to predict;
4. There must be validation of the model under local field conditions;
5. The system must be readily available to end-users.

Growers must be confident measurable benefits can be expected from using the model that would be unavailable without its use. Attributes that will ensure the success of a model include: (1) reliability, (2) cost effectiveness, (3) simplicity, i.e. a user friendly interface, (4) importance to the industry, (5) usefulness and (6) availability (Campbell and Madden 1990).

1.7.2 Current limitations of disease predictive models

A number of issues are associated with disease predictive models:
1. They predict sporulation or infection based on historical microclimatic data, which means that the response time to apply fungicides may be limited.
2. They can over-estimate sporulation or infection events. If the disease is not present in the crop and there are no obvious sources of spores in the field or farming area, the microclimate data can still predict sporulation or infection events.
3. They may require the tolerance of very low levels of symptoms in the field, as it may not be economically viable to completely eradicate the disease from the crop.
4. Most disease predictive models require leaf wetness, measured by a leaf wetness sensor, which, due to their position in the crop, are subject to weathering and corrosion from chemical sprays. Additionally, leaf wetness sensors can only represent a minuscule proportion of the foliage of a crop.

The accuracy of models could be improved by:
(i) Incorporating predicted microclimate or meteorological data into the model so it was truly a 'forecast' of expected events (e.g. Bureau of Meteorology real-time data).
(ii) Incorporating prediction of sporulation and infection into the model. Thresholds for spraying obviously need to be set below the actual sporulation and infection parameters of the pathogen so contact, preventative fungicide applications can be employed. Generally models predict either sporulation or infection. However the accuracy of models would be enhanced if they predicted both. Spore trapping alongside collection of microclimate data would enhance predictive models.
(iii) The use of systemic fungicides with curative activity to remove infections, which may have taken place due to the lag time between:
    (a) collection of microclimate data and output from the predictive model and
    (b) the output from the model and the time to organize spraying of the crop.

1.7.3 Alternative measurements for leaf wetness

Another means of measuring leaf wetness is Vapour Pressure Deficit (VPD). VPD is one measure of the ability air to release moisture. VPD measurement is used regularly as a key factor for predicting disease potential in greenhouse crops (Prenger and Ling on line). Other researchers are trying to develop models which estimate leaf wetness duration in the field.

These models are the fuzzy logic system and the CART/SLD/Wind model which is under evaluation using field data (Kim *et al.* 2002, 2006).

1.7.4 Brassica$_{spot}$™ disease risk predictive model for white blister

Prepared by Elizabeth Minchinton and Des Auer.

The Brassica$_{spot}$™ disease risk predictive model was developed in the UK by Kennedy and Giles (2003). It is an infection model based on temperature, leaf wetness, relative humidity and rainfall. The Brassica$_{spot}$™ disease predictive model is extensively used by UK consultants and growers where it has lead to a 50% reduction in fungicide sprays (Kennedy, pers. comm.). Preliminary evaluation of an older version of the Brassica$_{spot}$™ model (Brassica$_{spot}$I™) under Australian conditions was undertaken by Minchinton *et al.* (2007) and Petkowski (2008). Use of the model predictions reduced the number of sprays required for disease control by 8-10 and controlled white blister on broccoli heads as well as calendar spray programs.

Three versions of the Brassica$_{spot}$™ model were obtained under licence from HRI Warwick UK. These are referred to as Brassica$_{spot}$I™ (older) version of the model reported in HAL VG04013 (Minchinton *et al.* 2007) (Fig 1.10) and two versions of the newer model Brassica$_{spot}$II™ (5% infection and 50% infection) (Fig 1.11). Brassica$_{spot}$II™ retains the infection component of the Brassica$_{spot}$I™ model, but predicts when to spray based on lesion appearance and size in the field, lesion development and maximum sporulation from lesions in the field. Depending on the amount of disease that can be tolerated in the field, either the 5% or 50% disease development "track line" is followed.

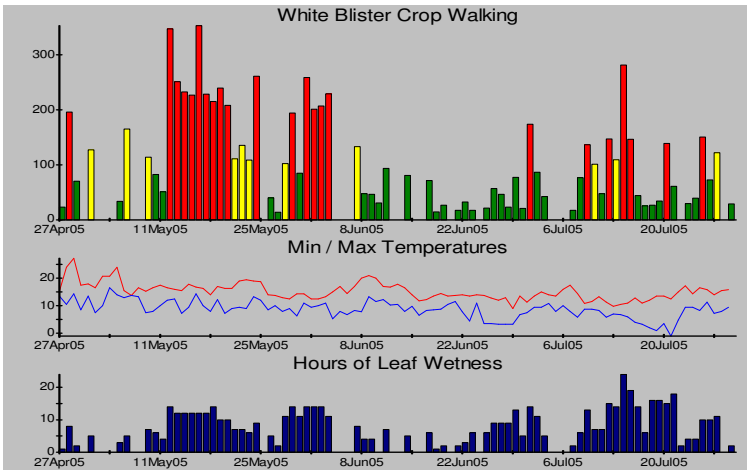

Fig 1.10 Brassica$_{spot}$I™ (older) version of the model.

Bottom graph: indicates leaf wetness duration (hrs),
Middle graph: indicates maximum and minimum temperatures (ºC),
Top graph: indicates the risk of conditions conducive to infection. Red bar, high risk of infection; yellow bar, moderate risk; green bar, low risk and no bar, no risk of infection from *A. candida*.
When a red bar or cluster of red bars occurs, the crop is walked 7, 14 and 21 days afterwards and if white blister lesions are observed, the grower decides to apply or withhold a fungicide spray.

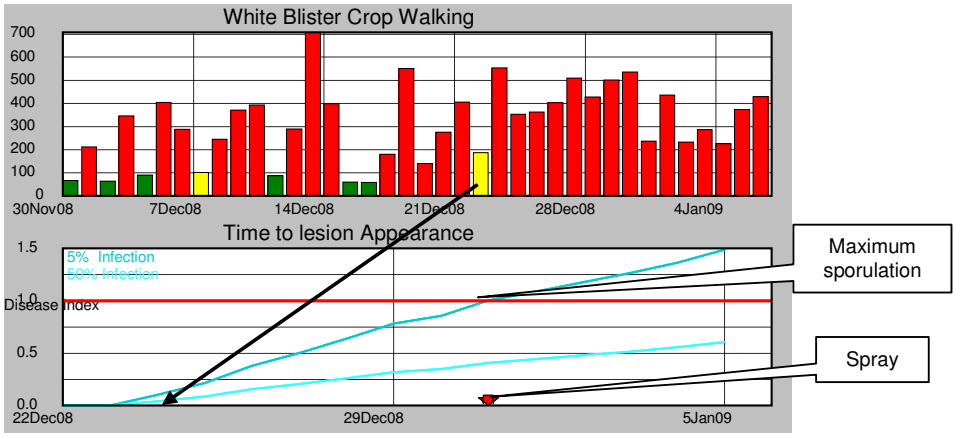

Fig 1.11 Brassica$_{spot}$II™ (new) version of the model.

Top graph: indicates the risk of conditions conducive to infection. Red bar, high risk of infection; yellow bar, moderate risk; green bar, low risk and no bar, no risk of infection from *A. candida*. When a red bar or cluster of red bars occurs the crop is walked 7, 14 and 21 days afterwards and if white blister lesions are observed, the grower decides to apply or withhold a fungicide spray.
Bottom graph (Time to lesion appearance): When lesions are first observed in the crop, the time of the observation and the lesion size are entered into the model, which generates two lesion development lines, 5% and 50% infection. When these lines cross the red "Disease Index" line, a red arrow appears to indicate that this is the time of maximum sporulation from these lesions and a spray should be applied. The 5% line denotes maximum sporulation from 5% of lesions in the crop and the 50% line indicates when maximum sporulation will occur from 50% of lesions in the crop.

In Fig 1.11 lesions first appeared on 22 December (arrowed). A fungicide spray was predicted and applied on 31 December with the Brassica$_{spot}$II™ 5% model.

1.7.5 Lettuce Downy mildew forecasting models

Prepared by Des Auer, Elizabeth Minchinton and Victor Galea.

A forecasting system or disease-predictive model for lettuce downy mildew (LDM) caused by *B. lactucae* has been researched for many years. This has occurred because of several issues: the large areas of lettuce production in the United States and elsewhere (Patterson *et al.* 1986), a lack of resistant cultivars to all races of *B. lactucae* (Crute and Johnson 1976; Ilot *et al.* 1987) and the resistance to the few fungicides that have been found to be effective against this disease (Cobelli *et al.* 1998; Shettini *et al.* 1991).

1.7.5.1 An overview of the parameters affecting *B. lactucae* sporulation and infection

One parameter that is essential for both spore germination and release in *B. lactucae* is light. The production of asexual spores occurs only at night when both a high relative humidity (RH) and low wind speeds are prevalent. Release of sporangia is associated with the onset of sunrise and a corresponding drop in RH (Su *et al.* 2000). It was shown that few spores were released in darkness and spore release reached maximum levels only 1-2 h after light initiation (i.e. dawn). Furthermore, the reduction in RH also initiated spore release independent of light levels (Su *et al.* 2000). In a two-year field study, Carisse and Philion (2002) demonstrated that large spore numbers were generated at night with RH values > 95%, with spores absent if the previous night was dry. This was also independent of leaf wetness, as long as the RH value was > 90%. Further, the peak release of spores occurred at about 10:00 h. This supported earlier work by Scherm and van Bruggen (1994a) who demonstrated that spore formation occurred at night and that infection by *B. lactucae* occurred if the

morning leaf wetness duration lasted until at least 10:00 h. In addition, they also attested that spore release was followed almost immediately by infection, as long as leaf wetness conditions were met (Scherm and van Bruggan 1995).

Spore survival after release is relatively short-lived and depends on both temperature and solar radiation (Wu *et al.* 2000). Sporangial viability was severely limited after exposure to light, in particular UVB, with even a short exposure of 2 h curtailing spore germination by 95% (Wu *et al.* 2000). Temperature also had a profound influence on spore viability and infection with an increase from 20°C to 25°C and 30°C decreasing viability from 60% to <20% to zero, respectively, and neither 25°C nor 30°C exhibited any evidence of infection (Scherm and van Bruggen 1993).

By analysing historical data and combining this with field observations, it was determined that the critical morning leaf wetness period had to be measured from the onset of dawn instead of at a specific time. In addition, higher temperatures also impeded infection. All of these parameters were integrated into the predictive model of Wu *et al.* (2000).

1.7.5.2 Validation of lettuce downy mildew disease-predictive models in the field

Wu *et al.* (2001) tweaked their own model to incorporate a pathogen survival component and a survival and sporulation component. Briefly, their original model focussed on leaf wetness duration only (LWD), stating that a fungicide spray should be applied if morning LWD was ≥4 h and no fungicides had been applied during the last seven days (OS). Modifications to the model included parameters which assumed spore survival during the previous day (Wu *et al.* 2000) (MS1) or assumed that infection only occurred if active inoculum was present (i.e. conditions overnight induced sporulation) and morning conditions were ideal for infection (MS2).

Over their two-year field evaluation, the disease incidence was low, reflecting the inconsistent nature of this disease (Scherm and van Bruggen 1994a). All three models predicted the same number of sporulation-infection periods (SIPs), but no model accounted for all SIPs, and this was attributed to the 4 h morning LWD period, prompting a change to ≥ 3 h in their model, reflecting observations by Scherm and van Bruggen (1993), who stated that *B. lactucae* caused infections in as short a time as 2 h under optimal temperature conditions. It was also found that post-penetration temperatures after the initial morning LWD had a profound effect on infection, with an upper limit of 22°C at midday (i.e. 10:00 h to 12:00 h) also used in the model. This latest version of their predictive model is used primarily in California (Wu *et al.* 2005).

1.7.5.3 Lettuce DownCast forecast model

Lettuce DownCast was adapted from the onion downcast system (Jesperson and Sutton 1987, Fitzgerald and O'Brien 1994) and modified later to include the work of Wu *et al.* (2001, 2002, 2005), which was based on sporulation and infection studies of Scherm and van Bruggen (1993, 1994a, 1994b, 1995a; Scherm *et al.* 1995). Factors involved in the in–house Lettuce Downcast model are listed in Table 1.3 and an example of the Excel program for the in-house model is shown in Fig 1.12.

Table 1.3 Definitions for the in-house Lettuce DownCast model.

Factor	Definition of parameter	Reference
Sporulation #1	Basis: Sporulation occurs at night (pre-dawn) when the following conditions are satisfied: • Temperature during the previous night must be within the range 4–20°C • If this range is exceeded, sporulation will not occur • Night range from Last light (previous day) to First light (current day)	Jesperson and Sutton 1987, Fitzgerald and O'Brien 1994
Sporulation #2	Basis: Sporulation occurs at night (pre-dawn) when the following conditions are satisfied: • Night RH of 90% and leaf wetness period of at least 3 hours	Kushalappa 2001
Infection #1	Basis: Leaf wetness period of at least 3 hours after sunrise	Scherm *et al.* 1995
Infection #2	Basis: Daytime temperature during the leaf wetness period must not exceed 20°C	Wu *et al* . 2001
Infection #3	Basis: Temperature of post-wetness period i.e. dry leaf temperature (of at least 4 hours) must not exceed 22°C	Wu *et al.* 2002
Infection #4	Basis: Temperatures between 100:00am and 2.00pm must not exceed 22°C	Wu *et al* . 2005

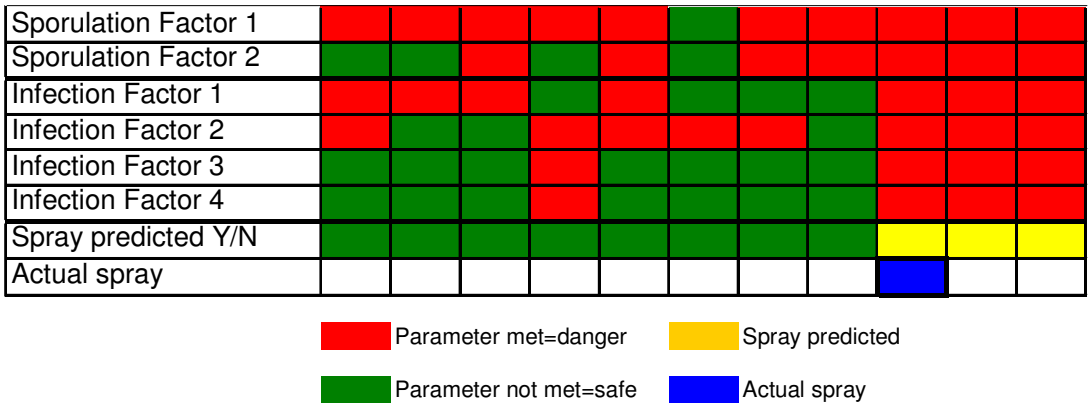

Fig 1.12 Example of the Excel in-house disease predictive model.
A fungicide spray is applied when all sporulation and infection are predicted to occur.

1.7.6 BREMCAST™ model

Prepared by Des Auer and Elizabeth Minchinton.

Another major disease-predictive system developed in Canada is BREMCAST™ that bases its predictions on sporulation, infection and survival of spores from one day to the next (Kushalappa 2000, 2001). BREMCAST™, is split into two components, sporulation and infection. Night is defined as the period between 18:00 and 6:00 h, and morning is defined as the period 6:00 to 13:00 h. For the sporulation component (SPOV), a range of 0-3 is determined by three attributes: night wetness duration (NWD), night average RH (NRH) and night average temperature (NT). The maximum value of 3 occurs if NWD > 4, NRH ≥ 91% and NT is in the range 7-20°C. For the infection component (INFV), a range 0-5 is determined by morning wetness duration (MWD) and morning temperature (MT), with a maximum value of 4-5 occurring if MWD ≥ 6 and MT ≤ 15°C. A disease severity value of 0-5 (DSV) is calculated from SPOV and INFV. DSVs of 4 and 5 are the result of INFV values of 3-5 and SPOV values of 2-3. DSV increases faster if an inoculum source (INOCS) is present. The study by Bhaskara Reddy *et al.* (1996) strongly implied that a considerable number of spores could survive extended periods of light exposure under Canadian conditions. With the presence of disease in a field, it was assumed that there would be many infection sites.

In the case of BREMCAST™, once a sporulation-infection period (SIP) is recorded (continuous leaf wetness period for 3-5 h post-dawn at 5-20 °C) a fungicide should be applied. Hovius *et al.* (2007) demonstrated that using BREMCAST™ in conjunction with a pre-symptom threshold (i.e. assuming an 8-day latent period for the disease) could significantly control lettuce downy mildew. Currently BREMCAST™ is used by lettuce growers in Canada to predict SIPs (*http://www.uoguelph.ca/muckcrop/forecasting.html*), advising them of the risk of lettuce downy mildew infection as well as that of other diseases and insect pests. An example of the BREMCAST™ model data input and output is shown in Fig 1.13.

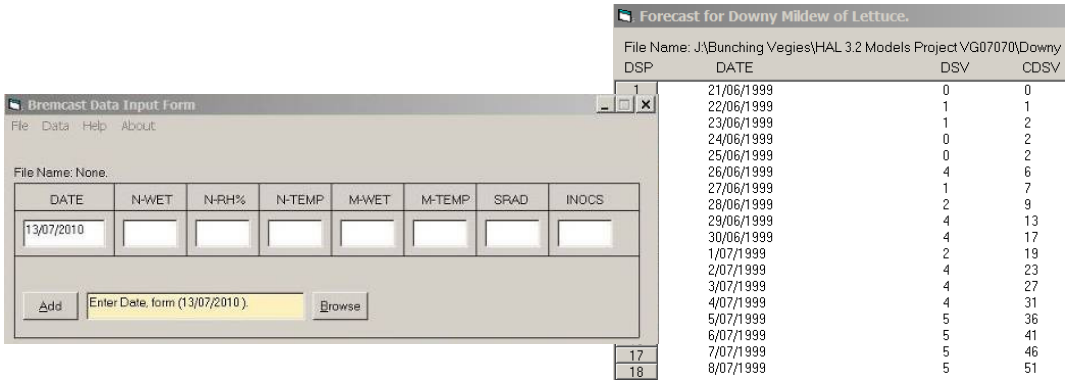

Fig 1.13 An example of the BREMCAST™ model.
The input data format is shown on the left and the output file is shown on the right. A DSV of 4 or 5 indicates a spray should be applied.

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Chapter 2

Evaluation of the efficacy and economics of irrigation management, a resistant variety and the Brassica$_{spot}$™ model as IPM tools for control of white blister on broccoli

Summary

A field trial on broccoli at Werribee run from July to November 2008 evaluated three IPM tools, variety, irrigation timing and spraying regimes including the Brassica$_{spot}$™ disease risk predictive model, for efficacy in the control of white blister and the economics of this control. The trial showed that growing the more resistant variety, Tyson, reduced the incidence of white blister on broccoli heads by 99% when compared to the susceptible variety Ironman whilst overhead irrigation at 04:00h rather than at 20:00h resulted in a 58% reduction. Neither version of the two versions of the Brassica$_{spot}$™ differed in its control of white blister nor were they different from the Weekly treatment. They were significantly better than doing nothing (Control). However, the use of the Brassica$_{spot}$™ models considerably reduced the numer of sprays applied by 12 to 13. An economic analysis of this trial showed an 11% increase in farm profits by growing the more tolerant variety, a 3% increase by choosing morning irrigation and a 3 to 4.5% increase in farm profits by using the models because fewer sprays were used.

2.1 Introduction

White blister caused by *Albugo candida* (Pers. Ex. Lev.) is a major disease of Brassica crops, especially broccoli (*Brassica oleracea* L). Since the white blister epidemic of 2001, the disease has become established in broccoli (*Brassica oleracea* var *italica*) and cauliflower (*B. oleracea* var *botrytis*) crops throughout Australia (Minchinton *et al.* 2005). In November 2010 it was identified on cabbage (*B. oleracea* var *capitata*) in Werribee South, Victoria.

The broccoli variety Greenbelt was the most commonly grown variety in Victorian production areas prior to 2001, often being produced year-round. It was highly susceptible to white blister and had up to 100% disease incidence, frequently rendering crops unmarketable (Minchinton et al. 2005). As a result of the white blister epidemic, Greenbelt is no longer grown in Victoria. Trials of broccoli varieties conducted in Tasmania, Victoria and Western Australia identified up to four other varieties which reduced the disease on heads by 44-100% compared to Greenbelt (Minchinton *et al.* 2007). Since 2001, some varieties with good resistance to white blister have been introduced into Victorian cropping regions.

Prior to the white blister epidemic, broccoli crops were sprayed with insecticides in summer to control diamondback moth and with fungicides in winter to control fungal leaf spots. As a consequence of the epidemic, there was a need to apply fungicides weekly and year-round for white blister control.

In Victoria, vegetable crops are grown under overhead irrigation. Systematic surveys showed that timing the application of irrigation onto radish crops affects the incidence of white blister (Minchinton *et al.* 2005). Albugo is an oomycete and free water on plant surfaces is required for infection by this pathogen (Develash and Sugha 1996; Gilijamse *et al.* 1998; Viranyi 1974). Zoosporangia of A. candida can be released at any time of day or night and require only 3 hours of leaf wetness for infection (Gilijamse *et al.* 1998; Lakra *et al.* 1989). Consequently the shorter the duration of leaf wetness, the lower the likelihood of white blister development in a crop.

Brassica$_{spot}$™ is a disease predictive model developed for white blister on brassicas by Kennedy and Gilles (2003) that accounts for the importance of moisture or leaf wetness in the life cycle of the Albugo pathogen. In the model, a reduction in leaf wetness, which can be associated with overhead irrigation practices, reduces the likelihood of infection by the pathogen and reduces disease pressure in crops. Model outputs for Brassica vegetable crops are generated using weather data, including temperature and leaf wetness period, collected half hourly by a weather station placed in the crop. Disease predictions made by the model are a decision support tool for optimising the timing of fungicide applications to reduce yield losses associated with the disease. In field trials in the UK, 50% fewer fungicide sprays were applied compared with calendar based spray programs when Brassica$_{spot}$™ was used to time sprays for the control of foliar diseases in Brussels sprouts crops (Kennedy & Gilles, 2003). Preliminary evaluations of the model on broccoli crops grown in Victoria showed that the number of sprays could be reduced by 8 to 10 (Minchinton *et al.* 2007). Developers of the UK model have recently upgraded the Brassica$_{spot}$™ model with a view to applying protectant fungicides rather than systemic fungicides when a spray prediction is forecast.

The aim of this study was to evaluate the efficacy and economics of modifying the time of overhead irrigations, growing resistant varieties and timing spray applications according to predictions of the Brassica$_{spot}$I™ disease predictive model (old version) and the Brassica$_{spot}$II™ disease predictive model (new version using the 5% disease appearance option), as IPM tools, in a field trial to control white blister on broccoli.

2.2 Materials and methods

2.2.1 Trial location and design

The trial site was located at Dairy Road, Werribee, Victoria. The trial plan was originally a general split-plot design with 6 replicates. Each of 3 blocks contained 2 replicates. Each replicate contained whole plots to which irrigation times in the morning or evening were randomly allocated. Within each whole plot there were 8 sub-plots. The 8 treatment combinations of variety (resistant or susceptible) and spray regime [unsprayed control, standard industry practice (weekly sprays), Brassica$_{spot}$I™ model, Brassica$_{spot}$II™ model] were randomly allocated to these 8 sub-plots. The trial was designed as 32 x 3 plots but was laid out as 12 x 8 plots with the treatment allocation as described above. Seedlings were planted 2 rows per bed and spaced 30 cm apart, on raised beds of 1.62 m width on 23 July 2008. Sub-plot dimensions of beds were 8 m long with a 1.5 m buffer of bed between each sub-plot. Each sub-plot contained approximately 52 plants and represented a treatment.

2.2.2 Broccoli varieties

Eight week old seedlings of the broccoli variety Tyson (Syngenta) designated resistant or the variety Ironman (Seminis) designated less resistant or susceptible to white blister, were supplied courtesy of Boomaroo Nurseries Ltd., Lara, Victoria.

2.2.3 Irrigation

An irrigation line was located in the middle of each replicate. There were 4 sprinkler heads per replicate with 2 adjacent sprinkler heads set to deliver irrigation at 04:00 h or 20:00 h for one hour, 2-3 times per week or more depending on need. Approximately 500,000 L of water was applied over the 15 week trial.

2.2.4 Weather station

A Model T weather station (Western Electronics Design, Loxton, SA) was placed in the middle of an irrigation line of the broccoli crop which received morning irrigation. The station recorded average leaf wetness, temperature, relative humidity and total rainfall at 30 min. intervals. The leaf wetness sensor was placed in the broccoli crop and its height adjusted according to crop growth. An additional weather station should have been placed in a plot receiving evening irrigation. Unfortunately this was not done.

2.2.5 The Brassica$_{spot}$™ model

The Brassica$_{spot}$™ model was purchased from Warwick HRI, Wellesbourne, Warwickshire, UK. The model is based on leaf wetness over a temperature range of 6-24ºC which determines high risk periods for infection by *A. candida* zoosporangia. The crop was inspected (walked) at 7, 14, 21 d after each high risk period and fungicides were applied if white blister symptoms were observed (Table 2.1). Systemic fungicides rather than protectant were applied to plants treated according to both versions of the disease predictive model because of the need to address high white blister disease pressure like that experienced in previous trials.

2.2.6 Application of chemicals

All chemicals were applied with a hollow cone nozzle TX-VK-8 at 30 psi by a Silvan Selctra 12v knapsack sprayer [Silvan Pumps and Sprayers (Aus) Pty. Ltd.]. Fungicides (Table 2.1) were applied at the low rate of 500L/ha during weeks 1-8 and at the high rate of 1000L/ha from weeks 9-14. The fungicide application program is given in Table 2.2.

Table 2.1 Chemicals and rates of application for the field trial.

Trade name	Active	Company	Rate
TriBase Blue®	Copper sulphate tribase (190g/L)	Nufarm	208ml/100L
Amistar®	Azoxystrobin	CropCare, Syngenta	280ml/100L
Du-Wett™	Trisiloxane ethoxylate	Nufarm	300ml/ha

Table 2.2. Spray program for the Werribee field trial to evaluate the effect of irrigation, variety and the Brassica$_{spot}$I™ and Brassica$_{spot}$II™ disease predictive models for control of white blister on broccoli.

	Week/ date of spray application/ dap													
Treatment	wk 1	wk 2	wk 3	wk 4	wk 5	wk 6	wk 7	wk 8	wk 9	wk 10	wk 11	wk 12	wk 13	wk 14
	8/08/2008	15/08/2008	22/08/2008	29/08/2008	5/09/2008	12/09/2008	19/09/2008	26/09/2008	3/10/2008	10/10/2008	15/10/2008	23/10/2008	31/10/2008	6/11/2008
	15	22	29	36	43	50	57	64	71	78	83	91	99	105
Evening Resistant Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Evening Susceptible Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Evening Resistant Model BSII	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Evening Susceptible Model BSII	-	-	-	-	-	-	-	-	-	-	A	-	-	-
Evening Resistant Model BSI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Evening Susceptible Model BSI	-	-	-	-	-	A	-	-	-	-	-	A	-	-
Evening Resistant Weekly	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD
Evening Susceptible Weekly	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD
Morning Resistant Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Morning Susceptible Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Morning Resistant Model BSII	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Morning Susceptible Model BSII	-	-	-	-	-	-	-	-	-	-	-	-	-	A
Morning Resistant Model BSI	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Morning Susceptible Model BSI	-	-	-	-	-	A	-	-	-	-	-	A	-	-
Morning Resistant Weekly	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD
Morning Susceptible Weekly	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD	TD

Evening irrigation commenced at 20:00 h; Morning irrigation commenced at 04:00 h; dap, days after planting; BSI, Brassica$_{spot}$I™ version of the disease predictive model; BSII, Brassica$_{spot}$II™ version of the disease predictive model; Resistant, broccoli variety Tyson; Susceptible, broccoli variety Ironman; TD, Tribase Blue and Du-Wett; A, Amistar; -, not sprayed.

2.2.7 Assessment and analysis of trial data

Foliage. The middle 20 plants per sub-plot were assessed for the incidence (presence or absence) of white blister on foliage at week 13 (29 October 2008). The incidence data consisted of the number of plants per sub-plot with disease symptoms on foliage. As no disease was present on the foliage in any of the plots containing the more resistant variety, these data were not included in the analysis. Analysis of Variance was carried out on the data using the arcsin transformation. The terms in the model consisted of an Irrigation main effect and the interaction of the Model with Irrigation. The analysis was done this way to allow for the situation that the Model treatments under the evening irrigation used the information from a weather station placed in a morning irrigation plot.

Heads. Approximately 50 plants per subplot were assessed for the incidence (presence or absence) of white blister on broccoli heads at week 15 (14 November, 2008). The data for analysis consisted of the percentage of assessed heads with disease. Analysis of Variance was carried out on the data using the arcsin transformation. The number of heads assessed varied considerably between subplots so this was used as a covariate in the analysis. The terms in the model consisted of Variety, Irrigation, their interaction and the interaction of Model with Irrigation (as described for foliage). The irrigation by model interaction was fitted using contrasts of the means of particular interest to the experimenter. This was necessary because the evening irrigation subplots under the Brassica$_{spot}$™ regimes did not get the correct information due to the lack of a weather station (Tables 2.3 and 2.4).

2.2.8 Economic analysis

Refer to Chapter 9.

2.3 Results

The incidence of white blister in the trial on both foliage and heads was low (Table 2.3).

2.3.1 Time of irrigation

At week 13 there was no significant difference in the incidence of white blister on variety Ironman between overhead irrigation in the morning or the evening (Table 2.3). Whilst at harvest week 15, overhead irrigation of the broccoli crop in the evening (20:00 h) more than doubled (P = 0.050), the incidence of white blister compared with overhead irrigation in the morning (04:00 h) in this spring maturing broccoli crop (Table 2.3). The 58% reduction in disease incidence on the morning irrigated broccoli crop corresponded to a 3% increase in yield.

2.3.2 Variety

Very few symptoms of white blister appeared on foliage of the resistant variety Tyson by week 13, consequently there was no data to analyse (Table 2.3). Growing this relatively resistant broccoli variety (Tyson) significantly reduced the incidence of white blister on broccoli heads by 99% compared with the susceptible variety Ironman (Table 2.3). The reduction in white blister incidence on the resistant variety of broccoli corresponded to a 10% increase in yield (Table 2.3).

Table 2.3 Efficacy of irrigation time, variety and the Brassica$_{spot}$™ model in controlling white blister on broccoli heads.

Parameter	Treatment	Incidence (%) of white blsiter on foliage of Ironman at week 13	Incidence of white blister on heads of Ironman and Tyson at week 15	Number of sprays	Estimated yield (t/ha)	Rank of profitability[2]
Time of irrigation[1]	Evening (20:00h)	32.2	4.5	31	10.74	2
	Morning (08:00h)	30.2	1.9	31	11.04	1
	F-test	ns	P-value<0.050			
Variety[1]	Susceptible (Ironman)	nt	10.1	34	10.11	2
	Restistant (Tyson)	nt	0.1	28	11.24	1
	F-test	nt	P-value<0.001			

[1]All data included in the analysis; [2] Profitability ranking where 1 = most profitable and 4 = least profitable, refer to Chapter 9. Yield estimates are based on 11.25 t/ha.

2.3.3 Brassica$_{spot}$™ models

On foliage at week 13 and within the morning irrigation treatment (08:00h), the weekly sprays were significantly better than the average of the models, or the control, at controlling white blister on foliage (Table 2.4). There was no significantl difference between the control and the average of the three spray regimes (Table 2.4).

At harvest week 15, for plants grown under the morning irrigation regime (08:00h), applying fungicide sprays based on predictions of the Brassica$_{spot}$™ models or on a weekly spray schedule significantly reduced the incidence of white blister on broccoli heads by approximately 77% compared with unsprayed broccoli heads (Table 2.4). The reduction in white blister incidence on heads corresponded to a 7% increase in yield. There were no significant differences in the incidence of white blister on heads between the average of the Brassica$_{spot}$™ models and the Weekly sprays (Table 2.4). The Brassica$_{spot}$I™ and Brassica$_{spot}$II™ models reduced the number of sprays by 12 and 13, respectively, compared with the weekly spray program of 14 sprays.

Table 2.4 Efficacy of two versions of the Brassica$_{spot}$™ model for timing fungicid sprays to control white blister on broccoli heads.

Treatment	Incidence (%) of white blsiter on foliage at week 13 (Ironman)		Incidence (%) of white blsiter on heads at week 15 (Ironman)		Number of sprays	Estimated yield (t/ha)		Rank of profitability[1]
	Time of Irrigation		Time of Irrigation			Time of Irrigation		
	Evening	Morning	Evening	Morning		Evening	Morning	
Control	58.4	38.8	11	8.3	0	10.02	10.32	3
Brassica$_{spot}$I™	na	38.7	na	2.0	2	na	11.03	2
Brassica$_{spot}$II™	na	46.6	na	2.3	1	na	10.99	1
Weekly sprays	3.4	7.4	0.9	1.4	14	11.25	11.23	4
Contrast tested for significance (Morning irrigation only)								
Difference between Control and the average of the 3 spray regimes	na	ns	na	P-value = 0.003				
Difference between Weekly sprays and the average of the two Brassica$_{spot}$™ models	na	P-value = 0.002	ns	ns				

[1] All data included in the analysis; [2] Profitability ranking where 1 = most profitable and 4 = least profitable, refer to Chapter 9. Yield estimates are based on 11.25 t/ha.

2.3.4 Comparison of two versions of the Brassica$_{spot}$™ model

Only one weather station was available for the trial and it was located in the morning irrigation plot. Consequently the Brassica$_{spot}$I™ and Brassica$_{spot}$II™ models could only be compared for the morning irrigation plots. Predictions of both models for "White blister crop

walking" agreed for the time and intensity of infection risks, represented by no bars, or green, yellow or red bars in Figures 2.1 and 2.2. Both models required crop inspections at 7, 14 and 21 d after high risk events. The Brassica$_{spot}$II™ model also required records of the time when new symptoms (non burst lesions) appeared in the crop.

In the Brassica$_{spot}$I™ model (Fig 2.1), high rainfall coincided with high risk periods for *A. candida* infection (red bars). Unfortunately the model did not present maximum and minimum temperatures, as there were problems with the data file. The Brassica$_{spot}$I™ model predicted 3 high risk periods (represented by clusters of red bars). After the first two high risk periods two flushes or cohorts of new white blister lesions appeared 14 d and 7 d later, respectively. Based on these predictions and the appearance of new lesions in the crop, two systemic fungicide sprays were applied. The crop was harvested before new lesions developed after the third high risk period.

The Brassica$_{spot}$II™ model predicted two sprays but only one was applied because of the close proximity to harvest of the predicted time of the second spray (Fig 2.2). A systemic fungicide spray was applied as soon as possible after the 5% infection line crossed the red line (Disease Index = 1). The Brassica$_{spot}$I™ model predicted a spray application early and late in the crop's life, whereas the Brassica$_{spot}$II™ model only predicted a spray application late in the crop's life. The late spray applications occurred between weeks 12 and 14.

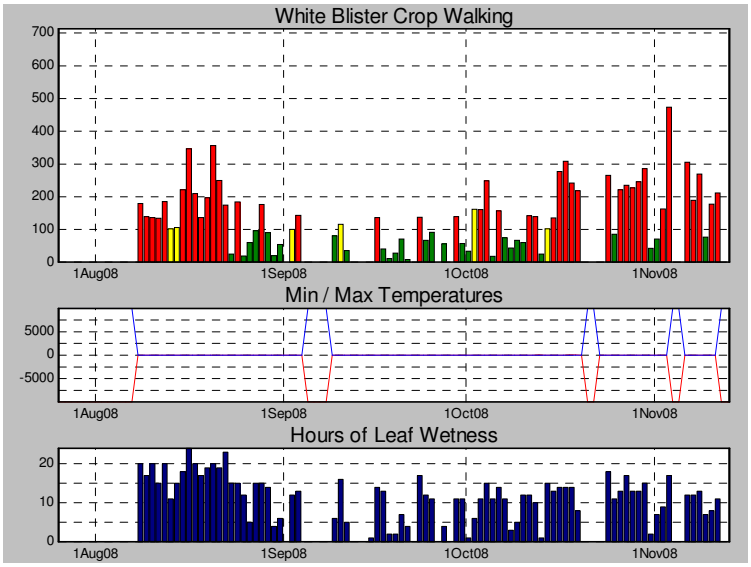

Fig 2.1 Predictions of risk of infection from *A. candida* based on the Brassica$_{spot}$I™ model at the Werribee trial site from 1 August to 10 November 2008.

Upper window: No bars = no risk; green bars = low risk; yellow bars = moderate risk and red bars = high risk of white blister appearing in a crop.
Middle window: Maximum and minimum temperatures.
Lower window: Hours of leaf wetness.

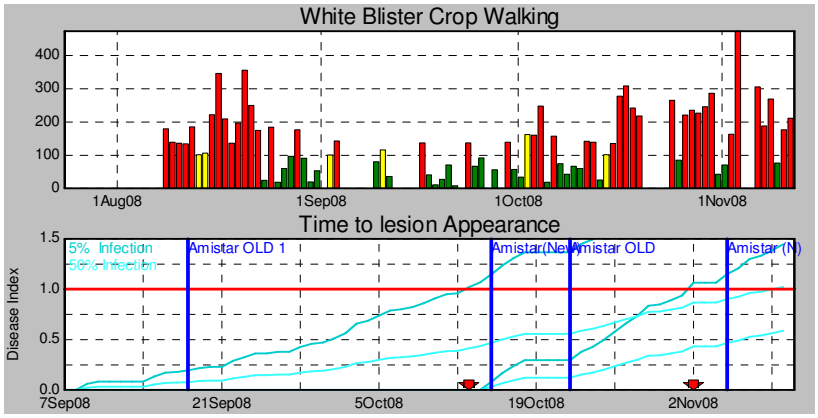

Fig 2.2 Predictions of risk of infection from *A. candida* based on the Brassica$_{spot}$II™ model at the Werribee trial site from 1 August to 10 November 2008.

Upper window: No bars = no risk; green bars = low risk; yellow bars = moderate risk and red bars = high risk of white blister appearing in a crop.
Lower window (Time to lesion appearance): When lesions are first observed in the crop, the time of the observation and the lesion size are entered into the model, which generates two lesion development lines, 5% and 50% infection. When these lines cross the red "Disease Index" line, a red arrow appears to indicate that this is the time of maximum sporulation from these lesions and a spray should be applied. The 5% line denotes maximum sporulation from 5% of lesions in the crop and the 50% line indicates when maximum sporulation will occur from 50% of lesions in the crop.

2.3.5 Economic analysis of the irrigation trial for white blister control of broccoli

Overhead irrigation of the broccoli crop in the morning (04:00 h) compared with overhead irrigation in the evening (20:00 h) increased farm profit bty 2.75% (Chapter 9). Growing a variety of broccoli resistant to white blister was more profitable than growing a susceptible variety and increased farm profit by 10.77% (Chapter 9). The highest to lowest ranking of spray regime profitability was the Brassica$_{spot}$II™ model, the Brassica$_{spot}$I™ model, the unsprayed Control and weekly sprays. In practice a resistant variety should never be left unsprayed as there is the risk that the pathogen will overcome the resistance. Use of the Brassica$_{spot}$II™ and Brassica$_{spot}$I™ models increased farm profit by 13.2% and 12%, respectively, when compared to the Weekly spray regime (Chapter 9).

2.4 Discussion

The field trial demonstrated that growing a variety of broccoli resistant to white blister provided the largest reduction in incidence of the disease on broccoli heads (99%), increased yield by 10% and increased farm profit by 11% (Chapter 9). Applying overhead irrigation to broccoli crops at 04:00 h instead of 20:00 h reduced disease incidence on heads by 58%, increased yield by 3% and increased farm profit by 3% (Chapter 9). Applications of fungicide sprays to control white blister on broccoli heads, based on predictions of either version of the Brassica$_{spot}$™ model or on a weekly spray regime, significantly reduced the incidence of white blister on broccoli heads of the susceptible variety by 77% when compared with unsprayed plants, but the incidence of the disease did not differ significantly between the various spray regimes. Use of the Brassica$_{spot}$I™ and the Brassica$_{spot}$II™ models and the Weekly sprays increased yield by an average of 7%. Compared to the unsprayed Control, Brassica$_{spot}$II™ increased farm profit by 4.5%, Brassica$_{spot}$I™ increased it by 3.33%, while Weekly sprays reduced it by 9.1%, however, given the dry conditions there were probably too many Weekly sprays of copper applied.

2.4.1 Varieties

While growing broccoli varieties resistant to white blister was the most efficient way to reduce impact of the disease, resistant varieties are not available for all production time slots. Resistance can also be unstable and lost. The summer broccoli variety Viper (Lefroy Valley) was resistant to white blister when it was introduced to Victorian cropping regions around 2001, but now the resistance has broken down and the variety is susceptible. More recently another white blister resistant variety Booster (Clause) also lost resistance after two years of cropping. While growers often complain that the cost of new varieties is exorbitant, this work has shown that crop production costs are lower. The resistant variety grown in our trial required no fungicide applications, but in practice a few preventative fungicides should be applied to resistant varieties to prolong their resistance. Economic analysis has shown that the cost of preventative fungicide applications, such as copper-based sprays, is very low compared to the enormous IPM benefit achieved by growing a resistant variety.

2.4.2 Irrigation

Crops in vegetable production areas around Melbourne are overhead irrigated because of low rainfall. Overhead irrigation applied proactively at a specific time (morning) created conditions less conducive to white blister development, compared with evening irrigation. Apparently evening irrigation created longer periods of leaf wetness more conducive to infection than the shorter periods of leaf wetness associated with morning irrigation.

Increased profits from improved timing of irrigations would make this IPM tool attractive to growers. A lower incidence of disease may enhance fungicide efficacy or create options for use of alternatives to fungicides which are safer for the environment. However, altering the timing of overhead irrigations may be a challenge logistically for some vegetable growers. Irrigation during daylight hours can compromise harvesting activities and it is often impossible to irrigate all of a farm's production areas at the same recommended time. Anecdotally, vegetable growers' who changed their irrigation times have reported less white blister on their broccoli.

2.4.3 Models

Disease predictive models are used to schedule fungicide sprays on crops when the microclimate in the crop is favourable for either sporulation, infection or both and to withhold sprays when the microclimate is unfavourable for pathogen development. The Brassica$_{spot}$II™ model allows a 5% tolerance of white blister in the crop and this may be unacceptable to some growers.

The Brassica$_{spot}$II™ model was designed for use with preventative or contact fungicides. The efficacy of this model with preventative fungicides is unknown under Australian conditions. Due to the frequency of 'high risk' infection conditions (red bars) generated under Australian conditions, the project team decided to use systemic fungicides with this version of the model to maximise the efficacy of disease control. Systemic fungicides were applied to the 'model' plots whist preventative fungicides were applied to the weekly spray plots. Persistent use of a systemic fungicide may increase the potential for *A. candida* to develop tolerance to these fungicides. Anecdotal evidence from growers indicated that Ridomil Gold MZ® had little efficacy in the Werribee South cropping area and glasshouse trials showed that this fungicide had less efficacy than expected for white blister control on broccoli seedlings (Minchinton *et al.* 2007). The efficacy of the Brassica$_{spot}$II™ model should be evaluated further to determine if use of the model with protectant fungicides is similar to its use with systemic fungicides.

The Brassica$_{spot}$II™ model is still under development and it was less 'user-friendly' than the Brassica$_{spot}$I™ model because: (i) it required more data entry; and (ii) the graphics were confusing. When the appearance of new white blisters symptoms are entered into the Brassica$_{spot}$II™ model, it produces two lines (Fig 2.2). For the purposes of this trial, the 5%

infection line was followed. The point at which the 5% infection line crosses the Disease Index Line is the time when the model predicts sporulation will occur from initial lesions associated with the 5% infection line. As new lesions appear, the new data are entered into the model and this generates new infection lines. The appearance of multiple cohorts of white blister symptoms generates multiple infection events, which can confuse interpretation of the data presented. Added confusion can arise because date data on the top graph (white blister crop walking) do not match date data on the bottom graph (time to lesion appearance).

2.5 Conclusion

Three IPM tools were evaluated, variety, irrigation timing and spray regimes using disease predictive models. Of these, growing a resistant variety was most effective because it produced the highest level of control of white blister without fungicide applications, but resistant varieties still need a few fungicide applications to delay loss of resistance. Using the Brassica$_{spot}$™ disease predictive models and growing resistant varieties were the most economical control measures. Use of the Brassica$_{spot}$™ models demonstrated that increased hours of leaf wetness enhanced the risk of white blister development in crops (Kennedy and Gilles 2003). Therefore, changing the time of overhead irrigation from evening to the morning would be expected to reduce periods of leaf wetness and the potential for white blister development.

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Chapter 3

Effect of nutrients on downy mildew and anthracnose in lettuce

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Summary
Only 4 of 21 lettuce cultivars, including Iceberg, Cos, Red and Green Oakleaf, Red and Green Coral and Mini Green Cos, were susceptible to a South Australian isolate of lettuce downy mildew, suggesting there is substantial resistance to downy mildew in lettuce cultivars. The cultivars Fortune and Sure Shot were the least susceptible, whilst Winter Select and Costanza were the most susceptible. Cotyledons were more susceptible to downy mildew than true leaves. Only 3 of the 20 cultivars evaluated showed tolerance to anthracnose, suggesting there is little resistance to anthracnose in these lettuce cultivars. Potassium nitrate solution at 40% and above inhibited germination of seeds, whilst a 1% solution was optimal for plant growth, although varietal differences were evident. Low, medium and high rates of nitrogen were identified. High rates of potassium nitrate made susceptible lettuce cultivars more susceptible to downy mildew, whilst all rates of ammonium nitrate produced a high incidence of downy mildew on all cultivars. Calcium nitrate, at any rate, had no affect on the incidence of downy mildew on lettuce seedlings. All rates of ammonium nitrate were associated with a high incidence of anthracnose on lettuce seedlings, whilst high rates of calcium nitrate reduced it. Ammonium nitrate should be avoided as a fertiliser for lettuce seedlings because it increased seedling susceptibility to downy mildew and anthracnose. Alternatively, high rates of calcium nitrate decreased seedling susceptibility to these diseases.

3.1 Introduction

There are reports of nutrients, particularly nitrogen, influencing plant susceptibility to disease. Growers were concerned that high levels of nitrogen can increase the susceptibility of lettuce seedlings to downy mildew or anthracnose. Nitrogen can influence disease by changing the anatomical and biochemical components of the plant. Nitrogen increases vegetative growth and promotes a higher proportion of young tissue which is more susceptible to disease. High nitrogen can also reduce the phenolic and lignin content of plants which in turn reduces their natural defence systems (Dordas, 2008). Nitrogen influences the severity of many diseases including those caused by foliar pathogens such as *Colletotrichum* on tomato and *Botrytis* on broad bean (Huber and Watson 1974). The source of nitrogen appears to be more important than the rate (Dordas, 2008; Huber and Watson 1974).

To assess lettuce susceptibility to downy mildew (*Bremia lactucae*) and anthracnose (*Microdochium panattonianum*), experiments were conducted to determine (i) varietal susceptibility, (ii) the role of nitrogen in plant growth, and (iii) the effects of the rate and form of nitrogen on susceptibility to infection.

3.2 Materials and methods

The experiments were conducted at the Waite Research Precinct, Urrbrae, South Australia (SARDI). Lettuce plants were grown in a controlled environment growth room at 14°C with a 12 hour (h) light and12h night cycle.

3.2.1 Maintenance of downy mildew and anthracnose

Isolates of downy mildew (*B. lactucae*) were obtained on fresh lettuce material cv. Cobham Green from DPI Vic, Knoxfield and cv. Sureshot from a commercial lettuce grower at Virginia, South Australia. An isolate of anthracnose (*M. panattonianum*) was collected from cv. Seneca derived from a commercial grower at Virginia, South Australia.

Leaves with symptoms of disease were placed in water and shaken to remove either *B. lactucae* and *M. panattonianum* spores. The solution (inoculum) was filtered through muslin cloth into a clean bottle to remove leaf debris and the spore concentration was checked with a haemocytometer prior to inoculation (>1 x 10^5 spores/ml).

Lettuce seedlings were grown in Coco peat at 14°C in a controlled environment growth room and inoculated with either *B. lactucae* at the two leaf cotyledon stage (7-14 days old) or with *M. panattonianum* at the 4-5 leaf stage. Leaves were sprayed with a spore suspension using a hand atomiser (Fig 3.1) and plants were enclosed in a plastic bag to induce near high humidity for a minimum of 24 h. Symptoms appeared between 8-20 days after inoculation. New lettuce seedlings were inoculated every 4-6 weeks as previously described to maintain isolates and sources of each disease.

Fig 3.1 Inoculation of lettuce seedlings with downy mildew (*B. lactucae*).

3.2.2 Varietal susceptibility to downy mildew and anthracnose

Experiments were conducted to determine the susceptibility of 21 lettuce varieties to downy mildew and 20 varieties to anthracnose (Table 3.1). *B. lactucae* and *M. panattonianum* were collected from field infected lettuce and maintained as described above.

Three plants of each variety were grown in each cell of a 6-cell seedling punnet (18 plants per variety).

Downy mildew: Plants at the two-leaf cotyledon stage were inoculated as described with *B. lactucae* (2.6 x 10^5 spores/ml) 12 days after sowing using a hand-held sprayer and assessed for disease incidence (number of plants diseased) and severity (leaf area diseased) 21 days after inoculation (Table 3.2).

Anthracnose: Plants at the 4-leaf stage were inoculated as described with *M. panattonianum* (1.6 x 10^6 spores/ml) 21 days after sowing and assessed 24 days after inoculation.

Table 3.1 Lettuce varieties assessed for downy mildew (*B. lactucae*) and anthracnose (*M. panattonianum).*

Lettuce	Variety	Source	Downy mildew race (DMR) resistance
Iceberg	Lily	South Pacific seeds	DMR 1-25
Iceberg	Kong	South Pacific seeds	DMR 1-25
Iceberg	Boost	South Pacific seeds	#
Iceberg	Seagull	South Pacific seeds	DMR 1-26
Iceberg	Roundhouse	South Pacific seeds	DMR 1-26
Iceberg	Boomerang	Seminis	DMR 1-26
Iceberg	Sure shot	Seminis	DMR 1-4
Iceberg	Constanza	Seminis	1-16, 19, 21 and 23
Iceberg	Winter Select	Seminis	none
Iceberg	Explore	Rijk Zwaan	#
Iceberg	Alpinas	Rijk Zwaan	#
Iceberg	Bernadinas	Rijk Zwaan	#
Cos	Quintus	Rijk Zwaan	#
Iceberg	Fortune	Terranova	none
Red Oak	Tekero	Syngenta Seeds	DMR 1-25
Green Oak	Sansula*	Syngenta Seeds	DMR 1-16, 18-24
Green Coral	Bellagio Curletta LE 290	Syngenta Seeds	DMR 1-24
Red Coral	Bellagio Robinio A	Syngenta Seeds	DMR 1-16, 18-24
Mini Green Cos	Tomos	Syngenta Seeds	DMR 1-16, 18-25
Iceberg	LE 291	Syngenta Seeds	#
Iceberg	LE 304	Syngenta Seeds	#

*Not assessed for anthracnose; # unknown resistance.

Table 3.2 Disease severity rating for assessment of downy mildew (*B. lactucae*) and anthracnose (*M. panattonianum).*

Rating	% leaf area diseased
0	0
1	1-10
2	11-25
3	26-50
4	51-75
5	76-90
6	91-100

3.2.3 Effect of nitrogen on plant growth

The aim of this study was to determine the effect of nitrogen (N) on growth of lettuce and assess what constitutes a low, medium and high rate of N.

The experimental design consisted of 4 Iceberg lettuce varieties x 1 N-source x 7 N-rates. The four varieties were cv. Fortune and cv. Constanza (susceptible to downy mildew) and cv. Boomerang and cv. Lily (resistant to downy mildew). Three seeds were planted in 100 ml coco peat in each pot (MK6, 7 cm x 7 cm). The coco peat was made to specification to remove calcium nitrate and Osmote® and ensure N was not present in the initial soil mix.

Five pots of each variety (15 replicate plants) were placed in a tray and each tray was allocated a different N treatment (Fig 3.2). Potassium nitrate (KNO_3), a common nursery fertilizer, was used at rates of 0, 1, 5, 12.5, 25, 40 and 70%. N was applied in solution at each watering time and the trays allowed the plant roots to be in contact with the solution at all times. Plants were watered 3 times per week. Seed germination and the fresh weight of plants were assessed after 4-6 weeks.

Fig 3.2 Experimental set-up of one tray containing four varieties watered with a solution of 5% potassium nitrate (KNO_3).

3.2.4 The effect of nitrogen on downy mildew and anthracnose

The aim of this study was to determine if the rate and form of N increases lettuce susceptibility to infection by downy mildew (*B. lactucae*) or anthracnose (*M. panattonianum)*.

The experimental design consisted of 4 Iceberg lettuce varieties x 3 N-sources x 4 N-rates (with water as a control treatment). For assessment of downy mildew, the four varieties were cv. Fortune and cv. Constanza (susceptible to downy mildew) and cv. Boomerang and cv. Lily (resistant to downy mildew).

For the assessment of anthracnose, the experiment was replicated using the varieties Kong, Explore, Seagull and Alpinas.

Five pots of each variety (15 replicate plants) were placed in a tray and each tray received a different N treatment. The sources of N were ammonium nitrate $(NH_4)_2$, calcium nitrate $Ca(NO_3)_2$, and potassium nitrate KNO_3. Rates were determined as high, moderate and low (Table 3.3). Untreated control plants (nil N) received water only.

Plants were grown in controlled environment growth rooms and N solutions were applied as previously described. Lettuce seedlings at the two-leaf cotyledon stage were inoculated with fresh downy mildew inoculum (3.4×10^5 spores/ml) or anthracnose (8×10^4 spores/ml) as described above 12 days after planting. The plants were assessed for (i) disease incidence (number of plants diseased), (ii) disease severity (leaf area diseased) of both cotyledons and true leaves separately and (iii) fresh weight.

Table 3.3 Rates and sources of N used to water lettuce plants.

Rate	N (based on % KNO_3)	% N	Total N KNO_3 (13.9 %)	Total N $Ca(NO_3)_2$ (11.9 %)	Total N $(NH_4)_2$ (21.2 %)
High	5	0.68	50 mM	57 mM	32 mM
Medium	1	0.137	10 mM	11.5 mM	6.4 mM
Low	0.2	0.02	2 mM	1.6 mM	0.9 mM

3.3 Results and discussion

3.3.1 Varietal susceptibility to downy mildew and anthracnose

Of the 21 lettuce varieties inoculated with downy mildew, only four developed disease symptoms, i.e. cvs. Fortune, Sureshot, Winter Select and Constanza. The other varieties were

resistant to the downy mildew race used in this experiment. Disease symptoms were observed 11-13 days after inoculation. Disease incidence on cv. Constanza was high compared to other varieties (Fig 3.3 and 3.5). All other varieties inoculated were deemed resistant to downy mildew. The disease was most severe on cotyledons and less severe on true leaves as each plant grew (Fig 3.6).

Anthracnose symptoms appeared 8 days after inoculation and caused damage to leaf development (Fig 3.4). All varieties tested were infected (Fig 3.7) yet, the severity of infection varied greatly between the varieties (Fig 3.8). In relation to disease severity, cv. Explore was most susceptible.

Fig 3.3 (a) Downy mildew on lettuce varieties cv. Constanza (left) and cv. Boomerang (right) inoculated with *B. lactucae*. (b) Downy mildew on cotyledons.

Fig 3.4 Anthracnose on lettuce cv. Kong 21 days after inoculation**.**

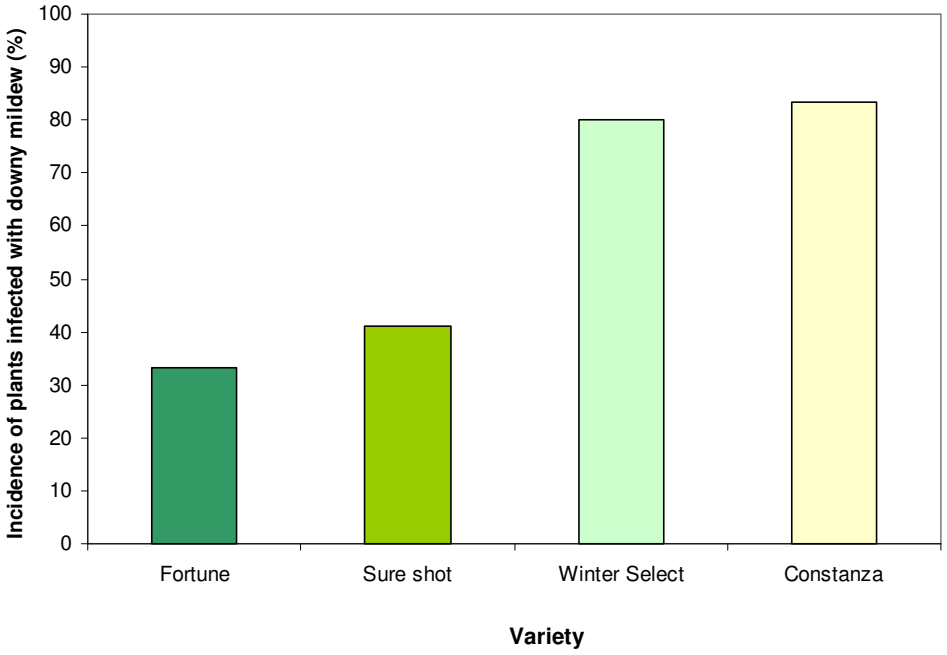


Fig 3.5 Incidence of downy mildew on lettuce varieties inoculated with *B. lactucae*.

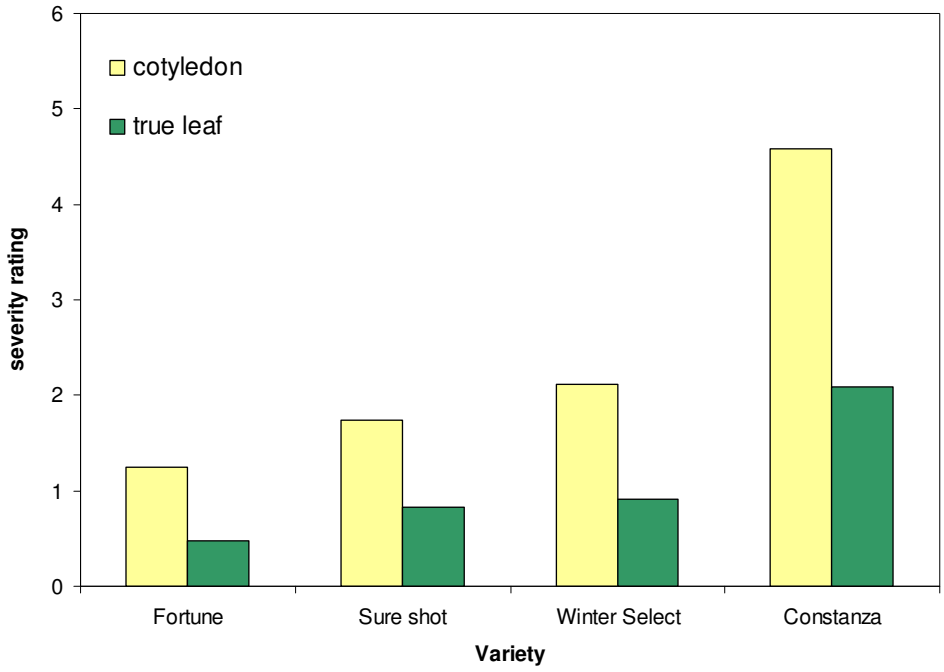


Fig 3.6 Severity of downy mildew (*B. lactucae*) on inoculated lettuce varieties whereby 0 = 0%; 1 = 1-10%; 2 = 11-25%; 3 = 26-50%; 4 = 51-75%; 5 = 76-90%; 6 = 90-100%.

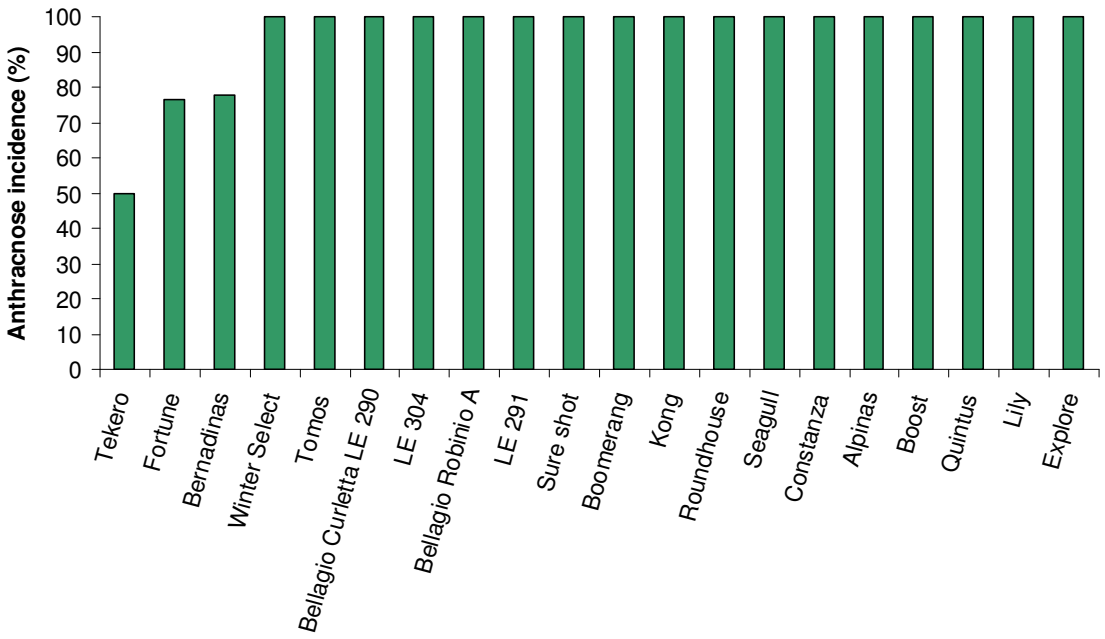


Fig 3.7 Susceptibility of lettuce varieties to infection by anthracnose (*M. panattonianum)*.

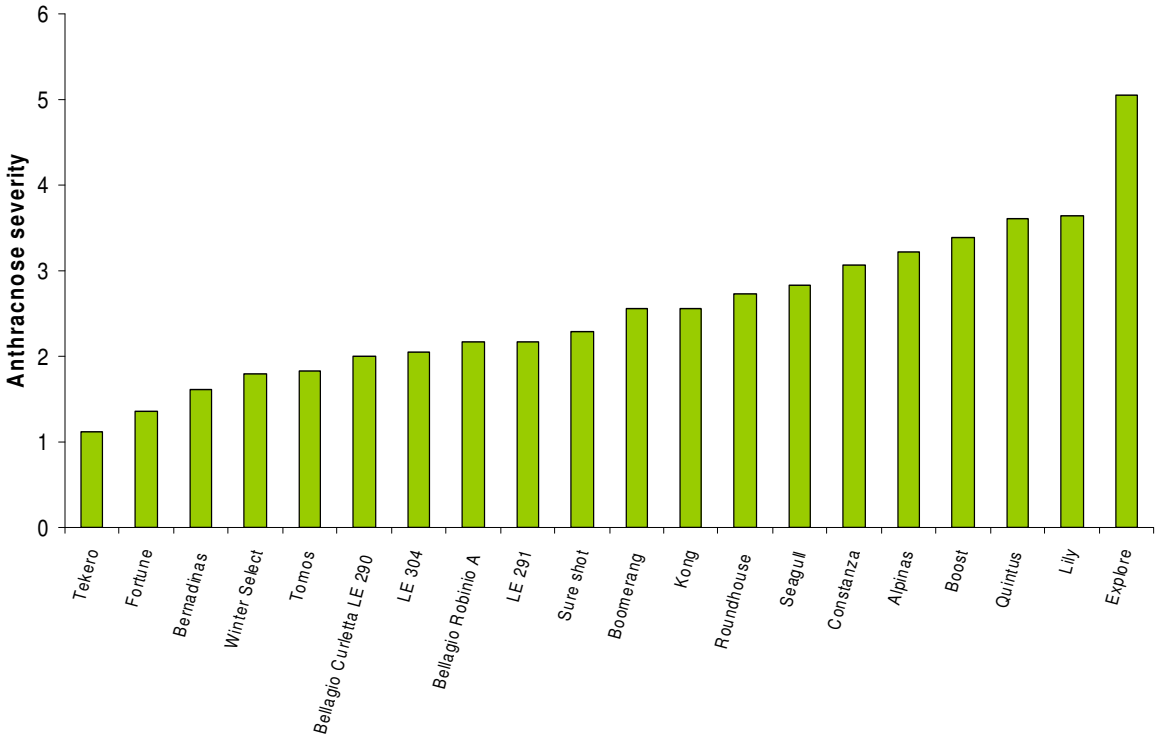


Fig 3.8 Severity of anthracnose on lettuce varieties based on area on leaf diseased whereby 0=0%; 1 = 1-10%; 2 = 11-25%; 3 = 26-50%; 4 = 51-75%; 5 = 76-90%; 6 = 90-100%.

3.3.2 Effect of nitrogen on plant growth

Nitrogen had a profound effect on germination of lettuce seeds. At 25% potassium nitrate, there was an obvious reduction in plant growth such that germination of cvs. Constanza and Fortune was inhibited. No germination occurred at 40 and 70% KNO_3 (Fig 3.9). Although there were varietal differences in growth of lettuce with KNO_3, the results showed that 1% KNO_3 was optimal for lettuce growth (Figure 3.10). Based on watering with KNO_3, 0.2, 1 and 5 % were low, medium and high rates, respectively when applied to lettuce grown in controlled environment conditions. This was equivalent to approximately 2, 5 and 50 mM nitrogen.

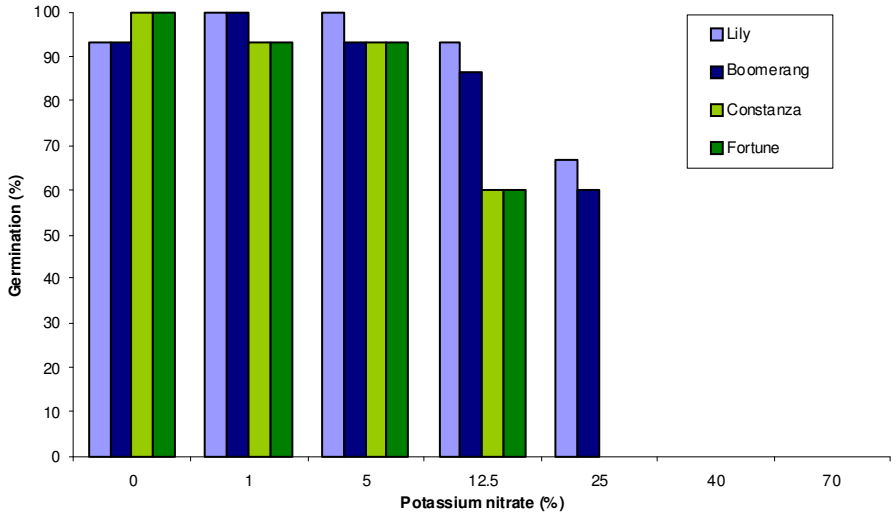


Fig 3.9 Germination of four lettuce varieties exposed to different rates of potassium nitrate applied during watering.

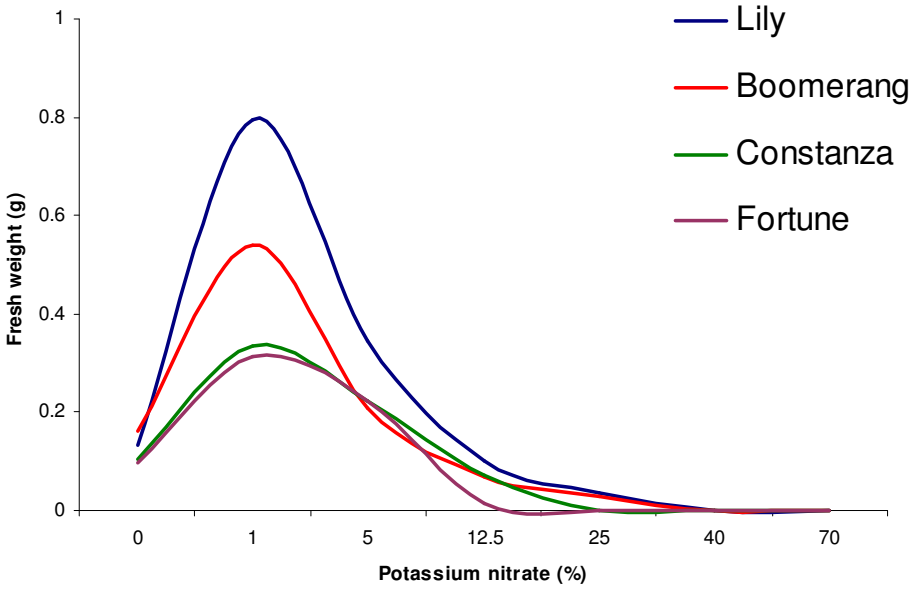


Fig 3.10 Fresh weight of four lettuce varieties after 26 days following watering with different rates of potassium nitrate.

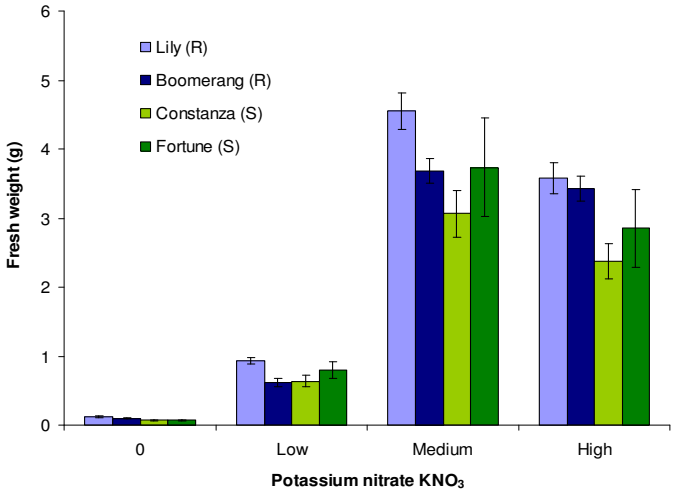
3.3.3 The effect of nitrogen on downy mildew and anthracnose infection

Potassium nitrate had the greatest effect on growth and disease incidence. Assessments of fresh weight indicated that there was a combined detrimental effect of high nitrogen and downy mildew infection on growth of lettuce. Growth of the plants inoculated with downy mildew was similar to those observed in experiment 3.3.2, i.e. at the high rate of KNO_3, growth was somewhat reduced (Fig 3.11a). Similarly, plants grown under different rates of $Ca(NO_3)_2$ had lower fresh weight at the high rate than at the other rates applied (Fig 3.11b). This was particularly obvious for cv. Constanza. Plants watered with $(NH_4)_2$ showed a high level of disease and for this reason, the resistant varieties Lily and Boomerang grew better than the susceptible varieties Constanza and Fortune (Fig 3.11c). Using higher rates of N did not improve plant growth.

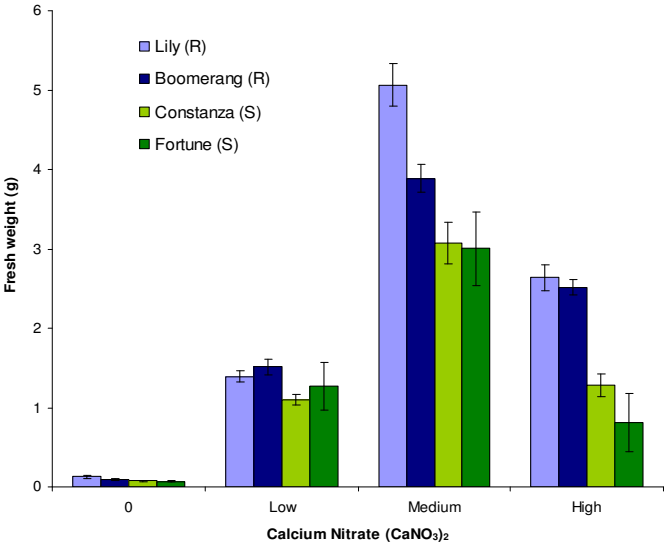
Although the varieties Lily and Boomerang had no symptoms of downy mildew, cv. Constanza was significantly more susceptible to the disease at the highest KNO_3 rate (Fig 3.12a). Disease development was reduced when plants were watered with a high rate of $Ca(NO_3)_2$ (Fig 3.12b). Infection was extremely high in plants watered with ammonium nitrate $(NH_4)_2$ although there was a significant reduction in disease incidence of cv. Constanza at the high rate (Fig 3.12b) when compared to the high rate of KNO_3.

Similar to the variety susceptibility trials, the incidence of anthracnose was high on plants watered with different sources and rates of nitrogen. The response of variety to rate of nitrogen was variable. Under medium rates of potassium nitrate, all varieties except Kong showed higher incidence of disease than at other rates (Fig 3.13a). Medium rates of ammonium nitrate also caused high disease levels (Fig 3.13b). In comparison, a high rate of calcium nitrate reduced incidence of the disease (Fig 3.13c).

a



b



c

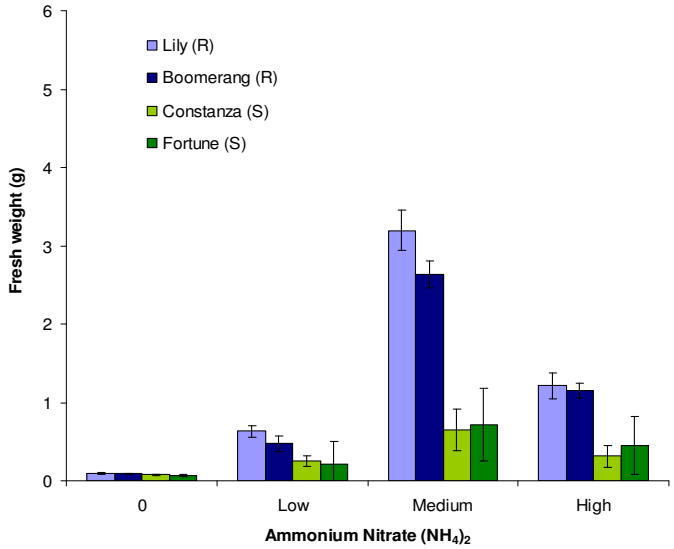
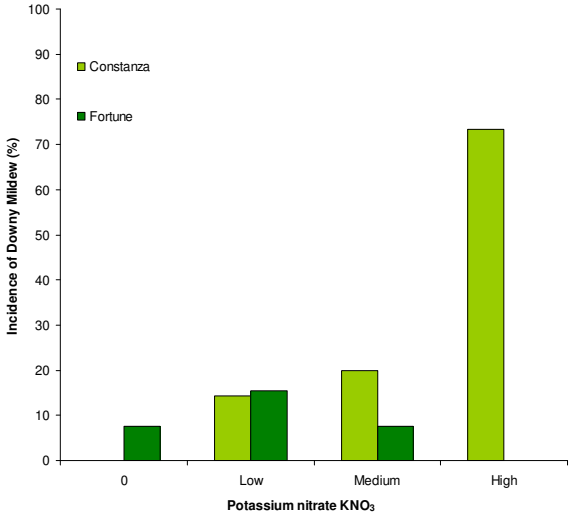
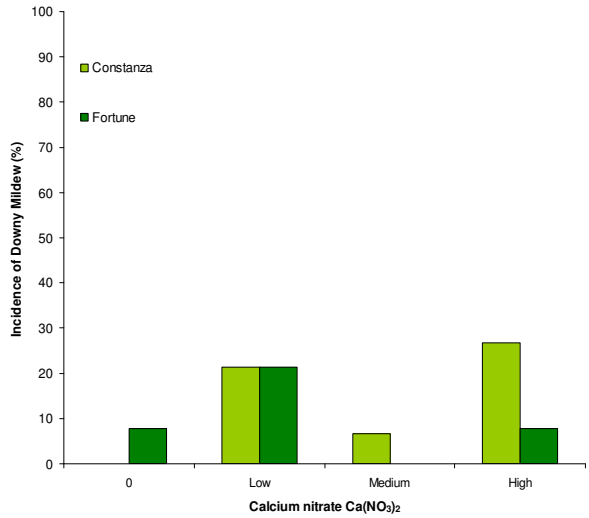


Fig 3.11 Fresh weight of plants of four lettuce varieties inoculated with downy mildew and watered with either (a) potassium nitrate, (b) calcium nitrate or (c) ammonium nitrate. R= resistant and S= susceptible to downy mildew.

a



b



c

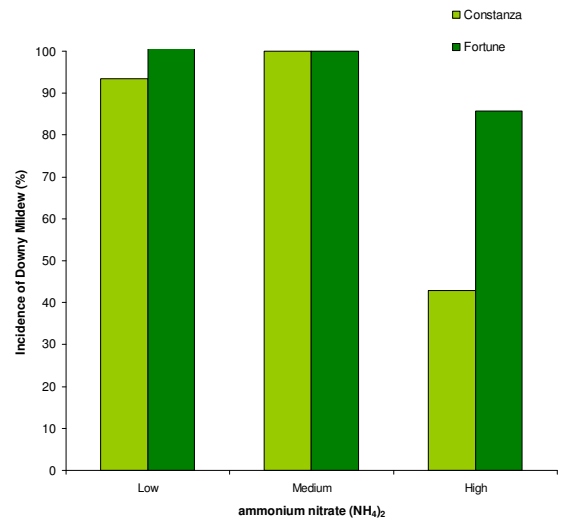


Fig 3.12 Incidence of downy mildew on susceptible varieties of lettuce cv. Fortune and cv. Constanza watered with low, medium and high rates of either (a) potassium nitrate, (b) calcium nitrate or (c) ammonium nitrate.

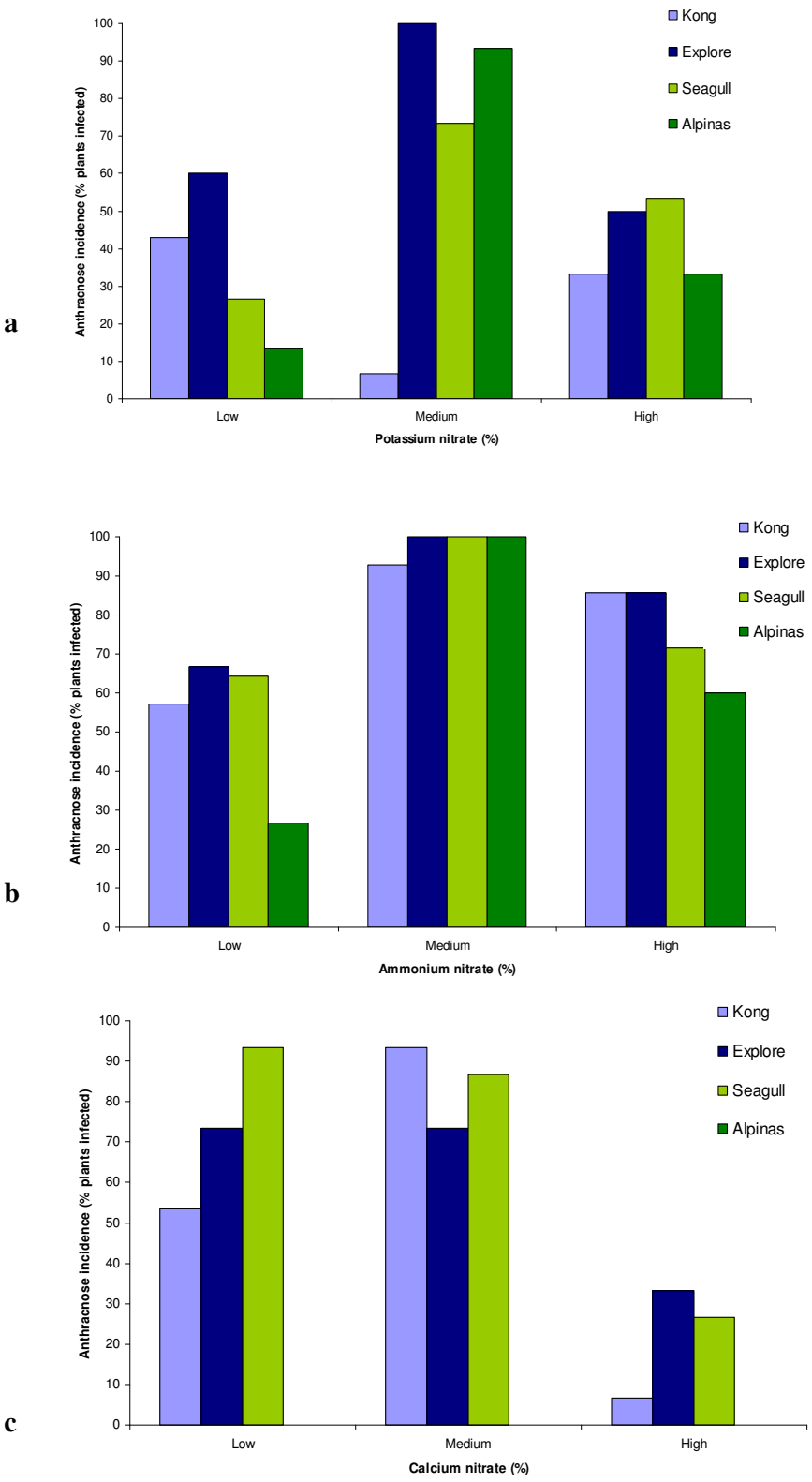


Fig 3.13 Incidence of anthracnose on inoculated lettuce plants watered with low, medium and high rates of either (a) potassium nitrate, (b) ammonium nitrate or (c) calcium nitrate.

3.4 Conclusion

The response to N was variable. Dordas (2008) reported that when there is a high N supply the severity of infection by obligate pathogens (e.g. downy mildew) is increased. Our findings showed downy mildew was more prevalent on plants watered with high rates of KNO_3 and there was little response to other sources of N. In comparison, high KNO_3 did not promote infection by anthracnose (a facultative pathogen). Application of high rates of $CaNO_3$ reduced the incidence of anthracnose. Calcium may assist in the plant's defence to disease by strengthening the cell wall. Applying more N can increase susceptibility of certain lettuce varieties to downy mildew but may reduce anthracnose development. In general, disease was observed when conditions were optimal for plant growth.

The findings support the conclusions of Dordas (2008), i.e. that the source of N appears to be more important than the rate of N applied in regard to susceptibility of the plant to disease. The ratio of NH_4-N to NO_3-N is critical for plant growth and the additional use of N can affect pH of the soil and availability of other nutrients.

There is a strong varietal response in susceptibility to disease and response to N. For this reason a balanced nutrient schedule needs to be tailored for particular lettuce varieties. For instance, high N may be required to promote vigorous growth in some varieties but can retard development in others.

3.5 Acknowledgements

Funding was provided by the Vegetable levy through HAL (project VG07070). The authors thank Angela Lush (SARDI) for technical assistance, the project team from DPI Vic and the University of Queensland for advice, lettuce growers for providing diseased plants and seed companies Rijk Zwaan, Seminis, South Pacific, Syngenta and Terranova for their generous supply of seed.

3.6 References

Dordas C (2008). Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agronomy for Sustainable Development* **28:1**, 33-46.

Huber DM and Watson RD (1974). Nitrogen form and plant disease. *Annual Review of Phytopathology* **12:** 138-165.

Chapter 4

Efficacy of two Brassica$_{spot}$™ disease predictive models for controlling white blister on broccoli, Chinese cabbage and cauliflower with systemic, protectant and fungicide alternatives

Summary

The efficacy and economics of using two versions of the Brassica$_{spot}$™ disease predictive model to time fungicide sprays for the control of white blister in crops of broccoli, cauliflower and Chinese cabbage were evaluated in 11 trials carried out in Tasmania, Victoria and Queensland. Spray programs based on use of the models were compared with alternative regimes: (i) weekly applications of a copper spray, (ii) grower-timed applications of a copper spray and (iii) applications of some alternative fungicides. An unsprayed regime was used as a control. Generally, the control of white blister on the foliage and heads of broccoli using the weekly and the grower-timed spray regimes was similar to or better than that of the regimes based on use of the two models. The number of sprays predicted by the models was the same or greater than the number in the grower-timed regime but less than the number in the weekly regime. Neither of the two models was consistently more effective than the other. Both lacked a sporulation component and it is possible that their effectiveness could be improved by use of an in-field spore detection kit currently under development. Of the fungicide alternatives, only *S. lyticus* and sodium lauryl sulphate (SLS) provided some disease control but both were not as effective as the weekly copper sprays. The trial results were used to compare the economics of the various regimes. Neither the weekly sprays nor the models were consistently superior. Trialling the models on Chinese cabbage identified the phenological age of the crop when the application of fungicide sprays will give the best control of white blister.

4.1 Introduction

White blister has been a problem on many *B. oleracea* crops since it appeared in the Werribee crop production area in 2001-02, although it has been present on *B. rapa* Chinese cabbage for many years (Minchinton *et al.* 2005). Methods of controlling the disease are calendar sprays of systemic and preventative fungicides; fungicide applications based on predictions of the Brassica$_{spot}$™ disease risk predictive model (Kennedy and Giles 2003); or applications of fungicide alternatives (Irish *et al.* 2002; Kaur and Kolte 2001). There is much interest in alternatives to fungicides which could reduce chemical use on crops (Akem *et al*. 2011). There is also concern about the emergence of fungicide resistance and the failure of some systemic fungicides to adequately control the disease (Tesoriero pers. comm. 2011).

The Brassica$_{spot}$™ disease predictive model is extensively used by consultants and growers in the UK (Kennedy pers. comm.). Disease predictive models use weather data collected in the crop to determine when pathogens will be active and indicate if a spray should be applied or conversely, to determine when pathogens will not be active and a spray should be withheld. Disease predictive models usually provide the greatest benefit when disease pressure is highest (Kable 1991; Maloy 1993; Polley 1983). Preliminary evaluation of the Brassica$_{spot}$™ disease predictive model under Australian conditions was undertaken by Minchinton *et al.* 2007.

Brassica$_{spot}$I™ was evaluated in Australia by Minchinton *et al*. (2007) and Petkowski (2008). This model was based on conditions conducive to infection and required the use of systemic

fungicide spray applications when white blister symptoms appeared in the field. Field trials demonstrated that the model was quite accurate in predicting the appearance of white blister 7, 14 or 21 days after a predicted high risk infection period. Subsequent timing of fungicides applications based on predictions of high risk infection periods of the model, reduced spray programs by 8-10 sprays and controlled white blister on heads as well as weekly spray programs. Economic benefits from using the model were inconsistent, because the major influence on profit was yield, not the cost of the model, weather station or systemic fungicides.

This chapter reports the results of field trials on broccoli, cauliflower and Chinese cabbage to determine the efficacy and economics of controlling white blister with spray programs based on two versions of the Brassica$_{spot}$™ disease risk predictive model (Brassica$_{spot}$I™ and Brassica$_{spot}$II™, Chapter 1); fungicide sprays applied weekly; "farm practice" and a selection of "fungicide alternatives" identified by Akem *et al.* (2011) and Minchinton *et al.* (2007).

4.2 Material and methods

Eleven field trials were conducted from 2008 to 2010 in Victoria (6), Tasmania (4) and Queensland (1) (Table 4.1). Only trials on broccoli covered all seasons. All crops were grown under overhead irrigation and those in Victoria were planted on raised beds. In northern Tasmania, the trials were conducted by Dr Hoong Pung of Peracto, while in Queensland the field trial was conducted by John Duff of DEEDI, Gatton. As there was a risk that the fungicide alternatives may not have the same efficacy as the fungicides, they were evaluated in a number of trials against weekly sprays or farm practice and the Brassica$_{spot}$I™ and Brassica$_{spot}$II™ disease risk predictive models, to avoid large crop losses on growers' properties. The economic evaluation of these trials is reported in Chapter 9.

Table 4.1 Trials locations, periods and crop varieties

Trial No	State	Location/Address	Season/Date	Crop	Variety
1	Vic	Diggers Road Werribee South	Winter/Spring Jul 08 to Oct 08	Broccoli	Bridge
2	Vic	Browns' Road Boneo	Winter/Spring Jun 08 to Sep 08	Broccoli	Grevillea
3	Vic	Crawford Rd Werribee South	Summer/Autumn Feb 09 to Apr 09	Broccoli	Viper
4	Vic	Diggers Road Werribee South	Autumn/Winter Apr 10 to Jun 10	Broccoli	Rhumba
5	Vic	North Road Devon Meadows	Summer Nov08 to Jan09	Chinese Cabbage	Matilda
6	Tas	Harford	Summer/Autumn Jan 09 to Apr 09	Broccoli	Shamrock
7	Tas	Wesley Vale	Winter Jul 09 to Oct 09	Broccoli	Ironman Tyson
8	Tas	Forthside	Summer Nov 09 to Jan 10	Cauliflower	Discovery
9	Tas	Lillico	Autumn/Winter Mar 10 to Jul 10	Broccoli	Prophet
10	QLD	Gatton Research Station Gatton	Spring Aug 10 to Oct 10	Chinese Cabbage	Matilda
11	Vic	O'Connor's Road Werribee South	Summer Jan 11 to Mar 11	Broccoli	Viper

4.2.1 The Brassica$_{spot}$™ model

The Brassica$_{spot}$™ model was purchased from Warwick HRI, Wellesbourne, Warwickshire UK in the early stages of the project for £700 and in the latter stages of the project for £2,000. Refer to section 1.4.4 for a description of the model. The model used relative humidity, rainfall and leaf wetness over a temperature range of 6-24ºC to determine high risk periods for infection by *A. candida* zoosporangia.

Plots in field trials grown with the Brassica$_{spot}$I™ spray regime were inspected (walked) at 7, 14 or 21 days after a high risk period and if white blister symptoms were observed, fungicides were applied. Plots in field trials grown with the Brassica$_{spot}$II™ spray regime were sprayed with a fungicide as soon as the weather permitted after the model indicated a spray was required, i.e. when the track line crossed the red index bar, which indicated when 5% of the crop will exhibit maximum spore release.

4.2.3 Weather station

A Model T weather station (Western Electronics Design, Loxton, SA) was placed in the middle of an irrigation line in Victorian crops, or set up within the trial site in Queensland and Tasmanian crops. The station recorded average temperature and relative humidity, the presence or absence of leaf wetness, daylight and total rainfall at 30 min. intervals. The leaf wetness sensor was placed in the crop at a 45 degree angle and its height was adjusted as the crop grew.

4.2.4 Chemicals and their application

The chemical treatments used in the field trials are listed in Table 4.2. In the Victorian trials, all chemicals were applied using a triple mounted boom with hollow cone nozzles SPX8 at 30 psi pressurised by a Silvan Selectra 12v knapsack (Silvan Pumps and Sprayers (Aus) Pty. Ltd.), except in Trial No. 11 where a single nozzle was used because of the wide beds. In the Queensland trials a motorised backpack with a hand held boom and four equally spaced twin jet nozzles was used to apply the fungicides. In the Victorian broccoli trials the fungicides were applied at a low rate of 500L/ha during weeks 1-8 and at a high rate of 1000L/ha from week 9 (Table 4.2), except for trial 11 which received one spray at 500L/ha and the remainder at 1000L/ha. In the Chinese cabbage trials, application rates in the Victorian trials were 500L/ha up to week 4 and 1000L/ha at week 5. In the Queensland trial sprays were applied at 588L/ha over the whole period.

Table 4.2 Chemical treatments used in the field trials.

Trade name	Active	Company	Rate	State	Trial No.
Agral™	nonyl phenol ethylene oxide condensate (60g/L)	Syngenta	0.13%	V	4,
Amistar WG®	azoxystrobin (500g/kg)	CropCare, Syngenta	400 ml/ha	T, Q	6, 7, 8, 10
Amistar WG®	azoxystrobin (500g/kg)	CropCare, Syngenta	500ml/ha	T	9
Amistar WG®	azoxystrobin (500g/kg)	CropCare, Syngenta	280ml/100L	V, T	1, 2, 3, 4, 5
Bion 50 WG	acibenzolar-*S* -methyl (500g/kg)	Syngenta	25g/ha	V	5
Bion 50 WG	acibenzolar-*S* -methyl (500g/kg)	Syngenta	1µM	Q	10
Bion 50 WG	acibenzolar-*S* -methyl (500g/kg)	Syngenta	10µM	Q	10
Cabrio®	pyraclostrobin (25g/L)	Syngenta	500ml/ha	Q	10
Designer™	organosilicone surfactant fluid+synthetic latex	Nufarm	200ml/ha	T	6
Du-Wett Rainfast™	trisiloxane ethoxylate (500g/L, non-ionic)	Nufarm			6
Du-Wett™	trisiloxane ethoxylate (500g/L, non-ionic)	Nufarm	300ml/ha	V	1, 2, 3, 5
Du-Wett™	trisiloxane ethoxylate (500g/L, non-ionic)	Nufarm	200ml/ha	T	6
Fulzyme™	*Bacillus subtilis*	Zadco	12ml/L	V	4
Fulzyme™	*Bacillus subtilis*	Zadco	24 ml/L	Q	10
Li-700™	soyal phospholipids (350g/L)+propionic acid (350g/L), nonylphenol ethoxulates (10-30%)	Nufarm	10ml/100L	T	6
Microplus™	*Streptomyces lyticus*	Organic Farming Systems	500g/ha	V	2,
Ridomil Gold Plus®	metalaxyl M (50g/kg)+copper hydroxide (600g/kg)	Syngenta	2kg/ha	T	6, 7, 8
Ridomil Gold Plus®	metalaxyl M (50g/kg)+copper hydroxide (600g/kg)	Syngenta	2.5kg/ha	T	9
Sodium laurel sulfate	Sodium laurel sulfate	Sigma	2 g/L	V	2
Tri-Base Blue®	copper sulphate tribase (190g/L)	Nufarm	280ml/100L	V, T	1, 2, 3, 4, 5, 6

Q, Queensland; T, Tasmania; V, Victoria.

4.2.5 Assessment

Broccoli and cauliflower

Incidence of white blister on broccoli in the Victorian trials was measured as a percentage of plants or heads with disease. Severity of white blister on foliage was measured on a scale of 0 to 5, where 0 was no blisters; 1 was one or two leaves with one or two blisters; 2 was more than one leaf with many blisters; 3 was leaves with blisters penetrating the upper leaf surface; 4 was swollen leaves or petioles; and 5 was hypertrophy (profuse sporulation on youngest leaves, swollen petioles). In some Victorian trials, white blister severity on heads was measured on a scale of 0 to 2, where 0 represented no blisters; 1 was fewer than 10 blisters per head (marketable) and 2 was more than or equal to 10 blisters per head (not marketable). In the Tasmanian trials, the incidence of white blister on foliage was assessed on the "5 top leaves", whilst severity on leaves was assessed on a scale of 0 to 5 where 0 was no leaves infected; and 5 was all five leaves infected.

Chinese cabbage

In the Victorian and Queensland trials incidence of white blister on Chinese cabbage was measured as a percentage of plants with disease. Severity of white blister was recorded as the number of wrap leaves showing symptoms of white blister on a scale of 0-4 where 0 was no wrap leaves with white blister; 1 was one wrap leaf with white blister and 4 was four wrap leaves with white blister. In the Victorian trial 20 plants per plot were assessed, while in the Queensland trial 40 plants per plot were assessed.

4.2.6 Trial No. 1

Trial No. 1 on broccoli variety Bridge (South Pacific Seeds) was located at 300 Diggers' Rd, Werribee South, Victoria. Plants were propagated by the grower and planted in two rows per bed and spaced 0.25m apart on 11 June 2008. The trial had a randomized block design with four blocks containing four plots each (Table 4.3). The plots were 8.5m long and 1.62m wide. The treatments were: (i) 'Control' (unsprayed); (ii) 'Weekly' sprays of Tri-Base Blue® and Du-Wett™; (iii) 'Brassica$_{spot}$I™', sprayed with Amistar® when predicted by the model; and (iv) 'Brassica$_{spot}$II™ 5%' incidence, sprayed with Amistar® when predicted by the model. The trial was assessed on 13 October 2008, 123 days after the day of planting (dap). In each

plot, the foliage of at least 20 plants and at least 40 broccoli heads were assessed for white blister incidence and severity as described above. Data were analysed by ANOVA (Analysis of Variance).

Table 4.3 Trial No. 1 treatment schedule on broccoli variety Bridge at Werribee South, winter to spring 2008

Treatment	Date/week												
	3/07/2008 wk 3 21 dap	10/07/2008 wk 4 28 dap	16/07/2008 wk 5 34 dap	24/07/2008 wk 6 42 dap	30/07/2008 wk 7 48 dap	8/08/2008 wk 8 59 dap	11/08/2008 wk 9 62 dap	22/08/2008 wk 10 73 dap	1/09/2008 wk 11 81 dap Button	5/09/2008 wk 12 85 dap	12/09/2008 wk 13 92 dap	19/09/2008 wk 14 99 dap	26/09/2008 wk 15 106 dap
Control	-	-	-	-	-	-	-	-	-	-	-	-	-
Weekly	+	-	+	+	+	+	+	+	+	+	+	+	+
BrassicaspotI™	-	-	-	-	-	-	+	-	-	-	-	-	-
Brassica$_{spot}$II™ 5%	-	-	-	-	-	-	-	-	-	-	+	-	-

Dap, days after planting; Control, unsprayed; Weekly, sprayed weekly with Tri-Base Blue and Du-Wett; Brassica$_{spot}$I™, sprayed with Amistar® when predicted by the model; Brassica$_{spot}$II™ (5% appearance), sprayed with Amistar ® when predicted by the model.

4.2.7 Trial No. 2

Trial No. 2 on broccoli variety Grevillea (Clause Vegetable Seeds) was located at 1155 Browns' Road, Boneo. Plants were obtained from a commercial seedling producer and planted in two rows per bed and spaced 0.33m apart on 2 June 2008. The trial had a randomized block design with eight blocks containing seven plots each (Table 4.4). The plots were 8m long and 1.62m wide. The treatments were: (i) 'Control' (unsprayed); (ii) 'Weekly' sprays of Tri-Base Blue® and Du-Wett™; (iii) fungicide applications of Amistar® based on predictions of the 'Brassica$_{spot}$I™' model; (iv) fungicide applications of Amistar® based on predictions of the 'Brassica$_{spot}$II™ 5%' incidence model of white blister in the crop; (v) fungicide applications of Amistar based on predictions of the 'Brassica$_{spot}$II™ 50%' incidence model of white blister in the crop; (vi) '*Streptomyces lyticus*' sprayed weekly from week 6 and (vii) Sodium laurel sulphate™ ('SLS') sprayed weekly from week 6. The final assessment was made on 22 September 2008, 112 dap. The foliage of 20 plants and heads of approximately 40 plants were assessed for incidence and severity of white blister as described above. Data were analysed by ANOVA, or using a logistic regression model.

Table 4.4 Trial No. 2 treatment schedule on broccoli variety Grevillea at Boneo from winter to spring 2008

Treatment	Date/week														
	09/06/08 wk 1 9 dap	16/06/08 wk 2 14 dap	23/06/08 wk 3 21 dap	30/06/08 wk 4 28 dap	07/07/08 wk 5 35 dap	14/07/08 wk 6 42 dap	21/07/08 wk 7 49 dap	28/07/08 wk 8 56 dap	04/08/08 wk 9 65 dap	11/08/08 wk 10 72 dap	18/08/08 wk 11 79 dap	25/08/08 wk 12 86 dap	01/09/08 wk 13 91 dap Button	08/09/08 wk 14 98 dap	15/09/08 wk 15 105 dap
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Weekly	11/06/08 T D	19/06/08 T D	26/06/08 T D	03/07/08 T D	-	16/07/08 T D	24/07/08 T D	30/07/08 T D	07/08/08 T D	15/08/08 T D	20/08/08 T D	28/08/08 T D	04/09/08 T D	11/09/08 T D	-
BrassicaspotII™ 5%	-	-	-	03/07/08 A	-	-	-	-	04/08/08 A	-	-	-	-	-	15/09/08 A
BrassicaspotII™ 50%	-	-	-	03/07/08 A	-	-	-	-	-	-	-	-	-	-	-
BrassicaspotI™	-	-	-	03/07/08 A	-	-	-	-	-	-	20/08/08 A	-	-	-	-
S. lydicus	-	-	-	-	-	16/07/08 S	24/07/08 S	30/07/08 S	07/08/08 S	15/08/08 S	20/08/08 S	28/08/08 S	04/09/08 S	11/09/08 S	
SLS	-	-	-	-	-	16/07/08 SLS	24/07/08 SLS	30/07/08 SLS	07/08.08 SLS	15/08/08 SLS	20/08/08 SLS	28/08/08 SLS	04/09/08 SLS	11/09/08 SLS	

Dap, days after planting; Control, unsprayed; T, Tri-Base Blue (copper fungicide); D, Du-Wett (adjuvant); A, Amistar (systemic fungicide); *S. lyticus, Streptomyces lyticus*; SLS, Sodium lauryl sulphate; -, no chemical application.

4.2.8 Trial No. 3

Field Trial. Trial No. 3 on broccoli variety Viper (Lefroy Valley) was located at Crawford Road, Werribee South. Plants were produced by the grower on site and planted in two rows

per bed and spaced 0.25m apart on 9 February 2009. The plots were 4m long and 1.62m wide. The trial had an incomplete block design with seven blocks containing five plots each. The treatments were: (i) 'Control' (unsprayed); (ii) 'Weekly' copper sprays; (iii) 'Best Bets', a spray of Amistar® at the first sign of white blister with a follow up spray of copper at button stage if necessary; (iv) Amistar® sprays based on predictions of the 'Brassica$_{spot}$I™' disease risk predictive model; (v) Amistar® sprays based on predictions of on the Brassica$_{spot}$I™ disease risk predictive model with an Amistar® spray at button stage ('Brassica$_{spot}$I™ + button') (Table 4.5). Button stage was defined as 50% of the plants showing buttons. The control was replicated only five times, the Brassica$_{spot}$I™ disease risk predictive model program with an Amistar® spray at button stage was replicated eight times and all other treatments were replicated seven times. The trial was monitored weekly with the final assessment and commercial harvest conducted at week 8 (19 April 2009, 72 dap). Broccoli heads of approximately 25 plants per plot were assessed for incidence of white blister. Data were analysed using Generalised Linear Mixed Models.

Table 4.5 Trial No. 3 Treatment schedule on broccoli variety Viper at Werribee South from summer to autumn 2009

	Date/week							
	23/02/2009	2/03/2009	9/03/2009	16/03/2009	23/03/2009	30/03/2009	6/04/2009	14/04/2009
Treatment	wk2	wk3	wk4	wk5	wk6	wk7	wk8	wk9
	14 dap	21 dap	28 dap	35 dap	42 dap	49 dap	56 dap	64 dap Button
Control	-	-	-	-	-	-	-	-
Weekly	T D	T D	T D	T D	T D	T D	-	T D
Best Bets	-	-	-	A	-	-	-	A
Brassica$_{spot}$I™	-	-	-	A	-	-	A	-
Brassica$_{spot}$I™ + button	-	-	-	A	-	-	-	A

Dap, days after planting; Control, unsprayed; Weekly, sprays applied weekly; T, Tri-Base Blue®; D, Du-Wett; A, Amistar®; Button, button stage of broccoli head development; -, no chemical application.

Post-harvest trial. After assessing the field trial at Werribee South on the 19 April 2009, a selection of broccoli heads at least 50mm in diameter without white blister symptoms were harvested from each treatment and plot, packed in Styrofoam boxes and placed in cold storage (5°C) at DPI Knoxfield for 12 days. Only a limited number of heads could be harvested because of the variable nature of the crop, but a representative sample from each treatment and plot was assessed. After cold storage, the heads were placed in plastic crates with two to three heads of broccoli from each treatment in each crate. Each of two crates was randomly assigned to three supermarket shelves for three days at 12 ºC from 1 May to 4 May 2009 and then assessed for incidence of white blister development. Dr Robert Holmes, a post harvest pathologist, was consulted for this trial.

4.2.9 Trial No. 4

Trial No. 4 on broccoli variety Rhumba (Clause Vegetable Seeds) was located at Diggers Road, Werribee South. Plants were produced by the grower on site and planted in 2 rows per bed and spaced 0.25m apart on 19 April 2010. The trial had a randomized block design with seven blocks containing five plots. Each block was a replicate (Table 4.6). Treatments were: (i) 'Control' (unsprayed); (ii) 'Grower' spray practice; (iii) fungicide applications based on predictions of the 'Brassica$_{spot}$I™' disease risk predictive model plus Amistar® at the button stage; (iv) '*Bacillus substilis*' and (v) 'Bion' 50WG™ applied at the first appearance of white blister symptoms and one week later. The plots were 6 m long and 1.62 m wide. The foliage and heads of 20 plants per plot were assessed for the incidence of white blister on 15 June 2010, 88 dap, and on 21 June 2010, 94 dap, respectively. Data were analysed by ANOVA.

Table 4.6 Trial No. 4 treatment schedule on broccoli variety Rhumba at Werribee South from

Traetment	Date/week 2/04/2010 wk 3 14 dap	9/04/2010 wk 4 21 dap	16/04/2010 wk 5 28 dap	23/04/2010 wk 6 35 dap	30/04/2010 wk 7 42 dap	7/05/2010 wk 8 50 dap	14/05/2010 wk 9 57 dap	21/05/2010 wk 10 64 dap Button	28/05/2010 wk 11 71 dap	4/06/2010 wk 12 77 dap	11/06/2010 wk 13 84 dap
Control	-	-	-	-	-	-	-	-	-	-	-
Weekly	1/4/10 T R	8/4/10 T R A	15/4/10 T R	22/4/10 T R	30/4/10 T R A	6/5/10 T R	13/5/10 T R	21/5/10 T R A	28/5/10 T R	4/6/10 T R	13/6/10 T R
Brassica$_{spot}$I™	-	-	-	-	30/4/10 T R A	-	-	21/5/10 T R A	-	-	-
B. subtilis	1/4/10	8/4/10	15/4/10	22/4/10	30/4/10	6/5/10	13/5/10	21/5/10	28/5/10	4/6/10	13/6/10
Bion™	-	8/4/10	15/4/10	-	-	-	-	-	-	-	-

Dap, days after planting; Control, unsprayed; Grower, sprays applied as per the grower's practice; T, Tri-Base Blue®; A, Amistar®; R, Agral; Button, button stage of broccoli head development; -, no chemical application.

4.2.10 Trial No. 5

Trial No. 5 on Chinese cabbage variety Matilda (SPS Seeds) was located at North Road Devon Meadows. The trial was direct seeded on 27 November 2008 in three rows per bed. The trial was laid out with six blocks containing six plots each (Table 4.7). There was unequal replication of four treatments in the blocks. The plots were 6m long and 1.62m wide and each contained about 60 plants. The treatments were: (i) 'Control' (unsprayed); (ii) 'Weekly' sprays of Amistar®; (iii) fungicide applications based on predictions of the 'Brassica$_{spot}$I™' model; and (iv) fungicide applications of Amistar® based on predictions of the 'Brassica$_{spot}$II™ 5%' model.

Plants were monitored for white blister weekly and assessed for symptoms on 24 December 2008 and 31 December 2008; and for symptoms on wrap leaves and heads on 19 January 2009, 53 dap. Incidence was recorded as the number of plants out of 20 per plot, showing symptoms of white blister. Incidence was analysed using GLM (Generalised Linear Models) due to the binomial nature of the data. Because there was 100% incidence of white blister on the Control plots, these data were omitted from the analysis. Severity was recorded as the number of wrap leaves showing symptoms of white blister on a scale of 0-4 where 0 was no wrap leaves with white blister; 1 was one wrap leaf with white blister; 2 was two wrap leaves with white blister; 3 was three wrap leaves with white blister and 4 was four wrap leaves with white blister. Average severity per plant was analysed using a mixed model and the REML method because there were unequal numbers of replications for the treatments.

Table 4.7 Trial No. 5 Treatment schedule on Chinese cabbage variety Matilda at Devon Meadows summer 2008-2009.

Treatment	Date/week 4/12/2008 wk 1 7 dap	11/12/2008 wk 2 14 dap	19/12/2008 wk 3 22 dap	24/12/2008 wk 4 27 dap	31/12/2008 wk 5 34 dap	8/01/2009 wk 6 42 dap	15/01/2009 wk 7 49 dap
Control	-	-	-	-	-	-	-
Weekly	-	-	T D	T D	T D	T D	T D
Brassica$_{spot}$I™	-	-	-	A	-	-	-
Brassica$_{spot}$II™ 5%	-	-	-	-	-	A	-

Dap, days after planting; Control, unsprayed; Weekly, sprays applied weekly; T, Tri-Base Blue®; D, Du-Wett; A, Amistar®; -, no chemical application.

4.2.11 Trial 6

Trial No. 6, conducted by Dr Hoong Pung on a processing broccoli variety Shamrock (Terranova), was located at Harford, Tasmania. Seedlings were transplanted on 17 January 2009. The trial was originally designed to include eight treatments. The original layout consisted of ten blocks of four plots each with a replicate made up of two adjacent blocks.

One treatment was dropped and those plots were not assessed. The trial design was a randomised block with five replicated blocks containing six plots, each representing one of six treatments (Table 4.8). The plots were 6m long and contained three rows of broccoli plants. The rows were spaced 0.8m apart and the plants were spaced 0.35m apart. The treatments were: (i) 'Control' (unsprayed); (ii) 'Grower', grower's spray program of Amistar® alternating with Ridomil Gold Plus®; (ii) weekly sprays of 'TriBase Blue® + Agral' commencing at week 7; (iv) weekly sprays of 'TriBase Blue® + Du-Wett' commencing at week 7; (v) weekly sprays of 'TriBase Blue® + Designer™' commencing at week 7; (vi) 'Brassica$_{spot}$I™' sprays of Amistar® alternating with Ridomil Gold Plus® based on predictions of the model; (vii) 'Brassica$_{spot}$II™ 5%' appearance sprays of Amistar® alternating with Ridomil Gold Plus® based on predictions of the model. The trial was monitored weekly and the final assessment was conducted on 31 March 2009, 73 dap. The latter was followed by the commercial harvest from 3-19 April 2009. Only the middle row was assessed. Incidence was recorded as the number of plants, out of 15-20 plants per plot, showing symptoms of white blister on foliage. The severity of white blister on foliage was rated on a 0-4 scale and analysed as a severity index. Data were analysed by ANOVA.

Table 4.8 Treatment schedule Trial No. 6 on the processing broccoli variety Shamrock at Harford, Tasmania from summer to autumn 2009.

	Date/week/dap					
Treatment	28/02/2009 wk 6 42 dap	6/03/2009 wk 7 48 dap	13/03/2009 wk 8 56 dap	20/03/2009 wk 9 63 dap	27/03/2009 wk 10 70 dap	31/03/2009 wk 11 73 dap
			Button			
Control	-	-	-	-	-	-
Grower		A	R	-	-	A
Brassica$_{spot}$I™	A	A	-	-	-	R
Brassica$_{spot}$II™ 5%	-	A	-	-	-	R
TriBase Blue + Agral	-	+	+	+	+	+
TriBase Blue + Du-Wett	-	+	+	+	+	+
TriBase Blue + Designer	-	+	+	+	+	+

Dap, days after planting; Control, unsprayed; Grower, sprays based on grower practice; A, Amistar; R, Ridomil Gold Plus; Button, button stage of broccoli head development; +. chemical application; -, no chemical application.

4.2.12 Trial 7

This trial was initially established on broccoli variety Ironman (Seminis) at Wesley Vale Tasmania but it was abandoned because of weather station reception issues and relocated and recommenced on broccoli variety Tyson (Syngenta). The trial was transplanted on 7 July 2009. The rows were spaced 0.8m apart and plants were spaced 0.35m apart. The trial design was a randomised block with five blocks, each containing three plots. The plots were 6m long and contained three rows of broccoli plants. The treatments were: (i) control (unsprayed); (ii) 'Grower', the grower's spray program of Amistar® alternating with Ridomil Gold Plus®; (iii) 'Brassica$_{spot}$I™', sprays of Amistar® alternating with Ridomil Gold Plus® based on predictions of the model. Only one spray of Amistar® was applied to the Grower spray program and the Brassica$_{spot}$I™ program on 29 September 2009 at week 12, 84 dap. The trial was checked weekly for white blister and the final assessment was made on 30 October 2009, 115 dap, just before the first commercial harvest. No data analysis was undertaken because there was no disease in the crop.

4.2.13 Trial 8

This trial was established on cauliflower variety Discovery (Seminis) at Forthside Tasmania. Seedlings were transplanted on 5 November 2009. The trial design was a randomised block with five blocks, each containing three plots. The plots were 6m long and contained three rows of cauliflower plants. The rows were spaced 0.8m apart and the plants were spaced 0.50m apart. The treatments were: (i) 'Control' (unsprayed); (ii) 'Grower', sprays based on

grower practice; (iii) 'Brassica$_{spot}$I™', sprays of Amistar® alternating with Ridomil Gold Plus® based on predictions of the model. No sprays were applied to the Grower practice but two sprays were applied to the Brassica$_{spot}$I™ treatment (Table 4.9). The trial was monitored weekly for white blister and the final assessment was made on 25 January 2010, 50 dap, a day before the commercial harvest. Data were assessed as for Trial No. 6.

Table 4.9 Treatment schedule on the cauliflower on variety Discovery at Forthside, Tasmanian during summer 2009-10

Treatment	Date of application/week/dap	
	2/12/2009	15/01/2010
	wk4	wk10
	27 dap	40 dap
Control	0	0
Grower	0	0
Brassica$_{spot}$I™	A	R

Dap, days after planting; Control, unspayed; Grower, sprays based on grower practice; Brassica$_{spot}$I™, A, Amistar; R, Ridomil Gold Plus; +. chemical application; -, no chemical application.

4.2.14 Trial 9

This trial on broccoli variety Prophet (Fairbanks) at Lillico, Tasmania was transplanted on 24 March 2010. The rows were spaced 0.6m apart and the plants were spaced 0.25 to 0.30m apart. The trial design was a randomised complete block with five blocks, each containing three plots. The plots were 6m long and contained three rows of broccoli plants. The treatments were: (i) 'Control' (unsprayed); (ii) 'Grower', sprays of Amistar® alternating with Ridomil Gold Plus® based on grower practice; (iii) 'Brassica$_{spot}$I™', alternating sprays of Amistar® and Ridomil Gold Plus® based on predictions of the model. The grower practice received two sprays on 11 and 23 June 2010, whilst three sprays were applied to the Brassica$_{spot}$I™ plots on 27 May, 11 June and 23 June 2010 (Table 4.10). Foliage and broccoli heads were assessed for white blister on 2 July (week 12, 100 dap), 16 July 2010 (week 14, 114 dap) and on 19 July (week 15, 117dap). Data were analysed by ANOVA and a permutation test was used to confirm the results.

Table 4.10 Trial No. 9 treatment schedule on processing broccoli variety Prophet at Lillico, Tas from autumn to winter 2010.

Treatment	Date/week						
	27/05/10	02/06/10	11/06/10	16/06/10	23/06/10	30/06/10	07/07/10
	wk 7	wk 8	wk 9	wk 10	wk 11	wk 12	wk 13
	64 dap	70 dap	79 dap	84 dap	91 dap	98 dap	105 dap
	Button						
Control (unsprayed)	-	-	-	-	-	-	-
Grower	-	-	A	-	R	-	-
Brassica$_{spot}$I™	A	-	R	-	A	-	-

Dap, days after planting; Control, unsprayed; Grower, sprays based on grower practice; Amistar; R, Ridomil Gold Plus; Button, button stage of broccoli head development; -, no chemical application.

4.2.15 Trial 10

Trial No. 10, located at Gatton Research Station, Gatton Queensland, was conducted by John Duff, Mary Firrell and Madaline Healey of DEEDI QLD. Seedlings of Chinese cabbage variety Matilda (SPS Seeds), which had been grown by a commercial nursery, were transplanted on 5 August 2010. This trial had a completely random design with seven treatments and seven replications. Plot size was 6m long by one bed with three rows of plants per bed and a plant spacing of 0.33m. Buffer beds were also grown between each treatment

bed. The treatments were: (i) 'Control' (unsprayed); (ii) 'Grower' spray program of Amistar® at week 4 after transplanting or at the first appearance of disease, which ever came first, and at two weeks before harvest; (iii) 'Bion' 50WG™ (1µM), applied at week 4 or at the first appearance of disease and one week thereafter; (iv) 'Bion' 50WG™ (10µM), at week 4 or at the first appearance of disease and one week thereafter; (v) 'Fulzyme Plus™', fortnightly; 'Cabrio®' applied two weeks before harvest; and (vi) the 'Brassica$_{SPOT}$I™' model, sprays of Cabrio® based on predictions of the model (Table 4.11). The crop was grown using conventional agronomic practices by the Gatton Research Station farm staff. Insecticides such as Success® were applied when required to manage insect pests such as Diamond Back Moth.

Table 4.11 Trial No. 10 treatment schedule on Chinese cabbage variety Matilda at Gatton Queensland during spring 2010

Treatment	Date of spray application/week/dap							
	12/08/10 wk 1 7 dap	19/08/10 wk 2 14 dap	26/08/10 wk 3 21 dap	02/09/10 wk 4 26 dap	06/09/10 wk 5 30 dap	16/09/10 wk 6 40 dap	22/09/10 wk 7 46 dap	30/09/10 wk 8 54 dap
Control	-	-	-	-	-	-	-	-
Grower (Amistar)	-	-	-	-	+	-	+	-
Bion (1µM)	-	-	-	-	+	+	-	-
Bion (10µM)	-	-	-	-	+	+	-	-
Bacillus subtilis	+	-	+	-	+	-	+	-
Cabrio	-	-	-	-	-	-	+	-
Brassica$_{spot}$I™	-	-	-	-	+	-	+	-

Dap, days after planting; Control, unsprayed; Grower (Amistar), sprays based on grower practice; +. chemical application; -, no chemical application.

The trial was monitored weekly for white blister by randomly checking a minimum of 40 plants across the trial site until the disease first appeared. Weekly surveys were used to determine the timing of the first application of Bion 50 WG® and Amistar®. Disease incidence and severity were assessed on the four innermost wrap leaves, for 40 of the 60 plants per plot, at harvest on 5 October 2010, 59 dap. Additionally *Alternaria brassicicola* was assessed at the same time as white blister using a disease severity rating scale of 0 to 3 where 0 was no obvious signs of *Alternaria* on the leaves; 1 was a low incidence of *Alternaria* on the leaves; 2 was a moderate incidence of *Alternaria* on the leaves; and 3 was a high incidence of *Alternaria* on leaves.

4.2.16 Trial 11

Trial No. 11 was located at O'Connors Rd, Werribee South. Seedlings of broccoli variety Viper (Lefroy Valley) produced by the grower, were transplanted into three rows per bed on 3 January 2011. The trial was a randomised block design with six treatments and six replications. The plot size was 6m long and 1.93m wide. The treatments were: (i) 'Control' (unsprayed); (ii) 'Weekly' sprays of TriBase Blue® and Agral; (iii) 'Grower' sprays of Ridomil Gold MZ® and Tribase Blue® alternating with Amistar®, alternating with TriBase Blue® and Agral™; (iv) the 'Brassica$_{SPOT}$I™' model predicted sprays with the grower's spray treatments alternated; (v) the 'Brassica$_{SPOT}$II™ 5%' model predicted sprays with the grower's spray treatments alternated; and (vi) 'Weekly + Sp', weekly sprays of TriBase Blue and Agral™ plus Sprayphos™ (Table 4.12). Pounce® and Rogor® insecticides were applied weekly to all treatments. The trial was assessed on 8 and 26 February 2011 and on 1 March 2011. The commercial harvest was on 27 February 2011, 56 dap, and on 2 March 2011, 58 dap (week 9).

Table 4.12 Treatment schedule for Trial No. 11 on broccoli variety Viper at Werribee South in summer 2011.

Treatment	7/01/2011 wk 1 4 dap	15/01/2011 wk 2 12 dap	22/01/2011 wk 3 19 dap	28/01/2011 wk 4 25 dap	4/02/2011 wk 5 32 dap	10/02/2011 wk 6 38 dap	17/02/2011 wk 7 45 dap Button	24/02/2011 wk 8 52 dap
Control	-	-	-	-	-	-	-	-
Brassica$_{spot}$I™	-	RT	-	-	5/2/2011 A	-	18/2/2011 RT	-
Brassica$_{spot}$II™ 5%	-	-	-	RT	A	-	21/2/2011 RT	-
Grower	RT	A	T	RT	A	T	RT	T
Weekly Cu	T	T	T	T	T	T	T	T
Weekly+Sp	TS	TS	TS	TS	TS	TS	TS	TS

Dap, days after planting; Control, unsprayed; A, Amistar®; T, TriBase Blue®; R, Ridomil Gold MZ®; S, Sprayphos™.

4.3 Results

4.3.1 Trial No. 1

This trial site was located between a shed and a hedge, and ventilation was poor. In addition the broccoli variety Bridge produced many auxiliary shoots, the plants were very close together and the foliage was very dense creating an ideal microclimate for white blister. The Brassica$_{spot}$ I™ disease risk predictive model indicated that microclimate conditions for white blister infection were intermittent, but a persistent infection period was predicted during early September 2008 at the button stage (Fig 4.1). White blister first appeared in the trial during week 9 (11 August 2008), prompting a spray alert by the Brassica$_{spot}$ I™ model. The symptoms may have developed from one of the earlier infection events. The Brassica$_{spot}$II™ 5% model predicted a spray that coincided with the predicted major infection events in early September and the button stage (4 September 2008). However application of the spray was delayed until 12 September 2008 because of unfavourable weather.

Weekly sprays of the preventative copper-based fungicide provided the most efficient control of white blister incidence and severity on foliage (Table 4.13). None of the treatments significantly reduced the incidence of white blister on heads which was high (over 60%). The severity of white blister on heads was significantly reduced by the Brassica$_{spot}$II™ 5% model spray program, compared with the unsprayed control. Only the Brassica$_{spot}$II™ 5% model predicted a spray at button stage, but the eight day delay and the two to four week period from the last sprays until harvest, could have contributed to broccoli heads being unprotected from infections. Use of the models reduced the number of weekly sprays from 12 to one but did not provide sufficient disease control. The models were, however, more economical than the Weekly treatment (Table 4.13). Whilst the economic analysis suggests that doing nothing (Control treatment) is as economical as using the models, such an approach runs the risk of exposing the variety to the loss of any resistance it had to *A. candida*.

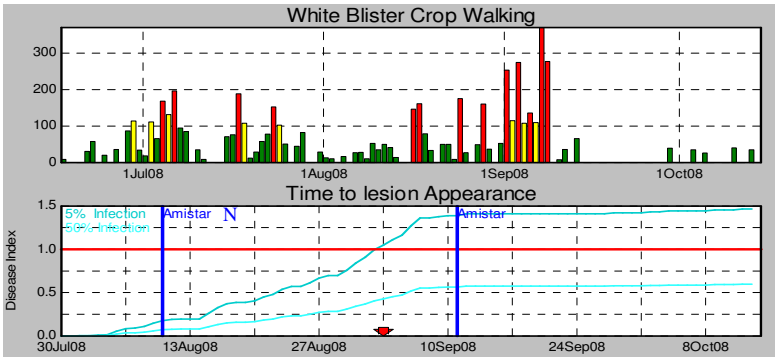


Fig 4.1 The Brassica$_{spot}$ I and II™ model graphs (top and bottom respectively) for Trial No. 1 at Werribee South on broccoli variety Bridge in winter to spring 2008.

Brassica$_{spot}$I ™ top; Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure;

Brassica$_{spot}$II™ bottom; dark blue line, predicted time for maximum spore release from 5% of the crop and a spray should be applied on the date indicated by the red arrow; Blue bars, time of fungicide (Amistar) applications.

O, Brassica$_{spot}$I ™ model predicted spray based on lesion appearance in the field.

N, Brassica$_{spot}$II™ 5% (infection) model predicted a spray based on maximum sporulation predicted from lesions when 5% of the crop is infected with white blister and a spray should be applied on the date indicated by the red arrow.

Table 4.13 Efficacy of treatments to control the incidence and severity of white blister on broccoli foliage and heads on variety Bridge at Werribee South from winter to spring 2008 in Trial No. 1.

Treatment	Mean incidence of white blister on foliage (%)	Mean severity of white blister on foliage (Scale 1-3)	Mean incidence of white blister on heads (%)	Mean severity of white blister on heads	No of sprays	Contribution to profit[1]
Control (no sprays)	100a	2.763a	74.9	1.139a	0	1
Brassica$_{spot}$II™ 5%	100a	2.463a	59.6	0.738b	1	2
Brassica$_{spot}$I™	98.8a	2.538a	66.1	1.034ab	1	3
Weekly	81.2b	0.988b	61.5	0.794b	12	4
lsd (5%)	13.2	0.314	ns	(ns)		
lsd (10%)				0.2855		

Severity scale (foliage): 0, no blisters; 1, one or two leaves with one or two blister; 2, more than one leaf with one or more blisters; 2.5, multiple leaves with numerous blisters; 3, Blister seen to penetrate upper surface of leaf; 4, swollen leaf petiole; 5, hypertrophy (profuse sporulation on youngest leaves, swollen petioles).

Severity scale (heads): 0, no blisters; 1, less than 10 blisters per head (marketable); 2, more than or equal to 10 blisters per head (not marketable).

[1], Refer to Chapter 9.

Values in the same column followed by the same letter were not significantly different

4.3.2 Trial No. 2

The Brassica$_{spot}$I™ model predicted that microclimate conditions for white blister development were intermittently high with eight potential infection periods (clusters of red bars, Fig 4.2). New blisters were observed in the crop on 3 July and 20 August 2008, but thereafter new lesions formed too close to harvest to apply fungicide sprays. At the final assessment, broccoli variety Grevillea was moderately susceptible to white blister with unsprayed control plants showing 60-70% incidence of the disease on foliage and heads, respectively (Table 4.14).

None of the treatments applied to the foliage significantly reduced the incidence or severity of white blister compared with the unsprayed control plants, but sodium lauryl sulphate significantly increased both incidence and severity of the disease. On broccoli heads the 13 weekly sprays of TriBase Blue plus Du-Wett had the most efficacy of all treatments and reduced the incidence of white blister on heads by 96% compared with the unsprayed control treatment. All the remaining treatments were worse than the weekly sprays, but better than the unsprayed control treatment for reducing white blister on heads. Of the remaining treatments, the two spray applications based on predictions of the Brassica$_{spot}$I™ model reduced white blister incidence on heads by 70%, while the nine applications of *S. lyticus* reduced it by 59%.

The level of incidence of white blister on heads was unacceptable to growers for all treatments except the weekly sprays of copper. Neither the Brassica$_{spot}$I™ nor the Brassica$_{spot}$II™ models predicted a spray at button stage, which could have protected the heads from infection. If sodium lauryl sulphate and *S. lyticus* had been applied earlier they may have had higher efficacy. The efficacy of *S. lyticus* and sodium lauryl sulphate may be of interest to organic growers. The application of these treatments may also be more suitable for varieties with higher resistance.

Of all the treatments, the 13 fungicide sprays applied to the Weekly treatment were more economical than the two fungicide spray applications based on predictions of the Brassica$_{spot}$I™ model (Chapter 9).

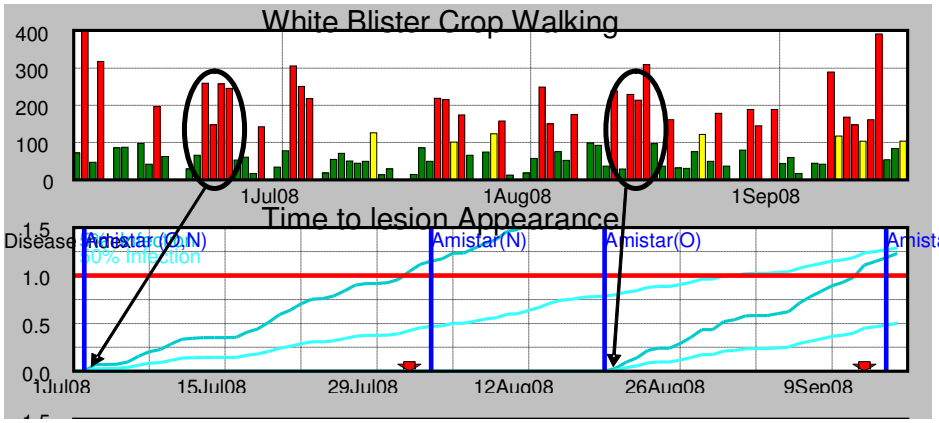


Fig 4.2 The Brassica$_{spot}$ I and II™ model graphs (top and bottom respectively) for Trial No. 2 at Boneo on broccoli variety Grevillia in winter to spring 2008.

Brassica$_{spot}$I ™ (top); Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure.

Brassica$_{spot}$II™ (bottom) 5% or 50% lesion appearance. Dark blue line (5%), predicted time for maximum spore release when 5% of the crop will show lesions and should be sprayed on the date indicated by the red arrow. Light blue line (50%); predicted time for maximum spore release when 50% of the crop will show lesions and should be sprayed on the date indicated by the red arrow, sprays not graphed. Blue bars, time of fungicide (Amistar) applications.

O, Brassica$_{spot}$I ™ model predicted a spray based on lesion appearance in the field.

N, Brassica$_{spot}$II™ 5% model predicted a spray based on predicted time when 5% of the crop will show lesions and should be sprayed on the date indicated by the red arrow.

Table 4.14 Efficacy of treatments to control the incidence and severity of white blister on broccoli foliage and heads of variety Grevillia in Trial No. 2 at Boneo in winter to spring 2008.

Treatment	Mean incidence of white blister on foliage (%)	Mean severity of white blsiter on foliage (scale 0-3)	Mean incidence of white blister on heads (%)	Standard error of mean incidence of white blister on heads	Number of sprays	Contribution to profit[1]
SLS	95.6 a	2.169 a	46.13	5.264	9	3
Brassica$_{spot}$II™ 5%	76.2 b	1.091 b	41.52	5.25	3	5
S. lyticus	68.1 bc	0.912 bc	31.04	4.893	9	4
Control	66.2 bc	0.941 bc	75.07	4.508	0	7
Brassica$_{spot}$II™ 50%	58.8 bc	0.850 bc	56.63	5.265	1	6
Brassica$_{spot}$I™	58.8 bc	0.788 bc	22.87	4.403	2	2
Weekly	48.1 c	0.531 c	2.97	5.265	13	1
lsd (5%)	20.9	0.482				

Control, unsprayed; SLS, Sodium lauryl sulphate; *S. lyticus, Streptomyces lyticus*; Severity scale (foliage): 0, no blisters; 1, one or two leaves with one or two blister; 2, more than one leaf with one or more blisters; 2.5, multiple leaves with numerous blisters; 3, Blister seen to penetrate upper surface of leaf. [1], Refer to Chapter 9. Values in the same column followed by the same letter were not significantly different.

4.3.3 Trial No. 3

The Brassica$_{spot}$I™ model predicted three extended periods where microclimate conditions were conducive for infection by *A. candida* and development of white blister symptoms (Fig 4.3). New lesions were observed in the crop during week 5 (16 March 2008) and week 8 (6 April 2008). No white blister symptoms were present in two blocks and another two blocks showed few symptoms. Viper is a summer broccoli variety considered to be resistant to white blister. The disease clubroot also affected plants in the trial and the crop was highly variable.

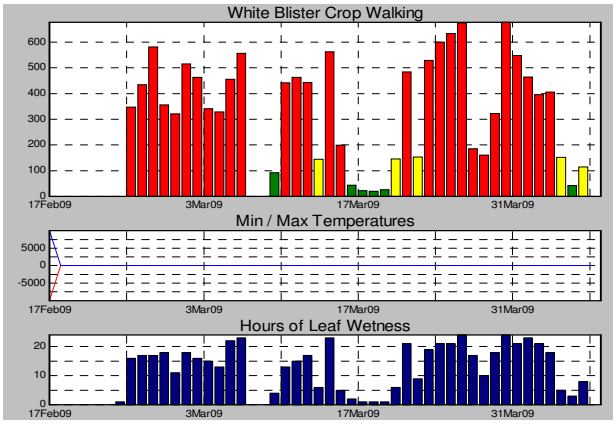


Fig 4.3 The Brassica$_{spot}$ I™ model graphs for Trial No. 3 at Werribee South on broccoli variety Viper in summer to autumn 2009.

No bars = no risk of white blister; green bars = low risk, yellow bars = moderate risk & red bars = high risk. Note: The temperature data was not graphed.

Field Trial. The incidence of white blister on foliage was too low to assess and analyse. Only 4.7% of unsprayed broccoli heads showed symptoms of white blister at harvest (Table 4.15). None of the treatments controlled the low incidence of white blister on the heads of broccoli variety Viper (Table 4.15). Unlike in past trials, the Brassica$_{spot}$I™ model predicted a spray within a week of button stage (50% of plants showing buttons). Harvest was one week after button stage and consequently additional sprays of copper could not be applied to the "Best Bets" treatment. The difference in timing of spray applications between the Brassica$_{spot}$I™, the Brassica$_{spot}$I™ + button and the "Best Bets" treatments was not sufficient to have any effect on the efficacy of disease control. The models and "Best Bets" reduced the number of sprays by 5 compared to the number of weekly sprays. Although the economic analysis indicated that the no spray treatment was the most economical (Chapter 9), this treatment would run the risk of Viper losing its high resistance to *A. candida*. Using a resistant variety in the trial may have reduced disease levels and differences between treatments but plants of a more susceptible variety were not available.

Post-harvest trial. Up to 25% of broccoli heads developed white blister after 12 days in cold storage at 12 ºC and two days of shelf life at 14 ºC, irrespective of fungicide treatment in the field and despite the absence of symptoms at harvest (Table 4.15, Fig 4.4). There were few blisters on heads and those that did develop were small. Because of variability in the crop, the size of samples of broccoli heads in the post harvest trial was too small for statistical analysis. The trial demonstrated proof of concept for post-harvest development of white blister on broccoli heads after cold storage and a short shelf life. Growers reported that the period in cold storage was excessive in comparison to normal industry practice.

Fig 4.4 Supermarket storage shelving showing boxed broccoli.

Table 4.15 Efficacy of treatments to control the incidence of white blister on broccoli heads of variety Viper in Trial No. 3 at Werribee South in summer to autumn 2009.

Treatment	Incidence of white blister on broccoli heads (%)	Number of sprays	Broccoli heads with white blister symptoms after post harvest storage (%)	Contribution to profit[1]
Control	4.682	0	25.0	1
Weekly	3.067	7	11.0	5
Best Bets	3.309	2	17.6	2
Brassica$_{spot}$I™	1.973	2	23.1	3
Brassica$_{spot}$I™ + button	1.785	2	25.0	4
lsd	ns			

Control, unsprayed; Weekly, sprayed with Tri-Base Blue® and Agral™; Best Bets, sprayed at first sign of white blister and at button stage (week five and nine, respectively); [1], Refer to Chapter 9.

4.3.4 Trial No. 4

Brassica$_{spot}$ I™ model predicted that microclimate conditions in the crop could lead to a high risk of white blister early and late in the trial with a low risk in the middle of the trial period (Fig 4.5). White blister symptoms, very small lesions surrounded by dark almost black boarders, appeared on leaves in control plots at week 7 (30 April 2010). The symptoms were similar to those that initially appeared on the broccoli variety Belstar (Minchinton *et al* 2007).

The weekly copper sprays and sprays based on predictions of the Brassica$_{spot}$ I™ model significantly reduced the incidence and severity of white blister on foliage compared with the unsprayed control treatments. Neither Bion™ nor *B. subtilis* reduced the incidence or severity of white blister symptoms on foliage (Table 4.16). No white blister symptoms appeared on broccoli heads of the variety Rhumba at harvest. Spray applications based on predictions of the Brassica$_{spot}$ I™ model, reduced the number of sprays applied by nine compared with the 11 weekly sprays (Table 4.16).

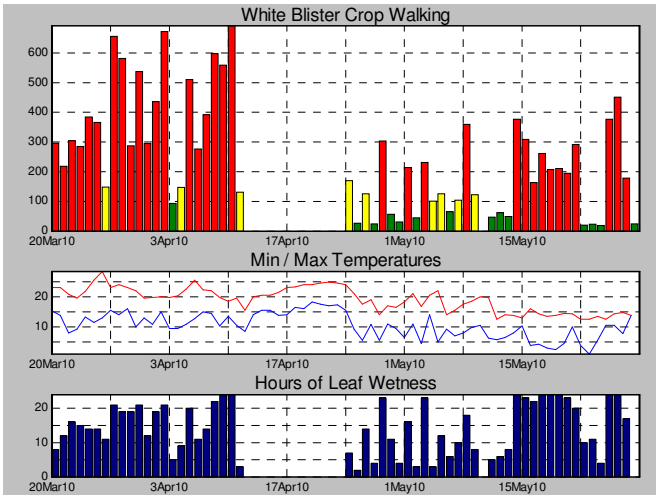


Fig 4.5 The Brassica$_{spot}$ I™ model graphs for Trial No. 4 Werribee South on broccoli variety Rhumba in autumn to winter 2010.

No bars, no risk of white blister; green bars, low risk; yellow bars, moderate risk and red bars, high risk of white blister developing in the crop 7, 14 or 21 days later.

Table 4.16 Efficacy of treatments to control the incidence of white blister on broccoli foliage of variety Rhumba in Trial No. 4 at Werribee South in autumn to winter 2010.

Treatment	Mean incidence of white blister on foliage (%)	Mean severity of white blister on foliage (scale 0-5)	Number of sprays
Control	50.0 a	0.686 a	0
Bion™	49.3 a	0.729 a	2
B. subtilis	43.6 ab	0.686 a	11
Brassica$_{spot}$I™	39.3 bc	0.543 ab	2
Weekly	20.0 c	0.257 b	11
l.s.d. (5%)	14.6	0.261	

Control, unsprayed; Scale, 0, on blisters; 1, one blister on 1 to 2 leaves; 2, multiple blisters on 1 to 2 leaves; 3, multiple blister on multiple leaves and or break through to the upper leaf surface; 4, swollen leaf petiole; 5, hypertrophy ie profuse sporulation on youngest leaves, swollen petioles. Values in the same column followed by the same letter were not significantly different.

4.3.5 Trial No. 5

Predictions of the Brassica$_{spot}$I™ model indicated that conditions consistently favoured white blister development throughout the trial (Fig 4.6). White blister symptoms, a few blisters on the abaxial surface of older leaves, appeared on plants in all of the treatment plots four weeks after sowing (24 December 2008). There after the symptoms developed rapidly (Fig 4.7). At harvest, infection appeared to have occurred from the oldest to youngest leaves. All unsprayed plants exhibited symptoms of white blister at harvest. No white blister symptoms were observed on the leaves covering heads.

Fig 4.6 The Brassica$_{spot}$ I™ and Brassica$_{spot}$II™ model graphs for Trial No. 5 at Devon Meadows on Chinese cabbage variety Matilda during summer 2008-2009.

Brassica$_{spot}$I ™ top; Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure.

Brassica$_{spot}$II™ 5% (bottom), dark blue line, predicted time for maximum spore release when 5% of the crop will show lesions and should be sprayed on the date indicated by the red arrow; Blue bars, time of fungicide (Amistar) applications.

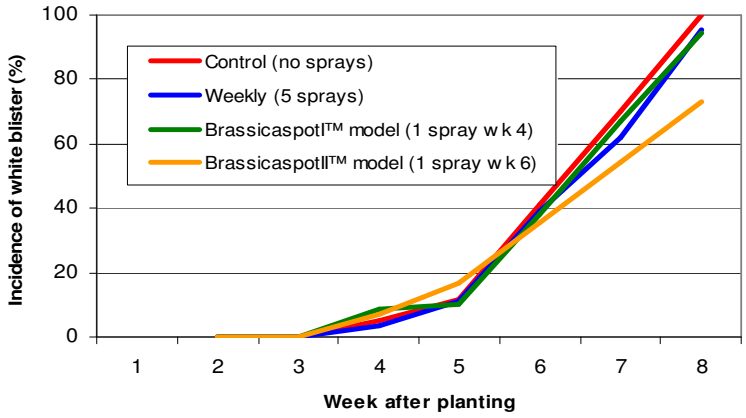


Fig 4.7 Development of white blister on Chinese cabbage variety Matilda with various treatments during the Trial No. 5 during summer 2008-2009.

Disease freedom on the outer four leaves of each harvested head is a critical commercial quality factor. Only the Brassica$_{spot}$II™ model treatment provided significant control of white blister on these outer wrap leaves (Table 4.17). The model recommended a single Amistar spray on 31 December 2008, based on disease progression data from the crop inspections and environmental data; but the spray was not applied until 8 January 2009, about a week later and 14 days before harvest. Co-incidentally spraying 14 days before harvest may be the best phenological time to protect the 4 unfolding wrap leaves. The economic analysis indicated that this was the most profitable treatment (Chapter 9).

Table 4.17 Efficacy of treatments to control the incidence and severity of white blister symptoms on Chinese cabbage variety Matilda in summer 2008-2009 at Devon Meadows in Trial No. 5.

Treatment	Mean incidence on plants (%)	Mean severity on 4 wrap leaves (scale 0-4)	Yield with wrap leaves (%)	Yield heads only (%)	Number of sprays	Contribution to profit[1]
Control	100	2.33a	41	100	0	2
Weekly	95.59 a	2.24a	44	100	5	4
Brassica$_{spot}$I™	94.48 a	2.17a	46	100	1 (wk 4)	3
Brassica$_{spot}$II™ 5%	72.9 b	1.17b		100	1 (wk 6)	1

Control, unsprayed; scale 0-4, 0, no symptoms on any of the four wrap leaves; 1, one wrap leaf showing white blister symptoms; 2, two wrap leaves showing symptoms of white blister; 3, three wrap leaves showing symptoms of white blister; 4, four wrap leaves showing symptoms of white blister. [1], Refer to Chapter 9. Values in the same column followed by the same letter were not significantly different.

4.3.6 Trial 6

The Brassica$_{spot}$II™ model predicted two spray applications on the basis of microclimate conditions within the crop. The Brassica$_{spot}$I™ model predicted that microclimate conditions within the crop would produce three major periods with a high risk of white blister development and a few minor ones (Fig 4.8). There were three flushes of new lesions. White blister was first observed on broccoli foliage in plots with the Brassica$_{spot}$I™ treatment on 24 February 2009 and by 3 March 2009, 67% of plants had new flushes of lesions. This is not surprising given that the microclimate conditions during the previous week were highly conducive for white blister development as indicated by the large cluster of red bars (Fig 4.8). New flushes of lesions were again observed on 10 March 2009 in both model plots and by 19 March new lesions also appeared in the plots sprayed with copper. The Brassica$_{spot}$II™ 5% model is not shown.

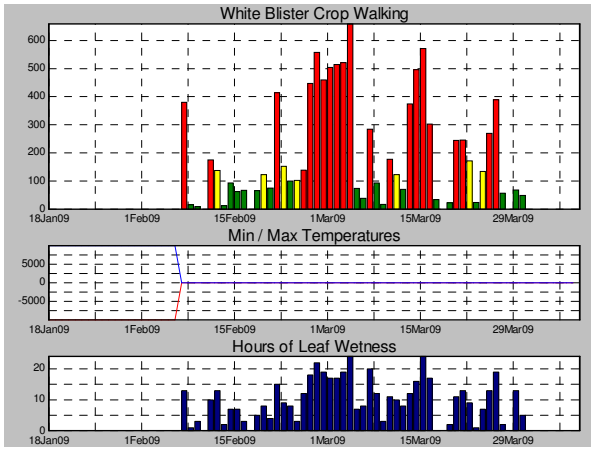


Fig 4.8 The Brassica$_{spot}$ I™ model graphs for Trial No. 6 on broccoli variety Shamrock at Harford Tasmania during summer to autumn 2009

Brassica$_{spot}$I ™ top; Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure. The temperature data was not graphed.

Incidence and severity of white blister on foliage was high (Table 4.18). The grower treatment with three sprays of a systemic fungicide, including one at button stage, produced the greatest reduction in incidence and severity of white blister on top leaves. There was no significant difference in the efficacy of the four adjuvant treatments for disease control on heads or leaves. As the incidence of white blister on heads was low or absent, a statistical analysis could not be undertaken (Table 4.18).

Table 4.18 Efficacy of treatments to control the incidence and severity of white blister on broccoli variety Shamrock in Trial No. 6 at Harford, Tasmania from summer to autumn 2009

Treatment	Incidence of plants with with infected top leaves (%)[1]	Severity of white blister symptoms on top leaves (%)	Incidence of white blister on heads (%)	No. of sprays
Control	100	91.6 a	2	0
Brassica$_{spot}$I™	100	69.7 b	0	3
TriBase Blue + Agral	99	60.6 bc	0	5
Brassica$_{spot}$II™ 5%	97	63.0 bc	0	2
TriBase Blue + Du-Wett	94	47.9 c	3	5
TriBase Blue + Designer	90	53.6 c	0	5
Grower	57	18.9 d	0	3
l.s.d. (5%)		15.3		

Severity scale 0-5; where 0, indicates no leaves infected; 5, all five leaves were infected. [1], no analysis was necessary as the result was obvious; [2], too little data to analyse Values in the same column followed by the same letter were not significantly different.

4.3.7 Trial 7

The Brassica$_{spot}$I™ model predicted that microclimate conditions within the crop produced about 10 periods with a high risk of white blister development alternated with low or no risk periods (Fig 4.9). No white blister was observed on foliage. Only one fungicide spray was applied at button stage in the Grower practice program and the Brassica$_{spot}$I™ program on 29 September 2009 at week 12. No white blister was observed on broccoli heads at harvest. This trial demonstrates the value of growing a resistant variety. Despite repeated periods with a high risk of white blister development, no disease developed in the crop.

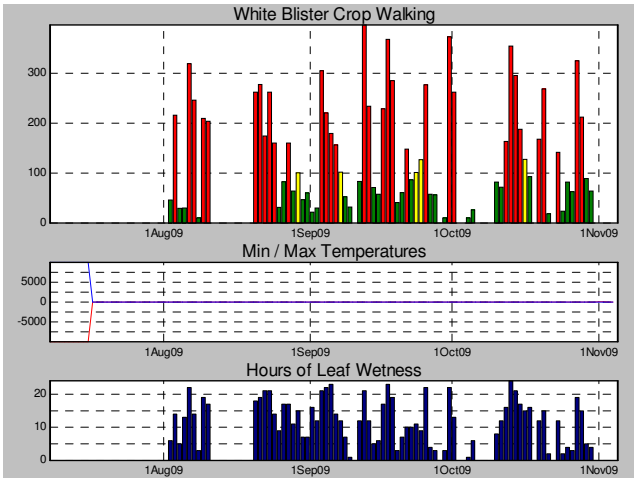


Fig 4.9 The Brassica$_{spot}$ I™ model graphs for Trial No. 7 on broccoli variety Tyson at Wesley Vale Tasmania during autumn to spring 2009.

Brassica$_{spot}$I ™ top; Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure. The temperature data was not graphed.

4.3.8 Trial 8

The Brassica$_{spot}$I™ predicted that microclimate conditions within the crop produced 2 major high risk periods and about 6 minor risk periods for white blister infection, alternated with low or no risk periods (Fig 4.10). White blister was first observed in the crop on 2 December 2009 (week 4). Symptom development was probably associated with the single high risk infection events early in the trial period. New white blister infections noted on the 11 December 2009 in week 5 were probably associated with the major high risk period indicated on the graph as a cluster of red bars (Fig 4.10). A third flush of white blister lesions observed on 15 January 2010 in week 10 was probably associated with the second major high risk period indicated on the graph as a cluster of red bars around the first week of January 2010.

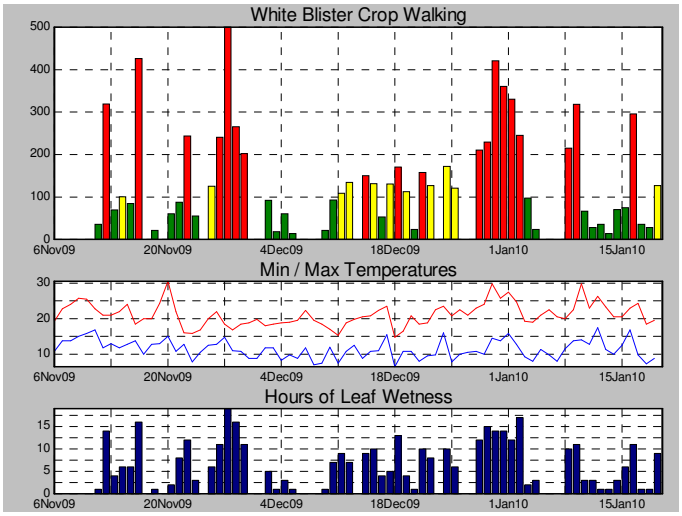


Fig 4.10 The Brassica$_{spot}$ I™ model graphs for Trial No. 8 on cauliflower variety Discovery at Forthside Tasmania during spring to summer 2009-2010.

Brassica$_{spot}$I ™ top; Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure.

The data were not analysed because there was no difference between disease incidence for any of the treatments and none of the treatments controlled white blister on plants or on top leaves (Table 4.19). No white blister symptoms were observed on cauliflower heads. The grower and his consultant agronomist were not concerned about white blister on cauliflower as their previous experience had shown that leaf infections were not severe and cauliflower heads were protected by the inner wrap leaves (Fig 4.11).

Table 4.19 Efficacy of treatments to control the incidence and severity of white blister on cauliflower variety Discovery in Trial No. 8 at Forthside Tasmania during spring and summer 2009-10.

Treatment	Incidence of white blister on plants (%)	Incidence of white blister on top leaves (%)	Incidence of white blister on heads (%)	No of sprays
Control	100	87	0	0
Grower	100	87	0	0
Brassica$_{spot}$I™	100	87	0	2

Fig 4.11 A, symptoms of white blister on a cauliflower leaf and B well grown crop of cauliflower.

4.3.9 Trial 9

The Brassica$_{spot}$I™ predicted that microclimate conditions within the crop produced about 3 periods with a high risk of white blister infection (clusters of red bars) alternated with low or no risk periods (Fig 4.12). New lesions were observed in the crop on 3 occasions (weeks 7, 9 and 11) and consequently the model plots received 3 sprays. The grower applied two fungicide sprays to the adjacent crop and consequently only two fungicides were applied to the grower treated plots. The incidence of white blister on plants was high and incidence of the disease on top leaves was moderate. The leaf coverage severity index was low (Table 4.20). At harvest on 19 July 2010 (week 16), the incidence of white blister on broccoli heads was very low. Although all treatments had at least one infected head, disease levels were too low for analysis. The Grower's two sprays reduced the disease on leaves more than the three sprays predicted by the Brassica$_{spot}$I™ model, although the difference was not significant.

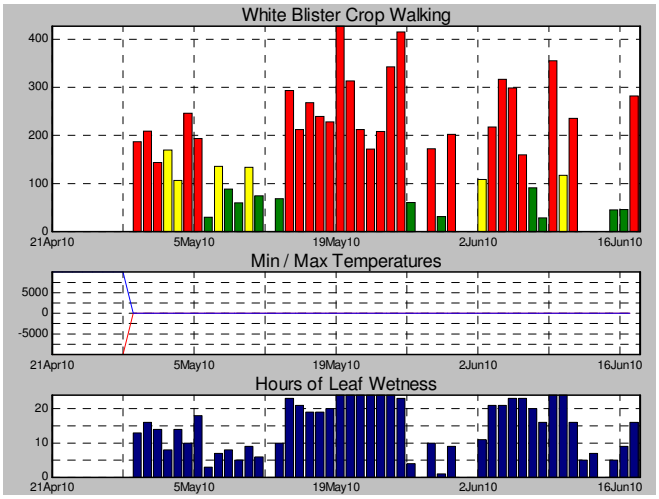


Fig 4.12 The Brassica$_{spot}$ I™ model output for Trial No. 9 on broccoli variety Profit at Lillico Tasmania during autumn to winter 2010

Brassica$_{spot}$I ™ top; Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure. The temperature data was not graphed.

Table 4.20 Efficacy of treatments to control the incidence and severity of white blister symptoms on broccoli variety Profit during autumn to winter 2010 at Lillico Tasmania in Trial No. 9.

Treatment	2/07/2010 wk 12		9/07/2010 wk 13		16/07/2010 wk 14		19/07/2011 wk 15	No. of sprays
	Predicted plants infected (%)	Incidence of white blister on top 5 leaves (%)	Heads infected (%)	Heads infected (%)	Leaf coverage severity index rating	Heads infected (%)	Heads infected (%)	
Brassica$_{spot}$I™	89.4	48.39b	0.0	0.0	2.6	0.0	1.7	3
Control (unsprayed)	88.63	50.48b	0.0	0.0	1.2	1.8	1.8	0
Grower	79.47	36.71a	0.0	0.0	1.2	2.3	0.6	2
lsd (5%)	ns	ns						

4.3.10 Trial 10

In Queensland, Brassica crops are grown primarily in the cooler southern regions, including the Lockyer Valley and the Granite Belt. Cabbage, cauliflower and broccoli are the major crops, while Chinese cabbage and other Asian vegetables also grown. Chemical control has to date been the option of choice by growers for the management of white blister and a number of products are often used on a crop with varying degrees of success.

White blister first appeared at the end of August after the plants were visually assessed in the field (Fig 4.13). The Brassica$_{spot}$I™ model predicted that microclimate conditions in the crop could lead to a high risk of white blister infection on four occasions and there were alternating high risk periods of short and long duration (Fig 4.14). The prediction of the onset of white

blister in the trial may have been erroneous because it was based on a leaf wetness sensor record indicating wetness for 4 consecutive days when the relative humidity was between 40% and 70%. The first spray application in the Brassica$_{spot}$I™ model program was not applied until a week after the first lesions were observed. Consequently the efficacy of the model program may have been enhanced if the spray had been applied when the lesions were observed.

Fig 4.13 The field trial site at DEEDI Gatton Research Station, Queensland.

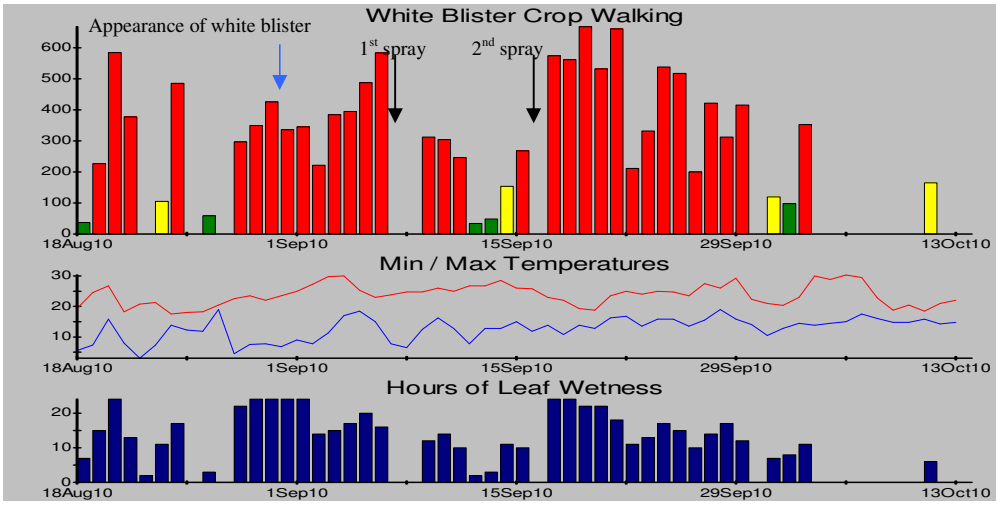


Figure 4.14 Brassica$_{spot}$ I™ model output for Trial No. 10 on Chinese cabbage variety Matilda at the Gatton Research Station Queensland from winter to spring 2010.

Brassica$_{spot}$I ™ top; Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure; blue arrow, first report of white blister lesions in the Trial; black arrow, application of fungicides.

Only four treatments are reported in Table 4.20 as the other treatment related to HAL project VG07125. The single application of Cabrio® and the two applications of Cabrio® based on predictions of the Brassica$_{spot}$I™ model provided the best control of white blister on the inner wrap leaves (Table 4.21). All other treatments were not significantly different from the

unsprayed control. All treatments containing either Amistar® or Cabrio® had the greatest efficacy for control of *A. brassicicola* (Fig 4.15).

Table 4.21 Efficacy of treatments to control the incidence and severity of white blister symptoms on Chinese cabbage variety Matilda in Trial No. 10 at the Gatton Research Station Queensland in winter to spring 2010.

Treatment	Mean incidence of white blister on wrap leaves (%)	Mean severity of white blister on 4 wrap leaves (scale 0-4)	% yield with wrap leaves	No. of sprays	Contribution to profit[1]
Control (unsprayed)	100	3.62a	9.5	0	3
Grower (Amistar)	97.8	3.25a	18.75	2	4
Cabrio (2 wks before harvest)	97.1	2.62b	34.5	1	1
Brassica$_{spot}$I™	95.7	2.61b	34.75	2	2
lsd (5%)		0.49			

[1], Refer to Chapter 9. Values in the same column followed by the same letter were not significantly different.

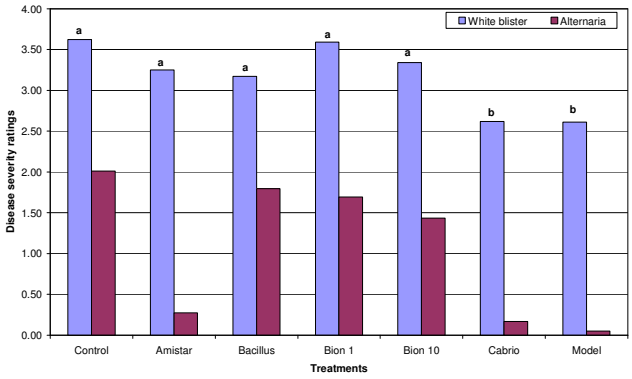


Fig 4.15 Incidence of white blister and target spot on Chinese cabbage at harvest on 5 October 2010. The white blister rating scale was from 0-4 and the *Alternaria* rating scale was from 0-3.

4.2.16 Trial 11

Some of the wettest conditions on record were experienced during this trial. At planting the foliage of seedlings showed symptoms of white blister lesions. There were three extended periods of microclimate conditions which were conducive for *A. candida* to infect plants and there were at least five flushes of new lesions (Fig 4.16). Some of the sprays were applied several days before the Brassica$_{spot}$II™ model predicted infection as wet weather was forecast and it would be impossible to get onto the ground to spray. Only copper based sprays of the Weekly and Weekly+S treatments and the Grower treatment significantly reduced white blister on foliage and heads and had the highest contribution to profit depending on the time of harvest (Table 4.22). The Brassica$_{spot}$I™ and Brassica$_{spot}$II™ models predicted slightly different timings for sprays and while both reduced the number of sprays by 5, neither controlled the disease as well as the Grower or Weekly treatments. No spraying occurred after week 8, 52 dap, on the grower's request, but more copper based sprays, which have a one day withholding period, may have been beneficial. Mild symptoms of phytotoxicity, expressed as a slight browning on foliage,

Fig 4.17 Mild symptoms of phytoxocity associated with copper sprays on broccoli variety Viper in Trial No. 11.

were associated with the copper based spray (Fig 4.17). Consultations with two crop consultants indicated the phytotoxicity was considered to be insignificant.

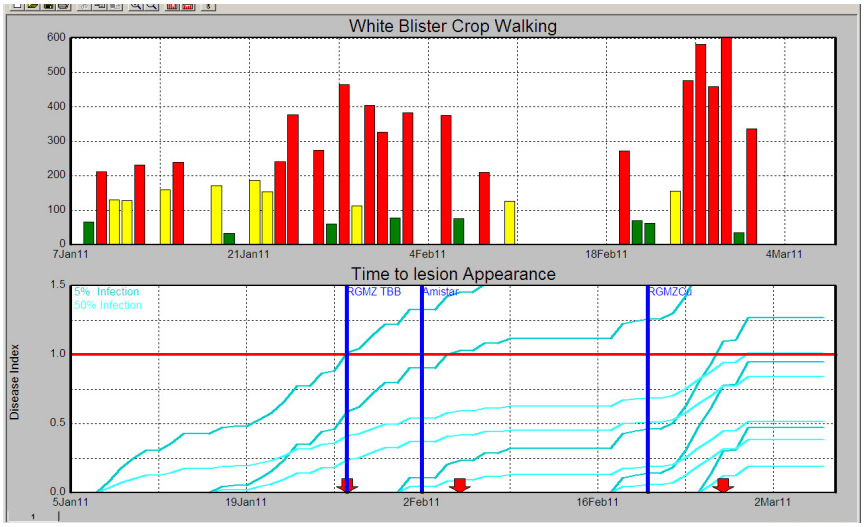


Figure 4.16 The Brassica$_{spot}$II™ disease risk predictive model output for Trial No. 11 on broccoli variety Viper from summer to autumn 2011, Werribee South Victoria.

Brassica$_{spot}$I ™ top; Red bars = high disease pressure, yellow bars = moderate disease pressure; green bars = low disease pressure and non bars = no disease pressure. Brassica$_{spot}$II™ (bottom) 5% lesion appearance, dark blue line, predicted time for maximum spore release when 5% of the crop will show lesions and should be sprayed on the date indicated by the red arrow; Blue bars, time of fungicide applications.

Table 4.22 Efficacy of treatments to control the incidence and severity of white blister symptoms on broccoli variety Viper in Trial No. 11 at Werribee South Victoria during summer 2011

Treatment	Average severity rating for white blister on foliage (scale 0-5)		Mean incidence of white blister on heads (%)		Number of sprays	Contribution to profit[1] (1st harvest)	Contribution to profit[1] (2nd harvest)
	8/02/2011	25/02/2011	25/02/2011	1/03/2011			
Control	2.8 a	3.575 a	26.67 a	95.0 a	0	2	4
Brassica$_{spot}$I™	2.68 ab	3.475 a	25.83 a	95.0 a	3	6	6
Brassica$_{spot}$II™ 5%	2.65 b	2.733 b	11.67 b	85.8 b	3	5	5
Grower	1.13 c	2.217 c	0.83 c	14.2 d	8	4	1
Weekly	1.06 cd	1.708 d	0.00 d	30.8 c	8	1	2
Weekly+S	0.98 d	1.708 d	0.00 d	30.8 c	8	3	3
lsd 5%	0.13	0.365	A	13.0			

Control, unsprayed; Scale, 0, on blisters; 1, one blister on 1 to 2 leaves; 2, multiple blisters on 1 to 2 leaves; 3, multiple blister on multiple leaves and or break through to the upper leaf surface; 4, swollen leaf petiole; 5, hypertrophy, i.e. profuse sporulation on youngest leaves, swollen petioles. Numbers followed by a different letter differ significantly. A, The logistic regression analysis produced a different lsd for each comparison so the lsd's were not presented. [1], Refer to Chapter 9. Values in the same column followed by the same letter were not significantly different.

4.3 Discussion

Generally weekly copper sprays or grower timed spray applications produced similar or better control of white blister on foliage and heads of broccoli than the spray applications based model predictions. The highest "Contribution to profit" of all the treatments varied between the Controls, the Weekly sprays, the Grower sprays and the Model-based spray applications (see Chapter 9). Whilst it may be tempting not to spray resistant varieties, this would increase the risk of the variety loosing any resistance it may have to *A. candida*. These trials were conducted during a period with extreme weather events, ranging from a 15 year drought to one of the wettest periods on record, which no doubt affected the epidemiology of a disease reliant on leaf wetness for infection. Modifications to the Brassica$_{spot}$™ model's spray threshold and the addition of a spore detection kit to enhance use of the infection model, may increase the model's efficacy and economic performance (Chapter 10).

The incidence of white blister on foliage of broccoli, cauliflower and Chinese cabbage was consistently high, but symptoms were generally lower or not present on heads. Anecdotally growers reported that broccoli varieties were more susceptible to white blister when grown out of their time slot and that the incidence of white blister on foliage may be high but this did not consistently translate to a high incidence of the disease on broccoli heads. As only young tissue is susceptible to infection, it is possible that when the buttons were formed (i) the microclimate weather conditions were not suitable for infection; (ii) *A. candida* zoospores were not present; or (iii) the foliage was more susceptible to infection than heads.

Comparison and use of the Brassica$_{spot}$I™ and Brassica$_{spot}$II™ models

The Brassica$_{spot}$I™, Brassica$_{spot}$II™ 5% and Brassica$_{spot}$II™ 50 % were compared. The Brassica$_{spot}$II™ 50% model produced an unacceptably high incidence of white blister on heads and its evaluation was discontinued. The Brassica$_{spot}$I™ and Brassica$_{spot}$II™ 5% models were compared for disease control in five trials (Trials Nos 1, 2, 5, 6 and 11). In three trials they were equivalent, in one trial the Brassica$_{spot}$I™ model was superior to the Brassica$_{spot}$II™ 5% model and visa versa in the other trial. Neither version of the model was economically more superior to the other.

The Brassica$_{spot}$I™ model is an infection model that predicts when microclimate conditions in the crop are conducive to the infection of plants by *A. candida*. If spores are not present, or a resistant variety is grown, infection may not take place. It appears that the Brassica$_{spot}$I™ model may over predict infection events. Conversely it may under predict infection events as in Trial No. 11. The Brassica$_{spot}$II™ 5% model appears to have been an attempt to introduce a sporulation component into the model. Similarly it also appears to have underestimated the "time to spray" or the "frequency of sprays" required to control white blister. In some cases this may be associated with the reliance of predictions on subjective data supplied from field observations. In Trial No. 11, spray predictions occurred in the middle of a sporulation period rather than at the beginning (Chapter 10). Sporulation of *A. candida* is difficult to predict, unlike downy mildew (Populer 1981). Consequently the evaluation of a lower spray threshold ("Disease Index" threshold) and the development of the in-field spore detection kit should enhance the model (Chapter 10).

The model was designed for use on seedlings with no symptoms of white blister at transplanting. Growers in Werribee South produce their own seedlings on-farm and generally there are symptoms of white blister on these transplants, a situation that does not enhance use of the model. In addition the model is an infection model that permits a low level of disease incidence later in the period of crop development, so planting infected seedlings shifts symptom formation to earlier in the period. Furthermore, some growers think that any white blister in the crop is unacceptable, even if it is not economical to treat.

Efficacy of the predictive models for white blister control on broccoli foliage
White blister was difficult to control on broccoli foliage. Fungicide spray applications timed according to the model reduced the incidence and severity of white blister on foliage but generally the Weekly and Grower timed sprays had higher efficacy. The latter spray programs did not differ from each other. Weekly sprays reduced the incidence of white blister on foliage by 17% to 50% and reduced severity by 43% to 64% (Trial Nos 1, 2 and 4). Grower timed fungicide applications produced the best control of incidence or severity of white blister on foliage in the three trials where it was tested (Trial Nos 6, 9 and 11). Incidence was reduced by up to 43% and severity by up to 80% (Trial No. 6). Fortunately, foliage is not marketed, only the heads, so a low disease incidence on foliage, unless it contributes inoculum for subsequent infections, is of less importance than disease on the heads.

Efficacy of the predictive model for white blister control on broccoli heads
The Brassica$_{spot}$™ models either showed:

(i) No significant difference in control of white blister on broccoli heads compared to Weekly or Grower spray regimes (Trials Nos. 1, 3 and 9); or
(ii) There was no disease on the heads (Trials Nos. 6 and 7); or
(iii) The model was less effective compared with Weekly sprays (Trial No. 2).

There were two previous trials which showed the Brassica$_{spot}$I™ model had the same or better efficacy for control of white blister and was more economical than weekly sprays. These were the Irrigation trial (Table 2.3) and Trial No. 9 (Tables 2.24 and 3.14) of VG04013 (Minchinton *et al* 2007). All of these trials were undertaken on the DPI trial site at Dairy Rd. Werribee, whereas all other trials were on grower properties. The irrigation trial used an estimated 250,000L of water applied over 15 weeks, whereas growers in Werribee South can apply around 500,000L of water to a crop of broccoli. It is possible that differences in the volume of water applied to the Werribee field site compared to the volume used for commercial crops, may have contributed to the difference in efficacy of the model. Interestingly, in the UK where the Brassica$_{spot}$™ models were developed, growers rely on rainfall to irrigate crops rather than overhead irrigation. Theoretically the UK cropping conditions would generate fewer periods of leaf wetness and would be less conducive to infection by *A. candida*.

Efficacy of the predictive model on Chinese cabbage
White blister on Chinese cabbage appears progressively, at a low severity, from older leaves to younger leaves. This suggests that microclimate conditions are consistently conducive for infection and spores are continually present. In Trial No. 5 the Brassica$_{spot}$II™ model treatment was sprayed 14 days before harvest, but spraying 14 days prior to harvest may co-incidentally have been the best phenological time to protect the four unfolding wrap leaves.

A later trial (Trial No. 10) tested the Brassica$_{spot}$I™ with a fungicide spray of Cabrio™ 14 days prior to harvest, but it was not possible to conduct the Brassica$_{spot}$II™ model as there were issues with the technology. Interestingly the single Cabrio™ spray had the greatest efficacy in the trial, which suggests phenological timing of fungicides is important especially as *A. candida* only infects young tissue. As the one spray 14 days prior to harvest did not completely control the disease, perhaps an additional spray three weeks prior to harvest may produce additional reductions in symptoms. It may be possible to achieve economical control of white blister on Chinese cabbage without using the model, which can be time consuming because it involves crop inspections and acquisition of a weather station.

Efficacy of the predictive model on cauliflower
The Brassica$_{spot}$I™ model was trialled in Tasmania on the cauliflower variety Discovery, which is moderately susceptible to the disease. White blister was difficult to control on the foliage of cauliflower (Trial No. 8), but the heads were not infected, possibly because the wrap leaves provided some protection. The model predicted two sprays, one which was near

harvest. However both were unnecessary because no blisters developed on the heads, even in the unsprayed Control treatment and even when there were numerous potential infection periods, indicating that spores were not present. It is possible the Brassica$_{spot}$I™ model may over predict spray events on cauliflower because it is only based on an infection model. Although the disease was not a problem in Tasmania, it was reportedly an issue on cauliflower heads in the Werribee South cropping area during the wet conditions of 2010.

Number of sprays

One of the aims of using the models was to reduce the number of weekly fungicide sprays by aligning the timing of spray applications with the activity of the pathogen. Both versions of the Brassica$_{spot}$™ model reduced the number of weekly sprays of a copper based fungicide. In Trial No. 1 the number of weekly sprays were reduced from 12 to one or two; in Trial No. 2 they were reduced from 13 to two or three; in Trial No. 3 there was a reduction from seven to two sprays; in Trial No. 4, 11 sprays were reduced to two; and in Trial No. 5, five weekly sprays were reduced to one. Some growers, however, also reduced the number of fungicide sprays they applied to crops, especially when the disease pressure was low. In Trial No. 6 the grower applied three fungicide sprays and the models predicted two or three sprays; in Trial No. 8 the grower did not spray whilst the model predicted two spays; and in Trial No. 9 the grower applied two sprays whilst the model predicted three sprays. More recently, the acceptability of models may need to be based on a similar number of fungicide sprays to that of the grower, but the model sprays will need to be timed more accurately to control pathogens than sprays in the grower's program. A comparison of the "contribution to profit" between weekly sprays and sprays based on model predictions was variable (Chapter 9).

Resistant varieties

Previous work (Minchinton *et al*. 2007) showed the Brassica$_{spot}$I™ model was less effective than weekly sprays for the control of white blister on resistant broccoli varieties. A possible explanation was that the low incidence of disease has little scope for further reduction. As this corresponds to only a small potential increase in yield, there is no justification for incurring the expense of purchasing a weather station and the licence for the model.

Varietial resistance or dry environmental conditions may account for the lack of, or the low incidence of white blister observed on broccoli heads of Tyson, Rhumba and Viper early in the project (Trials 3, 4 and 7). Later in the project when conditions were extremely wet, Viper was very susceptible to white blister and appeared to have lost its resistance (Trial 11). Blisters on Viper were surrounded by brown haloes, similar to the symptoms observed on Belatar, which has tolerance to white blister (Minchinton *et al.* 2007). Growers now are looking for a replacement for Viper because of its apparent loss of resistance. Although a newly introduced variety Kuba, is considered to be more tolerant to white blister than Viper, it does not have the same agronomic qualities a Viper. Broccoli varieties are sold with descriptions of "having strength against" white blister. It may be more informative if they were marketed with a list of resistance genes, similar to the DMR (downy mildew resistance) gene list of lettuce cultivar resistance to *B. lactucae*.

Post-harvest trial

The trial demonstrated that in principle white blister development could continue during post harvest storage, even on a "resistant" variety. The post harvest symptoms were small lesions and affected broccoli heads would probably be still acceptable for market under current conditions (March 2011). The trial demonstrated that post harvest treatment should be part of a complete white blister management package.

Efficacy of fungicide alternatives

White blister on broccoli foliage was not controlled by any of the selected fungicide alternatives: the detergent (Sodium lauryl sulphate), the biological control agents (*S. lyticus* and *B. subtilis*); the systemic acquired resistance promoting chemical (Bion™); nor the

adjuvants Agral™, Du-Wett™ and Designer™. On Chinese cabbage, neither Bion™ nor *B. subtilis* had efficacy for control of white blister (Petkowski pers. comm.).

On broccoli heads, nine sprays of *S. lyticus* had a similar efficacy for white blister control on broccoli heads to two fungicide sprays based on predictions of the Brassica$_{spot}$I™ model. The *S. lyticus* program reduced the incidence of white blister on heads by 59% compared with the unsprayed Control and was the third most economical treatment after the Weekly and Brassica$_{spot}$I™ model treatments (Trial No. 2). Interestingly, three sprays of *S. lyticus* had no efficacy for white blister control on broccoli heads in a Tasmanian trial (Hung Pung pers. comm.). Biological control agents may require weekly applications to produce significant control of the disease, similar to copper based protectant fungicides. Consequently the cost of these products, and their frequency of application and efficacy will need to be equivalent to that of copper based fungicides for the alternatives to be economical. The efficacy of the other biological control, *B. subtilis,* remains undetermined as no blister symptoms formed on broccoli heads in Trial No. 4.

Sodium lauryl sulphate reduced white blister on broccoli heads significantly, but only by 29% compared with the unsprayed control. It was not as effective as the fungicides or the disease predictive model in our trials. But anecdotally, a broccoli grower in a dryer region, Bathurst, reported good control of white blister with the detergent. Use of the agricultural preparation may have been more economical than the laboratory grade preparation used in our trials. A similar scenario was observed with its control of white blister on spinach in glasshouse conditions, where efficacy in reducing the incidence of disease was inconsistent when compared with the efficacy of fungicides (Irish *et al.* 2002). The efficacy of Bion™ and the adjuvants were undetermined due to either no, or low incidence of white blister on heads in the respective trials.

Copper fungicide

Weekly copper sprays generally gave good control of white blister on foliage and heads. Where they did not, either: (i) disease incidence was very low; (ii) weather conditions delayed spray applications and a spray was missed or (iii) harvest was delayed after spraying ceased. There was no difference in efficacy for white blister control between the grower spray program and the weekly copper treatments in Trial No. 11, but the copper treatments were more economical than the grower treatments. Although the weekly copper sprays caused some phytoxicity on foliage it was not considered to be detrimental to production at that time of the year. The situation may be different in winter, where the severity of copper phytotoxicity could be higher.

General conclusion

Under conditions of high disease pressure, such as high rainfall, either weekly copper sprays with a wetting agent such as Agral or weekly sprays of alternating systemic and protectant fungicides are necessary to control white blister on broccoli heads. Copper based sprays should be avoided during the colder months because of the higher risk of phytotoxicity. When disease pressure is low, such as during a drought or when overhead irrigation is reduced, either version of the Brassica$_{spot}$™ model can be used to control white blister and reduce fungicide use in comparison with weekly spray applications. The efficacy of the both versions of the model could be improved by:

1. Adding a sporulation component to the model, in the form of an “in-field” spore test kit to make the prediction system more robust.
2. Using the model up to expected button emergence and then changing to weekly fungicide sprays to provide protection when buttons develop into heads.
3. Reducing the “Disease Index” spray threshold (line) from 1 to 0.75 or 0.50. This would trigger an earlier spray that should coincide with early lesion sporulation observed on trap plants, instead of the current “maximum sporulation” (see Chapter 10). This should allow the use of protectant fungicides in Brassica$_{spot}$II™ model spray

programs instead of more expensive systemic fungicides, an aim of the model's original design.

With white blister on Chinese cabbage, disease progression data from crop inspections and environmental data indicated that the phenological age of the crop was important for control of white blister on harvested plant parts. As *A. candida* only infects young tissue, the same would apply to broccoli heads. Although spraying at button stage in the 'Best Bets' program did not produce any control benefits in this study or in the studies of Minchinton *et al.* (2007), it did have economic benefits in one of our trials. Management of white blister on broccoli heads would be enhanced if the period of head susceptibility to infection was known. Further studies of broccoli button development are warranted to determine the phenological time when buttons should be sprayed to provide the most effective protection of heads from white blister infection.

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Chapter 5

Effects of temperature on sporulation aspects of *B. lactucae*

Summary

Lettuce varieties with no downy mildew resistance (DMR genes) were inoculated with sporangiospores of *B. lactucae* at 14ºC, 16ºC, 18ºC and 22ºC. Infection and sporulation occurred at all temperatures, except 22ºC. At 22ºC aborted sporangiophores were evident. This data was incorporated into an in-house model for downy mildew on lettuce, which was trialled in Chapter 6.

5.1 Introduction

For many foliar pathogens, including *B. lactucae*, the infection process is dependant on temperature and the duration of leaf surface wetness or by the humidity of most environments. Using detailed studies of 52 foliar pathogens described in published reports, Magarey *et al.* (2005) formulated a generic model that could predict an infection event. In brief, temperature limits, leaf wetness durations and responses to interruptions in leaf wetness were assessed and analysed. In reviewing the literature up to that stage, it was found that fewer than 100 economically important pathogens had controlled temperature and wetness data necessary for the generation of a generic model. Depending on the pathogen involved, the infection process was sensitive, moderately sensitive or insensitive to leaf wetness interruptions. By either calculating or deriving parameters such as T_{min}, T_{opt}, T_{max} (minimum, maximum and optimal infection temperatures) from the various papers, as well as W_{min} and W_{max} (minimum and maximum wetness periods for infection), they were able to produce a simple mathematical model which fitted most of the published data.

Extensive studies of the life cycle of *B. lactucae* have been required to create an effective disease-predictive model. Generally disease predictive models or decision support tools require the input of meteorological data which is historical, rather than forecasted. The most reliable parameter forecasted by a Meteorological Bureau is temperature. It plays an important role in promoting or impeding spore germination, infection and sporulation. Sporangiospores of *B. lactucae* germinate from 5ºC to 20ºC. Germination is only 20% at 25ºC and there is none at 30ºC (Scherm and van Bruggen 1993). Infection of lettuce from germinated sporangiospores was observed over the temperature range of 2ºC to 20ºC but not above 25ºC (Scherm and van Bruggen 1993, Powlesland 1954). In a "Fungicide spray advisory" developed by Wu *et al.* (2002), temperature requirements for infection were defined as 20ºC for 3 hours (h) of leaf wetness followed by a post penetration period with 22ºC and 4 h of leaf wetness. The latest version of this predictive model is used primarily in California (Wu *et al.* 2005).

Temperature has a profound effect on the life cycle of *B. lactucae*. Lettuce leaves can remain wet for many hours during critical morning sporulation and infection periods because of overhead irrigation and especially where lettuce production is close to the coast (eg Werribee South). If the upper temperature limits of the local isolates of *B. lactucae* are the same as those overseas, it may be possible to use the Bureau of Meteorology daily or weekly forecasted temperature data to estimate the potential behaviour of *B. lactucae* for infection or sporulation in the field, especially during the wetter seasons of autumn, winter and spring.

This chapter reports on the upper temperature limits for sporulation of *B. lactucae* on seedlings of lettuce cultivars with varying sensitivities to *B. lactucae* inoculated with sporangiospores collected locally. Additionally the upper temperature limits for sporulation were incorporated into the in-house Lettuce DownCast model (Chapter 6).

5.2 Materials and methods

5.2.1 Selection of cultivars

Lettuce cultivars with varying sensitivities to downy mildew trialled in this study are listed in Table 5.1, along with their DMR gene designations.

Table 5.1 Origin of lettuce cultivars used in temperature studies.

Lettuce cultivar	Type	Seed company	DMR
Alligator	Cos	LeFroy Valley Seeds	1-16, 19, 21, 23
El Toro	Iceberg	Terranova Seeds	none
Fortune	Iceberg	Terranova Seeds	none
Grand Slam	Iceberg	Nunheim Seeds	18
Silverado	Cos	SPS Seeds	1-16, 21
Weston	Iceberg	Nunheim Seeds	none

5.2.2 Collection of *B. lactucae* sporangiospores

Sporangiospores of *B. lactucae* were collected from commercial field grown Iceberg lettuce in peak infection periods when required. Individual diseased leaves were sprayed with distilled water to dislodge old sporangiospores and then incubated in the dark at 14°C for 24-48 h to induce fresh sporulation. The sporulating leaves were then either immediately used for infecting seedlings, or stored at -20°C for no more than 3 months.

5.2.3 Growth of seedlings

Cultivars Weston and Grand Slam were supplied as pelleted seed coated in fertilisers. None of the cultivar seeds were treated with fungicides. Eleven lettuce seeds were germinated inside sealed round 600 mL containers on potting mix (Debco, Tyabb, Victoria) at 14 °C with a 12 h day and 12 h night cycle. The potting mix, pasteurised for 3 h at 70°C, had the following composition: 95% composted pine bark, coarse (5–10 mm) and medium (<5 mm) particle sizes; 5% sand; 1 kg/m^3 Saturaid®. Using a modification of a method supplied by Enza Zadec (Narromine, NSW), seedlings were grown for no more than 10 days before inoculation, depending on the cultivar.

5.2.4 Inoculation of seedlings

Leaf sections containing fresh sporangiospores were placed in a 50 mL centrifuge tube and suspended in 40 mL of distilled water. Tubes were vigorously shaken for 5 minutes and the suspension was placed in a universal 28 mL bottle. A spray trigger was attached to the bottle and three seedling containers were sprayed to saturation before sealing the containers to preserve a high humidity. Three containers were incubated at 14°C, 16°C, 18°C and 22°C with a 12 h day and 12 h night cycle and checked for the presence of lettuce downy mildew after 10-14 days. The trial was repeated three times with the cultivar Weston.

5.2.5 Assessing downy mildew on seedlings

Seedlings were checked after 10 days, and every day after that for a total of 21 days until visible sporulation was evident. Individual seedlings were scored for presence or absence of visible sporulation.

5.3 Results

Of all the cultivars trialled, Weston was the most consistent in terms of growth and infection (results not shown) and was used for the temperature studies. Weston also had the advantage

of being supplied as a 'pellets' allowing an accurate count of seeds to be dispensed and assessed. When data from three experiments were averaged, there was little difference in the number of seedlings showing active sporulation at either 14°C or 16°C, but there was a sharp decline in sporulation at 18°C, and no sporulation was evident at 22°C, only colonisation (Fig 5.1). Closer examination of lettuce leaves at 22°C indicated that the leaves had been colonised by *B. lactucae*, but sporulation had not occurred, in comparison with leaves at 14°C, 16°C and 18°C (Figs 5.2-5.4). To encourage sporulation, the containers at 22°C were transferred to 14°C, which was within the optimal temperature range for sporulation (Tchervenivanova 1995). After a further 2 weeks, limited sporulation (approximately 1 plant in 60) was evident (Fig 5.5).

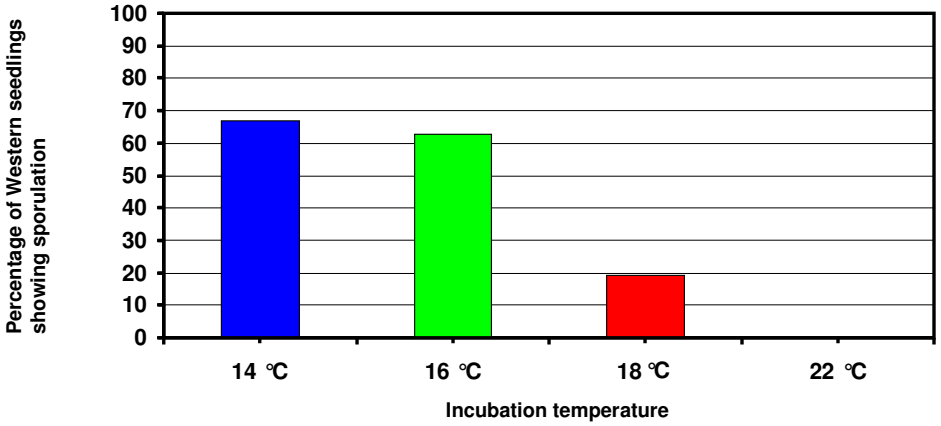
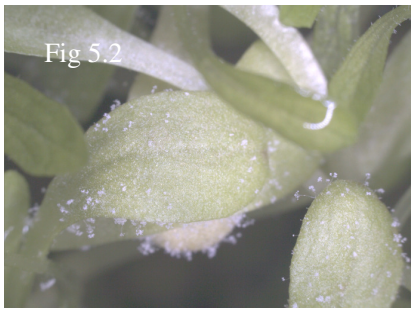
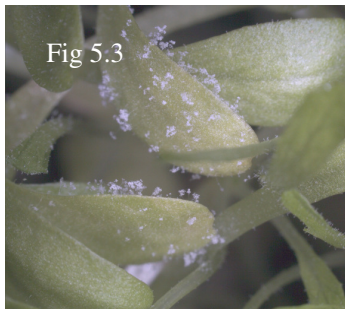
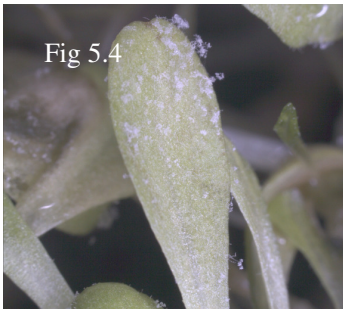


Fig 5.14 Percentage of cultivar Weston seedlings showing *B. lactucae* sporulation over increasing temperatures.







Figs 5.2, 5.3, 5.4 Profuse sporulation on seedlings of cultivar Weston at 14°C, 16°C and 18°C, respectively.

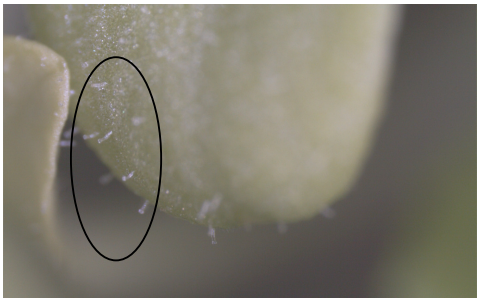


Fig 5.5 Seedlings of cultivar Weston at 22°C. Circle indicated shows *B. lactucae* colonisation, but not sporulation.

5.4 Discussion

The temperature limits of sporulation have been explored by several researchers. Tchervenivanova (1995) found that the optimal conditions for sporulation were between 10-15°C after 10 h leaf wetness and that very few sporangiospores were present at 25°C, regardless of the leaf wetness period. This corresponds well with reports by other workers such as Wu *et al.* 2002 who found that sporulation was suppressed at higher temperatures, with an upper limit of about 20-22°C when there was a wetness period after sunrise of about 3 h followed by a dry period of about 4 h. Local isolates of *B. lactucae* appear to have an upper temperature limit for sporulation similar to overseas isolates. The upper limit of 22°C could be used by growers. As a "rule of thumb", spray applications would not be required on days where 22°C was reached, especially during critical periods for sporulation and infection up to 14:00 h, as reported by Kushalappa (2001). Further studies are needed to determine how long a temperature of 22°C needs to occur to inhibit sporulation and infection.

The temperature limits were integrated into an in-house model that also used data on leaf wetness periods immediately after sunrise. Along with other considerations, use of the upper limit of 22°C confirmed in this study, is a good starting point for the development of a model for infection of *B. lactucae*.

The following parameters were used in the disease predictive model for lettuce downy mildew.

B. lactucae sporulation occurs if:

1) Night-time temperature is between 4-20°C (Powlesland 1953, Wu *et al.* 2002).
2) Night RH is ≥ 90% and leaf wetness is ≥ 3 h (Kushalappa 2001).

B. lactucae infection occurs if:

3) Leaf wetness period is ≥ 3 h after sunrise (Scherm and van Bruggen 1995).
4) Day-time temperature during the leaf wetness period is ≤ 20°C.
5) Temperature of the post-wetness period (≥ 4 hours), i.e. dry leaf temperature, is ≤ 22°C (Wu *et al.* 2002).

An addendum to (5) was: Midday temperature ≤ 22 °C [10:00 am -12:00 pm] (Wu *et al.* 2005). In addition to this, it was assumed that when lettuce downy mildew was present in the field, the conditions for infection only needed to be met, since there is evidence that viable sporangiospores can be carried over from the previous day (Wu *et al.* 2000, Le *et al.* 2008). The results of trials using this model as well as BREMCAST™ are reported in Chapter 4.

5.5 References

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Chapter 6

Efficacy of two disease predictive models for controlling downy mildew on lettuce compared with systemic, protectant and fungicide alternative

Summary

None of the three methods for timing fungicide sprays to control downy mildew on lettuce was consistently superior, but there was a trend for the BREMCAST model to reduce fungicide applications and produce a similar or better efficacy of disease control than Farm Best Practice and Lettuce DownCast. Generaly Lettuce DownCast was generally similar to Farm Best Practice. Under summer conditions BREMCAST reduced up to four sprays without significantly reducing yield of processing or fresh market lettuce. Bion had amazing efficacy and superior economics when it was effective, but it was unreliable because of its variable nature and the potential for phytotoxicity, was unreliable. More control of downy mildew was achieved when systemic fungicides were used late in the crop's life, which suggests they may have had some curative effect on *B. lactucae* infecting heads and wrap leaves.

6.1 Introduction

Downy mildew of lettuce (*Lactucae sativa* L.), caused by *Bremia lactucae* Regal, is a perennial problem in Iceberg, Cos and coloured leaf lettuce crops, especially in Victoria. Extensive research into downy mildew of lettuce has shown that the disease varies in severity from year to year, and develops erratically within seasons (Scherm and Bruggen 1994a, Kushalappa 2001). In Australia it is usually associated with cool wet weather in spring and autumn (Wicks *et al.* 1993). Growing resistant cultivars, calendar fungicide sprays and disease predictive models have been used to control downy mildew. Some models and delivery systems currently available are BREMCAST (Kushalappa 2001), PlanPlus (Dacom, Netherlands) Modell-analysis (Sweden, AS Forsberg in Nordskog *et al.* 2006) and Adcon Telemetry USA (www.adcon).

Resistant cultivars are used extensively, but only provide temporary control, as *B. lactucae* has the ability to rapidly overcome this resistance (Crute and Johnson 1976, Illot *et al.* 1989). The DMR number (Downy Mildew Resistance gene content) is known for all cultivars, but even resistant lines require spraying to avoid *B. lactucae* establishing on the cultivar and resistance breaking down.

Fungicide control relies on weekly sprays of contact or systemic fungicides. Systemic fungicides can provide good control, but are expensive and their overuse can lead to fungicide resistance in *B. lactucae* (Hovius *et al.* 2007; Crute 1987; Raid and Datnoff 1990; Cobelli *et al.* 1998; Brown *et al.* 2004). Epidemiological studies have indicated that conditions for sporulation and infection are very specific and could be used to develop a decision support system, suggesting weekly fungicide sprays may be unnecessary under certain conditions (Scherm and van Bruggen 1994a, 1994b).

The "Fungicide spray advisory", a type of disease predictive model, for downy mildew on lettuce developed in the USA is based on sporulation and infection data from studies of Scherm and van Bruggen (1993a, 1994a, 1994b, 1995; Scherm *et al.* 1995). Basically the

"Fungicide spray advisory" requires morning leaf wetness until 10.00 h within a temperature range of 5ºC to 25ºC preceded by a night leaf wetness period with temperatures ranging from 5ºC to 22ºC. Evaluations in the US indicated that while its use could reduce the number of fungicide sprays by one or two (67 %), it was inconsistent (Scherm *et al.* 1995). A dew simulation model (Scherm and van Bruggen 1993b) was omitted because of inaccuracies in leaf wetness forecasts (Wu *et al.* 2001a), while a degree-hours model had little advantage due to fluctuating temperatures (Scherm and van Bruggen 1994b). This spray advisory "model" was improved by addition of a temperature threshold of 20 ºC for a 3 hr wet period after sunrise and a threshold of 22ºC for a subsequent 4 hr post penetration period (2.00 pm) and validated by weather data and disease surveys (Wu *et al.* 2001b, 2002, 2005).

In Canada, the fungicide advisory of Scherm *et al*. (1995) was modified by Philion *et al.* (1998) to include degree days. In field evaluations use of the model reduced the number of fungicide sprays by one to four with only adequate disease control (Philion *et al.* 1998; Hovius *et al.* 2007). Kushalappa (2001) developed the model further to include continuous leaf wetness for 3-5 hrs preceding dawn until 10:00 h; presence or absence of disease in the field; sporulation criteria (duration of leaf wetness, average night RH and average night temperature) and infection criteria (duration of the morning leaf wetness and average temperature during the morning leaf wetness period). It had 84% accuracy for predicting sporulation and infection periods (Kushalappa 2001) and its use reduced the number of sprays by one to two, whilst improving control of downy mildew on lettuce above that of conventional sprays (McDonald *et al.* 2000).

The efficacy and economics of controlling downy mildew on lettuce was compared for two disease predictive models, Farm Best Practice of individual growers and two fungicide alternatives in five field trials undertaken in Victoria during 2009 and 2010. The disease predictive models evaluated were BREMCAST (Kushalappa 2001) and an in-house adaptation of DownCast (Jesperson and Sutton 1987) with modifications based on the work of Scherm *et al*. (1995) and Wu *et al*. (2001b, 2002 and 2005). The in-house model is referred to as Lettuce DownCast.

6.2 Materials and Methods

Field trials were undertaken in commercial crops of Iceberg, Cos and Coloured leaf lettuce varieties grown at Rosebud, Werribee South, Cranbourne and Skye, Victoria on raised beds under overhead irrigation. All crops were maintained by the growers.

6.2.1 Disease-predictive models

6.2.1.1 Lettuce DownCast

Lettuce DownCastI was constructed in-house and based on the work of authors listed in Table 6.1. It was evaluated in Trials 1, 2, 3 and 4 (see 6.2.5 to 6.2.8). It was further modified to Lettuce DownCastII based on the work of Wu *et al.* (2005), in an attempt to better reflect the optimal conditions for downy mildew infection, and evaluated in Trial 5 (see 6.2.9). There are two sporulation factors and three infection factors for the Lettuce DownCastI model, while Lettuce DownCastII has an additional infection factor. Both sporulation factors must be satisfied for sporulation to occur and all infection factors must be satisfied for infection to occur. Consequently for symptoms to develop, all sporulation and infection factors must be deemed to occur on the same day.

Table 6.1 Definition of factors for the in-house Lettuce Downcast models.

Factor	Definition of parameter	Reference	Lettuce DownCast model
Sporulation #1	Basis: Sporulation occurs at night (pre-dawn) when the following conditions are satisfied: • Temperature during the previous night must be within the range 4–20°C • If this range is exceeded, sporulation will not occur • Night range from Last light (previous day) to First light (current day)	Jesperson and Sutton 1987, Fitzgerald and O'Brien 1994	I, II
Sporulation #2	Basis: Sporulation occurs at night (pre-dawn) when the following conditions are satisfied: • Night RH of 90% and leaf wetness period of at least 3 hours	Kushalappa 2001	I, II
Infection #1	Basis: Leaf wetness period of at least 3 hours after sunrise	Scherm *et al.* 1995	I, II
Infection #2	Basis: Daytime temperature during the leaf wetness period must not exceed 20°C	Wu *et al* . 2001 (a,b)	I, II
Infection #3	Basis: Temperature of post-wetness period i.e. dry leaf temperature (of at least 4 hours) must not exceed 22°C	Wu *et al.* 2002	I, II
Infection #4	Basis: Temperatures between 100:00am and 2.00pm must not exceed 22°C	Wu *et al* . 2005	II

6.2.1.2 BREMCAST

BREMCAST was obtained from the original author (Kushalappa 2001). In comparison with Lettuce DownCast, BREMCAST has two additional parameters compared to determine when to spray: the length of sunlight period and the presence or absence of infection in the field. BREMCAST calculates a Sporulation Value and an Infection Value which are used to calculate a Disease Severity Value (DSV). The DSV has a scale of zero to five where 0 = no risk; 1 = light disease; 2 and 3 = moderate; 4 and 5 = severe. For the purpose of this work sprays were applied when the model predicted a DSV of 4 or 5. The inputs required for the BREMCAST model are:

1. Number of leaf wetness hours at night
2. Average night relative humidity
3. Average night temperature
4. Number of hours of morning leaf wetness
5. Average morning leaf wetness
6. Number of hours of solar radiation
7. Presence or absence of an inoculum source in the field

BREMCAST was evaluated in Trials 2, 3, 4 and 5 alongside the in-house Lettuce DownCastI and Lettuce DownCastII models.

6.2.2 Weather station

A ModelT weather station (Western Electronics Design, Loxton, SA) was placed in the middle of an irrigation line of the lettuce crop and adjacent to an unsprayed control plot. The station recorded average leaf wetness, temperature, relative humidity and total rainfall at 30 min. intervals. The leaf wetness sensor was placed within the lettuce crop at a 45 degree angle and the height was adjusted as the crop grew. Data from the weather station was imported into both disease-predictive models.

6.2.3 Application of chemicals

All chemicals in all trials were applied using a Silcan Selectra 12v knapsack sprayer [Silvan Pumps and Sprayers (Aus) Pty. Ltd.] with a triple boom assembly and a selection of TeeJet nozzles depending on availability and suitability at 30 psi by a Silvan Selectra 12v. Teejet nozzles used during these trials included TX-VK-8, TX-VK-12 (polymer, hollow cone) and TP8003E (polymer, fan) Fungicides were applied at a rate of 500L/ha over the life of the crop. A full list of all chemicals used is shown in Table 6.2. In some cases, chemicals were applied as tank mixes.

Table 6.2 Chemical treatments and application rates used in the field trials.

Trade name	Active	Company	Rate	Trial No.
Acrobat®	dimethomorph (500 g/kg)	Nufarm	360 g/ha	1, 2, 4, 5
Agral™	nonyl phenol ethylene oxide condensate (600g/L)	Syngenta	0.13%	4
Agriphos®	phosphorous acid (600g/L)	Agrichem	850 mL/ha	2, 4
Amistar 250EC®	azoxystrobin (500g/kg)	Syngenta	300 g/ha	1
Antracol®	propineb (700 g/kg)	Bayer	2 kg/ha	1, 3, 5
Fulzyme™	Bacillus subtilis (10^6 spores/mL)	confidential [1]	3–5 mL/L	1
Bion 50WG (half) ™	acibenzolar-s-methyl (500g/L)	Syngenta	25 g/ha	2
Bion 50WG®	acibenzolar-s-methyl (500g/L)	Syngenta	50 g/ha	1
Bozyl™	confidential [1]	confidential [1]	400 g/100L	2
Du-wett™	trisiloxane ethoxylate, non-ionic (500g/L)	Nufarm	300 mL/ha	1, 2
FoliCal Plus	fulvic acids	Omnia	7L/700L/ha	5
Pencozeb®	mancozeb (750 g/kg)	Nufarm	1600 g/ha	1, 4, 5
Polyram®	metiram (700g/kg)	Nufarm	2.2 kg/ha	2
Revus®1	mandipropamid (250 g/L)	Syngenta	600 mL/ha	4, 5
Seasol™	Seaweed extract	Seasol Int	833 mL/ha	2
Sprayphos 620 ™	phosphorous acid (620g/L)	Spraygro	170ml/100L	5
Synetrol™	vegetable oil (>60%), polyethoxylated oil (<10%)	OCP	300 mL/100L	2, 4
Tri-Base Blue®	copper (190g/L)	Nufarm	1400 mL/ha	1, 2, 5

[1], currently under development.

6.2.3.3 Timing of applications.

Spray applications based on the predictions of the Lettuce DownCastI or II models were made when the microclimate within the crop indicated that conditions were conducive to both sporulation and infection by *B. lactucae*. Spray applications based on the predictions of the BREMCAST model were made when it indicated a Disease Severity Value (DSV) of 4 or 5.

Fungicide sprays for the Farm Best Practice treatment were applied at the grower's direction. When weather conditions permitted, spray applications based on model predictions were performed on the same day of the prediction, but if conditions were not conducive for spraying, they were performed as close to the model predicted spray event as possible. For both models, it was assumed that the chemical regime applied in the model plots afforded protection against *B. lactucae* for a period of seven days. If the model predicted a spray within that seven-day period, spraying was delayed until the next predicted event.

Bacillus subtilis was sprayed in Trial 1 and the application rate was increased from 3 mL/L to 5 mL/L as of week seven in consultation with the manufacturer. Bozyl, a post harvest surface sterilizing agent was applied, weekly where possible, in Trial 2. Bion plots were sprayed at the first sign of downy mildew in the field, and twice more in Trial 1, at the rate recommended in the Unites States (50g/ha). The rate for Trials 2 and 4 was half that of Trial 1, and was applied twice at the first sign of downy mildew in the field. In Trial 5 it was applied at half the US rate, but when the canopy was starting to close up and before downy mildew symptoms were observed in the field.

6.2.4 Assessment

Incidence on whole plants or heads was recorded as presence or absence of downy mildew on the whole plant or on the head. Incidence on wrap leaves was recorded as the presence or absence of downy mildew on the four wrap leaves surrounding the heads. In Trial 4 severity was recorded as a Severity Index on a scale of 0 to 3 where 0 indicated no disease and 3 indicated severe disease with necrotic lesions and profuse sporulation. In Trial No 5 a yield estimate for fresh market production was made by recording weight of each head and 4 wrap leaves and width of the head at its widest point.

6.2.5 Trial 1 on Iceberg lettuce cultivar Marksman at Boneo from March to May 2009

Trial 1 on Iceberg lettuce cultivar Marksman (Terranova DMR Aus1) was located at Brown's Road in Boneo, Victoria. Seedlings were obtained by the grower from a commercial nursery and transplanted at two weeks of age, and spaced 20 cm apart in four rows on the 19th March 2009. Plant density was 64,220 plants/ha. The trial was a randomized block design with eight replicated blocks laid out on four beds, so there were two blocks per bed. Each block contained five plots representing one of five treatments, randomly allocated to a length of bed 8 m long by 1.62 m wide. Each plot contained approximately 90 plants. The treatments are listed in Table 6.3.

Table 6.3 Trial 1 treatment schedule for Trial 1 on Iceberg lettuce cultivar Marksman at Boneo during autumn 2009.

	Date of spray application/week/dap										
Treatment	19/03/2009	23/03/2009	31/03/2009	7/04/2009	14/04/2009	21/04/2009	28/04/2009	5/4/2009	12/5/2009	19/5/2009	25/05/2009
	0	1	2	3	4	5	6	7	8	9	10
	Planted	4 dap	12 dap	19 dap	26 dap	33 dap	40 dap	47 dap	54 dap	61 dap	67 dap
Control	-	-	-	-	-	-	-	-	-	-	
B. subtilis	+	+	+	+	+	+	+	+	+	+	-
Bion®	-	-	+	+	-	-	+	-	-	-	-
Lettuce DownCastI	-	AP	AP	AP	AP	CTD	CTD	CTD	CTD	CTD	-
Farm Best Practice	AP	AP	AP	AP	AP	CTD	CTD	CTD	CTD	CTD	-

Dap, days after planting; Control, unsprayed; A, Acrobat; C, Antracol; P, Pencozeb; T TriBase Blue; D, Du-wett; Lettuce DownCastI, sprays based on predictions of the Lettuce DownCast ModelI; + chemical applied; -, no chemical application.

The trial was assessed at week 6 and at week 10 (harvest). The first assessment recorded the incidence of downy mildew on at least 60 whole plants per plot, while at harvest 38–45 plants per plot were assessed for incidence on whole plants, heads and wrap leaves. At week 6 data were analysed with analysis of variance (ANOVA), while at week 10 data were analysed with a Generalised Linear Mixed Model (GLMM).

6.2.6 Trial 2 on Cos lettuce cultivar Amadeus at Devon Meadows from August to October 2009

This trial site was located in North Road, Devon Meadows, Victoria. The *B. lactucae* Cos lettuce cultivar Amadeus (Rijk Zwaan DMR 1-17, 21, 23) was transplanted on 24th August 2009. Seedlings were obtained by the grower from a commercial nursery. The trial was a randomized block design consisting of six blocks, each with six plots, laid out on six beds. The six treatments were randomly allocated to the plots in each replicated block. Plot dimensions were 6m x 1.62m and plants were transplanted at four rows per bed with approximately 100 plants per plot, spaced 10cm apart.

The treatments in Trial 2 are listed in Table 6.4. Fungicide sprays applied to the BREMCAST model plots and the Lettuce DownCast model plots were based on sprays applied to the Farm Best Practice treatments. The trial was sprayed for aphids on 7th September 2009 with Movento 240SC g/L (spirotetramat) at 200 mL/ha and 500 L/ha. Octave (prochloras) was sprayed weekly at the recommended rate (465 g/L) for anthracnose control, weekly commencing on the 5th October 2009. This trial was assessed at weeks 8 and 10 for the incidence of downy mildew on 60 whole plants per plot. A generalised linear mixed model (GLMM) was used to analyse the data because of the binomial nature of the data and the different numbers of plants assessed in the plots.

Table 6.4 Trial 2 treatment schedule for Trial 2 on Cos lettuce cultivar Amadeus at Devon Meadows during spring 2009.

Treatment	Date of spray application/week/dap							
	31/08/2009	07/09/2009	14/09/2009	21/09/2009	28/09/2009	05/10/2009	12/10/2009	19/10/2009
	1	2	3	4	5	6	7	8
	7 dap	14 dap	21 dap	28 dap	35 dap	42 dap	49 dap	56 dap
Control	-	-	-	-	-	-	-	-
Bozyl	+	+	+	-	+	+	+	+
Bion (half)	-	-	-	-	+	+	-	-
Lettuce DownCastI	AcPS	-	-	AmSnS	TDAgSn	AcPS	AmSnS	-
BREMCAST	AcPs	AmSnS	TDAgSn	-	AcPS	-	AmSnS	-
Farm Best Practice	AcPs	AmSnS	TDAgSn	-	AcPS	AmSnS	TDAgSn	-

Dap, days after planting; Control, unsprayed; Ac, Acrobat; Am, Amistar; Ag, Agriphos; D, Du-wett; P, Polyram; S, Seasol; Sn, Synertrol; T, TriBase Blue; + chemical applied; -, no chemical application.

6.2.7 Trial 3 on Red Oak Leaf lettuce cultivar Prunai at Skye, December to January 2009-2010

Trial 3 was located at Taylor's Road, Skye, Victoria. The Red Oak Leaf lettuce cv Prunai (Rijk Zwaan DMR 1-27) was transplanted into three rows per bed and spaced 25 cm apart, on 4th December 2009. The trial was a randomized block design of eight replicated blocks with four plots representing four treatments laid out across four beds. Plot dimensions were 6m long x 1.62m wide and contained approximately 45 plants per plot. The treatments and spray schedules are listed in Table 6.5. This trial was assessed at week 7 for the incidence of downy mildew on at least 30 whole plants per plot. No downy mildew was observed in the trial at harvest, consequently no analysis was undertaken.

Table 6.5 Treatment schedule for Trial 3 on Red Oak Leaf cv Prunai at Skye Boneo during summer 2010.

Treatment	Date of spray application/week/dap					
	9/12/2009	14/12/2009	21/12/2009	28/12/2009	4/01/2010	11/01/2010
	1	2	3	4	5	6
	5 dap	10 dap	17 dap	24 dap	31 dap	38 dap
Control	-	-	-	-	-	-
Lettuce DownCastI	+	-	-	-	-	-
BREMCAST	+ (10/12)	-	-	-	-	-
Farm Best Practice	-	-	-	-	+ (2/1)	-

Dap, days after planting; +, Antracol fungicide applied; -, no fungicide application; numbers in brackets refer to date of application.

6.2.8 Trial 4 on Iceberg lettuce cultivars Kong and Marksman at Werribee South, March to April 2010

Trial 4 was located at Cayley's Road in Werribee South, Victoria. Iceberg lettuce cultivars Kong (SPS, DMR 1-26) and Marksman (Terranova, DMR Aus1) were transplanted at four rows per bed, spaced 33cm apart, in adjacent trial sites which were separated by an irrigation line, on 5th March 2010. Seedlings had been produced by a commercial nursery. The trial was a randomized block design of six replicated blocks with five plots representing five treatments laid out on three beds, with each bed containing two blocks. Plot dimensions were 6m long by 1.62m wide. Each plot contained approximately 100 plants. The treatments are listed in Table 6.6. Although cv. Kong showed no signs of infection in the unsprayed control plots, Bion was applied in error, and thus was applied twice on cv. Kong to conform to the treatment on the sensitive cv. Marksman.

The trial was assessed at week 6 for the incidence of downy mildew on 10 plants per plot. These data were not analysed because it was clear that Farm Best Practice, BREMCAST and Lettuce DownCast were effective early in the trial. At harvest, week 8, the trial was assessed for incidence of downy mildew on whole plants, heads, and four wrap leaves around the head, on 20 to 40 plants per plot. Due to the high incidence of downy mildew in the trial on the susceptible cultivar Marksman, a severity assessment was also performed. A severity index on a scale of 0–3, with 0 indicating no infection, and 3 indicating severe infection with necrotic lesions and profuse sporulation was calculated. At week 8, 20 to 40 plants per plot were assessed. Data were analysed by ANOVA.

Table 6.6 Treatment schedule for the Trial 4 on Iceberg lettuce cultivars Marksman and Kong at Werribee South during autumn 2010.

	Date of spray application/week/dap						
Treatment	10/03/2010	15/03/2010	23/03/2011	31/03/2010	05/04/2010	12/04/2010	22/04/2010
	1	2	3	4	5	6	7
	5 dap	10 dap	18 dap	26 dap	31 dap	38 dap	48 dap
Control	-	-	-	-	-	-	-
Lettuce DownCastI	AR	-	AgP	AR (1/4)	-	AgP (10/4)	AR
BREMCAST	-	-	R	-	AgP	R	-
Farm Best Practice	-	-	AgP	AcAgP	-	-	AgAn
Bion (half)	-	-	-	M	MK (8/4)	K (15/4)	-

Dap, days after planting; A, Agral; Ac, Acrobat; Ag, Agriphos; An, Antracol; R, Revus; P, Pencozeb; K, Kong: M, Marksman; -, no fungicides applied; -, no fungicide application; numbers in brackets refer to date of application.

6.2.9 Trial 5 on Iceberg lettuce cultivar Silverado at Boneo, November to December 2010

Trial 5 was located at Brown's Rd, Boneo, Victoria. Iceberg lettuce cultivar Silverado (SPS DMR 1-16, 21, 23) was transplanted at 3 rows per bed and spaced 30cm apart on 14th November 2010. A commercial nursery supplied the seedlings. The trial was a randomized block design of eight blocks with beds as blocks. Each bed contained eight plots, to which the eight treatments were randomly allocated. Plot dimensions were 6m long by 1.62 m wide and contained approximately 54 plants. On this site, there were approximately 64,220 plants per ha. The treatments are listed in Table 6.7. On 8th November 2010 the whole trial was inadvertently sprayed with Agriphos plus Pencozeb plus potassium nitrate.

At week 8, 40 to 50 plants per plot were assessed for incidence of downy mildew on whole plants and the data were analysed using a generalised linear mixed model (GLMM). On the 29th of December 2010, 15 plants per plots were assessed for disease incidence on whole plants, wrap leaves, heads and marketable yield for processing and fresh market. Marketable yield for processing was defined as no symptoms of downy mildew on heads. Marketable yield for fresh market was defined as no downy mildew on heads or wrap leaves and a minimum weight of 600g per head and a minimum heart width 144mm at the widest point. A generalised linear mixed model (GLMM) was used to analyse marketable heads and incidence of downy mildew on whole plants. Incidence of downy mildew on wrap leaves and the average yield per head in grams for each treatment at harvest, was analysed using ANOVA.

Table 6.7 Treatment schedule for Trial 5 on Iceberg lettuce cultivar Silverado at Boneo summer 2010.

Treatments	Date of spray application/week/dap							
	1	2	3	4	5	6	7	8
	8/11/10	15/11/10	24/11/10	1/12/11	9/12/10	13/12/10	18/12/10	25/12/2010
	4 dap	11 dap	20 dap	27 dap	35 dap	39 dap	44 dap	51 dap
Control (unsprayed)	PPnS	-	-	-	-	-	-	-
Bion (half)	AgPPn	-	-	+	+	-	-	-
Farm Best Practice	AgPPn	PS	AcP	AcP	AcP	AAnT	AAnT	-
Farm Best Practice + FoliCal Plus	AgPPn	F	F	AcP	F	AAnT	F	-
BREMCAST	AgPPn	-	-	AcP	-	-	AAnT	-
DownCastII	AgPPn	PS	AcP (26/11)	AcP (3/11)	-	-	AAnT	-
DownCast R	AgPPn	R	AcP (26/11)	-	-	-	R	-
DownCast BOM	AgPPn	PS(12/11)	AcP (26/11)	-	AcP	-	-	-

Dap, days after planting; A, Agral; Ag, Agriphos; Ac, Acrobat, An, Antracol; F, FoliCal Plus; P, Pencozeb; Pn, potassium nitrate; S; Sprayphos; T, TriBase Blue; + chemical applied; -, no chemical application; numbers in brackets refer to dates of application; DownCast R, DownCast model sprayed with Revus; DownCast BOM, DownCast model sprayed on Bureau of Meterology weather predictions.

6.3 Results

6.3.1 Trial 1 on Iceberg lettuce cultivar Marksman at Boneo from March to May 2009

Microclimate conditions were very conducive to downy mildew in the trial and the disease was first observed in a Control plot in week 2. In Control plots symptoms progressed from a single lesion at week 2 to over 90% incidence on whole plants by week 7. At week seven the Bion treatment was significantly better than all other treatments in relation to downy mildew control on lettuce, reducing disease incidence by 98% (Table 6.8). The Lettuce DownCast ModelI and The Farm Best Practice were significantly better than the Control, reducing incidence by 83% and 88%, respectively. Downy mildew was not controlled with *B. subtilis*. At harvest week 10, only Bion significantly reduced symptoms of downy mildew on whole plants, heads and wrap leaves, by 48%, 87% and 90%, respectively, but it was phytotoxic. Phytoxicity was expressed as immature and distorted canopy shape (Fig 6.1). The Lettuce DownCast ModelI predicted both a sporulation and an infection event at least once a week for the duration of the trial, resulting in only one less spray than the Farm Best Practice (Table 6.8). Not surprisingly the Farm Best Practice had the highest rank for contribution to farm profit from this autumn grown lettuce crop which was subject to high disease pressure from *B.lactucae*.

Table 6.8 Trial 1: Efficacy of treatments to control downy mildew on Iceberg lettuce cultivar Marksman at Boneo during autumn 2009.

Treatment	Mean incidence of downy mildew on Iceberg lettuce cultivar Marksman (%)								Number of sprays	Rank of farm profit[1]
	Week 6, 27 April 2009		Week 10, 25 May 2009							
	Whole plant		Whole plants		Heads		Wrap leaves (No. leaves with symptoms out of 4 leaves)			
	Log	Back transformed	Fitted	Back transformed	Log	Back transformed	Log	Back transformed		
BION	0.967 a	1.63	0.029 a	50.72	1.615 a	5.03	-1.375 a	0.253	3	na
Farm Best Practice	2.502 b	11.20	2.881 b	94.69	3.387 b	29.57	0.771 b	2.162	10	1
Lettuce DownCastI	2.834 b	16.02	2.850 b	94.53	3.636 b	37.95	0.680 b	1.973	9	4
B. subtilis	4.287 c	71.78	3.472 b	96.99	3.601 b	36.63	0.583 b	1.792	10	3
Control	4.594 c	97.92	3.566 b	97.25	3.738 b	42.02	0.931 b	2.536	0	2
lsd	0.4764		0.68		0.462		0.3316			

Numbers which are followed by a different letter differ significantly at 5%
[1], Refer to Chapter 9.

Fig 6.1 Phytotoxicity symptoms with the Bion treatment on lettuce.

Symptoms included immature and distorted head shapes.

6.3.2 Trial 2 on Cos lettuce cultivar Amadeus at Devon Meadows from August to October 2009

Downy mildew was first observed in the trial at week five and its incidence on Cos lettuce cultivar Amadeus remained low throughout the trial, barely reaching 10% in unsprayed Control plots by harvest (Table 6.9). The trial was complicated by a high incidence of anthracnose (*Microdochium panattonianum*) which developed rapidly after week 7.

At week 8, the incidence of downy mildew in the trial was low, with less than 2.5% of plants with symptoms (Table 6.9). Bion (half) had significantly more downy mildew than all other treatments. BREMCAST and the unsprayed Control did not differ significantly, while Bozyl, Farm Best Practice and the Lettuce DownCastI model did not differ significantly from each other, but had significantly lower incidence of symptoms. At harvest in week 9, the unsprayed Control was significantly worse than all the other treatments, however, the overall disease incidence was still low, reflecting low disease pressure from *B. lactucae* (9.45%). This was a marked contract to previous years on the same property, where downy mildew incidence on the same cultivar was up to 90%. All other treatments were not significantly different from each other (Table 6.9).

Both BREMCAST and the Lettuce DownCastI model used one spray less than the Farm Best Practice treatment. The half rate of Bion was effective and economical against downy mildew, using four less sprays and with no evidence of phytotoxicity. The surface disinfectant chemical, Bozyl, was also effective at this low disease pressure, but required weekly sprays.

Table 6.9 Trial 2: Efficacy of treatments to control downy mildew on Cos lettuce cultivar Amadeus at Devon Meadows during spring 2009.

Treatment	Incidence of downy mildew (%)		Number of	Rank of farm
	Week 8 (22/10/2009)	Week 9 (27/10/2009)	sprays	profit[1]
Bion (half)	2.466a	1.27b	2	1
BREMCAST	1.852b	1.83b	5	3
Control (unsprayed)	1.374b	9.45a	0	5
Bozyl	0.272c	1.58b	7	na
Farm Best Practice	0.271c	0.33b	6	4
Lettuce DownCastI	0.267c	2.14b	5	2

N umbers followed by a different letter differ significantly at 5%.
[1], Refer to Chapter 9.

6.3.3 Trial 3 on Red Oak Leaf lettuce cultivar Prunai at Skye, December to January 2009-2010

No downy mildew symptoms were evident in the Red Oak Leaf cultivar Prunai at harvest, which is probably not surprising as it has a DMR 1-27. Additionally, microclimatic conditions in the crop were not conducive to downy mildew development, as was evident by the paucity of predictions to apply fungicide sprays made by the models. This situation may have been associated with the elevated summer temperatures and unlike other properties, the plants were well spaced further apart, avoiding microclimates suited to the disease (Fig. 6.2).

The BREMCAST model predicted one infection event on 8/12/2009, and the Lettuce DownCast ModelI predicted an infection event on 9/12/2009 and 14/12/2009. Since the second prediction was within the 7-day window, no additional sprays were applied. Thus there were the same number of sprays applied to both model treatments and the Farm Best Practice treatment (Table 6.10). Economically it would be tempting not to spray this variety because of its resistance. However, a few preventative sprays should be applied to prevent break down of the resistance.

Table 6.10 Trial 3: Efficacy of treatments to control downy mildew on Red Oak Leaf lettuce at Skye during summer 2009 to 2010.

Treatment	Incidence of downy mildew at harvest	Number of sprays	Rank of farm profit[1]
Control	0	0	1
Lettuce DownCastI	0	1	3
BREMCAST	0	1	3
Farm Best Practice	0	1	2

[1], Refer to Chapter 9.

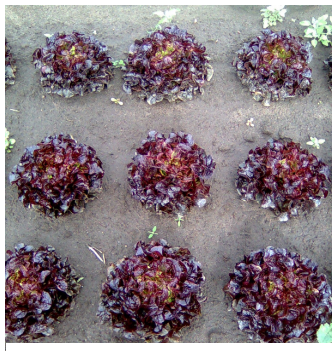

Fig 6.2 Lettuce plants well spaced apart in the plot.

6.3.4 Trial 4 on Iceberg lettuce cultivars Kong and Marksman at Werribee South, March to April 2010

Throughout the trial, the resistant cultivar Kong did not exhibit symptoms of downy mildew. Downy mildew symptoms first appeared on the susceptible cultivar Marksman in week 4 and developed rapidly late in the crop's life. By harvest, nearly 100% of plants in the unsprayed Control plots had disease symptoms. Both BREMCAST and DownCast predicted seven infection events each. However, on no day did their predictions agree.

At week 6 in the susceptible cultivar Marksman, both BREMCAST and the Lettuce DownCast ModelI were significantly more effective at controlling downy mildew symptoms compared with the Farm Best Practice, whilst Bion (half) was not effective (Table 6.11). By harvest at week 8, in all parameters assessed, the BREMCAST treatment was significantly better in relation to efficacy and economics for downy mildew control compared with all other treatments (Table 6.11). It reduced the downy mildew incidence on whole plants by 16%, on heads by 68%, on wrap leaves by 59% and plant downy mildew severity by 68% compared with the unsprayed Control treatment. This was achieved with three sprays fewer than the Lettuce DownCast ModelI. Although BREMCAST had more efficacy than the Farm Best Practice with the same number of sprays applied, they cannot be directly compared, as the fungicides used differed between these treatments. The Lettuce DownCast ModelI, to a lesser extent, showed efficacy in relation to reducing disease on wrap leaves and disease severity on plants. Farm Best Practice significantly reduced downy mildew severity on plants.

Table 6.11 Trial 4: Efficacy of treatments to control downy mildew on Iceberg lettuce cultivar Marksman at Werribee South during autumn 2010.

Treatment	Week 6 (12/04/2010)	Week 8 (26/04/2010)				Number of sprays	Rank of farm profit[1]
	Plant incidence (%)	Plant incidence (%)	Head incidence (%)	Wrap leaf infections (scale 0-4)	Severity on plant (scale 0-3)		
Control	86.7	99.6 a	22.1 a	3.20 a	3.00 a	0	2
Bion (half)	85.2	98.3 a	19.2 a	2.91 ab	2.83 a	2	3
Farm Best Practice	36.1	97.5 a	25.4 a	2.65 ab	1.83 b	3	4
DownCastI™	8.2	97.5 a	23.8 a	2.93 b	1.92 b	5	5
BREMCAST™	13.1	83.8 b	7.1 b	1.32 c	1.00 c	3	1
l.s.d. (5%)		9.44	10.7	0.43	0.31		

Numbers followed by a different letter differ significantly at 5%.
[1], Refer to Chapter 9.

6.3.5 Trial 5 on Iceberg lettuce cultivar Silverado at Boneo, November to December 2010

Downy mildew first appeared in the trial at week seven and by harvest approximately 50% of unsprayed plants in Control plots showed symptoms of the disease. No downy mildew formed on heads during the trial. The disease developed rapidly near harvest. An additional spray of Antracol plus TriBase Blue, which has a three day withholding period, may have reduced symptoms at harvest.

At week 8, BREMCAST, Farm Best Practice plus FoliCal Plus, Lettuce DownCastII with Revus, Farm Best Practice and Bion (half) all significantly reduced the incidence of downy mildew on whole plants by 78% to 95% compared with the unsprayed Control plants (Table 6.12). The farm manager confirmed that the canopy of Farm Best Practice plus FoliCAl Plus treatment looked smaller than those of other treatments.

By harvest at week 9 BREMCAST, Farm Best Practice and Bion (half) all significantly reduced the incidence of downy mildew on whole plants by 54% to 96% compared with the unsprayed control plants (Table 6.12). The average number of wrap leaves per plant with downy mildew was significantly reduced by BREMCAST, Lettuce DownCastII and Bion (half) by 92% to 100%. None of the treatments affected the weight of lettuce (g) at harvest as indicating that FoliCal Plus did not reduce head weight. Interestingly, at harvest Lettuce DownCastII produced heads with significantly higher weights at harvest. For processing lettuce there was no significant difference between any of the treatments and the unsprayed Control plants, but there were significant differences between treatments, with the Lettuce DownCastII model treatment producing a significantly lower weight for processing than the other treatments. The DownCastII model treatment produced less lettuce than other treatments for the fresh market. The Bion (half) treatment produced significantly more fresh market lettuce than all other treatments except the Farm Best Practice.

Bion (half) resulted in a similar level of downy mildew control to Farm Best Practice but with four fewer sprays (Table 6.12). BREMCAST produced a similar level of downy mildew control to Farm Best Practice but with four fewer sprays. Sprays applied according to the predictions of the BREMCAST model were two fewer and had more efficacy for downy mildew control, than the four sprays applied based on predictions of the Lettuce DownCastII model. The replacement of fungicides with FoliCal Plus, a calcium supplement, in the Farm Best Practice treatment reduced the control of downy mildew, but not significantly, when compared with the Farm Best Practice treatment. Bion (half) followed by Farm Best Practice was the most economical treatment.

Table 6.12 Trial 5 Efficacy of treatments in to control downy mildew on Iceberg lettuce cultivar Silverado at Boneo during summer 2010.

Treatment	Week 8 (23/12/2010)	Week 9 (29/12/2010)						
	Percent probability of plants having the disease (whole plants)	Percent probability of the incidence of downy mildew on plants at harvest	Average no. wrap leaves with downy mildew per plant out of 4	Average yield per head at harvest (gms)	Percentage probablility of marketable heads - no disease (processing)	Percentage probablility of marketable heads - no disease/size OK (fresh market)	Number of sprays minus the inadvertant spray	Rank of farm profit1
Control	5.352 a	47.50 ab	0.3012 a	792.6 a	51.67 ab	30.73 ab	0	5
Lettuce DownCastII BOM	3.586 ab	-	-	-	-	-	3	-
Lettuce DowncastII	3.517 ab	57.50 a	0.5063 a	890.5 b	42.5 a	29.89 a	4	6
BREMCAST	1.202 bc	21.67 b	0.0250 b	827.8 ab	78.33 b	60.07 b	2	3
Farm Best Pracrice/ FoliCal Plus	0.954 bc	26.67 ab	0.1502 ab	868.4 ab	73.33 ab	53.36 b	6	4
Lettuce DownCastII R	0.949 bc	-	-	-	-	-	3	-
Farm Best Practice	0.716 bc	4.17 b	0.0042 b	822.7 ab	95.83 b	70.11 bc	6	2
Bion (half)	0.239 c	1.67 b	0.0010 b	844.0 ab	98.33 b	85.95 c	2	1
lsd				80.1				

Numbers followed by a different letter differ significantly at 5%; -, not applicable.
[1], Refer to Chapter 9.

6.4 Discussion

None of the three methods for timing fungicide sprays to control downy mildew on lettuce was consistently superior in all trials, but overall it was apparent that the BREMCAST model reduces fungicide applications and produces a similar or better efficacy of disease control than Farm Best Practice and Lettuce DownCast. BREMCAST compared with Farm Best Practice resulted in 0, 1 or 4 fewer sprays. These reductions are in the same order as those achieved by McDonald *et al.* (2000) and Kushalappa (2001). The spray forecast advisory of Scherm *et al.* (1995) also resulted in a similar number of spray reductions.

The highest reductions in spray numbers were in a summer crop of fresh market Iceberg lettuce (Trial 5), when disease pressure was low. This was consistent with Scherm *et al.* (1995) who obtained a 67% reduction in spray number with low disease pressure. BREMCAST, with the same number of fungicide sprays as Farm Best Practice, produced significantly better control of the disease, as the timing of sprays was also aligned with the epidemiology of *B. lactucae*. Fungicide sprays were predicted in the two weeks after the initial infection in the former but were not applied in the latter. Bion had amazing efficacy and economics when it was effective, but was unreliable because of its variable nature and the potential for phytotoxicity. Bozyl, if it is ever registered for downy mildew control on lettuce, could provide an alternative to fungicides under conditions of low disease pressure, but its economics would determine its uptake.

Iceberg lettuce is not grown through late autumn, winter and early spring in Victoria, no doubt due to high disease pressure and frosts. In Trial 1 the disease pressure from *B. lactucae* in a late autumn crop (Marksman Aus 1) was so high that neither the conventional weekly fungicides sprays of the Farm Best Practice nor the timing of conventional fungicides based on the predictions of the Lettuce DownCastI model slowed the progress of downy mildew. Failure may have been associated more with the fungicides used rather than with a lack of predictions of the model. More control of downy mildew may have been achieved if systemic fungicides had been applied late, rather than early, in the crop's life. Failure of the downy mildew forecast spray advisory under high disease pressure was also observed by Scherm *et al* (1995). Fortunately the crop was grown for processing and despite the high levels of disease, all was ll harvested. Disease predictive models may be more suitable for use in

seasons with a low incidence of downy mildew, while in seasons with a high incidence, control measures should revert to calendar fungicide sprays.

BREMCAST compared with DownCast
BREMCAST predicted the timing of two fewer fungicide sprays, which had more accuracy for controlling downy mildew on Iceberg lettuce than similar fungicide sprays based on the predictions of the Lettuce DownCast models I and II, in Trials 4 and 5, respectively. In Trial 5, the efficacy of BREMCAST may be related to the timing of the sprays at week four, which was the only difference between the two models. In Trial 2 on Cos lettuce, both models produced similar efficacy with a similar number of sprays. In Trial 3 on Red Oak Leaf, both models over predicted one or two sprays. BREMCAST produced better control of downy mildew with fewer sprays compared with DownCast, on susceptible and resistant cultivars when disease pressure was moderate (20 to 50% incidence) on whole plants.

BREMCAST compared with Farm Best Practice
BREMCAST had a similar efficacy to Farm Best Practice in two trials (Trials 2 and 5), and better efficacy in a third (Trial 4) in controlling downy mildew with a reduction by one or four sprays, respectively. It was more accurate in timing spray applications based on microclimate data than Farm Best Practice in Trial 4 on Iceberg lettuce, even though both treatments had the same number of fungicide sprays. In this trial fungicide were applied at weeks 3, 5 and 6 to the BREMCAST model treatment; whereas the Farm Best Practice treatment received more fungicides earlier in the crops life at weeks 3, 4 and 7. The addition of Revus to the model treatment was unlikely to have affected the result as there was no difference in incidence, with and without Revus in Trial 5, whe applied on predictions of the downcast model.

DownCast compared with Farm Best Practice
The Lettuce DownCastI model had the same efficacy in controlling downy mildew as the Farm Best Practice treatment in four trials (Trials 1, 2, 3, and 4) but not in Trial 5, where it was less efficacious. In two of these trials, Trials 1 and 2, it reduced the number of sprays by one to two, but increased the number of sprays by two in Trial 4 and sprays by two in Trial 5. The Lettuce DownCastI model treatment predicted a reduction in the number of sprays compared to Farm Best Practice only when Farm Best Practice consisted of weekly applied fungicides. Modifications which produced Lettuce DownCastII, did not enhance the model's performance and led to additional fungicide sprays early in the crop's life.

Lettuce DownCast disease predictive model
The delivery systems for the Lettuce DownCast models were easier to use than the symptoms for BREMCAST. Issues which arose with Lettuce DownCastII may be related to the: (i) manual manipulation of the model where a period of 2 hrs dryness was deemed necessary to interrupt a wet period; or (ii) half hourly measurements of leaf wetness which were rounded up not down. Lettuce DownCast tended to produce more spray events early in the crop's life compared with BREMCAST. Unlike BREMCAST, it does not require knowledge of the presence or absence of the disease in the field. Inclusion of a parameter on the status of downy mildew in the field may enhance the model.

BREMCAST disease predictive model
The version of BREMCAST employed in the trials was not user-friendly. Once the inputs had been calculated from the microclimate weather data they had to be manually entered into the model, which was time consuming. BREMCAST had two additional parameters compared with the Lettuce DownCast models, the day length and the presence or absence of downy mildew in the field. Additionally the BREMCAST disease predictive model requires crop scouting, which growers do not necessarily have the time to undertake. Consequently the model is more likely to be suitable for crop scouts when its user-friendliness is improved.

Bion

Although use of Bion can reduce up to four fungicide sprays, have efficacy and be very economical, its effectiveness was highly variable. The product is very expensive, but only small quantities are used. On Iceberg lettuce it: (i) was phytotoxic when three applications at 50g/ha were applied when the disease was first observed; (ii) had no efficacy at half the rate, applied when the disease was first observed; but (iii) two applications at half rate applied before the disease was observed had a similar efficacy to Farm Best Practice and BREMCAST in most categories and had a superior efficacy to BREMCAST for fresh market lettuce. On Cos lettuce two applications of Bion at half rate and at the first sign of the disease, produced a similar level of disease control but it was more economical than other treatments. Variability of Bion may be associated with cultivars, timing of application or some other factor. It is sold by Syngenta as Actigard 50WG in the USA but is only registered as a seed treatment for cotton in Australia and will not be registered in Australia as a stand alone product (Dal Santo pers. comm.). Other SAR products could be worth investigating, especially newly developed ones such as DL-3-amino-butyric acid (BABA) which had efficacy and curative activity against downy mildew of lettuce in the field in other studies (Cohen *et al.* 2010, 2011).

Fungicide alternatives and comparisons with models

B. subtilis had no efficacy under the high disease pressure of Trial 1. Bozyl, a surface sterilizing agent, applied to Cos lettuce as weekly sprays under low disease pressure had a similar efficacy to the models and Farm Best Practice, but cost of the product and the frequency of application may ultimately determine uptake of these products. Perhaps under low disease pressure, these fungicide alternatives could be considered. It would be interesting to trial their application based on a disease predictive model, especially during a period of low disease pressure.

Calcium nitrate

Enhanced calcium nitrate resulted in lettuce seedlings being less susceptible to downy mildew than those treated with ammonium nitrate or potassium nitrate (Chapter 3). Replacement of fungicide sprays with a calcium supplement (FoliCal Plus) in Trial 5 reduced yields of fresh market and processing lettuce but not significantly, in comparison with the Farm Best Practice. There was no evidence to support suppression of downy mildew with the calcium supplement at harvest. A week before harvest the frame of plants appeared to be smaller, but by harvest weights of these heads did not differ from the Farm Best Practice. Use of FoliCal Plus may be more suited to early stages of crop production when disease pressure is low. The site on which this trial was conducted normally receives applications of calcium nitrate, which may have compounded the results.

Lettuce cultivars

The on-farm trials used cultivars suited to their time slot. Generally all the cultivars used in these trials had a good DMR except Marksman (DMR Aus1) and despite high incidences of downy mildew on heads and wrap leaves; the whole crop was harvested for processing. Cos lettuces are grown throughout the year in Victoria. The cultivar Amadeus (DMR 1-17, 21, 23) grown in autumn had a lower incidence of downy mildew at harvest, 9.45%; whilst the summer grown Iceberg cultivar Silverado (DMR 1-16, 21, 23) had a higher incidence, 47.5%. The autumn-grown cultivar would be expected to have more downy mildew. Perhaps the presence of one resistance gene may make a lot of difference to susceptibility and additionally the upright and open nature of the Cos lettuce frame may provide additional advantages by not holding moisture. Interestingly cv Prunai DMR 1-27 and Kong DMR 1-26 did not show symptoms of downy mildew in our trials, but a later planting of Kong displayed a high incidence of downy mildew symptoms. Even though these varieties have a good collection of resistance genes they still need to be sprayed with fungicides to prevent establishment of *B. lactucae* and prolong cultivar resistance. The disease predictive models can adequately

forecast when *B. lactucae* will be active in the field and thus encouraging timely fungicide sprays for these resistance cultivars instead of the *ad hoc* application of fungicides.

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Chapter 7

Epidemiological studies of powdery mildew on cucurbits in north Queensland from 2008 to 2010 production seasons

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Summary
Development of powdery mildew, caused by *P. fusca* (formally *P. xanthii)*, was measured on a susceptible cucurbit host (zucchini cv. Congo) grown at two different locations, the Ayr and Bowen DEEDI Research Stations in Queensland, during 2008 to 2010. In field data loggers recorded weather parameters (temperature, relative humidity and dew point) throughout each growing season. The incidence and severity of the disease varied between the two sites during the two years of monitoring. Powdery mildew was more severe on the lower leaf surface compared with the more exposed upper leaf surface. Stem lesions were prevalent late in the season rather than early in the season. In the field, temperature did not appear to be a limiting factor, unlike free moisture in the form of dew.

7.1 Introduction
7.1.1. Forecasting plant diseases
The study of disease epidemics and the factors that influence them is called epidemiology (Agrios, 2005). A thorough understanding of the dynamics of these factors (host, environment and pathogen), which make up the classic disease triangle, can be useful in developing a disease forecasting system to devise rational management strategies for powdery mildew in cucurbits (Jarvis *et al,* 2002).

Forecasting plant disease is an attempt to estimate the future state of a disease in a crop from observations of the current or recent state of the disease, or from measurement of related factors which include the host, pathogen and environment (Parry, 1990). A plant disease model is a mathematical description of the interaction of these variables that can result in the expression of disease. A model may be presented as a simple rule, an equation, a table or a graph, with the output being a numerical index of the disease risk, predicted inoculum development, and/or predicted disease incidence or severity (California PestCast, 1998).

Forecasting systems have been developed for powdery mildew in grapevines, apples, tomatoes, barley, rubber, sugar beet and roses. Various environmental factors such as temperature, relative humidity, vapour pressure deficit (VPD), duration of leaf wetness, hours of sunshine, daily rainfall and wind, etc. have been used to devise these models.

7.1.2 Powdery mildew of cucurbits
Powdery mildew of cucurbits is generally favored by moderate temperatures, dry conditions, high relative humidity, reduced light, fertile soils and succulent plant growth.

7.1.2.1 Temperature and Relative Humidity
Favorable temperatures for disease development in the field occur in the range of 20-30ºC, while under controlled conditions, development was promoted by temperatures in the range of 15 to 25ºC. A study of the effects of humidity on the epidemiology of *P. fusca* on squash under controlled conditions showed that the colonization, sporulation and dispersal of the pathogen were favored by dry conditions with 45-50% humidity, while infection and conidial survival were promoted by high humidity conditions of 90-95% (Reuveni and Rotem, 1974).

Cheah *et al.* (1996) reported that in laboratory tests, germination of conidia on slides was greatest at temperatures of 25ºC under high humidity, while there was no germination below 15ºC and above 30ºC or at relative humidity ≤ 94%. Under field conditions, infection of squash plants occurred 5-7 days after a prolonged period of continuous leaf wetness (about 12 h) and high humidity (~95%) in the summer when temperatures frequently rose above 22ºC.

Temperature and humidity are best considered together as they are components of water vapour pressure deficit (VPD) which is a more meaningful parameter than relative humidity in describing water dynamics in agricultural pathosystems (Jarvis *et al.* 2002).

7.1.2.2 Free Water

Conidia of *P. fusca* are able to germinate and infect plants under high temperature and low humidity conditions, in the absence of free water. Excessive water is often detrimental to the development of powdery mildews and colony development is impaired by rain and overhead irrigation (Jarvis *et al.* 2002).

7.2.2.3 Light

Shade rather than full light favors powdery mildew development. The disease was more profuse under large overlapping leaves of cucumber grown in greenhouses. Tissue susceptibility to infection is likely to be influenced more by the indirect effects on photosynthesis and flowering and fruiting and fruiting stress in the host, which alters sink-source relationships, than the direct effects of light (Jarvis *et al.* 2002).

7.2.2.4 Study Objectives

The purpose of this study was to measure the development of powdery mildew on a susceptible cucurbit host (zucchini cv. Congo) at two different locations, namely, Ayr and Bowen Research Stations and record weather parameters (temperature, relative humidity and dew point) using data loggers placed in the crops throughout the growing season. From these data a forecasting model which predicts disease development in relation to favorable environmental conditions for infection would be developed. The model could be used to determine when to schedule appropriate fungicide sprays, with a view to limiting fungicide use only to times when needed, rather than applying sprays on a calendar basis as is common practice on most commercial farms in Australia.

7.2 Materials and Methods

Field trial sites were established at two locations with very distinct micro-climates, namely, Ayr and Bowen in North Queensland, which are large cucurbit production areas. These trials were conducted at both locations in 2008 and 2009 and only at Ayr in 2010. Congo (SPS) seed were sown into 96 cell seedling trays using a 3:1 ratio of peat to vermiculite 3 mix and raised in an evaporative-cooled glasshouse for 2 weeks. When 2-3 true leaves had developed, the seedlings were transplanted into raised beds (1.52 m centres) under black plastic and trickled irrigated (2 L/min). Plants were spaced at 55 cm within each row. Standard agronomic practices for weed control, fertigation and pest management were implemented throughout the growing season.

7.2.1 Disease Assessments

Disease severity assessments were made weekly on leaves within the lower third of the canopy using a 0-6 scale as follows: 0=Nil, 1=1-5%, 2=6-10%, 3=11-25%, 4=26-50%, 5=51-75% and 6=76-100% of leaf covered with sporulating lesions. The upper and lower surfaces of two leaves per plant were assessed. Later in the season stem disease severity ratings in the lower third of the canopy were also taken using a 0-5 scale as follows: 0=Nil, 1=≤20%, 2=21-40%, 3=41-60%, 4=61-80%, 5=81-100%.

7.2.2 Field Trials 2008 at Ayr and Bowen

Ayr Plantings

In all there were 10 sequential plantings of zucchini seedlings arranged in a randomized complete block design with four replicates. Each plot contained 8 plants with the inner 5 data plants being sampled for disease severity on a weekly basis. The planting dates are listed in Tables 7.1 and 7.2.

Bowen Plantings

There were 4 sequential plantings at Bowen, namely: (1) 26th June; (2) 11th July; (3) 24th July; and (4) 30th July. Each plot contained 7 plants with the inner 5 being data plants.

Disease Assessments

Disease assessments were conducted as described in 7.2.1

7.2.3 Field Trials 2009 at Ayr and Bowen

Congo was again used at both field sites. At Ayr the trial was composed of 4 rows of 4 data plants/row, while at Bowen there were 2 rows of 4 data plants/row.

Ayr Plantings

There were 8 sequential plantings at fortnightly intervals, as follows:
(1) Sown into seedling trays on 18 May, raised in a glasshouse and transplanted on 5th June. Subsequent plantings were all direct-seeded. (2) 10th June; (3) 22nd June; (4) 6th July; (5) 20th July; (6) 3rd August; (7) 17th August and (8) 31st August.

Bowen Plantings

There were 4 sequential plantings at fortnightly intervals, as follows: (1) Sown into seedling trays on 15th June and transplanted on 6th July. Later plantings were all direct-seeded; (2) 21st July; (3) 11th August; and (4) 25th August.

Disease Assessments

There were weekly disease ratings of data plants at each site. Leaf ratings were used at the Bowen site and stem ratings were used at Ayr where downy mildew was the predominant disease on foliage. Rating dates are listed in Tables 7.3 and 7.4 for Ayr and in Tables 7.5 and 7.6 for Bowen.

7.2.4 Field Trial 2010 at Ayr

In the absence of the predictive disease model that was to be tested under field conditions, it was agreed that a sequential planting trial would be planted at Ayr Research Station and disease incidence and severity would be assessed on a regular basis.

Sequential plantings began on 15th June 2010 and extended through to 20th September 2010, with a total of 10 planting dates. Planting dates were as follows:
(1) 15th June; (2) 28th June; (3) 13th July; (4) 27th July; (5) 16th August; (6) 23rd August; (7) 1st September; (8) 7th September; (9) 13th September; and (10) 20th September. The first four planting dates were on a fortnightly basis with the latter six being on a weekly basis from 16th August.

Disease Assessments

Disease incidence and severity were recorded on a regular basis using five data plants, from within the middle of the row. At each sampling date four actively growing leaves from the lower third of the plant canopy were assessed for incidence and severity (% leaf area diseased) on the undersurface of leaves. Assessments were made on the dates listed in Tables 7.7 and 7.8.

7.3 Results

7.3.1 Ayr 2008

No powdery mildew was observed on the first 5 plantings at Ayr, until 1 August. There was a very low level of disease severity on the leaves, but disease was more prominent on the stems (Table 7.1). The general trend for stem disease was that earlier plantings were more severely affected than the later plantings. Downy mildew was the predominant foliar disease, with severe disease being observed on all data plants for all plantings.

Table 7.1 Mean disease severity ratings for leaves (upper and lower surfaces) and stems of zucchini at Ayr on 1 August 2008 for plantings 1 to 5.

Planting		Mean disease severity rating		
Number	Date	Upper leaf surface (scale 0-6)	Lower leaf surface (scale 0-6)	Stem (scale 0-5)
1	29 May	0.042	0.083	2.92
2	5 June	0	0	2.08
3	12 June	0.042	0	2.17
4	19 June	0	0	1.11
5	26 June	0	0.042	0.08

In the second group of plantings (plantings 6 to 10), powdery mildew was first noted on 5th September on the undersurface of leaves although this was at a very low level, while stem disease only became apparent a week later on 12 September, showing a similar trend to the first 5 plantings (Table 7.2). Weather conditions were suitable for the development of powdery mildew (Fig 7.1), but downy mildew was the predominant disease.

Table 7.2 Mean disease severity for leaves (lower surfaces) and stems of zucchini at Ayr on 5 and 12 September for plantings 6 to 10.

Planting		Mean severity of powdery mildew			
		Lower leaf		Stem	
Number	Date	5 Sept	12 Sept	5 Sept	12 Sept
6	10 July	0.83	0.58	0	1.78
7	17 July	0.75	0.46	0	1.17
8	28 July	0.50	0.71	0	0.33
9	8 August	0.33	0.46	0	0
10	11 August	0	0	0	0

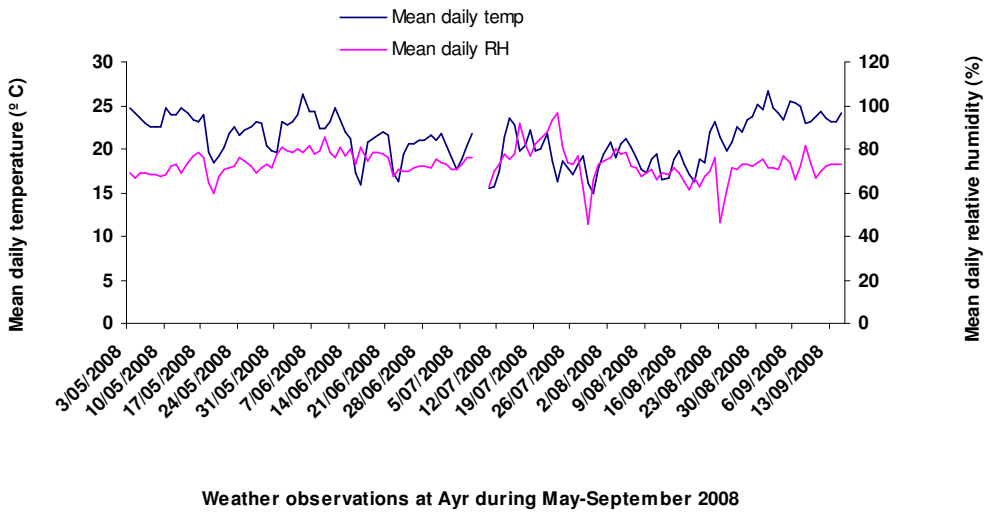

Fig 7.1 Weather observations at Ayr field site from May-September 2008.

7.3.2 Bowen 2008

Disease was fist observed on data plants on 6th August 2008 in plantings 1 and 2 (41 and 26 days after transplanting (DAT) respectively, and on 20th August for plantings 3 and 4 (27 and 21 DAT), respectively. The slopes of the graph in Fig 7.2 demonstrate that in the later plantings the rate of disease development was greater than for the two earlier plantings.

Environmental conditions in Bowen favored the rapid development of powdery mildew regardless of planting date (Fig 7.5). This is clearly demonstrated in the graphs (Fig 7.2- 7.4) for disease severity on leaves (lower and upper surfaces) and stems. The values for disease severity were always higher for the lower leaf surfaces at the different sampling dates, except later in the season when the maximum disease severity rating score of 5 was reached at the second last and last rating dates for plantings 1 and 2, respectively. Early in the season the stems showed no disease symptoms. These only became apparent later in the season for all planting dates (Fig 7.4).

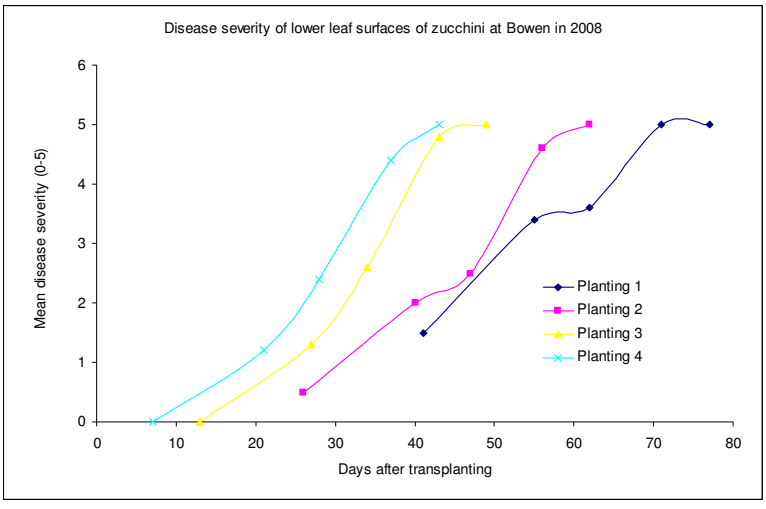

Fig 7.2 Development of disease on lower leaf surfaces of zucchini at Bowen in 2008.

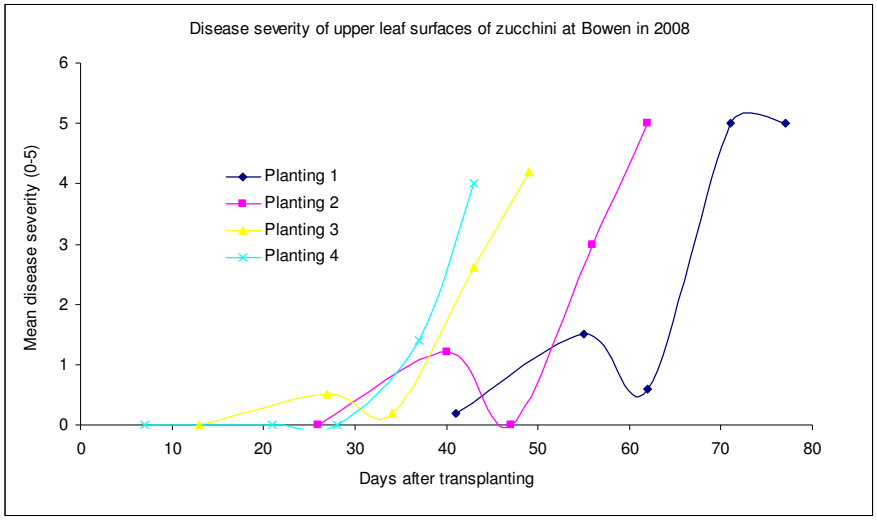

Fig 7.3 Development of disease on upper leaf surfaces of zucchini at Bowen in 2008.

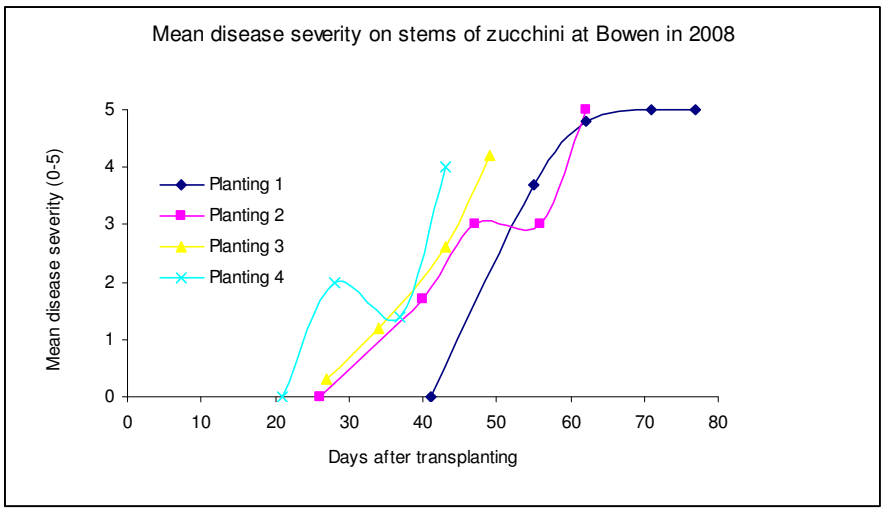

Fig 7.4 Development of disease on stems of zucchini at Bowen in 2008.

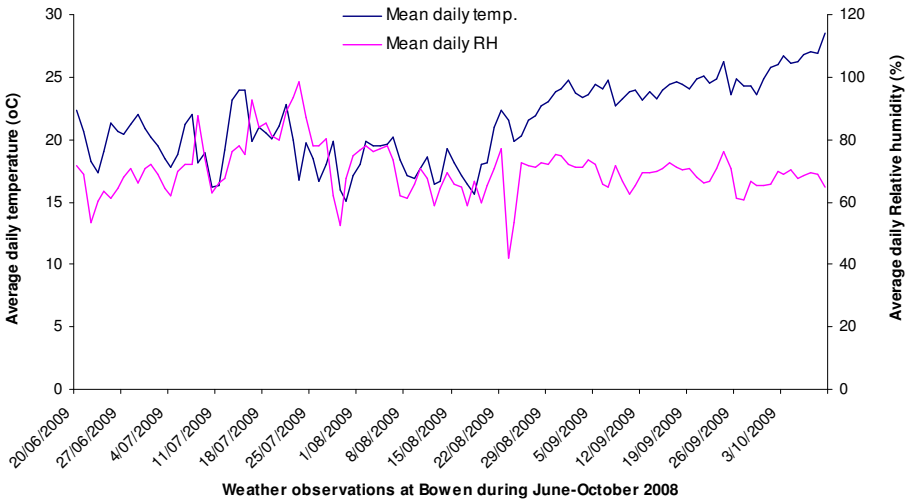

Fig 7.5 Weather observations at Bowen field site from June-October 2008.

7.3.3 Ayr 2009

Disease was first recorded at Ayr on 17 July 2009 on data plants from the first planting. Generally, there was a rapid increase in disease incidence and severity with progressive rating dates, particularly for plantings 1 to 6 (Tables 7.3 and 7.4).

Table 7.3 Disease incidence over time on plantings (P) 1 to 8 at Ayr in 2009.

Rating Date	Disease incidence (% infected plants)							
	P1	P2	P3	P4	P5	P6	P7	P8
12 Jun-10 Jul	0	0	0	0				
17 July	13*	0	0	0				
24 July	0	0	0	0				
31 July	88	0	0	0				
7 August	100	100	0	0				
15 August	100	100	100	0	0	0		
21 August	100	100	100	6	44	0		
28 August			100	100	100	0		
5 September			100	100	100	0	0	
18 September					100	100	0	0
25 September						100	31	31
2 October						100	19	6
9 October							13	0
16 October							69	31

Note: The shaded grey areas at the bottom left-hand side of Tables 7.3 to 7.6 indicate that the data plants had senesced and were removed and hence no data entries. *Infection on 2 plants each with one powdery mildew colony (on a leaf, not stem).

Table 7.4. Disease severity over time plantings (P) 1 to 8 at Ayr in 2009.

Rating Date	Disease severity on stems (scale 0-5)							
	P1	P2	P3	P4	P5	P6	P7	P8
12 Jun-10 Jul	0	0	0	0				
17 July	0	0	0	0				
24 July	0	0	0	0				
31 July	1.4	0	0	0				
7 August	2.3	1.1	0	0	0			
15 August	3.6	3.7	1.1	0	0	0		
21 August	4.0	4.0	1.4	1.0	0.7	0		
28 August			3.2	1.1	1.0	1	0	
5 September				2.9	1.4	0	0	
18 September					3.3	1.1	0	0
25 September						1.0	1	1
2 October						2.4	1	1
9 October							1	0
16 October							1	1

7.3.4 Bowen 2009

Disease was first recorded on data plants at Bowen on 25 August 2009 on data plants from the first planting. Generally, there was a rapid increase in disease incidence and severity with progressive rating dates, particularly for plantings 1, 2 and 3 (Tables 7.5 and 7.6), a similar trend to that noted for the Ayr trial (Tables 7.3 and 7.4).

Table 7.5 Disease incidence over time (Plantings 1 to 4) at Bowen in 2009.

Rating Date	Disease incidence (% infected plants)			
	P1	P2	P3	P4
25 August	90	78	25	0
1 September	100	100	100	90
8 September	100	100	100	100
15 September				100
22 September				100

Table 7.6 Disease severity over time (Plantings 1 to 4) at Bowen in 2009.

Rating Date	Disease severity (% Leaf area infected)			
	P1	P2	P3	P4
25 August	18	5	0	0
1 September	47	21	9	6
8 September	74	51	15	<1
15 September				23
22 September				21

7.3.5 2010 Ayr

The 2010 season in Ayr, was similar to previous years, in that downy mildew was the predominant disease, even in the later plantings, following regular dewy mornings and occasional overcast and rainy days. Downy mildew tended to colonise the foliage more effectively than powdery mildew. To try and overcome this, Acrobat (which is registered for downy mildew control), was applied on 3 September and additionally, infected leaves were removed and discarded, to limit spread of the disease.

Data plants from plantings 1 and 2 (P1 and P2) showed no powdery mildew symptoms. Downy mildew severely affected the leaves, with >50% of the leaf area diseased in the lower two thirds of the canopy. Later plantings were also severely affected by downy mildew. Powdery mildew was first observed on 30 July on data plants from planting 3 (Table 7.7).

Tables 7.7 and 7.8 summarize the development of disease over time. Generally there was an increase in incidence and severity with successive rating dates, unless there was a heavy rainfall event that reduced disease incidence, e.g. from 27 September to 4 October, as was the situation for plantings 5, 6, 7 and 8 (highlighted in yellow in Table 7.7). Bureau of Meteorology data showed that rainfall up until 9:00 a.m. on 29 September was 27.8 mm.

Disease severity was also reduced significantly from 6 August to 3 September (Table 7.8), following a number of rainfall events, namely, 55.8 mm, 0.2, 8.4 and 0.2 mm recorded on 11, 21, 27 and 30 August 2010, respectively.

Table 7.7 Disease incidence over time (Plantings 1 to 10) at Ayr in 2010.

Rating Date	Disease incidence (% data leaves infected)									
	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
30 July	0	0	95	0	0					
6August	0	0	95	95	50	0				
3 September	0	0	100	75	75	100	0			
13 September			100	75	100	100	0	0	0	
20 September			100		90	25	5	10	0	
27 September					100	80	25	40	0	0
4 October					90	55	0	5	0	0

Table 7.8 Disease severity over time (plantings 1 to 10) at Ayr in 2010.

Disease severity (% leaf area infected)										
Rating Date	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
30-Jul	0	0	22	0	0					
6-Aug	0	0	62	59	29	0				
3-Sep	0	0	8	3	5	2	0			
13-Sep			16	8	15	5	0	0	0	
20-Sep			23		21	11	15	8	0	
27-Sep					25	41	3	4	0	0
4-Oct					39	23	0	10	0	0

7.4 Discussion

There was only minor disease expression of powdery mildew on foliage at Ayr throughout the whole season of 2008, while downy mildew was severe. Late in the season powdery mildew was most noticeable on the stems in the lower third of the canopy. The situation was very different in Bowen where there were very high levels of disease expression on both leaves and stems. Characteristically, stem infection only occurred late in the season at both sites. The shorter duration to reach the maximum disease severity readings recorded in the third and fourth plantings at Bowen in 2008 were to be expected as there would have been a larger inoculum load from infected plants in the adjacent earlier plantings.

The rapid disease development at Bowen in 2008 and 2009 demonstrates the polycyclic nature of the pathogen. Our earlier studies under controlled glasshouse conditions showed that the life cycle took between 5-7 days, with the process being accelerated by physical rubbing of infected leaves on uninfected leaves, compared to natural infection from exposure of seedlings overnight in cucurbit fields. These results are similar to those reported by other researchers (Cheah *et al.* 1996). Horlock and McGrath (2004) reported that symptoms can occur within 3-7 days, affecting older leaves first, which is what was observed in these trials also. Under favorable conditions the powdery mildew fungus may produce masses of spores, up to 2 million spores per square inch (2 x 10^6/6.5 cm^2) of leaf surface within 7-10 days (Eastburn and Ortiz-Ribbing 1999).

Temperature is not normally a limiting factor in the development of powdery mildew in commercial production areas in Australia, occurring between 10-35ºC, with the optimum around 23-26ºC. These were the conditions experienced in our trials (Figs 7.1 and 7.5; Appendices 1, 2 and 3). This being the case, the major determinant of the development of

powdery mildew is likely to be the presence of free moisture, from heavy dews and/or rainfall events as the crops were trickle irrigated.

Unlike most fungal pathogens of cucurbits, powdery mildew does not require a film of water on plant surfaces for infection. Free moisture inhibits spore development which could explain checks in epidemics during wet weather (Blancard *et al* 2004). It was noted that conditions in Ayr, characterized by dews that persisted until 9:00 a.m., were common throughout the growing seasons, to the detriment of powdery mildew development, but encouraging downy mildew infection. This was not the situation in Bowen which is characteristically windy throughout the day, even early in the morning, which would assist in rapid drying of leaf surfaces. The sudden decline in disease severity on the upper leaf surfaces from 20 to 27 August at Bowen in 2008 appeared to be due to a rain event, however, the meteorological data did not show this.

Infection and spore survival is promoted by dense plant growth, low light intensity and high relative humidity. However, infection can take place at a relative humidity as low as 50% (Horlock and McGrath 2004). Our results also concur with these observations and not surprisingly, the lower surfaces of the leaves gave higher disease severity ratings, being more sheltered from the elements, as demonstrated in Figs 7.2 and 7.3.

Data obtained from these field trials will be used by a PhD student (Ms Zaiton Sapak), at the University of Queensland, to develop the disease model which will be used to predict powdery mildew infection events during the 2011 cropping season. This information will then be used to improve spray management programs, with a view to reducing the number of sprays. This has the potential to reduce input costs and impacts on the environment as well as ensuring the longevity of the systemic fungicides. The latter are under threat because of overuse leads to the development of resistant strains of the powdery mildew pathogen.

7.5 References

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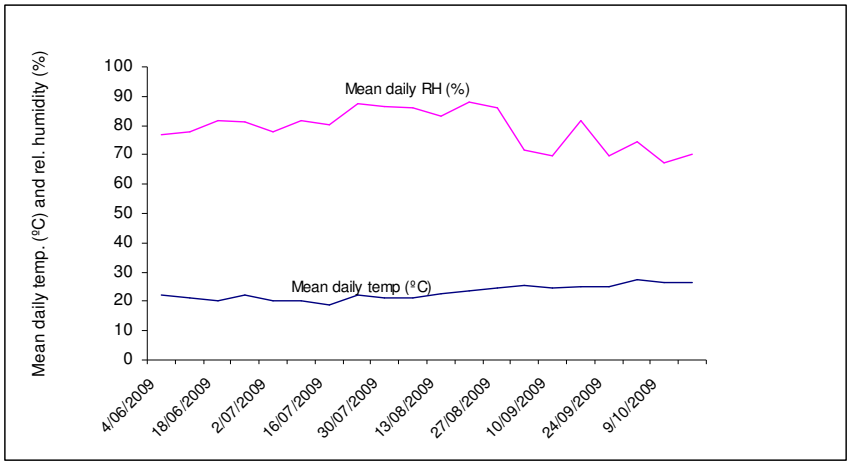
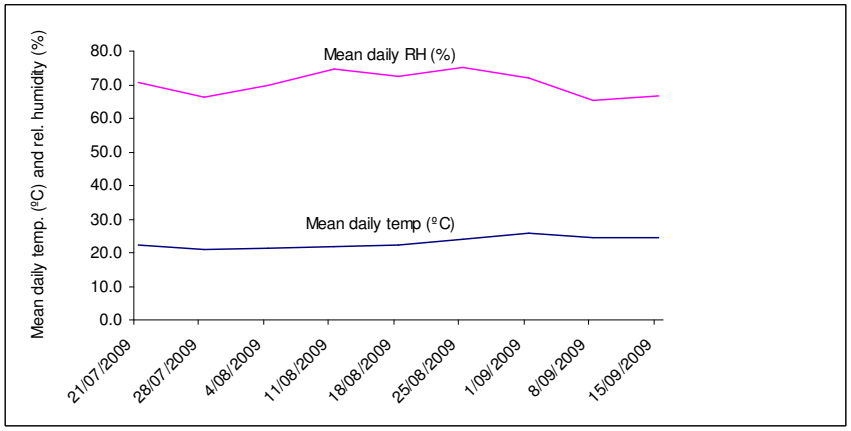
7.6 Appendices

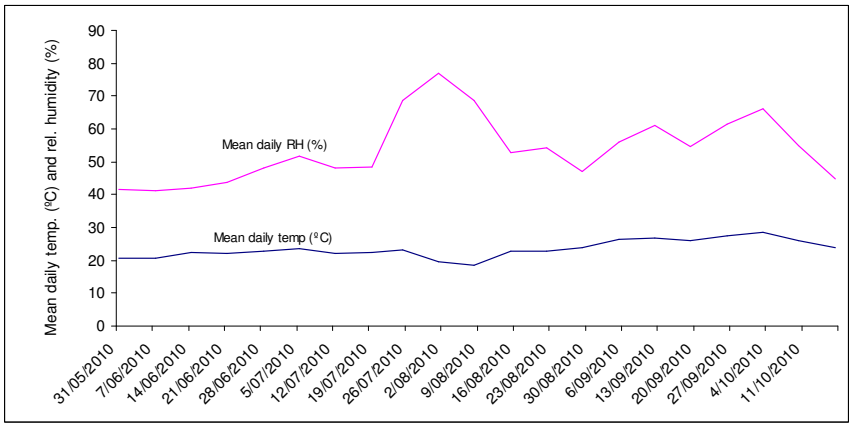
Appendix 1. Weather observations at Ayr from June to October 2009.



Appendix 2. Weather observations at Bowen from July to September 2009.



Appendix 3. Weather observations at Ayr from June to October 2010.



Chapter 8

Development of a disease predictive model for powdery mildew of cucurbits

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Summary
A disease predictive tool for powdery mildew of cucurbits was developed by using a modified spore settling tower to inoculate plants and conducting growth chamber experiments to determine the effects of temperature and vapour pressure deficit (VPD) on infection by *P. fusa*. *P. fusca* needs at least three consecutive days with at least 12 consecutive hours of temperatures between 20 and 30°C and moisture between 0.03 (near saturation) and 0.20 KPa for spore germination and host infection. The pathogen also needs another two consecutive days with at least 12 consecutive hours of temperatures between 20 and 30°C and moisture of between 0.03 and 2.56 KPa to promote leaf colonisation and fungal sporulation processes. Exposure to temperatures of 35°C and above for more than 30 minutes can damage spores and stop the infection process. This information was used to develop a cucurbit 'powdery mildew risk index' (PMRI). Subsequent use of the PMRI to time fungicide sprays for the control of powdery mildew on zucchini in a field trial reduced the standard weekly spray program by one spray without affecting yield.

8.1 Introduction

8.1.1 Powdery mildew
Powdery mildew caused by *Podosphaera fusca* (Fr.) U. Braun & N. Shishkoff is a common and often serious disease of cucurbits in Queensland and worldwide. The disease manifests as white powdery colonies on both upper and lower leaf surfaces, petioles and stems. Under certain environmental conditions, the disease can grow rapidly and cover the surface of the entire leaf. Severe infection, normally leads to defoliation of crops.

Powdery mildew of cucurbits is an obligate parasite, unable to be cultured and maintained on agar media. Thereby, it provides a challenge to researchers studying the infection processes in the laboratory. More problematic is that the pathogen is sensitive to free water, which damages conidia by negatively affecting their viability and infectivity (Sivapalan, 1993). Thus, conidia cannot be applied to plants using water suspensions. Use of dry conidia applied by dusting or blowing from infected leaves onto test plants is commonly used in artificially inoculating plants. This approach allows for deposition of conidia onto plant surfaces, but with little uniformity of distribution (Reifschneider and Boiteux, 1988). Techniques such as the use of paint brushes and cotton swabs provide improved uniformity of conidial distribution, but are lacking in convenience and accuracy. A spore-settling tower applying Stoke's law of sedimentation as proposed by Reifschneider and Boiteux (1988) has provided a more convenient and repeatable method for inoculation of powdery mildews. These authors constructed a plywood tower and demonstrated successful use of a low vacuum induced air inrush to dislodge conidia from infected leaves, and to disperse them effectively over test plants.

The disease is currently managed by applying chemical fungicides and by developing resistant cultivars. However, the ability of the pathogen to develop new resistance genes to overcome the effectiveness of chemical fungicides is problematic. Also, relatively limited

availability of resistant cultivars of cucurbits has made this disease management approach unsustainable. A more strategic fungicide-based disease management program needs to be developed to minimise the pathogen fungicide resistance and host disease susceptibility problems.

8.1.2 Powdery mildew risk index

A disease forecasting system based on an understanding of the inter-relationship among the pathogen, host and environmental conditions offered an improved approach to powdery mildew management on cucurbits. A Powdery Mildew Risk Index (PMRI) was developed by Thomas *et al*. (1994) and Gubler *et al*. (1999) in California, for managing powdery mildew of grapes. In this study, the concepts of PMRI, which are based on temperature effects of powdery mildew development, were used with some modifications to suit powdery mildew of cucurbits. The PMRI assumed that the infection of *P. fusca* on cucurbits only occurred with favourable temperatures and VPD. The model aimed to manage the fungicides usage in the cucurbit field by stretching intervals between sprays when the possibility of disease was low.

8.1.3 Aim

The research aimed to identify methods to develop and test a disease forecasting model for powdery mildew of cucurbits since none exists. In order to undertake this work the authors improved the design of a spore settling tower to enhance useability for inoculation of powdery mildews onto test plants. Quantitative data on the effects of temperature and vapour pressure deficit (VPD) on *in vitro* conidial germination were obtained. A modified PMRI was developed and its effectiveness was evaluated under field conditions.

8.2 Materials and Methods

8.2.1 Background techniques and resources

8.2.1.1 Pathogen source

The pathogen of powdery mildew was collected from naturally infected cucurbit leaves from fields located at Gatton S.E. Queensland. The pathogen was maintained throughout this study on cucumber seedlings (cv. Crystal salad) grown in the glasshouse at The University of Queensland, Gatton. The seedlings were grown in 16.0 cm diameter pots containing potting mix (composted bark ($1m^3$), Osmocote plus 8-9 months (2kg), Osmocote plus 3-4 months (1kg), Nutricote 7 months 7 months (2kg), coated iron (1.3kg), Saturaid (1.2kg), Polimite (1.2kg), Osmoform (1.3kg) and watered daily using an irrigation system.

8.2.1.2 Plant material

Plant material for growth chamber studies was produced as follows. Healthy cucumber seedlings were grown in the nursery at The University of Queensland, Gatton until the two to three leaf stage. Fully expanded leaves were then collected, washed with sterile distilled water and dried on filter paper before they were cut into 22 mm- diameter disks using a cork borer.

8.2.1.3 Identification of the pathogen

Two techniques were applied in identifying of the pathogen. The first technique used a cellotape strip as described by Amsalem *et al.* (2006). A piece of cellotape was stripped from leaf surfaces of the infected cucumber leaves and mounted on a glass slide containing a drop of lactophenol-cotton blue (LCB) solution for staining the pathogen. The second technique used leaf clearing as proposed by Liberato *et al.* (2005). With this technique, chloral hydrate was applied to remove leaf chlorophyll and aniline blue was used to stain the structures of the pathogen. The stained leaf segments were then mounted on glass slides containing a drop of lactic acid (85%) and examined microscopically. Samples of infected leaves were also sent to expert mycologists at the Department of Primary Industry (DPI) in Brisbane to confirm the species of pathogen.

8.2.2.1 The spore settling tower

The spore settling tower was constructed from a flanged Perspex cylinder (100 cm in height, 50 cm diameter, 20 mm thick). The complete tower consisted of the cylinder, a top cover, and a base plate. The top cover incorporated a vacuum line and air valve, a vacuum gauge, and a small removable lid with a 25mm inlet valve leading to an internal inoculum platform suspended below. The vacuum line and air valve was used to connect the tower to a vacuum pump, and the internal vacuum applied was measured by the attached gauge. The inlet valve in the removable lid was used to sharply break the vacuum inside the tower, dislodging conidia from the inoculum source (freshly harvested infected leaves). The internal inoculum platform (12 cm diameter) was constructed under the top cover with three holes, each with a 9.0 cm diameter in its side walls (20 cm deep) to allow inoculum to be expelled and shower the test plants placed on the base plate at the bottom of the tower. The whole construction was designed and fabricated to maintain an internal vacuum by the use of rubber gaskets and sealants.

8.2.2.2 The distribution of conidia by the spore settling tower

The distribution of conidia dispersed through the operation of the spore settling tower was examined using water agar (2%) trap plates. The base of the trap plates were marked with a 1.0 cm grid using a marker pen. Three open trap plates were used as replicates per inoculation run, and arranged on the base plate of the spore settling tower. Powdery mildew (*P. fusca*) maintained on cucurbit plants in the glasshouse, was used as the inoculum source. Leaves were cut into disks 2.0 cm^2 in diameter. In each inoculation run, 20 leaf disks (~ 2.5 g f.w.) were placed in an open Petri plate and covered with a layer of tape-fastened open plastic mesh (1 cm^2 pore size). The inoculum was placed on the inoculum platform, the water agar trap plates placed on the base plate and the unit sealed. A vacuum of 20 kPa (taking ~ 10 sec) was applied followed by closing of the air valve. Sudden opening of the inlet valve in the removable lid resulted in a sharp break in the vacuum causing a sudden inrush of air onto the inoculum source and dislodgment of conidia. The trap plates at the base of the unit plate were then exposed to the resulting shower of conidia. The unit was left undisturbed for a minimum of 120 seconds after breaking of vacuum to optimise conidial distribution (Reifschneider and Boiteux 1988). After each inoculation run, the media in the water agar trap plates was cut into 1 cm squares and fifteen randomly selected sections placed on glass microscope slides, stained with lactophenol cotton blue and observed under a light microscope to evaluate the distribution of conidia by counting. The data were analysed using a statistical analysis system (SAS).

8.2.2.3 Inoculation of cucumber leaves with the pathogen in the spore setting tower

Leaf disks (adaxial surface face up) were placed on top of moistened sponges that were kept in the 30ml McCartney bottles. The leaf disks in McCartney bottles were then placed on the basal plate of a settling tower and infected leaf parts with the powdery mildew were placed in the inoculum platform. Within the tower, conidia were dislodged from the infected leaf parts and dispersed on the disks because of the inrush air over the inoculum in the platform. Conidia were allowed to settle on the disks for 2 minutes before assessing conidial deposition and density under a light microscope. Inoculum density was assessed by counting the number of conidia deposited on the leaf disks. A piece of cellotape used to strip the conidia from the disk surface was mounted on a glass slide containing a drop of LCB solution and examined microscopically.

8.2.3 Identification of model parameters

8.2.3.1 Effect of temperature on conidial germination and formation of germ tubes at -0.03 kPa

Conidial germination and formation of *P. fusca* germ tubes at VPD -0.03 kPa were studied at seven temperatures (17, 19, 22, 25, 28, 31 and 35^{o}C). For each temperature, conidial germination was assessed at 4 incubation times (12, 24, 36 and 48 h). At each time point, nine discs were randomly selected and carefully removed from the humidity chambers. Four discs were used to examine conidial germination under a light microscope by using a piece of cellotape (4 cm × 2 cm) to remove the conidia from the leaf surfaces. For each leaf disc examined, 100 conidia were counted. A conidium with a primary germ tube whose length was at least half the diameter of the conidium was classed as a germinated conidium, as proposed by Lacy (1994). The remaining five discs were cleared using a leaf clearing technique to observe the formation of the infection structures and count the number of germ tubes. For each cleared leaf examined, 50 conidia were counted at 36 h incubation to assess the formation of germ tubes. The cellotape and leaf clearing techniques that were used in this experiment followed the same procedures described in the confirmation of the pathogen experiment. Unless stated otherwise, the examination procedures and recording of germinated conidia described here were applied in all experiments.

8.2.3.2 Effect of VPD levels on conidial germination at constant temperatures

The effect of VPD on conidial germination was evaluated at three constant temperatures. In this study, temperatures of 22, 25 and 28^{o}C were used as favourable temperatures for conidial germination of *P. fusca*. The combination of six different levels of relative humidity with three temperatures provided 18 VPD values, as listed in Table 8.1. Each VPD value was calculated using an online VPD calculator (http://www.autogrow.com/vpd_calc.php). Germination was assessed at all of these VPD levels and at four incubation time points followed the same procedures as described in the previous study.

Table 8.1 Saturated salt solutions used to give different levels of relative humidity at three temperatures.

Temperature (^{o}C)	22		25		28	
Solution	*RH	*VPD	RH	VPD	RH	VPD
Distilled water (H_2O)	99	-0.03	99	-0.03	99	-0.03
Potassium hydrogen phosphate ($KHPO_4$)	95	-0.13	95	-0.16	95	-0.19
Potassium chloride (KCl)	85	-0.40	85	-0.50	85	-0.60
Sodium chloride (NaCl)	75	-0.70	75	-0.80	75	-0.90
Calcium nitrate ($Ca(NO_3)_2.4H_2O_4$	55	-1.20	51	-1.55	50	-1.90
Magnesium chloride ($MgCl_2.6H_2O$)	33	-1.80	32.5	-2.10	32.5	-2.60

*RH value (%) is a mean value of data recorded every 15 min by Smart Logger sensors. *VPD (kPa) is an approximate value calculated using an online VPD calculator.

8.2.3.3 Effect of incubation time on conidial germination

The amounts of time required for conidia to produce a primary germ tube when exposed to the optimal condition for germination were examined at 6 different time points (2, 4, 6, 8, 10 and, 12 h). Preparation of leaf discs, inoculation, incubation and germination assessment were followed the same procedures as described previously. All the inoculated leaf discs were incubated at a temperature of 28^{o}C and VPD -0.03 kPa as this condition was considered to be favourable condition for the highest germination rate of *P. fusca*. At each time point, 4 leaf discs were used to examine conidial germination under a light microscope by using a cellotape technique. For each disc, 100 conidia were counted.

8.2.3.4 Data analysis

The effects of temperature, VPD and incubation time on conidial germination and formation of germ tubes were analysed using an analysis of variance (ANOVA) with a general linear model procedure (GLM) in the statistical analysis system® (SAS) software package. A

factorial ANOVA model was applied to explore interaction effects of temperature, VPD, and incubation time on conidial germination. Variation between replicates within treatments was used to assess the effect of leaf age on conidial germination.

8.2.7 Development of a powdery mildew predictive tool

8.2.7.1 Powdery Mildew Risk Index (PMRI) development from the laboratory data

A modified PMRI used temperature and VPD (calculated from relative humidity) environmental data from the laboratory studies. The model assumed that the powdery mildew pathogen (*P. fusca*) requires at least 2 consecutive days with at least 12 consecutive h of temperatures between 20 and 30°C and VPD between -0.03 and -0.13 kPa to trigger occurrence of conidial germination and infection. The pathogen needs another 3 consecutive days with at least 12 consecutive h of temperatures between 20 and 30°C and VPD between -0.03 and -2.55 kPa to promote fungal colonisation and sporulation processes. The concepts of the model are described in the Fig. 8.1. With the use of PMRI it is possible to estimate the risk of powdery mildew infection of cucurbits. The index can be used to successfully guide fungicide spray applications in cucurbits.

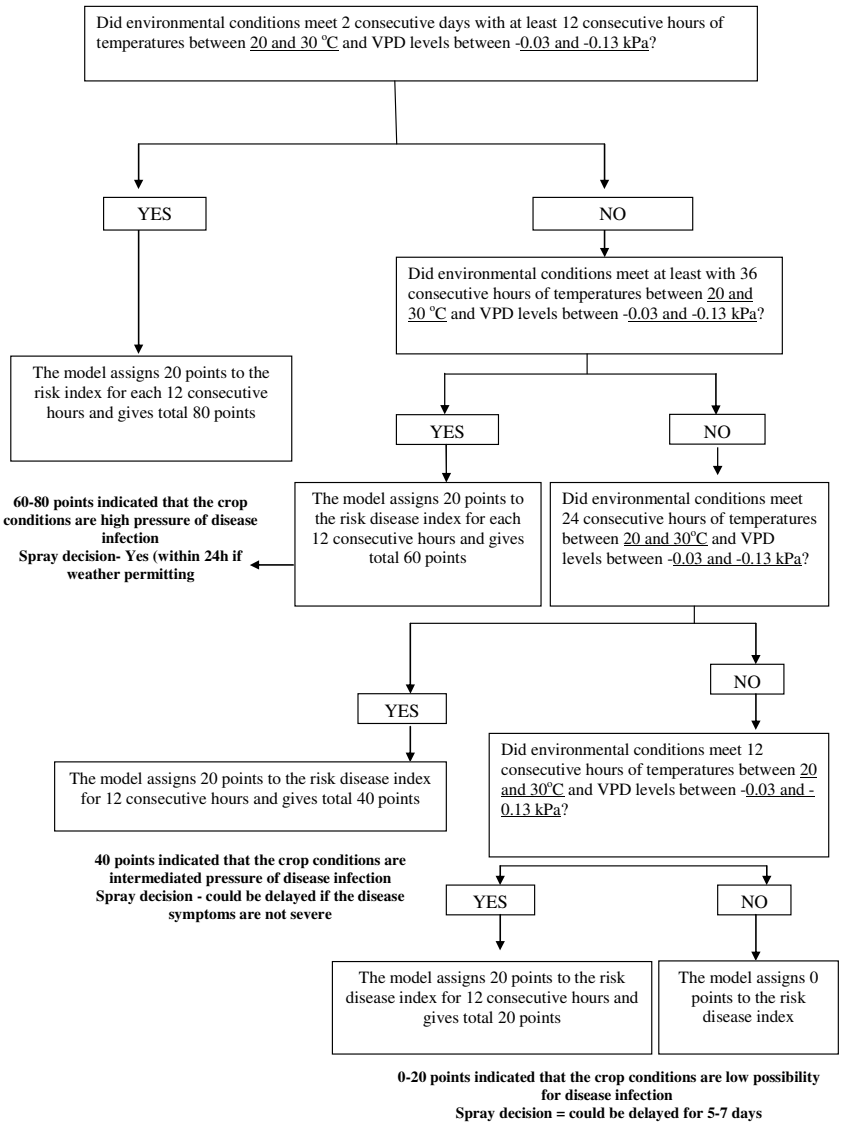


Fig 8.1 Flow chart for the PMRI in the field.

8.2.7.2 Testing and refining the model in the field.

Trial design: The field trial was conducted at the DEEDI Agri-Science, Queensland Gatton Research Station with zucchini being direct seeded on 7th February 2011. The final assessment was conducted on 19th April 2011. Zucchini cv. Amanda (Henderson Seed Co Pty Ltd), a cultivar susceptible to powdery mildew was selected as planting material. Twenty-one experimental plots were prepared using plastic mulch and accompanied by a drip irrigation system. These plots were assigned in a completely randomised design with three treatments and seven replicates per treatment. The three treatments were: (i) an unsprayed control; (ii) fungicides sprayed according to grower practice; and (iii) sprays timed on the predictions of the PMRI. Each plot contained 4 beds and each bed (5 m × 1.5 m) was planted with 10 plants spaced 55 cm apart. The two central beds were used for data plant rows with the outer two beds being buffer plant rows. Standard agronomic practises for irrigation, weed control, insect pest management and fertigation were undertaken by the DEEDI Gatton farm staff. In the early stage of plant growth, no fungicide was applied to allow natural infection of powdery mildew to develop on the plants.

Trial treatments: Three treatments were tested in this study. The first treatment was a standard industry program, which is a weekly based spray program with rotating fungicides. When the first disease inoculum was found in the field, a systemic fungicide (azoxystrobin or bupirimate) was applied using a tractor mounted boom spray delivering 600 L /ha of water. After 7 days, a contact fungicide (microgranule sulfur) was introduced into the treatment. The rotating fungicide treatment that is systemic and contact, was applied every 7 days for 7 weeks. The second treatment applied was the same rotating fungicide method but the application interval was based on powdery mildew risk index (PMRI) points. The third treatment was a control where no fungicide treatment was used.

Weather data: A portable weather station (Monitor Sensor, Caboolture, QLD, Australia) was used to record seven environmental parameters, including temperature, relative humidity, leaf wetness, wind speed, daylight intensity and cumulative rainfall every 15 minutes. These data (including the calculated VPD) and disease incidence and severity data, were used to fine-tune the PMRI.

PMRI in the field: After the early symptoms of powdery mildew were observed in the zucchini field, the PMRI was applied to predict the disease pressure and spraying time based on risk index points. The PMRI point was calculated from meteorological data (hourly average temperatures and VPD) that were recorded every 15 minutes by a temperature/humidity sensor. This PMRI model assumed that *P. fusca* requires at least 36 consecutive h of temperatures between 20 and 30°C and vapour pressure deficit (VPD) levels between -0.03 and -0.13 kPa to trigger the infection processes. If the environmental conditions in the field had experienced hourly average temperatures and VPD that were suitable for infection for 36 consecutive h, the model assigned 20 points for every 12 consecutive h giving a total of 60 points. These 60 points indicated that the environmental conditions were very conducive for infection. If the environmental conditions in the field continuously had the favourable temperatures and VPD for 60 consecutive h, the model assigned 100 points. These maximum points indicated that the conditions were very conducive for *P. fusca* to produce a high number of infection structures (a dense of mycelium with the presence of appressoria and penetration pegs) and for it to start to colonise the plant surfaces. When the model assigned points between 60 and 100, the crops need to be sprayed within 24 h (weather permitting).

When the environmental conditions in the zucchini field had not experienced hourly average temperatures and VPD levels that were suitable for infection for 36 consecutive h or had experienced only 12 consecutive h, the model assigned 0 or 20 points, respectively. This range of points indicated that the crop conditions were low pressure for infection and no chemical fungicide was recommended. However, if the conditions were met with these

favourable temperatures and VPD for 24 consecutive h, the model assigned 40 points to the index. These points indicated that the crop conditions were intermediate pressure for infection and the model recommended that applying chemical fungicides could be delayed if the disease symptoms were not severe.

8.2.7.3 Disease severity assessments

Disease severity on zucchini plants was assessed on leaves of 4 tagged data plants within each replicate (data row 1). Powdery mildew on the leaves was given a rating on a 0-5 scale as indicated in Table 8.2. Ratings were given to 2 upper canopy leaves that were fully expanded, but not the youngest ones, and 2 lower canopy leaves that were not showing signs of senescing or necrosis. Disease symptoms were assessed on both the upper and lower surfaces of the leaves.

Table 8.2 Powdery mildew disease severity ratings on the leaves of zucchini.

Severity level	Area covered (%)
0	0
1	0.3 – 4.7
2	5 – 9.7
3	10 – 29.7
4	30 – 74.7
5	75 - 100

8.2.7.4 Data analysis

The effects of 3 treatments on the disease severity and yield assessments were analysed using an analysis of variance (ANOVA) with general linear model procedure (GLM) in the statistical analysis system® (SAS) software package.

8.3 Results and Discussion

8.3.1 Spore settling tower

The spore settling tower developed in this study is relatively easy to operate for inoculation of plants with powdery mildew pathogens. The transparent Perspex construction allows users to monitor the process inside the tower. Provision of a vacuum gauge allows for more consistent conditions to be achieved, resulting in higher repeatability among inoculation runs. The incorporation of an air inlet valve (not present in the unit designed by Reifschneider and Boiteux, 1988) allows for easy breaking of vacuum, while modifications to the inoculum platform further improved useability (Fig 8.2).

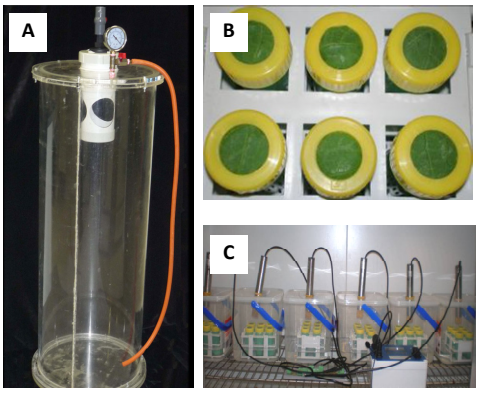


Fig 8.2 Inoculation and incubation of inoculated cucumber leaf discs. (A) A spore settling tower for disease inoculation, (B) Leaf discs retained beneath the cap of McCartney bottles, (C) small humidity chambers with smart logger sensors placed in an illuminated constant temperature chamber.

The distribution of conidia observed in this study was uniform with no significant difference between the numbers observed within and between trap plates $P<0.05$ (Table 8.3). The results showed that each trap plate received an average of 27±2 conidia / cm^2. The results of this study agreed with those of Reifschneider and Boiteux (1988), who determined that a settling tower was a more practical method, and ensuring improved uniformity in the distribution of conidia on test plants than dusting techniques using paint brushes.

Table 8.3 Actual conidial counts from trap plate squares.

Random square	Plate 1	Plate 2	Plate 3
R1	29	28	28
R2	25	26	27
R3	29	28	30
R4	24	27	29
R5	27	27	28
Means	26.8	27.2	28.4

Variation among and between plates was not significantly different at the 0.05% level.

8.3.2 Effect of temperature on conidial germination and formation of germ tubes at -0.03 kPa

Germination: Germination of *P. fusca* was observed at all temperatures tested, except at temperature of 35°C where there was no evidence of germination at any of the incubation time points. From these results, it is evident that temperatures of 35°C and above can inhibit conidial germination and prevent powdery mildew infection on cucurbits. This pathogen germinated well across temperature range of 20 to 30°C with an optimum growth at 28°C (Fig. 8.3). This result was similar to that reported by Hashioka (1936) in Formosa, Taiwan. There was a significant ($P < 0.05$) interaction between temperature and incubation time on conidial germination, with germination at all temperatures (below 35°C) increasing with time. At 28°C, more than 50% of conidia produced germ tubes after 12 h and then this proportion increased by another 10% after 24 h before peaking at 85% after 48 h (Fig 8.3). Germination at temperatures between 22 and 25°C was greatly increased after 24 h with 48% and 52% of conidia germinating, respectively, and reached 75-80% after 48 h. In contrast, germination was significantly reduced at temperatures of 19°C and below, or at 31°C and above, across all incubation times. Hashioka (1936) and Cheah *et al*. (1996) reported the absence of germination at temperatures of 10°C and below. In this study, at the lowest temperature tested (17°C), less than 10% of conidia germinated after 12 h, but the proportion increased to 25% after 48 h. There were no significant differences between replicates within all treatments. This result indicated the absence of variation in germination due to leaf age. Moreover, germination at 0 h (control discs) ensured that no germination occurred before incubation.

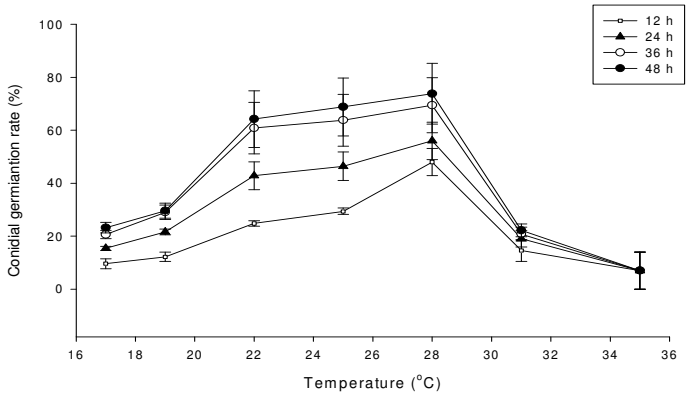


Fig 8.3 The effects of various temperatures on conidial germination of *P. fusca* at -0.03 kPa and at 4 incubation time points. Each value is a mean of 4 replicates. Standard errors are indicated by error bars.

Germ tubes: Observations of cleared leaf discs after 36 h of incubation showed that temperature had a significant effect ($P < 0.05$) on the formation of germ tubes. Production of a third germ tube peaked at temperatures between 25 and 28ºC with more than 50% of conidia showing germ tubes (Fig 8.4). Meanwhile, production of a second germ tube was higher at a temperature of 22ºC than at other temperatures. The decrease in the number of the second germ tubes at 25 and 28ºC suggested that after 36 h of incubation under optimum temperatures, a germinated conidium tended to produce a third germ tube while the first and second germ tubes established their infection sites on the host tissues. In contrast, production of a third germ tube was not observed at 17ºC and 31ºC and only 5% conidia produced the third germ tube at 19ºC. At the lowest temperature (17ºC) and the highest temperature (35ºC) tested, a conidium was observed to consistently produce a short first or second germ tube after 36 h of incubation. Observation of cleared leaf discs that were incubated at 28ºC and -0.03 kPa revealed that *P. fusca* started to produce a primary germ tube after 12 h of incubation. After 24 h incubation, a second germ tube emerged on the other side of the conidium. This germ tube then elongated to form a long hypha, with attendant development of appressoria

Appressoria: Appressoria of *P. fusca* develop without a specific shape, like other powdery species, and can be differentiated from hyphae only by the widening of the hyphae, as suggested by Boesewinkel (1977). From beneath the appressoria, an infection structure, known as a penetration peg develops and penetrates the epidermal host tissues. This structure could be observed after 24 h on cleared leaf tissues and its goal is to penetrate the epidermal cells. After a successful penetration, a haustorium develops at the end of the peg. This haustorium is used to absorb nutrients from cucurbits and it supplies these to the other fungal structures that remain on cucurbit leaf surfaces. Once a parasitic relationship is established with the host, a conidium was able to produce more germ tubes and branching hyphae, which can be clearly observed after 36 and 48 h incubation. This study assumed that as a conidium increases its formation of germ tubes, it would have more appressoria and infection sites.

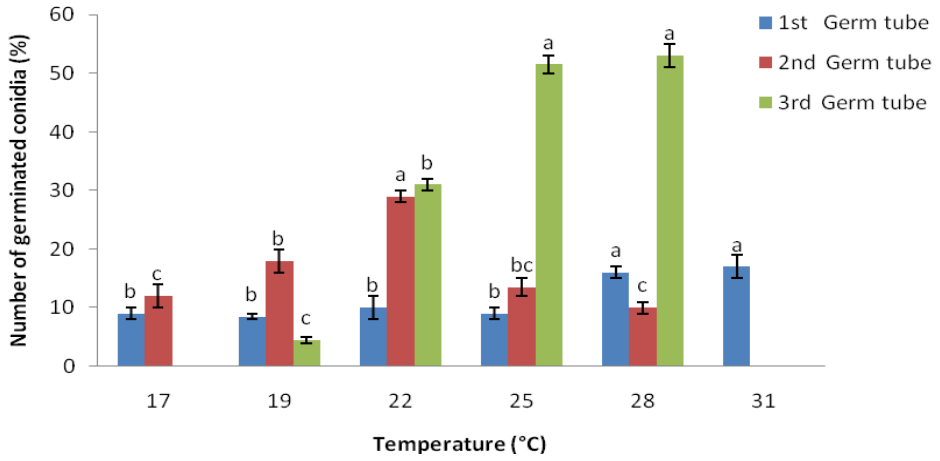


Fig 8.4 The effect of various temperatures on formation of *P. fusca* germ tubes on leaf discs at -0.03 kPa after 36 h incubation. Each value is a mean of 5 replicates. Standard errors are indicated by error bars.

8.3.3 Effect of VPD on conidial germination at constant temperatures

Besides temperature, VPD had a significant effect on the germination of *P. fusca* on cucurbit leaf discs. Germination was favoured at the VPD -0.03 kPa where the conditions were nearing saturation. This was also reported by Hashioka (1936), Sitterly (1978) and Cheah *et al.* (1996). The highest germination was obtained at the VPD -0.03 kPa and significantly dropped

when the VPD values decreased across all incubation points, regardless of temperature (Figs 8.4, 8.5 and 8.6). Observations at 22 ^{o}C and at 4 incubation time points showed that the rate of conidial germination declined significantly ($P < 0.05$) when the VPD level decreased to -0.13 kPa in all incubation time points (Fig 8.5). The same pattern was also found at temperatures of 25 ^{o}C (Fig 8.6) and 28 ^{o}C (Fig 8.7), at which conidial germination dramatically dropped with decreasing VPD values below -0.03 kPa. It can be reliably assumed that dry conditions (VPD -0.13 kPa and below) can reduce disease severity on cucurbit leaves. However, in this study at the lowest VPD tested (-2.55 KPa), a low number of conidia (5%) was able to produce germ tubes after 24 h incubation and the number increased to 7% after 48 h incubation. This result could be explained by the findings by Yarwood (1957) who suggested that powdery mildew of cucurbits is able to survive at VPD as low as -2.6 kPa without the presence of free water because the conidia contain internal water which can be used for germination.

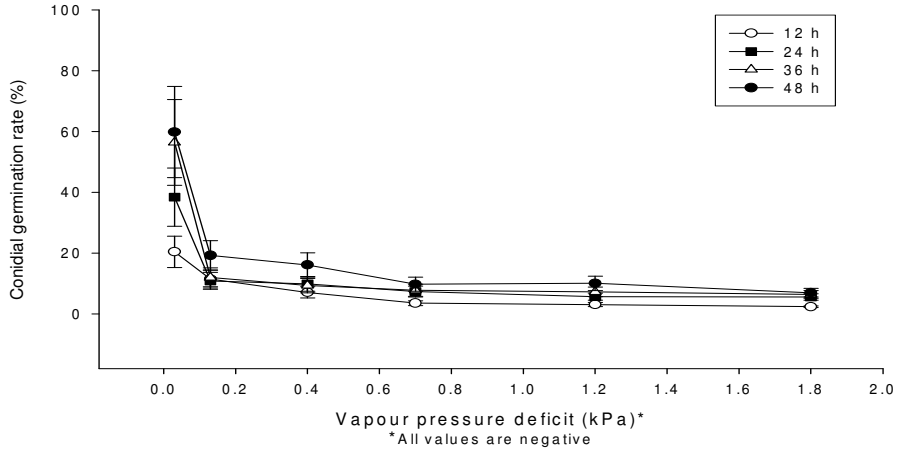


Fig 8.5 The effect of different VPD levels on conidial germination of *P. fusca* at 22^{o}C and at 4 incubation time points. Each value is a mean of 5 replicates. Standard errors are indicated by error bars.

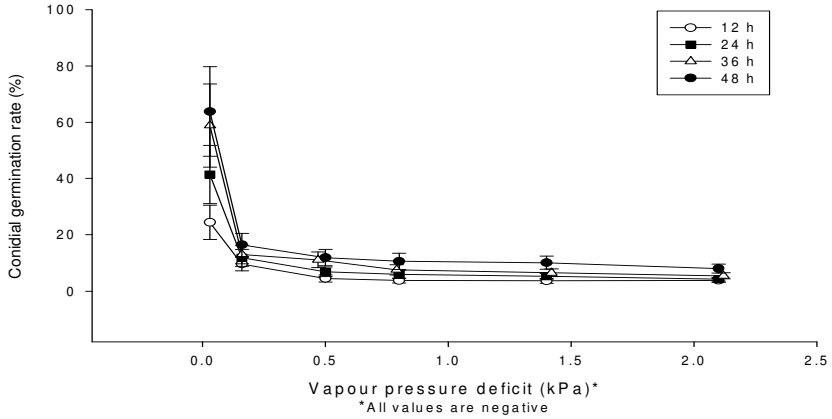


Fig 8.6 The effect of different VPD levels on conidial germination of *P. fusca* at 25^{o}C and at 4 incubation time points. Each value is a mean of 5 replicates. Standard errors are indicated by error bars.

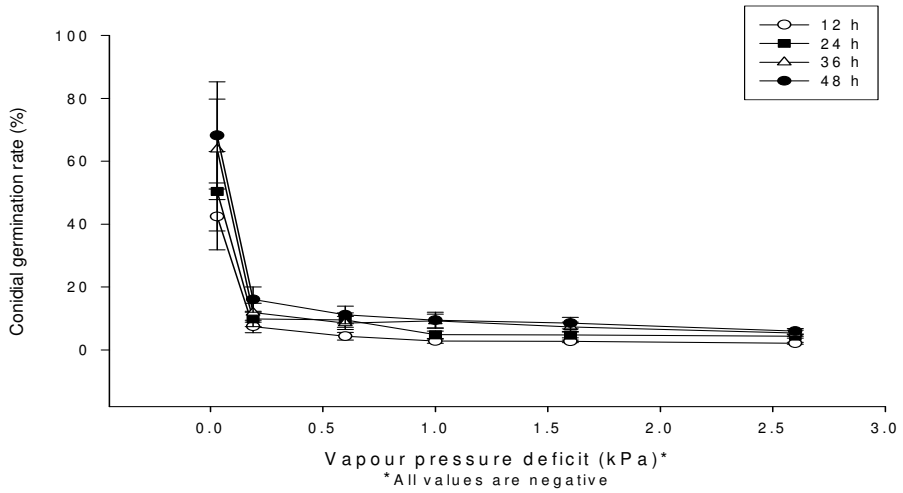
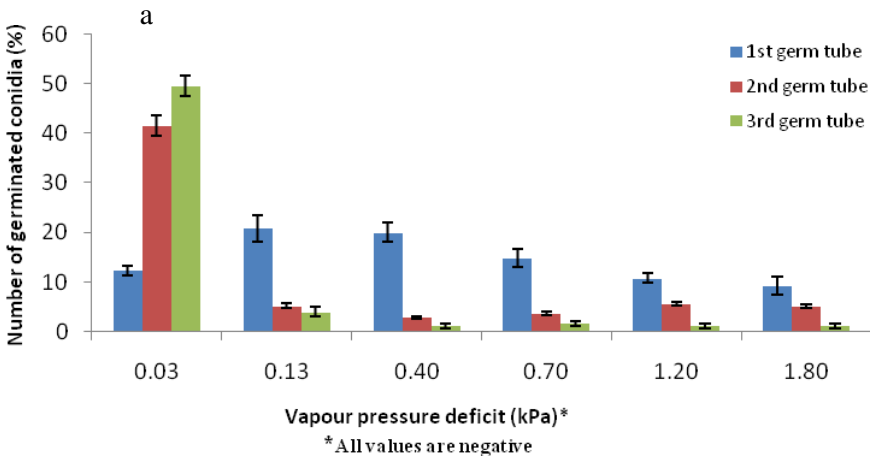


Fig 8.7 The effect of different VPD levels on conidial germination of *P. fusca* at 28ºC and at 4 incubation time points. Each value is a mean of 5 replicates. Standard errors are indicated by error bars.

8.3.4 Effect of VPD levels on conidial germination at constant temperatures

Observation of cleared leaf discs after 36 h incubation revealed that conidia exposed to the VPD between -0.03 to -1.80 kPa were able to produce a third germ tube but this significantly dropped ($P < 0.05$) when the VPD decreased below -0.03 kPa, regardless of temperature (Fig 8.8a-c). At VPD -0.13 kPa, only 4% of germinated conidia were able to produce a third germ tube. This finding indicated that VPD levels - 0.13 kPa and below can reduce disease severity on cucurbits by inhibiting the production of germ tubes, appressoria and penetration pegs. No production of a third germ tube was observed at the lowest VPD tested (– 2.55 kPa).



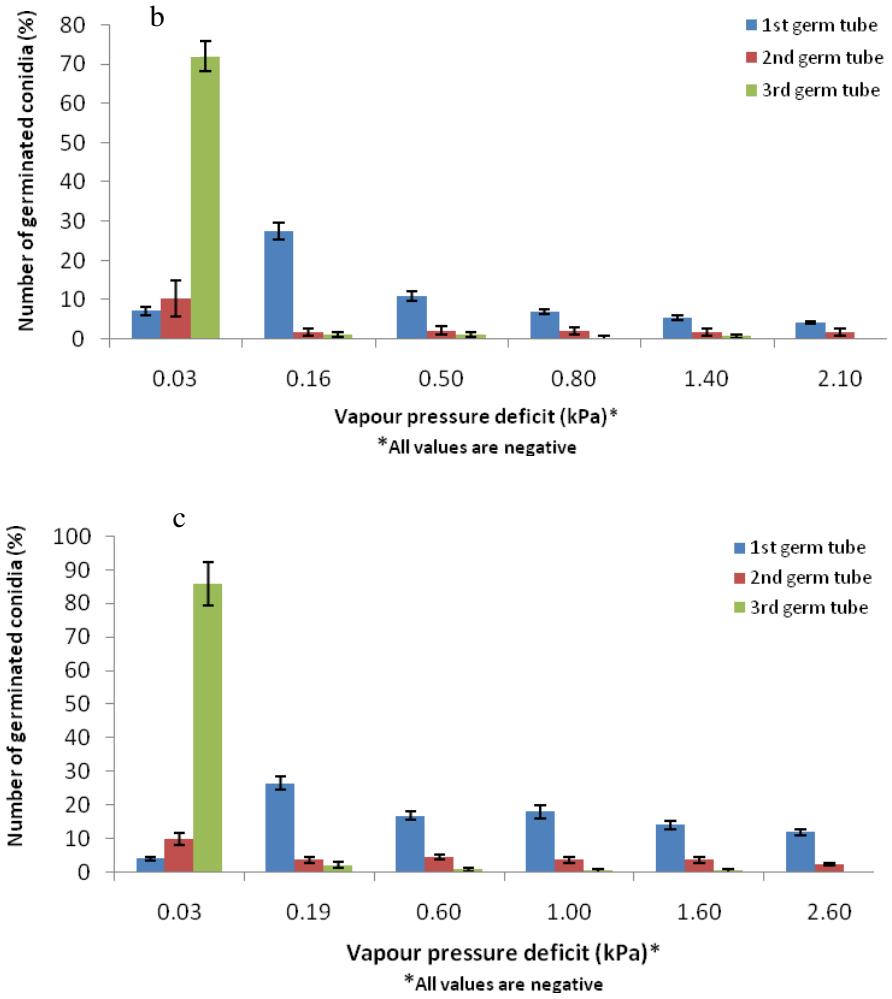


Fig 8.8 The effect of different VPD levels on formation of *P. fusca* germ tubes on cucumber leaf discs incubated at 3 different temperatures. (a) 22ºC, (b) 25ºC and, (c) 28ºC. Each value is a mean of 5 replicates. Standard errors are indicated by error bars.

8.3.5 Evaluation of a powdery mildew predictive tool in the field trial

The early symptom of white colonies of powdery mildew was observed 4 weeks after the planting date. This observation indicated the availability of disease inoculum but the level of disease severity was extremely low. No chemical spraying was decided for all treatments. For the first week (4th -10th March, 2011), the PMRI model also predicted that the conditions were low pressure for infection. The model only gave 20 points to the risk index for the week (Table 8.4). This range of points (0-20) indicated that the daily temperatures and VPD levels were not favourable for the formation of infection structures. The model predicted no infection and low disease pressure. No chemical fungicide was applied for all treatments.

The second observation of disease severity (week 2) was conducted on 14th March, 2011. The plants were not severely affected by the disease. Disease severity was very low and was below level 1. The low severity was explained by the unfavourable environmental conditions predicted by the model. The model assigned 0 points to the risk index (Table 8.4). No chemical fungicide was applied for all treatments.

The third observation of disease severity (week 3) was carried out on 21st March, 2011 and the zucchini leaf area covered by the disease was between 0.3 – 4.7%. The standard industry

treatment was sprayed with a systemic fungicide (azoxystrobin) within 24 h. Meanwhile, the model assigned 40 points to the risk index (Table 8.4) and predicted that the environmental conditions were intermediate pressure for infection. Due to the low disease severity recorded, the model recommended to delay the spraying time about 2-3 days for the PMRI treatment.

After 7 days, the fourth observation (week 4) was conducted on 28th March, 2011. The disease severity was low in the treatments sprayed with azoxystrobin and both were significantly different ($P < 0.05$) to the untreated treatment (control). There was no significant difference between severity levels in the standard industry treatment and the PMRI treatment (Fig. 8.9 and 8.10). In order to keep disease inoculum low, a spray with a contact chemical (microgranular sulfur) was applied to the standard industry treatment within 24 h. The spray was not applied to the PMRI treatment because the model predicted that environmental conditions were not conducive for infection and assigned 0 points to the index (Table 8.4). This point level suggested that the spraying time could be delayed for 5-7 days. The contact chemical was applied to the PMRI treatment on 4th April, 2011.

The observation of disease severity for week 5 was conducted on 5th April, 2011 and recorded in all treatments. The control plants were covered with the disease (10%). In contrast, the plants treated with microgranular sulfur had low disease severity (0.3%) on both upper and lower leaf surfaces and there was no significant difference between these two treatments. This suggested that the PMRI can be used to delay fungicide spraying if conditions were not conductive for infection. For week 5, a systemic fungicide (bupirimate) was applied to the standard industry treatment on 6th April, 2011 and the PMRI treatment was sprayed with the same chemical 5 days later, as the PMRI assigned 0 points to the index (Table 8.4).

The sixth observation of disease severity (week 6) was done on 12th April, 2011 and statistical analysis of the results showed that there was no significant difference between levels of disease severity in the standard industry treatment and the PMRI treatment. However both treatments were significantly different to the untreated control. After the week 6 treatment evaluation, the standard industry treatment was sprayed with a contact chemical (microgranular sulfur) on 13th April, 2011 and the PMRI treatment was sprayed with the same chemical after 5 days (18th April, 2011) as the model assigned 0 to the index (Table 8.4).

The final disease assessment was carried on 19th April, 2011. The disease severity observation showed there was no significant difference between the standard industry treatment and the PMRI treatment.

Table 8.4 Powdery Mildew Risk Index (PMRI) calculated for weeks 1 to 6 after powdery mildew inoculum was found on crops.

Week	Day	Date		Hour	Point	Prediction
1	Friday	4/03/2011	d-1	1st 12	0	unfavourable
				2nd 12	20	germination
	Saturday	5/03/2011	d-2	3rd 12	0	unfavourable
				4th 12	0	
	Sunday	6/03/2011	d-3	5th 12	0	unfavourable
				6th 12	0	
	Monday	7/03/2011	d-4	7th 12	0	unfavourable
				8th 12	0	
	Tuesday	8/03/2011	d-5	9th 12	0	unfavourable
				10th 12	0	
	Wednesday	9/03/2011	d-6	11th 12	0	unfavourable
				12th 12	0	
	Thursday	10/03/2011	d-7	13th 12	0	unfavourable

Week	Day	Date		Hour	Point	Prediction
				14th 12	0	
2	Friday	11/03/2011	d-1	1st 12	0	unfavourable
				2nd 12	0	
	Saturday	12/03/2011	d-2	3rd 12	0	unfavourable
				4th 12	0	
	Sunday	13/03/2011	d-3	5th 12	0	unfavourable
				6th 12	0	
	Monday	14/03/2011	d-4	7th 12	0	unfavourable
				8th 12	0	
	Tuesday	15/03/2011	d-5	9th 12	0	unfavourable
				10th 12	0	
	Wednesday	16/03/2011	d-6	11th 12	0	unfavourable
				12th 12	0	
	Thursday	17/03/2011	d-7	13th 12	0	unfavourable
				14th 12	0	
				10th 12	0	
	Wednesday	16/03/2011	d-6	11th 12	0	unfavourable
				12th 12	0	
	Thursday	17/03/2011	d-7	13th 12	0	unfavourable
				14th 12	0	
3	Friday	18/03/2011	d-1	1st 12	0	unfavourable
				2nd 12	0	
	Saturday	19/03/2011	d-2	3rd 12	20	germination
				4th 12	20	high germination
	Sunday	20/03/2011	d-3	5th 12	0	unfavourable
				6th 12	0	
	Monday	21/03/2011	d-4	7th 12	0	unfavourable
				8th 12	0	
	Tuesday	22/03/2011	d-5	9th 12	0	unfavourable
				10th 12	0	
	Wednesday	23/03/2011	d-6	11th 12	0	unfavourable
				12th 12	0	
	Thursday	24/03/2011	d-7	13th 12	0	unfavourable
				14th 12	0	
4	Friday	25/03/2011	d-1	1st 12	0	unfavourable
				2nd 12	0	
	Saturday	26/03/2011	d-2	3rd 12	0	unfavourable
				4th 12	0	
	Sunday	27/03/2011	d-3	5th 12	0	unfavourable
				6th 12	0	
	Monday	28/03/2011	d-4	7th 12	0	unfavourable
				8th 12	0	
	Tuesday	29/03/2011	d-5	9th 12	0	unfavourable
				10th 12	0	
	Wednesday	30/03/2011	d-6	11th 12	0	unfavourable
				12th 12	0	
	Thursday	31/03/2011	d-7	13th 12	0	unfavourable
				14th 12	0	
5	Friday	1/04/2011	d-1	1st 12	0	unfavourable
				2nd 12	0	
	Saturday	2/04/2011	d-2	3rd 12	0	unfavourable
				4th 12	0	

Week	Day	Date		Hour	Point	Prediction
	Sunday	3/04/2011	d-3	5th 12	0	unfavourable
				6th 12	0	
	Monday	4/04/2011	d-4	7th 12	0	unfavourable
				8th 12	0	
	Tuesday	5/04/2011	d-5	9th 12	0	unfavourable
				10th 12	0	
	Wednesday	6/04/2011	d-6	11th 12	0	unfavourable
				12th 12	0	
	Thursday	7/04/2011	d-7	13th 12	0	unfavourable
				14th 12	0	
6	Friday	8/04/2011	d-1	1st 12	0	unfavourable
				2nd 12	0	
	Saturday	9/04/2011	d-2	3rd 12	0	unfavourable
				4th 12	0	
	Sunday	10/04/2011	d-3	5th 12	0	unfavourable
				6th 12	0	
	Monday	11/04/2011	d-4	7th 12	0	unfavourable
				8th 12	0	
	Tuesday	12/04/2011	d-5	9th 12	0	unfavourable
				10th 12	0	
	Wednesday	13/04/2011	d-6	11th 12	0	unfavourable
				12th 12	0	
	Thursday	14/04/2011	d-7	13th 12	0	unfavourable
				14th 12	0	

The 0 point indicated that the environmental conditions were not conducive for disease infection.

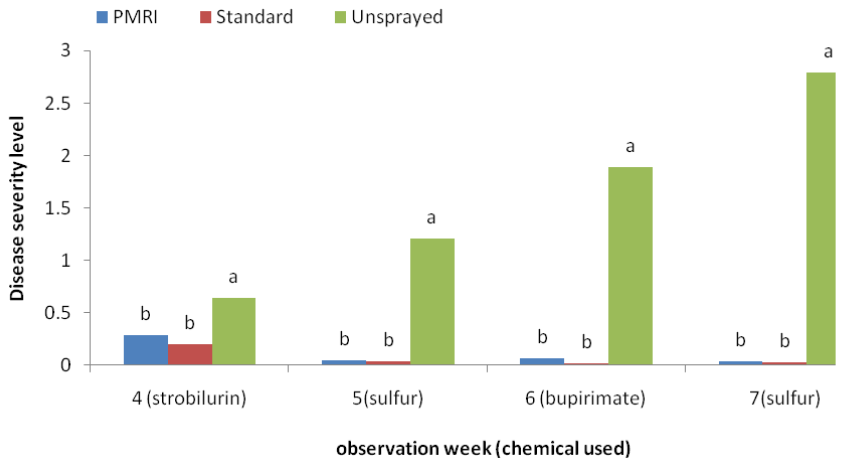


Fig 8.9 Disease severity levels on the upper surface of zucchini leaves recorded after fungicide treatments were applied. PMRI is an abbreviation of powdery mildew risk index. Means with the same letter between treatments were not significantly different.

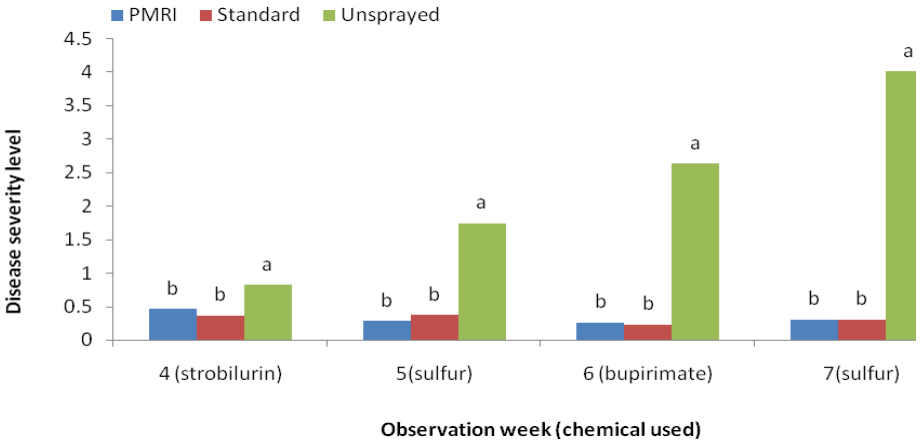


Fig 8.10 Disease severity levels on the lower surface of zucchini leaves recorded after chemical treatments were applied. PMRI is an abbreviation of powdery mildew risk index. Means with the same letter between treatments were not significantly different.

Fig 8.11 Field site, equipment and PhD student, Ms Zaiton Sapak.

8.4 References

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Chapter 9

Economic analyses of trials reported in the previous chapters

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Summary
Economic analyses of treatments for the management of white blister on brassicas and downy mildew on lettuce using chemical sprays based on (i) a weekly routine; (ii) grower timing; (iii) strategic growth stages of the host; (iv) fungicide alternatives; (v) irrigation timing or (vi) disease predictive models and their associated software, were undertaken in relation to field evaluations. Results for the treatments were expressed as their "contribution to farm profit" and ranked. No treatment for each disease consistently had the best ranking.

9.1 Introduction

The approach used in conducting economic analyses for the various trials on brassicas and lettuce reported in previous chapters was to rank the economic results for the various treatments as their contribution to profitability for the whole farm involved in the production of vegetables.

Changes in contribution to profitability for the treatments were calculated by deducting their variable expenses from gross income. Variable expenses included the cost of applying chemicals, the cost of crop inspections, and costs for harvesting and packaging vegetables. All other variable expenses such as costs for tillage and bedding, herbicide costs for weed control, costs of fertiliser and any other variable costs were assumed to be the same for the various treatments.

Other costs deducted from gross income were overhead expenses for weather stations and costs of using Brassica$_{spot}$™ disease predictive models on Brassica crops.

9.2 Methods

9.2.1 Costs of chemicals applied for the various treatments
Application rates for chemicals used in the various treatments and their costs per hectare are shown in Table 9.1.

9.2.2 Assumptions used in the analyses
- The cost of applying chemicals was $50 per hectare.
- Harvesting cost for broccoli and lettuce was $2.50 per 10 kg box.
- Harvesting cost for Chinese cabbage was $2.50 per box containing 10 plants.
- Packaging cost for broccoli, Chinese cabbage and lettuce was $1.50 per box.
- Weekly crop inspections for treatments took 20 minutes per 4 hectare at a cost of $70 per hour.
- The number of hectares of crop per weather station was 5 hectares.
- The costs of repairs and maintenance of weather stations were $10 per hectare.
- The cost of using the Brassica$_{spot}$™ Model was initially $1,500 per 10 hectares of crop or $150 per hectare. The cost per model for trial 11 for broccoli was later increased to $4,000 per 10 hectares or $400 per hectare. On reflection it is possible

that the area (ha) the model would be used over is an underestimate as a grower producing only 10 ha of broccoli is unlikely to purchase the model, consequently a $1,500 per 100 ha or $150/10ha may be a more reasonable estimate. However, this is unlikely to make any difference to the outcomes of the analyses.

- Gross income per 10 kg box of broccoli was $16.50. For Chinese cabbage the price per box containing 10 plants was $14.00. Iceberg lettuce was marketed in 10 kg boxes for $12.78 per box. The price for Cos lettuce in 4 kg boxes was $13.45 per box and Red Oakleaf lettuce realised $12.00 per 2 kg box.
- The overhead cost for weather stations was $75 per hectare as shown in Table 9.2.

Table 9.1 Application rates for chemicals used in the various treatments and their costs per hectare[a].

Trade name	Active ingredient	Application rate per ha	Cost per unit (L or kg)	Cost per ha
			$	$
Acrobat®	Dimethomorph	360g	315.00	113.40
Agral™	Nonyl phenol ethylene oxide condensate (60 g/L)	0.13%	16.28	10.58
Agriphos®	Phosphorous acid (600 g/L)	850 ml	7.06	6.00
Amistar 250 EC®	Azoxystrobin (500 g/kg)	300 g	235.96	70.79
Amistar SC®	Azoxystrobin (500 g/kg)	400 ml	235.96	94.38
Amistar SC®	Azoxystrobin (500 g/kg)	500 ml	235.96	117.98
Amistar WG®	Azoxystrobin (500 g/kg)	60 g/100L	357.00	107.10
Antracol®	Propineb (700 g/kg)	2.0 kg	25.90	51.80
Bion 50 WG™	Acibenzolar-*S*-methyl (500 g/L)	25.0 g	1,000	25.00
Cabrio®	Pyraclostrobin (25 g/L)	500 ml	102.96	51.48
Designer™	Organosilicone surfactant fluid + synthetic latex	200 ml	52.31	10.46
Du-Wett™	Trisiloxane ethoxylate (500 g/L, non-ionic)	300 ml	56.28	16.88
Du-Wett™	Trisiloxane ethoxylate (500 g/L, non-ionic)	200 ml	56.28	11.26
FoliCal Plus	Fulvic acids	7 L/700 L	6.30	31.50
Fulzyme™	*Bacillus subtilis*	4 ml/L	23.25	46.50
Li-700™	Soyal phospholipids (350 g/L) + propionic acid (350 g/L)	10 ml/100L	11.46	0.57
	Ethoxulates (10-30%)			
Microplus™	*Streptomyces lyticus*	500 g/ha	240.00	120.00
Pencozeb®	Mancozeb (750 g/kg)	1.6 kg	10.81	17.30
Polyram®	Metiram (700 g/kg)	2.2 kg	13.23	29.11
Revus®[1]	Mandipropamid (250 g/L)	600 ml	100.00	60.00
Ridomil Gold Plus®	Metalaxyl M (50 g/kg) + copper hydroxide (600 g/kg)	2.0 kg	60.00	120.00
Ridomil Gold Plus®	Metalaxyl M (50 g/kg) + copper hydroxide (600 g/kg)	2.5 kg	60.00	150.00
Ridomil Gold MZ®	Metalaxyl M (40 g/kg) + mancozeb 640 g/kg	250 g/100L	57.72	72.15
Seasol™	Seaweed extract	833 ml	10.45	8.70
Sodium lauryl sulfate™	Sodium lauryl sulfate	2.0 g/L	9.50	9.50
Sprayphos 620™	Phosphorous acid (620 g/L)	170 ml/100L	5.79	4.92
Synetrol™	Vegetable oil (>60%), polyethoxylated oil (<10%)	300 ml/100L	8.48	12.71
Tri-Base Blue®	Copper sulphate tribase (190 g/L)	280 ml/100L	13.85	19.38

A,The cost per hectare for the various chemicals was for applications in the first 8 weeks of the trials where the amount of wash applied was 500 litres per hectare. The cost per hectare after 8 weeks would be doubled when 1,000 litres of wash was applied per hectare.

Table 9.2 Overhead costs for weather stations.

Year	Investment at start of year	Annual depreciation	Investment at end of year	Average investment	Interest at 10% per annum
	$	**$**	**$**	**$**	**$**
1	2,500	250	2,250	2,375	238
2	2,250	250	2,000	2,125	213
3	2,000	250	1,750	1,875	188
4	1,750	250	1,500	1,625	163
5	1,500	250	1,250	1,375	138
6	1,250	250	1,000	1,125	113
7	1,000	250	750	875	88
8	750	250	500	625	63
9	500	250	250	375	38
10	250	250	0	125	13
Average		250			125

Overhead cost is equal to $75 per hectare beig depreciation and interest equal to $250 + $125 = $375÷ 5 = $75

9.4 Results of economic analyses

The results of the trials reveal the contribution per hectare for the various treatments with their rankings and graphs showing the relative contributions per hectare for the various treatments. The cost of chemicals applied for the treatments in the various trials are shown in the appendices.

9.4.1 Effect of time of irrigation for the control of white blister on broccoli.

Table 9.3 Contribution to profit per hectare for timing of irrigation to reduce the incidence of white blister on broccoli.

Treatment	Total cost of applying chemicals	Yield	Harvesting @ $2.50 per 10kg box	Packaging @ $1.50 per box	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[a]	Annual cost for use of Brassicaspot™ Model[b]	Farm gate income @ $16.50/box	Contibution to farm profit
	$/ha	t/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha
Evening	4,936	10.74	2,685	1,611	150	20	300	17,721	13,255
Morning	4,936	11.04	2,760	1,656	150	20	300	18,216	13,630

Note: The cost for crop inspections was the same for all treatments.
a, cost of repair and maintenance of the weather station was assumed to be $10 per hectare per annum.
b, Brassica$_{spot}$™ models cost $1,500 per 10 hectares of crop.

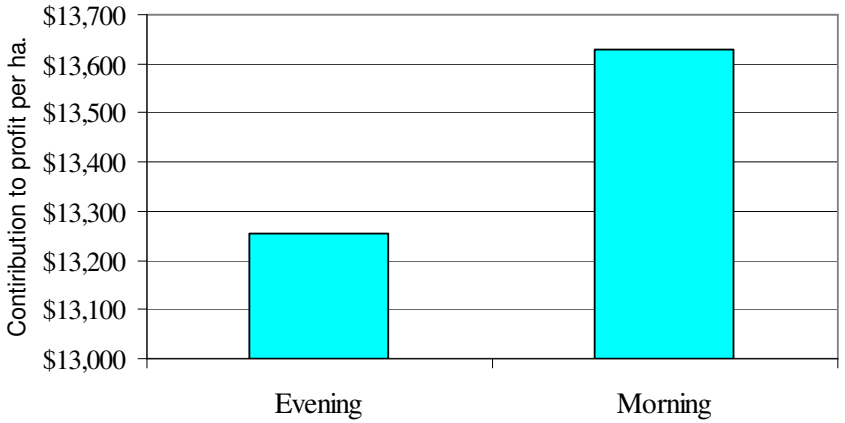

Figure 9.1 Contributions to profit per hectare for times of irrigation on resistant and susceptible varieties to reduce the impact of white blister on broccoli

9.4.2 Effect of resistant variety on reducing the incidence of white blister on broccoli.

Table 9.4 Contribution of profit per hectare for resistant compared with susceptible variety of broccoli.

Treatment	Total cost of applying chemicals	Yield	Harvesting @ $2.50 per 10kg box	Packaging @ $1.50 per box	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[b]	Annual cost for use of Brassicaspot™ Model[b]	Farm gate income @ $16.50/box	Contibution to farm profit
	$/ha	t/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha
Resistant variety	4,251	11.24	2,810	1,686	300	40	600	18,546	13,110
Susceptible variety	5,622	10.11	2,528	1,517	300	40	600	16,682	11,698

Note: The cost for crop inspections was the same for all treatments.
a, cost of repair and maintenance of the weather station was assumed to be $10 per hectare per annum.
b, Brassica$_{spot}$™ models cost $1,500 per 10 hectares of crop.

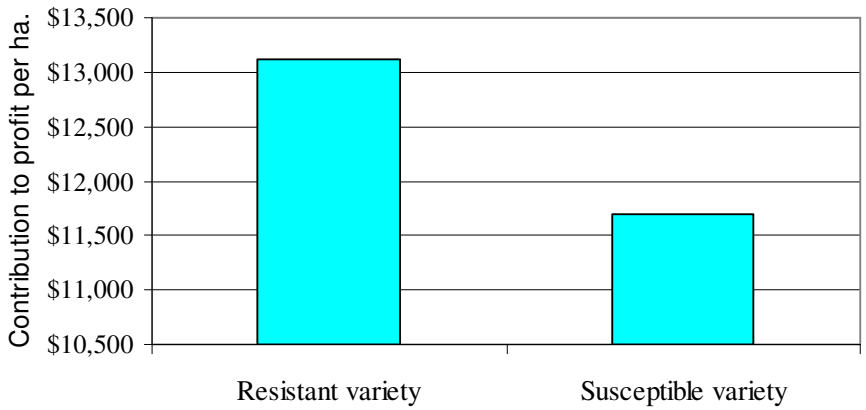

Fig 9.2 Contribution to profit per hectare for resistant and susceptible varieties in reducing the incidence of white blister on broccoli.

9.4.3 Efficacy of the Brassica$_{spot}$™ Models compared with weekly sprays in reducing the incidence of white blister on broccoli.

Table 9.5 Contribution of profit per hectare for Brassica$_{spot}$™ Models and weekly sprays on broccoli.

Treatment	Total cost of applying chemicals	Yield	Harvesting @ $2.50 per 10kg box	Packaging @ $1.50 per box	Crop inspections[a]	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[b]	Annual cost for use of Brassica$_{spot}$™ Model[c]	Farm gate income @ $16.50/box	Contibution to farm profit	Rank
	$/ha	t/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	
Control	0	10.32	2,580	1,548		0	0		17,028	12,900	3
Brassica$_{spot}$I™ Model	421	11.08	2,770	1,662	82	75	10	150	18,282	13,344	2
Brassica$_{spot}$II™ Model	264	11.08	2,770	1,662	82	75	10	150	18,282	13,501	1
Weekly sprays	2,125	11.08	2,770	1,662	82	0	0		18,282	11,725	4

Note: The cost for crop inspections was the same for all treatments.
a, cost of repair and maintenance of the weather station was assumed to be $10 per hectare per annum.
b, Brassica$_{spot}$™ models cost $1,500 per 10 hectares of crop.

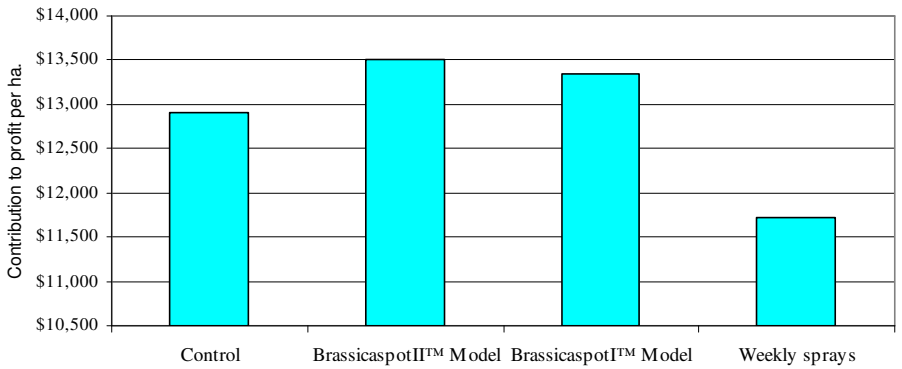

Fig 9.3 Contribution of profit per hectare for Brassica$_{spot}$™ Models and weekly sprays on broccoli.

9.4.4 Efficacy of Brassica$_{spot}$™ disease predictive models for controlling white blister on broccoli and Chinese cabbage.

Table 9.6 Trial 1: Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models and weekly sprays on Bridge broccoli in Trial 1 at Werribee. South

Treatment	Chemical	Total cost of applying chemicals	Yield	Harvesting @ $2.50 per 10kg box	Packaging @ $1.50 per box	Crop inspections[a]	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[b]	Annual cost for use of Brassicaspot™ Model[c]	Farm gate income @ $16.50/box	Contibution to farm profit per hectare	Rank
		$/ha	t/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	
Control		0	3.88	970	582					6,402	4,850	1
Weekly	Tri-base Blue® + Du-Wett™	1,289	3.88	970	582	88				6,402	3,473	4
Brassica$_{spot}$I™	Amistar WG®	157	3.88	970	582	88	75	10	150	6,402	4,370	2
Brassica$_{spot}$II™	Amistar WG®	264	3.88	970	582	88	75	10	150	6,402	4,263	3

Note: The cost for crop inspections was the same for all treatments.
a, cost of repair and maintenance of the weather station was assumed to be $10 per .hectare per annum.
b, Brassica$_{spot}$™ models cost $1,500 per 10 hectares of crop.

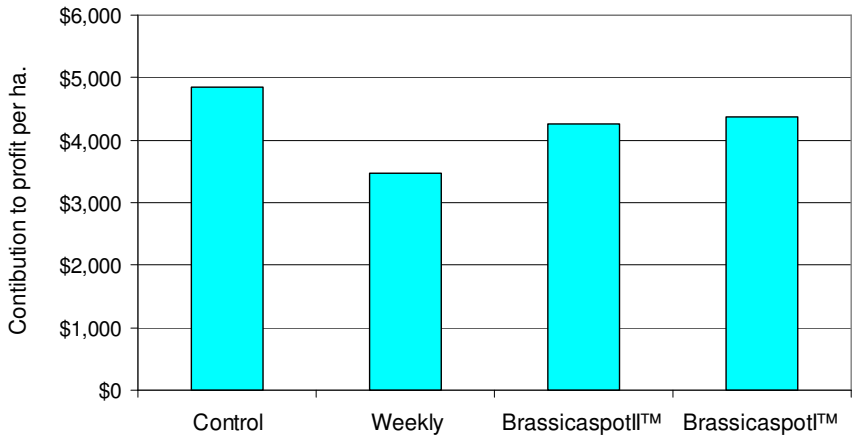

Fig 9.4 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models and weekly sprays on Bridge broccoli in Trial 1 at Werribee South.

Table 9.7 Trial 2: Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with weekly, *Streptomyces lyticus* and Sodium lauryl sulfate ™ sprays in Trial 2 on Grevillea broccoli at Boneo.

Treatment	Total cost of applying chemicals	Yield	Harvesting @ $2.25 per 10kg box	Packaging @ $1.50 per box	Crop inspections[a]	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[b]	Annual cost for use of Brassica$_{spot}$™ Model[c]	Farm gate income @ $16.50/box	Contibution to farm profit per hectare	Rank
	$/ha	t/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$	$/ha	$/ha	
Control	0	2.80	630	420					4,620	3,570	7
Weekly	1,339	10.92	2,456	1,637	88				18,011	12,491	1
Brassica$_{spot}$II™ 5%	686	6.32	1,422	948	88	75	10	150	10,428	7,050	5
Brassica$_{spot}$II™ 50%	170	4.88	1,098	732	88	75	10	150	8,051	5,728	6
Brassica$_{spot}$I™	421	8.22	1,849	1,233	88	75	10	150	13,559	9,734	2
Streptomyces lyticus	2,250	8.22	1,849	1,233	88				13,559	8,140	3
Sodium lauryl sulfate™	593	6.32	1,422	948	88				10,428	7,378	4

a, Crop inspections: All crops inspected weekly for 20 minutes per 4 ha at a cost of $70 per hour.
b, Cost of repair and maintenance of the weather station was assumed to be $10 per hectare per annum.
c, Brassica$_{spot}$™ models cost $1,500 per 10 hectares of crop.

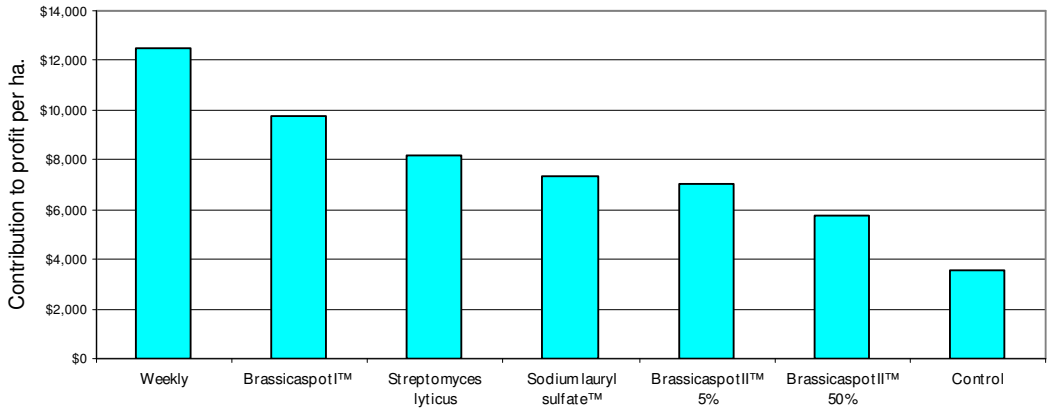

Fig 9.5 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with weekly, *Streptomyces lyticus* and sodium lauryl sulfate ™ sprays on Grevillea broccoli in Trial 2 at Boneo.

Table 9.8 Trial 3: Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with weekly and best bet sprays in Trial 3 on Viper broccoli at Werribee South.

Treatment	Total cost of applying chemicals	Yield	Harvesting @ $2.50 per 10kg box	Packaging @ $1.50 per box	Crop inspections[a]	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[b]	Annual cost for use of Brassicaspot™ Model[c]	Farm gate income @ $16.50/box	Contibution to farm profit per hectare	Rank
	$/ha	t/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	
Control	0	10.92	2,729	1,637					18,012	13,646	1
Weekly	990	10.92	2,729	1,637	88				18,012	12,568	5
Best Bets	421	10.92	2,729	1,637	88				18,012	13,137	2
Brassica$_{spot}$I™	314	10.92	2,729	1,637	88	75	10	150	18,012	13,009	3
Brassica$_{spot}$II™	421	10.92	2,729	1,637	88	75	10	150	18,012	12,902	4

a Crop inspections: All crops inspected weekly for 20 minutes per 4 ha at a cost of $70 per hour.
b Cost of repair and maintenance of the weather station was assumed to be $ 10 per per hectare per annum.
c Brassica$_{spot}$™ models cost $1,500 per 10 hectares of crop.

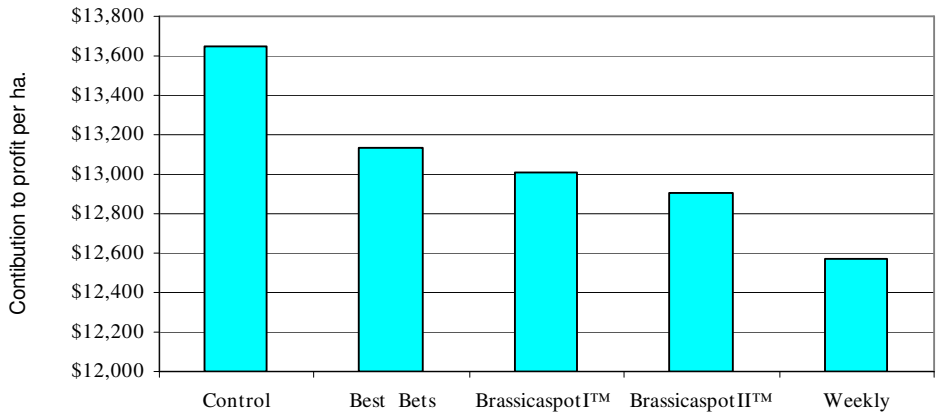

Fig 9.6 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with weekly and best bet sprays on Viper broccoli in Trial 3 at Werribee South.

Table 9.9 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with weekly sprays on Chinese cabbage in Trial 5 at Devon Meadow.

Treatment	Total cost of applying chemicals	Yield	Harvesting @ $2.50 per box[a]	Packaging @ $1.50 per box	Crop inspections[b]	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[c]	Annual cost for use of Brassicaspot™ Model[d]	Farm gate income @ $14.00/box	Contibution to farm profit per hectare	Rank
	$/ha	Plants/ha	$/ha	$/ha	$/ha	$/ha	$/ha		$/ha	$/ha	
Control	0	16,200	4,050	2,430					22,680	16,200	2
Weekly	431	16,200	4,050	2,430	41				22,680	15,728	4
Brassica$_{spot}$I™	157	16,200	4,050	2,430	41	75	10	150	22,680	15,767	3
Brassica$_{spot}$II™	157	26,149	6,537	3,922	41	75	10	150	36,609	25,716	1

a, 10 plants per box
b, Crop inspections: All crops inspected weekly for 20 minutes per 4 ha at a cost of $70 per hour.
c, Cost of repair and maintenance of the weather station was assumed to be $ 10 per per hectare per annum.
d, Brassicaspot models cost $1,500 per 10 hecatres of crop.
d, Based on an average price per box from the Melbourne market data for 2007-2008.

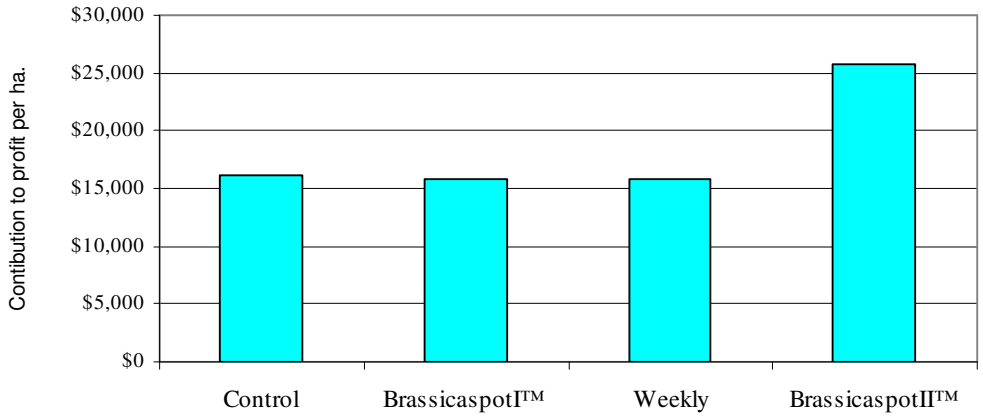

Fig 9.7 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with weekly sprays on Chinese cabbage in Trial 5 at Devon Meadow.

Table 9.10 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive model compared with grower and Cabrio® sprays on Chinese cabbage in Trial 10 at Gatton.

Treatment	Total cost of applying chemicals	Yield	Harvesting @ $2.50 per box[a]	Packaging @ $1.50 per box	Crop inspections[b]	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[c]	Annual cost for use of Brassica$_{spot}$ ™ Model[d]	Farm gate income @ $14.00/box	Contibution to farm profit per hectare	Rank
	$/ha	plants/ha	$/ha	$/ha			$/ha	$/ha	$/ha	$/ha	
Control	0	5,221	1,305	783					7,309	5,221	3
Grower	314	5,221	1,305	783	47				7,309	4,907	4
Cabrio®	101	12,297	3,074	1,845	47				17,216	12,196	1
Brassica$_{spot}$ I™	314	12,297	3,074	1,845	47	75	10	150	17,216	11,748	2

a, 10 plants per box.
b, Crop inspections: All crops inspected weekly for 20 minutes per 4 ha at a cost of $70 per hour.
c, Cost of repair and maintenance of the weather station was assumed to be $10 per hectare per annum.
d, Brassicaspot models cost $1,500 per 10 hecatres of crop.

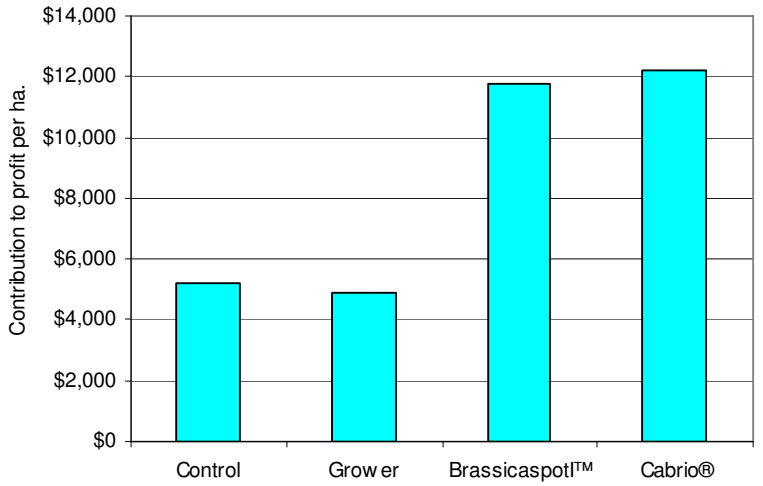

Fig 9.8 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive model compared with grower and Cabrio® sprays on Chinese cabbage in Trial 10 at Gatton.

Table 9.11 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with grower, Weekly Cu and Weekly Cu plus Sprayphos™ sprays on Viper broccoli in Trial 11 at Werribee.

Treatment	Total cost of applying chemicals	Yield Combined 1st & 2nd pick	Harvesting @ $2.50 per 10kg box	Packaging @ $1.50 per box	Crop inspections[a]	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[b]	Annual cost for use of Brassicaspot™ Model	Farm gate income @ $16.50/box	Contibution to farm profit per hectare	Rank
	$/ha	t/ha	$/ha	$/ha	$/ha		$/ha	$/ha	$/ha	$/ha	
Control		1.93	435	290					3,189	2,464	4
Brassica$_{spot}$I™	540	1.95	439	293	47	75	10	400	3,220	1,463	6
Brassica$_{spot}$II™	540	2.83	636	424	47	75	10	400	4,667	2,581	5
Grower	1,097	7.45	1,675	1,117	47				12,287	8,397	1
Weekly Cu	555	6.45	1,451	968	47				10,643	7,669	2
Weekly + Sprayphos 620™	994	6.45	1,451	968	47				10,643	7,230	3

a, Crop inspections: All crops inspected weekly for 20 minutes per 4 ha at a cost of $70 per hour.

b, Cost of repair and maintenance of the weather station was assumed to be $ 10 per per hectare per annum

c, Brassica$_{spot}$ ™ models cost $4,000 per 10 hecatres of crop

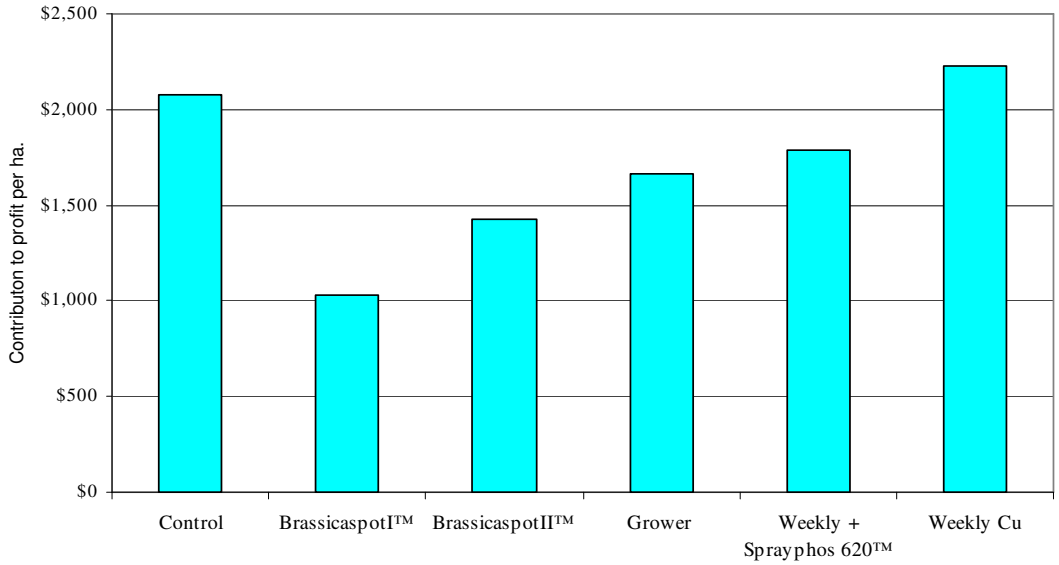

Fig 9.9 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with grower, Weekly Cu and Weekly Cu plus Sprayphos™ sprays on Viper broccoli in Trial 11 at Werribee South over the 8 week period of the trial for the first pick.

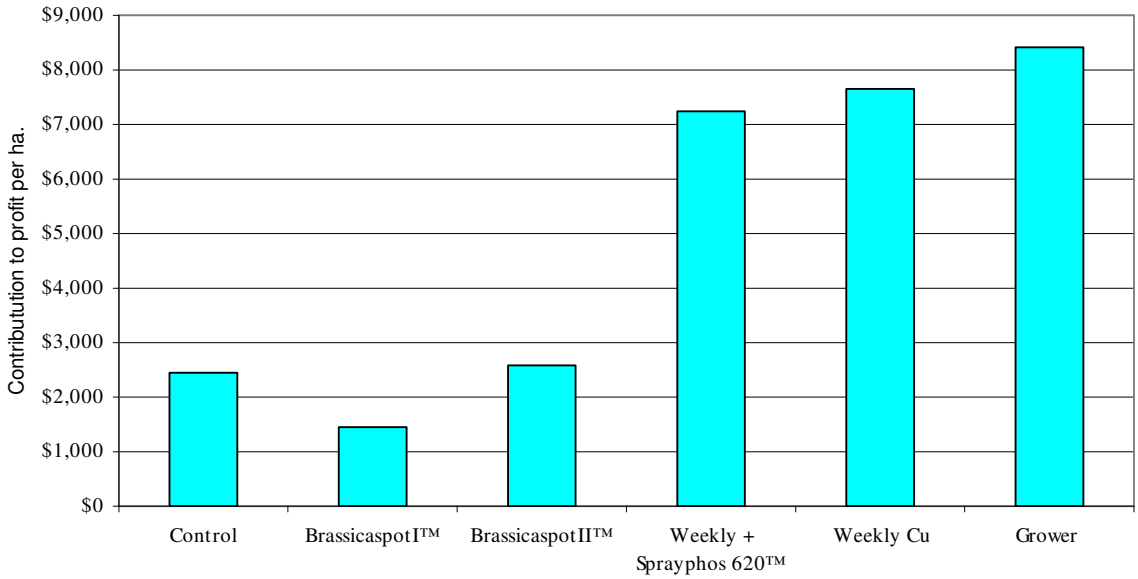

Fig 9.10 Contribution to profit per hectare for Brassica$_{spot}$™ disease predictive models compared with grower, Weekly Cu and Weekly Cu plus Sprayphos™ sprays on Viper broccoli in Trial 11 at Werribee South for the second pick part of the trial.

9.4.3 Efficacy of the disease predictive models for controlling white blister on lettuce compared with systemic, protectant and fungicide alternative.

Table 9.12 Contribution to profit per hectare for DownCast Model compared with *Bacillus subtilis*, weekly and Bion 50 WG™ sprays on Iceberg lettuce cultivar Marksman in Trial 1 at Boneo.

Treatment	Total cost of applying chemicals	Yield fresh	Harvesting @ $2.50 per 10 kg box	Packaging @ $1.50 per box	Extra labour cost for packaging	Crop inspections	Annual depreciation & interest on weather station	Repairs and maintenance of weather station[a]	Farm gate income @ $12.78/box	Contibution to farm profit	Rank
	$/ha	t/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	
Control	0	20.50	5,125	3,075	674				26,197	17,323	2
B. subtilis	1,174	21.31	5,327	3,196	701	58			27,229	16,774	3
DownCast model	1,329	20.06	5,015	3,009	660	58	75	10	25,635	15,480	4
Weekly	1,509	26.33	6,582	3,949	866	58			33,647	20,682	1

a, The cost for repairs and maintenance of the weather station was estimated to be $50 per annum for 5 ha. of lettuce or $10 per hectare

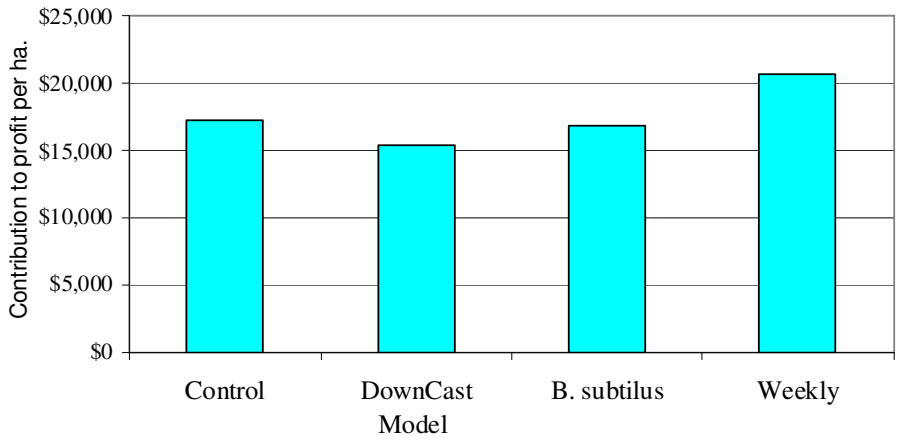

Fig 9.11 Contribution to profit per hectare for DownCast Model compared with *Bacillus subtilis*, weekly and Bion 50 WG™ sprays on Iceberg lettuce cultivar Marksman in Trial 1 at Boneo. Note Weekly here is Farm Best Practice in Chapter 4.

Table 9.13 Contribution to profit per hectare for BREMCAST and DownCast models compared with Bion® and grower standard sprays for Cos lettuce variety Amadeus in Trial 2 at Devon Meadows.

Treatment	Total cost of applying chemicals	Yield	Yield	Harvesting @ $2.50 per 4 kg box	Packaging @ $1.50 per box	Crop inspections	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[a]	Farm gate income @ $13.45/box	Contibution to farm profit per hectare	Rank
	$/ha	t/ha	Boxes/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	
Control		23.91	5,978	14,944	8,966				80,397	56,487	5
Bion	200	25.67	6,418	16,044	9,626	53			86,315	60,393	1
BREMCAST	744	25.67	6,418	16,044	9,626	53	75	10	86,315	59,763	3
DownCast	530	25.67	6,418	16,044	9,626	53	75	10	86,315	59,978	2
STD	892	25.67	6,418	16,044	9,626	53			86,315	59,701	4

a, The cost for repairs and maintenance of the weather station was estimated to be $50 per annum for 5 ha of lettuce or $10 per ha.

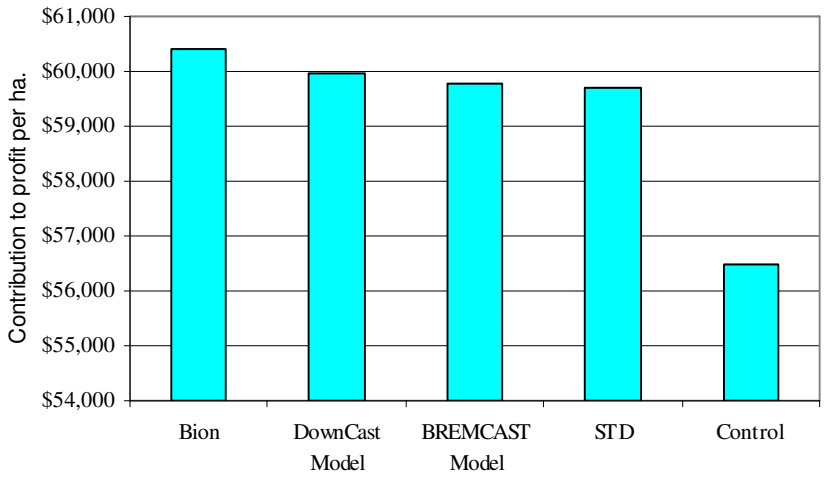

Fig 9.12 Contribution to profit per hectare for BREMCAST and DownCast models compared with Bion® and grower standard sprays for Cos lettuce variety Amadeus in Trial 2 at Devon Meadows.

Table 9.14 Contribution to profit per hectare for BREMCAST and DownCast models compared with grower standard sprays for Red Oak Leaf lettuce in Trial 3 at Skye.

Treatment	Total cost of applying chemicals	Yield	Harvesting @ $2.50 per 2 kg box	Packaging @ $1.50 per box	Crop inspections	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[a]	Farm gate income @ $12.00/box	Contibution to farm profit[b]	Rank
	$/ha	t/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	
Control	0	14.83	18,538	11,123	0			88,980	59,320	1
BREMCAST model	102	14.83	18,538	11,123	35	75	10	88,980	59,098	3
DownCast model	102	14.83	18,538	11,123	35	75	10	88,980	59,098	3
STD	102	14.83	18,538	11,123	35			88,980	59,183	2

a, A cost for repairs and maintenance of the weather station was estimated to be $50 per annum for 5 ha. of lettuce or $10 per ha.
b, Differences in contribution to farm profit for the Control and Treatments were insignificant.

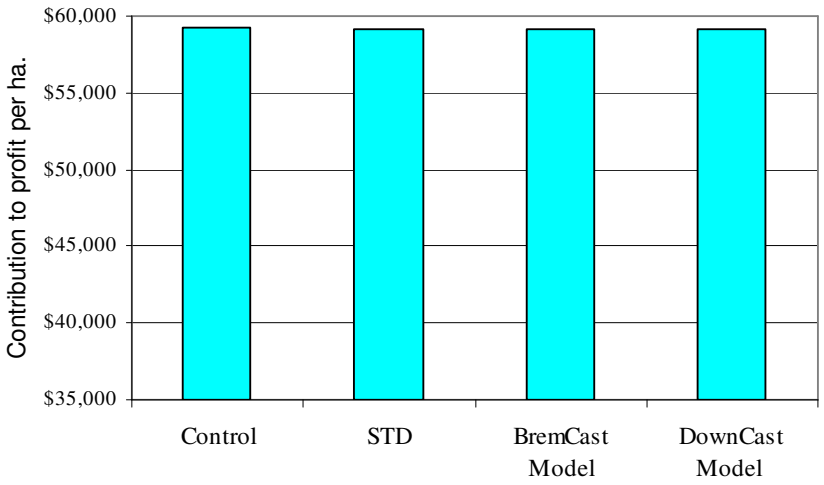

Fig 9.13 Contribution to profit per hectare for BREMCAST and DownCast models compared with grower standard sprays for Red Oak Leaf lettuce in Trial 3 at Skye.

Table 9.15 Contribution to profit per hectare for DownCastI and BREMCAST models compared with Farm Best Practice and Bion™ sprays on Iceberg lettuce variety Kong in Trial 4 at Werribee.

Treatment	Cost of seedlings	Total cost of applying chemicals	Yield	Yield	Harvesting @ $2.50 per 10 kg box	Labour to to remove wrap leaves	Packaging @ $1.50 per box	Crop inspections	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[a]	Farm gate income @ $13.45/box	Contibution to farm profit	Rank
		$/ha	**t/ha**	**Boxes/ha**	**$/ha**	**$/ha**	**$/ha**		**$/ha**	**$/ha**	**$/ha**	**$/ha**	
Control	4,674		46.93	4,693	29,331	1,135	7,040				63,121	20,942	1
Lettuce DownCastI	4,674	508	46.93	4,693	29,331	1,135	7,040	41	75	10	63,121	20,307	4
BREMCAST	4,674	298	46.93	4,693	29,331	1,364	7,040	41	75	10	63,121	20,288	5
Farm Best Practice	4,674	377	46.93	4,693	29,331	1,135	7,040	41			63,121	20,524	3
Bion (half)	4,674	150	46.93	4,693	29,331	1,135	7,040	41			63,121	20,751	2

a, The cost for repairs and maintenance of the weather station was estimated to be $50 per annum for 5 ha. of lettuce or $10 per ha.

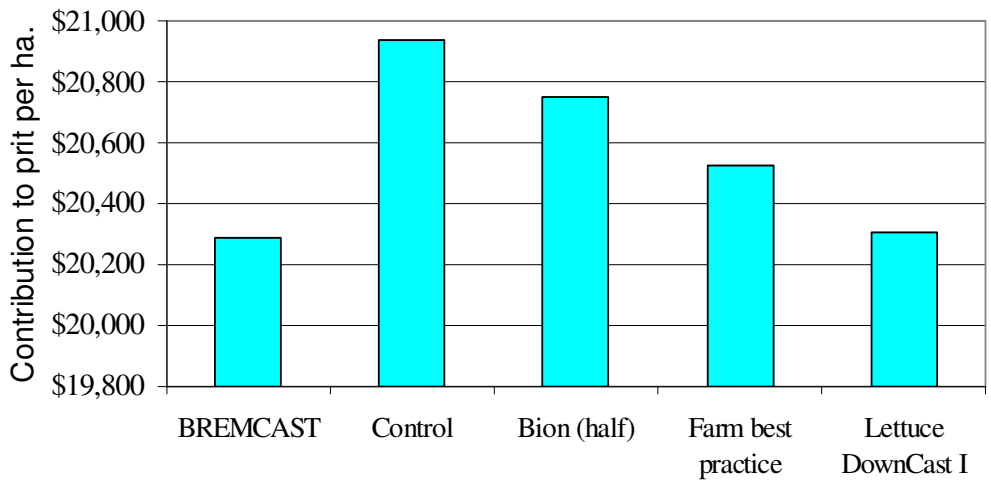

Fig 9.14 Contribution to profit per hectare for DownCastI and BREMCAST models compared with Farm Best Practice and Bion™ sprays on Iceberg lettuce variety Kong in Trial 4 at Werribee.

Table 9.16 Contribution to profit per hectare for DownCastI and BREMCAST models compared with Farm Best Practice and Bion™ sprays on Iceberg lettuce variety Marksman in Trial 4 at Werribee.

Treatment	Cost of seedlings	Total cost of applying chemicals	Yield	Yield	Crop inspections	Harvesting @ $2.50 per 10 kg box	Labour to to remove wrap leaves	Packaging @ $1.50 per box	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[a]	Farm gate income @ $13.45/box	Contibution to farm profit	Rank
		$/ha	t/ha	Boxes/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	
Control	4,098		36.31	3,631		22,691	1,135	5,446			48,830	15,461	2
Lettuce DownCastI	4,098	508	36.31	3,631	41	22,691	1,135	5,446	75	8	48,830	14,829	5
BREMCAST	4,098	298	43.60	4,360	41	27,249	1,364	6,540	75	8	58,639	18,967	1
Farm Best Practice	4,098	377	36.31	3,631	41	22,694	1,135	5,447			48,837	15,046	4
Bion (half)	4,098	150	36.31	3,631	41	22,694	1,135	5,447			48,837	15,273	3

a , The cost for repairs and maintenance of the weather station was estimated to be $50 per annum for 5 ha. of lettuce or $10 per ha.

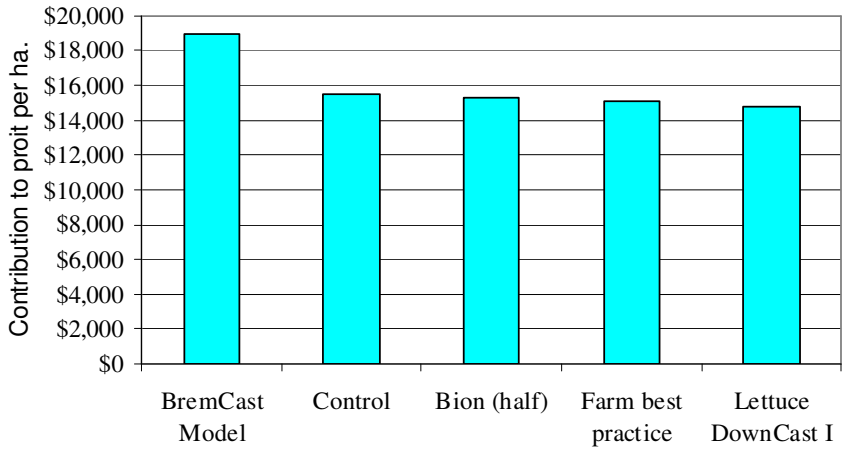

Fig 9.15 Contribution to profit per hectare for DownCastI and BREMCAST models compared with Farm Best Practice and Bion™ sprays on Iceberg lettuce variety Marksman in Trial 4 at Werribee.

Table 9.17 Contribution to profit per hectare for DownCastII and BREMCAST models compared with Bion™, Farm Best Practice and Farm Best Practice + FoliCal Plus sprays on Iceberg lettuce variety Silverado in Trial 5 at Boneo.

Treatment	Total cost of applying chemicals	Yield	Yield	Crop inspections	Harvesting @ $2.50 per 10 kg box	Packaging @ $1.50 per box	Annual depreciation and interest on weather station	Repairs and maintenance of weather station[a]	Farm gate income @ $12.78/box	Contibution to farm profit	Rank
	$/ha	t/ha	Boxes/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	$/ha	
Control		23.64	2,364		5,910	3,546			30,213	20,756	5
Bion (half)	150	38.74	3,874	47	9,686	5,812			49,515	33,821	1
Farm Best Practice	912	29.46	2,946	47	7,364	4,418			37,645	24,904	2
Farm Best Practice + FoliCal Plus	650	25.93	2,593	47	6,483	3,890			33,142	22,073	4
BREMCAST	324	25.93	2,593	47	6,483	3,890	75	10	33,142	22,314	3
DownCastII	588	14.67	1,467	47	3,668	2,201	75	10	18,753	12,164	6

a, The cost for repairs and maintenance of the weather station was estimated to be $50 per annum for 5 ha. of lettuce or $10 per ha.

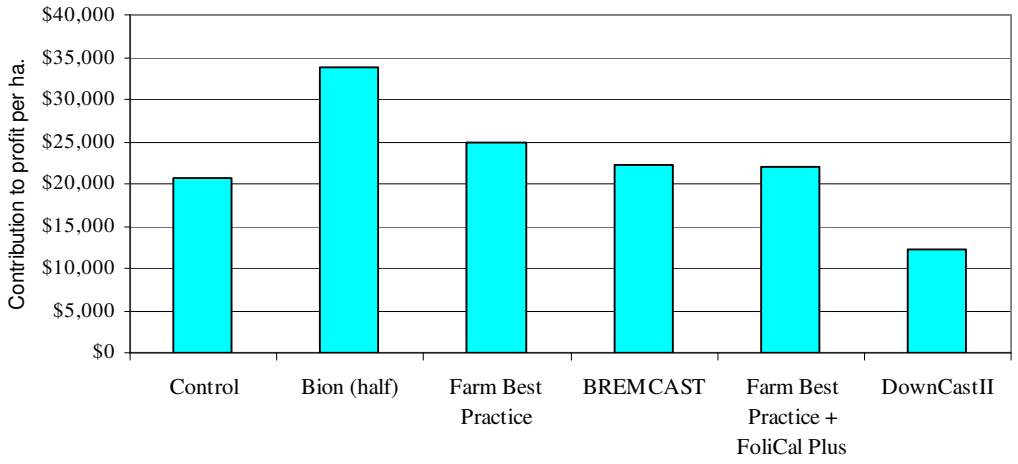

Fig 9.16 Contribution to profit per hectare for DownCastII and BREMCAST models compared with Bion™, Farm Best Practice and Farm Best Practice + FoliCal Plus sprays on Iceberg lettuce variety Silverado in Trial 5 at Boneo.

9.5 Conclusions

The following conclusions were drawn from the results of the trials described above.

9.5.1 Chapter 1 trials

Trials to evaluate reducing the incidence of white blister on broccoli by using morning versus evening irrigation, resistant instead of susceptible varieties, and Brassica$_{spot}$™ disease predictive models revealed the following:

- Effect of time of irrigation for the control of white blister on broccoli

  There was an economic benefit for irrigating broccoli in the morning rather than in the evening. Figure 9.1 showed that contribution to profit per hectare was 2.8 percent higher for crops irrigated in the morning rather than in the evening.

  The number of sprays used in the trial was the same for irrigation in the morning and evening. Therefore, an environmental benefit did not occur by reducing the number of sprays for irrigating crops in the morning rather than the evening.

- Effect of resistant variety on reducing the incidence of white blister on broccoli

  The contribution to profit per hectare for growing the resistant variety, Tyson, was 10.7 percent greater than growing the susceptible variety, Ironman.

  Since the number and type of sprays used for spraying the two varieties were identical, an environmental benefit between the resistant versus the susceptible variety did not occur.

- Efficacy of Brassicaspot™ Model's versus weekly sprays for controlling white blister on Ironman broccoli

  The Brassica$_{spot}$II™ Model and the Brassica$_{spot}$I™ Model produced respectively 13.2 percent and 12 per cent higher profitabilities per hectare than the weekly sprays. However, the profitability per hectare for the Brassica$_{spot}$™ Model on average was 3.8 percent more than that of the Control. Therefore, there was advantage in using the Brassica$_{spot}$II™ Model to reduce the incidence of white blister on broccoli.

9.5.2 Chapter 4 trials brassicas

Trials to determine the efficacy of the Brassica$_{spot}$™ disease predictive models for controlling white blister on brassica crops using systemic, protectant and soft option fungicides.

- ***Trial 1*** was on Bridge broccoli at Werribee South. The Brassica$_{spot}$™ disease predictive models produced larger contributions to profit per hectare than weekly sprays. However, the contributions were lower than that of the Control which did not have any sprays.

- ***Trial 2*** was on Grevillea broccoli at Boneo. The treatment that produced the highest contribution to profit per hectare was the Weekly Sprays. The contribution to profitability per hectare for Weekly Sprays was 28 per cent higher than the contribution to profit per hectare for BrassicaspotI™ which was the next best treatment.

  The environmental benefit for Weekly Sprays was less than that of BrassicaspotI™ because it had 13 applications of Tri-Base blue® + Du-Wett™ compared with two sprays of Amistar® for BrassicaspotI™.

- ***Trial 3*** was on Viper broccoli at Werribee South. The Best Bet treatment contributed greater profit per hectare than the Brassica$_{spot}$™ Models or Weekly Sprays. However, the contribution per hectare for the Best Bet treatment was 4 per cent lower than the Control with no sprays.

  Apart from providing the highest contribution per hectare, the Control with its absence of sprays also had the highest environmental benefit.

- ***Trial 5*** was on Chinese cabbage variety Matilda at Devon Meadow. BrassicaspotII™ had a contribution to profit per hectare which was 59 percent better than the Control. The contribution to profit per hectare for the Control was slightly higher than that of BrassicaspotI™ and Weekly sprays.

  BrassicaspotII™ had only one spray of Amistar® and compared favourably with the control for environmental benefits.

- ***Trial 10*** was also on Matilda Chinese cabbage at Gatton. BrassicaspotI™ had a slightly lower contribution to profit per hectare than Cabrio® (3.7 per cent) and had two sprays of Amistar® compared with one spray of Cabrio®.

- ***Trial 11*** was the final trial in this series on broccoli variety Viper at Werribee South. For the complete trial over eight weeks, the treatment that produced the largest contribution to profit per hectare was the Grower comprising two sprays of Amistar® which was 69 per cent greater than BrassicaspotI™. It was also 9 per cent higher than weekly sprays of Tri-Base Blue® and 14 per cent higher than weekly sprays of Tri-Base Blue® plus Sprayphos™.

  The BrassicaspotI™ was less environmentally damaging, however, with only two sprays compared with eight spays for Grower, Weekly and Weekly plus Sprayphos™.

9.5.3 Chapter 6 trials on lettuce

Efficacy of the disease predictive models for controlling downy mildew on lettuce compared with systemic, protectant and fungicide alternative.

- ***Trial 1***, was on Iceberg lettuce variety Marksman at Boneo. The highest contribution to profit per hectare was the Weekly sprays treatment that was 28 per cent greater than the contribution by the Lettuce DownCastI treatment. The next best was the Control without any sprays followed by *Bacillus subtilis* with 10 sprays.

  Although Weekly sprays made the greatest contribution to profitability per hectare they were not as environmentally sound as the Control with no sprays.

- ***Trial 2*** was on Cos lettuce variety Amadeus at Devon Meadows. In this trial the contribution to profit per hectare was almost identical for the Bion®, DownCast model and the BREMCAST model. However, Bion®, a promoter of systemic acquired resistance would have been superior based on its environmental benefit and because it had only two sprays compared with five fungicide sprays for the DownCast and BREMCAST models.

- ***Trial 3*** was on Red Oak Lettuce at Skye. Contributions to profit per hectare for the Control and treatments were almost identical. Since the Control did not have sprays it had the highest environmental benefit.

- ***Trial 4*** was on Iceberg lettuce for cultivars Kong and Marksman. For Kong lettuce, the Control had the largest contribution to profitability followed by Bion, Farm Best Practice, and DownCastI with BREMCAST making the lowest contribution.

  The Control had the highest environmental benefit because sprays were not applied.

  Contrarily, for Marksman, BREMCAST made the largest contribution to profit per hectare. The increase was approximately 25 per cent greater than that of the Control, Bion®, Farm Best Practice and DownCastI which had about the same contributions to profit per hectare.

  The environmental benefit for BREMCAST with 3 sprays was greater than for DownCastI which required 5 sprays.

- ***Trial 5*** was on Iceberg lettuce cultivar Silverado at Boneo. Bion® applied at a half rate of 25 grams per hectare produced the largest contribution to profitability. The contribution by Bion® was followed by Farm Best Practice, BREMCAST, Farm Best Practice with FoliCal plus and the Control with DownCastII contributing the lowest contribution to profitability per hectare.

  The Bion® had only two sprays and its environmental benefit was slightly lower than that of the Control which was not sprayed.

Appendices

Appendix 1 Cost of treatments for the Werribee field trial to evaluate the effect of time of irrigation, resistant versus susceptible varieties and the Brassica$_{spot}$™ disease predictive models for the control of white blister on broccoli.

Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application[a]	Total cost of chemicals applied per treatment
			$/ha	$/ha	$/ha	$/ha	$/ha
Evening Susceptible Model BSII	Amistar®	1	214.20	214.20	214	50	264
Evening Susceptible Model BSI	Amistar®	1	107.10	107.10	321	100	421
		1	214.20	214.20			
Evening Susceptible Weekly	Tri-Base Blue®	8	19.38	155.06	725	1,400	2,125
		6	38.77	232.60			
	Du-Wett™	8	16.88	135.07			
		6	33.77	202.61			
Morning Susceptible Model BSII	Amistar®	1	214.20	214.20	214	50	264
Morning Susceptible Model BSI	Amistar®	1	107.10	107.10	321	100	421
		1	214.20	214.20			
Morning Susceptible Weekly	Tri-Base Blue®	8	19.38	155.06	725	1,400	2,125
		6	38.77	232.60			
	Du-Wett™	8	16.88	135.07			
		6	33.77	202.61			

a The cost per application was $50 per ha.

Appendix 2 Cost of chemicals applied per treatment for Trial 1 on broccoli variety Bridge at Werribee South.

No.	Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
				$/ha	$/ha	$/ha	$/ha	$/ha
1	Control							0
2	Weekly	Tribase blue®	5	19.38	97	689	600	1,289
			7	38.77	271			
		Du-Wett™	5	16.88	84			
			7	33.77	236			
3	Brassica$_{spot}$I™	Amistar	1	107.10	107	107	50	157
4	Brassica$_{spot}$II™	Amistar	1	214.20	214	214	50	264

Appendix 3 Cost of chemicals applied per treatment for Trial 2 on broccoli variety Grevillea at Boneo.

No.	Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
				$/ha	$/ha	$/ha	$/ha	$/ha
1	Control							0
2	Weekly	Tri-Base blue®	7	19.38	136	689	650	1,339
			6	38.77	233			
		Du-Wett™	7	16.88	118			
			6	33.77	203			
3	Brassica$_{spot}$II™ (5% appearance)	Amistar	1	107.10	107	536	150	686
	Brassica$_{spot}$II™	Amistar	2	214.20	428			
4	Brassica$_{spot}$II™	Amistar	1	120.00	120	120	50	170
5	Brassica$_{spot}$I™ (5% area)	Amistar	1	107.10	107	321	100	421
	Brassica$_{spot}$I™	Amistar	1	214.20	214			
6	*Streptomyces lyticus*		3	120.00	360	1,800	450	2,250
	Streptomyces lyticus		6	240.00	1,440			
7	Sodium lauryl sulfate™		3	9.50	29	143	450	593
	Sodium lauryl sulfate™		6	19.00	114			

Appendix 4 Cost of chemicals applied per treatment for Brassica$_t$™ Trial 3 on broccoli variety Viper at Werribee South.

No.	Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
				$/ha	$/ha	$/ha	$/ha	$/ha
1	Control							0
2	Weekly	Tribase blue®	6	19.38	116	290	700	990
			1	38.77	39			
		Du-Wett™	6	16.88	101			
			1	33.77	34			
	Best Bets	Amistar WG®	1	107.10	107	321	100	421
		Amistar WG®	1	214.20	214			
3	Brassica$_{spot}$I™	Amistar WG®	2	107.10	214	214	100	314
4	Brassica$_{spot}$II™	Amistar WG®	1	107.10	107	321	100	421
		Amistar WG®	1	214.20	214			

Appendix 5 Cost of chemicals applied per treatment for Trial 5 on Chinese cabbage variety Matilda at Devon Meadows.

No.	Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
				$/ha	$/ha	$/ha	$/ha	$/ha
1	Control							0
2	Weekly	Tribase blue®	5	19.38	97	181	250	431
		Du-Wett™	5	16.88	84			
3	Brassica$_{spot}$I™	Amistar	1	107.10	107	107	50	157
4	Brassica$_{spot}$II™	Amistar	1	107.10	107	107	50	157

Appendix 6 Cost of chemicals applied per treatment for Trial 10 on Chinese cabbage variety Matilda at Gatton.

Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
			$/ha	$/ha	$/ha	$/ha
Control						0
Grower	Amistar®	2	107.10	214.20	100	314
Cabrio®	Cabrio®	1	51.48	51.48	50	101
Brassica$_{spot}$I™	Amistar®	2	107.10	214.20	100	314

Appendix 7 Cost of chemicals applied per treatment for Trial 11 on broccoli variety Viper at Werribee South.

Treatment	No. of applications	Cost of chemical per application	Total cost of chemicals per treatment	Total cost of application[a]	Total cost of chemicals applied per treatment
		$/ha	$/ha	$/ha	$/ha
Control					0
Brassica$_{spot}$I™	2	72.15	290.17	250	540
	2	19.38			
	1	107.10			
Brassica$_{spot}$II™	2	72.15	290.17	250	540
	2	19.38			
	1	107.10			
Grower	3	72.15	546.95	550	1,097
	3	19.38			
	2	107.10			
	3	19.38			
Weekly Cu	8	19.38	155.06	400	555
Weekly + Sprayphos 620™	8	19.38	194.40	800	994
	8	4.92			

Appendix 8 Cost of chemicals applied per treatment for Trial 1 on Iceberg lettuce cultivar Marksman at Boneo.

Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
			$/ha	$/ha	$/ha	$/ha	$/ha
B. subtilis	Fulzyme®	6	34.88	209	674	500	1,174
	Fulzyme®	4	116.25	465			
DownCast model	Acrobat®	4	113.40	454	523	200	1,329
	Penncozeb®	4	17.29	69			
	Antracol®	5	51.80	259	356	250	
	Tri Base Blue	5	19.38	97			
Weekly	Acrobat®	5	113.40	567	653	250	1,509
	Penncozeb®	5	17.29	86			
	Antracol®	5	51.80	259	356	250	
	Tri Base Blue	5	19.38	97			
Bion	Antracol®	3	50.00		150	150	300

Appendix 9 Cost of chemicals applied per treatment for Trial 2 on Cos lettuce cultivar Amadeus at Devon Meadows.

Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
			$/ha	$/ha	$/ha	$/ha	$/ha
Bion	BION®	2	50	100	100	100	200
Bozyl	Unknown	7	na	na	101	350	451
	Du-wett®	6	16.88	101			
BREMCAST	Acrobat®	2	113.40	227	302	100	744
	Polygram®	2	29.11	58			
	Seasol®	2	8.70	17			
	Amistar®	2	70.79	142	164	100	
	Synertrol®	2	2.54	5			
	Seasol®	2	8.70	17			
	Tri-Base Blue™	1	19.39	19	28	50	
	Synertrol®	1	2.54	3			
	Agriphos®	1	6.00	6			
DownCast model	Acrobat®	2	6.00	12	88	100	530
	Polygram®	2	29.11	58			
	Seasol®	2	8.70	17			
	Amistar®	2	70.79	142	164	100	
	Synertrol®	2	2.54	5			
	Seasol®	2	8.70	17			
	Tri-Base Blue™	1	19.39	19	28	50	
	Synertrol®	1	2.54	3			
	Agriphos®	1	6.00	6			
Standard grower	Acrobat®	3	113.40	340	454	150	892
	Polygram®	3	29.11	87			
	Seasol®	3	8.70	26			
	Amistar®	1	70.79	71	82	50	
	Synertrol®	1	2.54	3			
	Seasol®	1	8.70	9			
	Tri-Base Blue™	2	19.39	39	56	100	
	Synertrol®	2	2.54	5			
	Agriphos®	2	6.00	12			

Appendix 10 Cost of chemicals applied per treatment for Trial 3 on Red Oak Leaf lettuce cultivar Prunai at Skye.

Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
			$/ha	$/ha	$/ha	$/ha	$/ha
BremCast model	Antracol®	1	51.80	52	52	50	102
DownCast model	Antracol®	1	51.80	52	52	50	102
STD	Antracol®	1	51.80	52	52	50	102

Appendix 10 Cost of chemicals applied per treatment for Trial 4 on Iceberg lettuce cultivars Kong and Marksman at Werribee South.

Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
			$/ha	$/ha	$/ha	$/ha	$/ha
Lettuce DownCastI	Agral	3	10.58	32	258	250	508
	Revus	3	60.00	180			
	Agriphos	2	6.00	12			
	Pencozeb	2	17.29	35			
BREMCAST	Revus	2	60.00	120	148	150	298
	Agral	1	10.58	11			
	Pencozeb	1	17.29	17			
Farm Best Practice	Agriphos	1	6.00	6	227	150	377
	Pencozeb	1	17.29	17			
	Acrobat	1	113.40	113			
	Agral	1	10.58	11			
	Pencozeb	1	17.29	17			
	Agral	1	10.58	11			
	Antracol	1	51.80	52			
Bion (half)	Bion	2	25.00	50	50	100	150

Appendix 10 Cost of chemicals applied per treatment for Trial 5 on Iceberg lettuce cultivar Silverado at Boneo South.

Treatment	Chemical	No. of applications	Cost of chemical per application	Total cost of chemicals	Total cost of chemicals per treatment	Total cost of application	Total cost of chemicals applied per treatment
			$/ha	$/ha	$/ha	$/ha	$/ha
Bion	Bion	2	25.00	50	50	100	150
Farm Best Practice	Pencozeb	1	17.29	17	612	300	912
	Sprayphos	1	4.92	5			
	Acrobat	3	124.74	374			
	Pencozeb	3	17.29	52			
	Agral	2	10.58	21			
	Antracol	2	51.80	104			
	Tri-Base Blue	2	19.38	39			
Farm Best Practice + FoliCal Plus	FoliCal Plus	4	31.50	126	350	300	650
	Acrobat	1	124.74	125			
	Pencozeb	1	17.29	17			
	Agral	1	10.58	11			
	Antracol	1	51.80	52			
	Tri-Base Blue	1	19.38	19			
BREMCAST	Acrobat	1	124.74	125	224	100	324
	Pencozeb	1	17.29	17			
	Agral	1	10.58	11			
	Antracol	1	51.80	52			
	Tri-Base Blue	1	19.38	19			
DownCast II	Pencozeb	1	17.29	17	388	200	588
	Sprayphos	1	4.92	5			
	Acrobat	2	124.74	249			
	Pencozeb	2	17.29	35			
	Agral	1	10.58	11			
	Antracol	1	51.80	52			
	Tri-Base Blue	1	19.38	19			
DownCast R	Revus	2	60.00	120	262	150	412
	Acrobat	1	124.74	125			
	Pencozeb	1	17.29	17			
DownCast BOM	Pencozeb	1	17.29	17	306	150	456
	Sprayphos	1	4.92	5			
	Acrobat	2	124.74	249			
	Pencozeb	2	17.29	35			

Chapter 10

Development of a detection kit for airborne zoosporangia of A. candida for use with a disease forecast model for optimal crop protection regimes

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Summary

This chapter reports on the development and preliminary testing of a 'lateral flow' (immunochromatographic test strips) to detect the availability of *A. candida* in-field airborne inoculum in order to enhance the white blister risk forecast. The lateral flow is based on monoclonal antibodies generated from recognition sites on *A. candida* Race 9 zoosporangia. Of the two fusions conducted, five *A. candida* zoosporangia positive cell lines were identified and cloned from a mouse immunized with >30 KDa *A. candida* soluble material. These cell lines were tested against a range of fungal material including *A. candida* races, but none were specific for *A. candida* Race 9. Although two cell lines had a useful level of specificity only one, EMA256, was used to develop the competitive lateral flow. Visual readings required concentrations of 1 x 10^6 zoosporangia ml^{-1}, but quantitative readings required an electronic reader device. Prior to quantification of the lateral flow, airborne zoosporangia of *A. candida* were monitored in three commercial broccoli crops in Victoria using three air samplers: a Microtitre immunospore trap (MTIST), a volumetric 7 day sampler and a multi-vial cyclone air sampler, but only the former two had a good association between the concentration of zoosporangia. A batch of competitive lateral flow kits were produced using EMA 256 for quantification of *A. candida* inoculum in field aerosols collected in commercial Brussels sprout crops in the UK. There was an association between weekly values of the optical density readings of the lateral flow devices and the MTIST weekly results, indicating low disease transmission events or zoosporania load. Low transmission events corresponded to crop inspections where no visible symptoms of white blister were observed in the Brussels sprout crop. Information on inoculum availability could be used directly with environmental disease forecast models for optimal crop protection.

10.1 Production of Monoclonal antisera

10.1.1 Introduction

The white blister pathogen *Albugo candida* is an obligate parasite which cannot be cultured on agar and will only grow and develop on host tissue. The pathogen can lie dormant by the formation of oospores on contaminated seed, in soil or on infected host material. During favourable periods oospore germination and sporangia production give rise to pustule formation on the host. Zoosporangia are dispersed by wind, rain, or insects to neighboring plants.

The zoosporangial stage of the pathogen's life cycle was identified for monoclonal antibody production (MAb). Antibodies are proteins produced by the immune system in response to foreign proteins, such as those which occur on the cell walls of zoosporiangia. Antibodies are highly specific and by labeling the antibody with e.g. a fluorescent dye, they can be used to

rapidly identify zoosporangia of *A. candida*. Monoclonal antibodies have been extensively developed to rapidly identify diseases affecting both human and animals.
The present study aims to improve the environmental white blister disease risk forecast by including information on availability of *A. candida* airborne disease inoculum. Monoclonal antibodies, with recognition sites to *A. candida* Race 9 zoosporangia, were produced and used in an immunological chromatographic test strip (lateral flow) to provide 'in-field' information on *A. candida* in collected field air samples.

10.1.2 Materials and methods

Collection of zoosporangia. A hand held Burkard surface cyclone sampler (Burkard Manufacturing Co., Rickmansworth, Herts., UK) was used to collect zoosporangia of *A. candida* from the leaf surface of sporulating infected *B. oleracea* host material. One ml of chilled phosphate buffered saline (PBS) (0.01M phosphate buffer, 0.0027M potassium chloride, 0.137M sodium chloride, pH 7.4) was added to the air sampler collection vessel. Using a spin mix (Gallenkamp Ltd, Cheshire) at high speed for five minutes the zoosporangia were suspended in the aqueous phase. The spore suspension was filtered through a polyester Spectra mesh membrane (47µm pore size; NBS Biologicals Ltd, Huntingdon, UK) to remove any large contaminating material. The liquid phase was collected and, by using a membrane of 10µm pore size, bacteria and other small leaf contaminants were removed after filtration. The filtrate was collected and resuspended in 2ml chilled PBS and, presence of *A. candida* zoosporangia was confirmed by bright field microscopy (x 400). A zoosporangial concentration of $1x10^7$ ml^{-1} was determined.

Immunogen preparation. The *A. candida* zoosporangia were disrupted using a Fast Prep 120 instrument (www.qbiogene.com) at an operating speed of 5 for 25 seconds. The sample was rested on ice for 3 minutes and the process repeated twice. After microfugation in an MSE Microcentaur (www.mseuk.co.uk), operated at 10,000 r.p.m for five minutes, the soluble phase of the disrupted *A. candida* sample was retained and transferred to a YM30 microcon centrifugal unit (www. millipore .com). The sample was separated into two fractions of> 30 KDa and < 30 KDa according to manufacturer's guidelines. The protein concentration of the >30 KDa fraction was adjusted to 2 mg ml^{-1} and stored at -20°C. Using a mini slide-a-lyzer dialysis cassette of molecular weight cut off 2 KDa (www.thermoscientific.com) the *A.candida* < 30 KDa fraction was dialysed according to manufacturers guidelines in ½ strength chilled PBS buffer. The protein concentration of the recovered dialysed sample was determined, adjusted to 2mg ml^{-1} and stored at -20°C.

Immunization. Three Balb C mice were immunized, by intraperitoneal injection, each with 50µl of > 30 KDa *A. candida* zoosporangia preparation mixed with an equal volume of Titermax adjuvant (Sigma T-2684). An additional three mice were immunized with the < 30 >2 KDa *A. candida* zoosporangia preparation. The process was repeated monthly over an 8 week period. To determine the immune response of each mouse to the homologous immunogen preparation, tail bleeds (50µl blood sample / mouse) were taken 7 days after the third immunization.

Tail bleeds. Using a standard plate-trapped antigen enzyme-linked ELISA (PTA-ELISA) polysorp microtitre wells (Catalogue No. 469957, Life Technologies Ltd, Paisley, Scotland) were each coated with 100µl of 10µg homologous antigen in PBS and incubated overnight at 4°C. The PTA-ELISA was carried out as described by Wakeham et al. (2004) but at the primary antibody stage, doubling dilutions of each tail bleed were made in PBSTwC (PBS, 0.05% Tween 20 and 0.1% Casein [wt/vol]) to an end point of 1 in 128,000 and, each dilution was probed against the replicate homologous well-trapped antigen. A Strept ABC complex DAKO duet amplification system, (Cat.No. KO492; DAKO Ltd, Cambridge, UK) was used

according to the manufacturers guidelines at the secondary Ab stage. Two weeks later a pre fusion immunogen boost was given, as described previously, to two mice. Mouse 1 received an immunogen preparation of *A. candida* <30KDa >2KDa and Mouse 6 >30 KDa. Four days later the two mice were killed.

Fusion. The spleens of two mice were removed and, for each, a fusion was carried out according to Warwick HRI standard protocol using a modified method of Kennet (1978). Antibody producing B cells isolated from the spleen were fused *in vitro* with lymphoid tumour cells (myeloma). The cell hybrids (hybridomas) were supported in Dulbecco's Modified Eagles's Medium (Code No. D5976, www.sigmaaldrich.com) containing 10% Foetal calf serum (DME). On day 6, 100µl DME fresh medium was added to each hybridoma and, the medium changed on day 10. Cell tissue culture supernatants (TCS) were screened by PTA ELISA and immunofluorescence (Kennedy *et al*. 1999) 14 days after cell fusion for the presence of antibodies which recognized zoosporangial epitopes of *A. candida*. Cell lines which gave a positive results (> 3 times the negative control in PTAE ELISA) were selected and twice cloned three times.

10.1.3 Results

Tail bleeds. The two immunogen preparations (*A. candida* <30KDa >2KDa and, > 30 KDa *A. candida* zoosporangia soluble antigen) elicited an immune response in each of the mice, producing antibodies that reacted with either *A. candida* preparation as determined by PTA ELISA (Fig. 10.1.). Of the two types of immunogen preparation used, Mouse 1 and Mouse 6 were selected for fusion, as each proved optimal in the immune response to its homologous antigen.

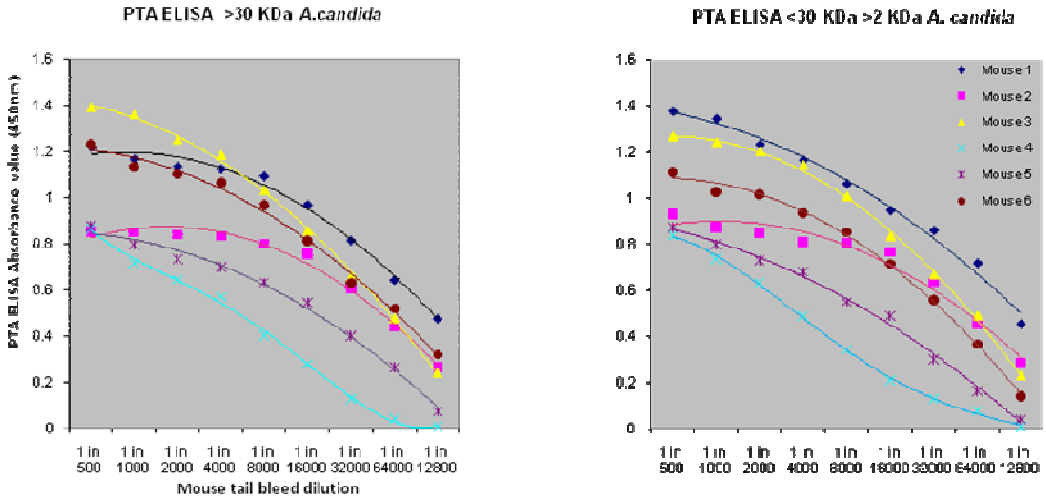


Fig. 10.1 Immune response (polyclonal serum) of immunized mice to *A. candida* zoosporangia preparations, as tested by PTA ELISA.

Fusion. Five cell lines which produced antibodies positive to *A. candida* by either PTA ELISA or IF were identified from Fusion 1 (Mouse 6 – immunized with >30KDa *A. candida*) and cloned. The cell lines were coded, the class and subclass determined (Table 10.1) and, for long term storage, retained in vials in liquid nitrogen at -185°C. Tissue culture supernatants (monoclonal antibodies) of each cell line were stored at -20°C in 250µl lots. Few positive cell hybridomas were identified from Fusion 2 (Mouse 1 – immunized with <30 KDa > 2 KDa) and, each proved unstable during cloning.

Table 10.1 MAb cell lines raised to *Albugo candida*

Fusion	Cloned cell line	Class	Subclass	Code Name
1	4E1 D1 A2	IgM		EMA 256
1	5C5 E2 C4	IgG	1	EMA 257
1	3A10 D2	IgG		EMA 258
1	5F1 C10 A4	IgG		EMA260
1	F1 F8 C4			EMA 251

10.1.4 Discussion

From two fusions, five *A. candida* zoosporangia positive cell lines were identified and cloned. All of the cloned cell lines were from Fusion 1 (mouse immunized with >30 KDa *A. candida* soluble material). Although preliminary studies (PTA ELISA tail bleeds) indicated a strong immune reponse to immunogen <30 KDa > 2 KDa *A. candida*, post fusion few positive hybridoma cell lines were identified and, during cloning all proved unstable. Additional fusions would be required to determine whether this result was characteristic of the immunogen used.

10.2 Reactivity of selected Monoclonal antibody cell lines (MAbs)

10.2.1 Introduction

To determine specificity of the five selected MAb cell lines, the MAbs were screened by immunfluorescence (IF) against selected *Albugo candida* races and a range of fungal species (Table 10.2). Where the fungal species was cultivated on host material, spore collection was as described previously, using a hand held cyclone sampler and the spore sample made into suspension with the addition of PBS. Spore numbers were adjusted to a final concentration of 1 $x10^6$ spores ml^{-1}. The remaining fungal species were inoculated onto a synthetic agar medium (Table 10.2), which had been pre-covered with a PN60206 Supor 450 90 mm diameter membrane disc (www.pal.com). Fourteen days after inoculation the membranes were removed, and 5 ml PBS was added to each. Surface washings were taken by gently stroking the surface of the membrane with a sterile glass rod and were retained on ice whilst spore counts were made by bright field microscopy (x 400). Spore numbers were adjusted to a final concentration of 1 $x10^6$ spores ml^{-1}.

Table 10.2 Fungal species used to assess reactivity of the five MAb cell lines.

Fungal species	Code Name/Origin	Host / Growth Mdeium	Race
Albugo candida	UK	Cabbage	9
Albugo candida	AU03	Broccoli	9
Albugo candida	AU04	Broccoli	9
Albugo candida	AU05	Broccoli	9
Albugo candida	AU06	Chines cabbage	7
Albugo candida	AU	Pak Choi	7
Albugo candida	AU	Soi Choi	7
Albugo candida	AU	Green stem pakChoi c.v. Mijako	7
Albugo candida	AU	Radish	1
Albugo candida	AU	Radish c.v. radar	1
Albugo candida	AU15	Broccoli	9
Albugo candida	UK	Garden weed	
Albugo candida	UK	Shepherds purse	4
Albugo candida	AU	Shepherds purse	4
Albugo candida	AU	Rocket	
Albugo laibachii	Norway	Arabidopsis thaliana	
Ramularia	UK	Malt extract agar	
Fusarium culmorum	UK	V8 juice agar	
Puccinia allii	UK	Onion	
Oideum neolycopersici	UK	Tomato	
Erysiphe cruciferarum	UK	Brussels sprout	
Hyaloperenospora brassicae	UK	Lettuce	
Bremia lactucae	UK	Lettuce	
Pyrenopeziza brassicae	UK	Brussel sprout	
Botrytis cinerea	UK	Potato Dextrose Agar	
*Stemphylium botryosum*AU	AU	Agar	
Cladosporium cladosporioides	AU	Agar	
Alternaria alternata	AU	Agar	
Phoma lingam	AU	Agar	

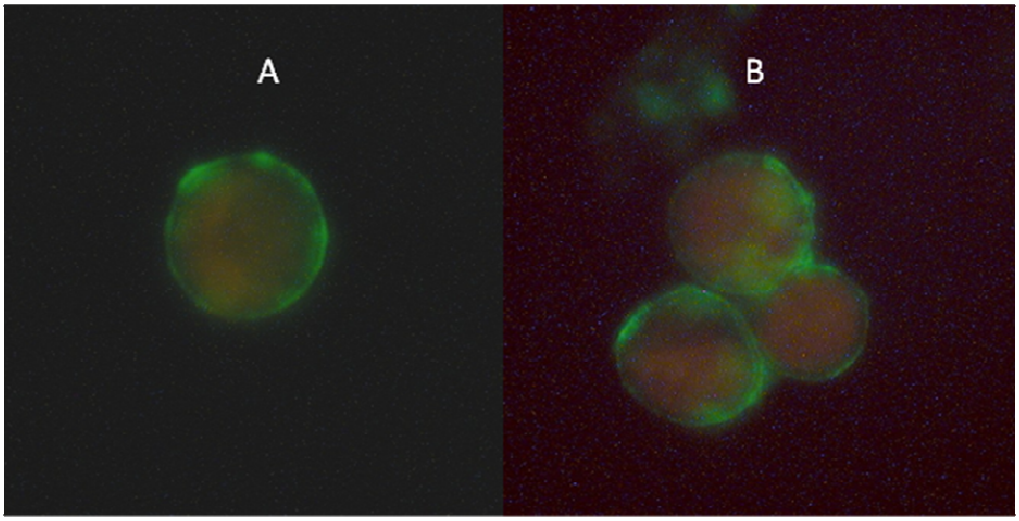
10.2.2 Materials and methods

Microwells of 29 Teflon coated 6 multiwell (8 mm diameter) glass slides (Code 63423-08, Electron Microscopy Services, Hatfield) were each coated with 30µl poly-l-lysine (Code No. www.sigmaaldrich.com) in PBS (1mg ml^{-1}). Following a 10 min incubation period at 18 to 20°C, the slides were rinsed in distilled water and air-dried under a constant air-flow. To each well of a coated multiwell slide, 30µl of a fungal suspension was aliquoted and, retained in a humid environment for a 12 hr period. The glass slide was removed and allowed to air dry overnight. The slide was rinsed in PBS and air dried in a laminar flow cabinet. Tissue culture supernatants of each MAb cell line (Table 10.1) were diluted 1:2 in PBSTwc and, each applied at a rate of 30µl to a multiwell. The sixth well received PBSTwC alone. The slide was incubated for a period of 30 mins at 18 to 20°C in a moist chamber. The slide was gently rinsed with PBS and air-dried as described previously. A 30µl of anti-mouse (whole molecule) fluorescein isothiocyanate conjugate (Cat. No. F-0527 www. sigmaaldrich.com) diluted 1:60 in PBSTwC Evans blue counterstain (Kennedy *et al*. 1999) was then aliquoted to each well and, incubated as previously described, in darkness. The slide was then rinsed, air dried in darkness and mounted in DAKO fluorescent mounting medium (Code no. S3023 www.dako.co.uk). This process was repeated for each of the fungal species listed in Table 10.2. The slides were viewed under U.V. with a fluorescent microscope using selective fluorescein excitation at wavelength 490 and a barrier filter at 520 wavelength.

10.2.3 Results

Each of the MAb cell lines bound to epitopes present on the zoosporangial wall of *Albugo candida* race 9 (Plate 10.1A) and, to *A. candida* zoosporangia isolated from a garden weed (Plate 10.1B). Initial studies suggest that EMA 256 is *A. candida* species and is race(s) specific, binding strongly to Races 9 and 1 and weakly to Race 4 and 7. All other fungal species and *A. candida* races tested (Table 10.2) were negative by IF. EMA 258 gave a similar profile but did not react with Race 4 or 7. Nevertheless, some recognition by EMA 258 was observed to the germ tube of *Erysiphe cruciferarum*. With the exception of EMA 256, all the MAbs bound to germ tube epitope sites of *E. cruciferarum* and, EMA 259 to the conidial stage. No reactivity to the other fungal species was observed for any of the MAbs tested (Table 10.2). Only EMA 257 reacted with *A. candida* zoosporangia isolated from Rocket (*Eruca sativa*).

Plate 10.1. *A. candida* zoosporangia labeled with EMA 256 conjugated to a fluorescein anti-mouse label as viewed by fluorescence microscopy (x 400).



10.2.4 Discussion

None of the cell lines selected proved *A. candida* race 9 specific. However MAb cell lines EMA 256 and 258 exhibited a level of specificity that may prove useful in the development of a rapid field based test for monitoring epidemiological significant levels of airborne inoculum of *A. candida,* in horticultural areas of Australia. Of the five MAbs, four bound to epitopes present on the germ tube of *Eryisphe cruciferarum* (*Brassica* powdery mildew), however no recognition to *Oidium neolycopersici* (tomato powdery mildew) was observed. Additional studies could look to determine whether these MAb recognition sites relate to a protein / structure involved in the infection process / immune response of *Brassica* host material.

10.3 Competitive Lateral flow development

10.3.1 Introduction

The technical basis of the lateral flow immunoassasy test (lfd) was derived from the latex agglutination assay (Plotz and Singer, 1956). However establishment of the technology for the lateral flow test was not available until the late 1980's. Pioneering work in the development of a 'home test' for determination of human pregnancy assisted this technology to the wider market place, enabling complex laboratory processes to be carried out on-site by non-laboratory personnel. The simplicity of the design, requiring addition only of the sample and, the compact and portable capability of the test, make it popular for development of a wide range of assay tests. The application of these tests has expanded beyond clinical diagnostics to areas as diverse as veterinary, agriculture, bio-warfare, food, environmental health and safety, industrial testing, as well as newer areas such as molecular diagnostics and theranostics. Different configurations of the lateral flow assay exist, however, all require the basic elements of a solid membrane phase, a fluid transport and, a test specific labelled antibody.

For the purpose of this work, a competitive lateral flow assay format was used to develop a prototype field test for semi-quantification of trapped airborne inoculum of *A. candida*. A competitive lateral flow device (clfd), in the absence of a target sample (*A. candida* zoosporangia), will give rise to the formation of a test line. Rate of test line depletion will relate directly to target levels in the test sample. In the competitive format, the test line depletion is generally measured using a portable optical device.

10.3.2 Materials and methods

Lateral flow construction. Competitive lateral flows (clfds) (Fig 10.2), comprising a Millipore 180 HiFlow^{TM} cellulose ester membrane direct cast onto 2ml Mylar backing (Millipore Corp, USA), an absorbent pad (www.whatman.com), sample pad (www.millipore.com) and a filter pad (www.whatman.com) were constructed for the detection of *A. candida* zoosporangia. A control line of an anti-mouse serum at 0.5 mg ml $^{-2}$ (www.sigmaaldrich.com) was sprayed directly on to the membrane surface using a flat bed air jet dispenser (Biodot Ltd,West Sussex, UK) at a constant rate of 50 m/s. A collected soluble fraction of an *A. candida* zoosporangial sample was adjusted to 0.5 mg ml^{-1} in ¼ strength PBS and applied as a test line to the membrane surface, as previously described. Membranes were air-dried overnight at 18 to 20°C and sectioned in to 5mm strips before being individually housed in a plastic case (Advanced Microdevices, Ambala, India).

A 5µl gold anti-mouse IgM solution (Code BA GAMM 40, British Biocell International, Cardiff, UK) was pre-mixed with 30µl EMA 256 (Lot 1 diluted 1 in 50 conjugate buffer) before application to a pre cut 5mm conjugate pad (www.millipore.com). After this conjugate pad was laid horizontal and air-dried at 35°C for a period of 10 mins (or until dry). After which the conjugated antibody pads were stored individually in non-stick 0.5ml microfuge tubes.

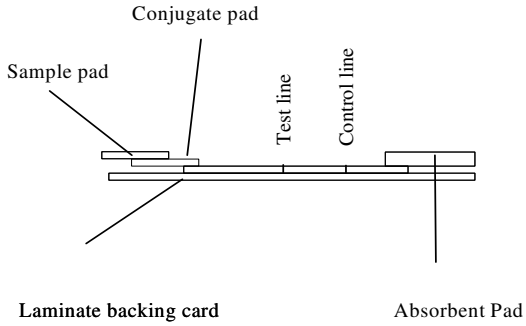


Fig 10.2 Lateral flow cross section (5mm strip)

Lateral flow test. Ten fold dilutions of $1x10^8$ to 100 *A. candida* zoosporangia (race 9) were prepared in Warwick HRI PVPC buffer. To 0.5ml non-stick microfuge tubes, each of which contained a pre prepared gold conjugate pad, 100µl aliquots of the zoosporangia dilution series were dispensed. Following a 1 minute incubation period to rehydrate the gold conjugated MAb, the contents of the microtube were mixed and transferred drop-wise to a competitive lateral flow device (clfd). After 10 minutes the clfds were observed visually for the development of a control and test line. The test line area of each clfd was electronically measured using a Quadscan device (BioDot, Chichester, UK).

10.3.3 Results

With the exception of *A. candida* suspensions at $1x10^8$ and $1x10^7$ zoosporangia ml^{-1}, test and control lines were observed on each of the competitive lateral flow strips (clfd). Electronic readings of the test line area however showed a correlation (r^2=0.9777) (Fig 10.3) between the optical density of the test line area and the *A. candida* zoosporangia suspension applied to the clfd.

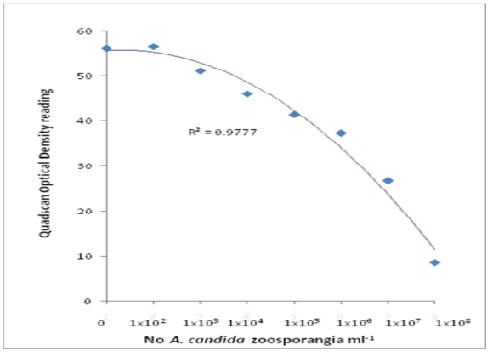


Fig 10.3 Change in optical density of test line with *A. candida* concentrations in test samples.

10.3.4 Discussion

The developed competitive lateral flow device enabled quantitative readings to be made of *A. candida* zoosporangial numbers when an electronic reader device was used. However test visual readings only enabled samples in excess of 1x 10^6 zoosporangia ml^{-1} to be identified. Further work will be carried out to optimize the assay format to increase visual test sensitivity whilst retaining good discrimination using an electronic reader. A competitive lateral assay format will be developed using EMA 258 labelled to a gold carrier.

10.4 Monitoring airborne conidia of Albugo candida in commercial Brassica crops in Australia using aerial spore traps.

10.4.1 Introduction

Accurate information about the presence of sufficient pathogenic inoculum is required to predict plant disease occurrence in field settings (Scherm *et al.* 1995). Traditionally, plant disease forecasting systems have relied upon environmental data singularly to predict disease occurrence in crops (Magarey *et al.* 2004; Scherm *et al.* 1995). Mathematical models describing the effect of temperature and wetness on pathogen infection have been developed for many types of plant pathogen. Magarey (Magarey *et al.* 2004) provides a comprehensive review of these systems. However, detecting and quantifying airborne spores could augment disease forecasting systems by determining airborne spore numbers during a time period when environmental risk for a disease is high, and when protective disease control strategies could be implemented. This study reports on monitoring airborne inoculum of *Albugo candida* in commercial Brassica crops in Australia using air samplers placed within the crop canopy.

Commercial broccoli crops c.v. Ironman and Steel, located on a property at Cunninghams Rd, Werribee, South Victoria were monitored for airborne disease transmission events by *Albugo candida* for two, two month periods between May 2010 and December 2010. Another commercial broccoli crop c.v. Viper located on a property at O'Conners Road, Werribee South Victoria was also monitored for airborne disease transmission events by *Albugo candida* for a further 2 month period in January-February 2011. Three air samplers: a Microtitre immunospore trap (MTIST), a volumetric 7 day sampler and a multi-vial cyclone air sampler, were operated within the cropping system. An environmental white blister disease forecast model was evaluated for use in determining periods of white blister risk.

10.4.2 Materials and methods

Monitoring airborne disease transmission of *Albugo candida* in a commercial field broccoli cropping system was conducted using three air samplers.

Microtitre Immunospore Trap (MTIST). A detailed description of the MTIST device, which is manufactured by Burkard Manufacturing Company (Rickmansworth, Herts, UK) can be found in Kennedy *et al.* 2000. In the outdoor version, air is drawn thorough a manifold consisting of a plastic tube with a right angle bend placed over the sampler inlet. The manifold samples air through a 9cm diameter vertical circular inlet and directs it into the sampler body that is held horizontally. For field use, the sampler (including the manifold) is mounted on a wind vane so that the manifold inlet faces into the wind. Within the sampler the airflow is channelled through 32 trumpet shaped nozzles each directed at the base of a

microtitre well. The sampler contains four microtitre strips each containing 8 wells. The MTIST air sampler uses a suction system and particulates in the airstream are impacted on the base of each collection well of the four microtitre strips. The collected impacted target particulates may, if appropriate antibodies are available, be immunoquantified by plate trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA).

Monitoring *A. candida* spores in MTIST collected field aerosols. An MTIST spore trap was placed within a commercial field broccoli crop. Held within the base plate of the machine were four coated eight well microtitre strips [0.1mg ml-1 Poly-L-Lysine (Sigma P-1524) in distilled water and sodium azide (Sigma S-2002)]. The MTIST spore trap was operated for 12 h periods from 06:00h to 18:00 h daily. The coated microtitre strips were changed daily and stored at -20^{o}C prior to analysis.

Enumeration of MTIST trapped *A. candida* spores. Visual examination of the base of field exposed microtitre wells determined that a high concentration of air particulates would prevent accurate enumeration of *A. candida* spores. The wells of two, eight well strips of each field sampling period were processed by PTA ELISA (Kennedy *et al.* 2000). Field trapped *A. candida* spores were identified and labelled using a monoclonal antiserum (MAb EMA 256).This process was amplified using an anti-mouse biotinylated conjugate linked to a streptavidin horseradish peroxidase system (Dako K-0492) and visualised by adding 100µl to each microtitre well of 3,3',5,5'-tetramethylbenzidene substrate (Sigma T-3405 and P-4922). The reaction was stopped by adding 25µl of a 20% 1M H_2SO_4 solution and the generated absorbance values were read at 450nm using a Biotek ELISA plate reader (EL800). This process was repeated using the remaining field exposed microtitre strips in conjunction with MAb EMA 258 (raised to *A. candida*) used in the assay format.

Burkard 24hr Volumetric glass slide air sampler A Burkard 24 h volumetric air sampler which contained a glass slide coated with silicone (BC 380S, Basildon Chemical Co, Kimber road, Abingdon, Oxon, UK) operated at an air flow of 10 L of air per minute. Sampled air particulates impacted directly onto an area of the glass slide which corresponded to time intervals by movement of the glass slide over a 24 h period.

Monitoring *A. candida* spores in collected field air samples. The Burkard 24 h glass slide volumetric spore trap was placed 2 m from and adjacent to the MTIST spore trap. The trap was loaded daily with a silicone coated glass slide and air particulates were impacted directly onto the surface. The glass slide was changed daily after 18:00 h and stored at -20ºC.

Enumeration of trapped *A. candida* spores. Each glass slide was examined for the presence *A. candida* spores under bright field microscopy.

Burkard multi-vial cyclone air sampler. The characteristics of a cyclone air sampler are described by Ogawa and English (1995). Air is drawn through the sampler using a vacuum pump in the form of a cyclone. The height of the cyclone and air inlet, along with the width of the air inlet, air exhaust diameter and the diameter of the cyclone within the length of the exhaust pipe influence the relative efficiency of the trap. These characteristics have been drawn together and standardised within the Burkard cyclone sampler (Burkard Manufacturing Co.). The cyclone air sampler operates at an air flow rate of 10 to 15 L air / min.

Monitoring *A. candida* spores in collected field air cyclone samples. The multi-vial cyclone spore trap was placed 2 m from and adjacent to the MTIST spore trap in the commercial broccoli field crop. The trap was loaded weekly with eight 1.5ml microfuge tubes (Sarstedt 2013/4). By an integrated automated mechanism each tube was exposed once for a 12 h period for collection of field air partici;ates. Each sampling exposure period was between 06:00 to 18:00 h daily. After each eight day period the field exposure tubes were collected and stored at -20ºC.

Enumeration of trapped *A. candida* spores. To each exposed microtitre tube 100µl of NPARU B2 buffer was added and agitated using a Gallenkamp Spin Mix for 5 seconds at high speed. A lateral flow device developed for field assessment risk of the white blister pathogen (Section 10.3.2) was identified to semi-quantify trapped airborne disease inoculum of *A. candida*. A 100ul aliquot of the spore suspension was applied to the sample pad of the lateral flow device (Plate 10.2A) and test line development was assessed 15 min. later using an ESE Quant reader (Plate 10.2B).

Plate 10.2. A lateral flow device for evaluation of field crop risk to white blister (A) and a hand held reader (B).

A B

10.4.3 Results

Microtiter Immunospore Trap (MTIST). The PTA ELISA carried out on the field exposed wells for the sampling periods May - June 2010, November - December 2010 and January - February 2011 identified periods of when the Broccoli crops were at risk to infection by *A. candida* (Fig 10.4). Low level disease potential by MTIST PTA-ELISA was determined within the May – June, 2010 sampling period. During November and December 2010 a high risk period was noted between 19th- 21st November 2010. A number of moderate risk periods were identified throughout the period (Fig 10.4). For the third field sampling phase, the broccoli field crop was determined to be at low level risk of *A. candida* until the end of January. A high risk period was then identified in the middle of February 2011.

Each field exposure period was monitored for white blister disease transmission using two different *A. candida* specific monoclonal antibody cell lines. Both exhibited a similar response pattern but with EMA 258 providing an enhanced signal. However this may be due to the activity used as EMA 258 was optimised at 1:2 whereas EMA 256 is used at a 1:200 dilution.

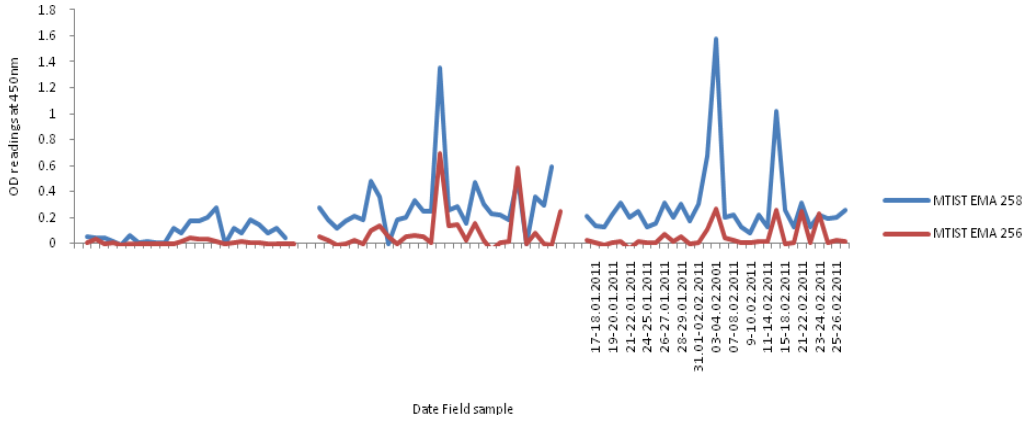


Fig 10.4 Monitoring in commercial field broccoli cropping systems for white blister airborne disease transmission periods using an MTIST air sampler and immunoquantification of *A. candida* by PTA ELISA.

24 h Volumetric glass slide air sampler. Enumeration of *A. candida* spores on the field exposed glass slides was carried out for the periods May-June 2010, November-December 2010 and January-February 2011. During May – June the airborne concentration of *A. candida* spores was limited to one period at a low concentration (Fig 10.5). For the second field trapping period, inoculum was identified in the airborne environment from the 7th - 13th November 2010 and then at a notably increased concentration during the 21st - 22nd November 2010 (Fig 10.5). *A. candida* exposure periods were observed at the end of November / beginning of December 2010 but at a much reduced concentration. During January (third sampling period) limited or no airborne *A. candida* spores were observed. However from February 2011 onwards, high amounts of inoculum were again observed (Fig 10.5).

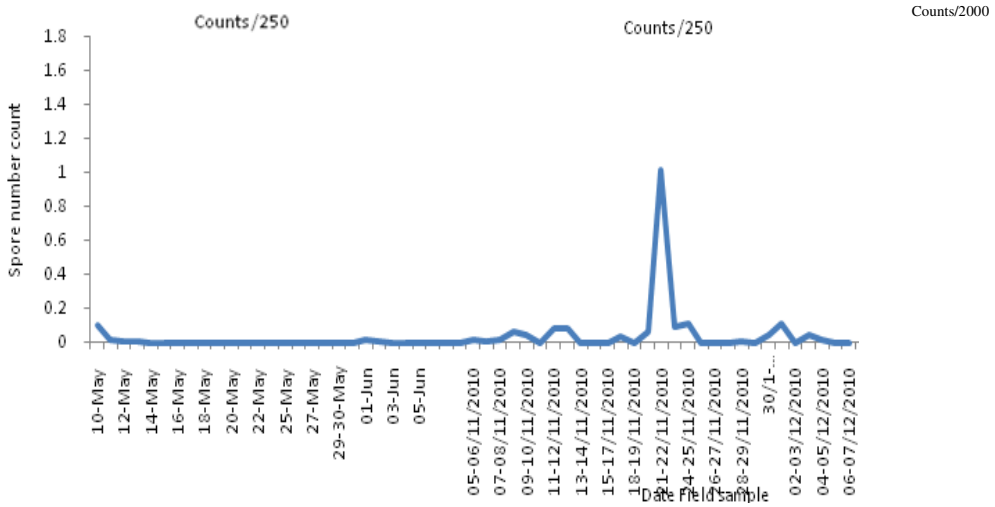


Fig 10.5 Quantification of airborne spore numbers of A. *candida,* collected daily using a volumetric air sampler onto glass slides and identified by bright field microscopy.

10.4.4 Discussion

For the three field monitoring periods, a good association was observed between the airborne concentration of *A. candida* spores trapped using the volumetric air sampler and the MTIST PTA ELISA (Fig 10.6).

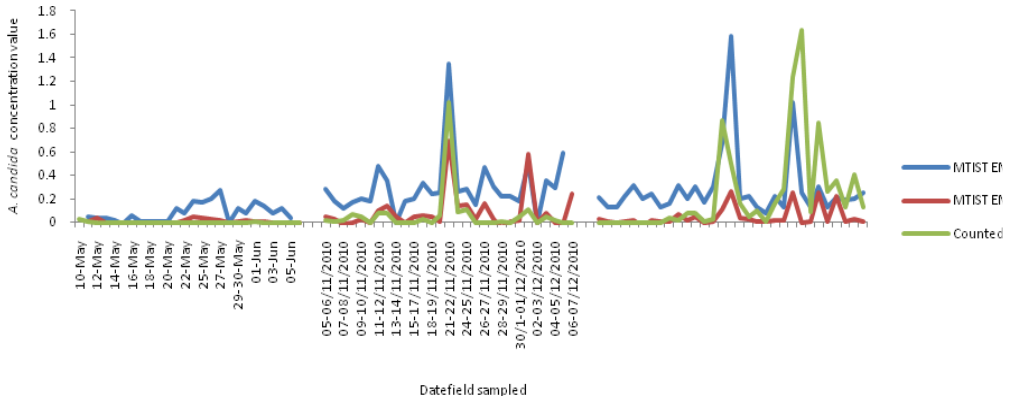


Fig 10.6 An overlay of daily field sampling periods in a commercial broccoli crop of *A. candida* airborne spore concentrations as derived from a Volumetric glass slide sampler and a MTIST PTA ELISA spore trap system.

However the sampling period of the 26th- 27nd November 2010 showed variability where the MTIST PTA ELISA system identified a concentration of airborne *A. candida* spores within the crop canopy when employing either of the two *A. candida* specific monoclonal cell lines. However for this period a glass slide was not made available for evaluation and a 0 value was recorded.

Using bright field microscopy to determine the presence of *Albugo candida* spores impacted on to glass slides is extremely time consuming, requiring expertise and results that are often available days after the event. Alternatively, the MTIST ELISA process enables results to be made available within two hours of the laboratory receiving the field exposed microtitre wells and using staff who only require basic training. The ability to produce results quickly provides information on *A. candida* inoculum availability. When this information is used in conjunction with an environmental based forecast for infection risk, growers can make an informed decision on an appropriate crop protection regime.

10.5 Development of a lateral flow field test for quantification of A. candida in air samples.

10.5.1 Introduction

Previous work describes the successful development of a competitive lateral flow for semi-quantitative detection of *A. candida* spores using EMA 256 as the specific antibody label (Section 10.3). A conclusion of this work was to extend test development to a competitive lateral flow which used EMA 258 as a specific antibody label and makes a comparative study. In earlier studies (Section 10.2) the two monoclonal antibody cell lines of EMA 256 and 258 each exhibited a level of specificity suitable for use in a field diagnostic test for *A. candida* [Race 9 (AC9) *Brassica oleracea*)]. Reactivity tests with a wide range of fungal spora

determined EMA 256 to be *A. candida* species specific, reacting strongly with Races 9 and 1 and weakly with Races 4 and 7. EMA 258 was determined *A. candida* race specific for 9 and 1 only but did however react to the germ tube of *Erysiphe cruciferarum* (powdery mildew).

This study describes comparative studies of two competitive lateral flow assay systems for quantitative detection of *A. candida* using either EMA 256 or 258 as a specific *A. candida* label.

10.5.2 Materials and methods

Lateral flow test development. Competitive lateral flows were prepared as described in Section 1.4.2. However in these experiments EMA 256 monoclonal antiserum (Lot 1) was pre-mixed with gold anti-mouse at activities of either of 1:50, 1:100 or 1:500. This process was repeated for EMA 258 (Lot 1) ,however the monoclonal antiserum was used undiluted, 1:2 and 1:4 in conjugate buffer.

Lateral flow test line activity. To each of the prepared competitive lateral flow tests 100µl of buffer (no *A. candida* zoosporangia present) was applied to the conjugate pad (Plate 10.2A). Test line readings were taken after 15 min. using an ESE Quant LFR reader (Qiagen UK) (Plate 10.2B).

10.5.3 Results

Lateral flow test line activity. As previously observed (Sect 1.4.3) EMA 256 proved useful for detection of *A. candida* zoosporangia when incorporated in a competitive lateral flow test format (Fig 10.7). However at a dilution of 1:500 (EMA 256, Lot 1) activity was reduced and limited with poor binding at the test line observed. Poor test line formation was observed when EMA 258 was employed in the competitive lateral flow assay format and an optical density reading could only be generated when the monoclonal antiserum was used undiluted (Fig 10.7).

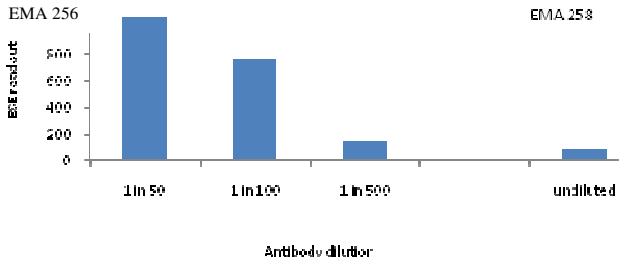


Fig 10.7 Competitive lateral flow test line values as generated using an ESE Quant portable reader.

10.5.4 Discussion

The competitive lateral flow format incorporating EMA 256 enabled production of a clear test line but MAb activity was limited at a dilution of 1 in 500. EMA 258 proved sensitive to *A. candida* zoosporangia when used in a PTA ELISA format (Fig 10.4) but not when incorporated within a lateral flow assay. This contrasted with EMA 256, which demonstrated

increased sensitivity to *A. candida* applied to a lateral flow test membrane than in a PTA ELISA format. If appropriate *i.e.* increased specificity of the lateral flow test is required, different label identifiers (latex, iron, carbon) could be investigated to determine optimal test labelling for EMA 258. The use of a double antibody system (combination of EMA 256 and 258) as a capture and identifier may prove useful and provide increased specificity than the current competitive lateral flow assay format. Given the field MAb profiles generated in each of the ELISA assays, there would appear to be little difference in specificity (Fig 10.4). This may relate to the pre- coating of microtitre wells with sodium azide which has been shown to limit germination of trapped air spora (Wakeham and Kennedy 2010). For the purpose of this study, a batch of competitive lateral flow devices were produced using EMA 256 as the label identifier for immunoquantification of *A. candida* inoculum in collected field aerosols.

10.6 Test predictions using the developed lateral flow kits for within crop and between crop spread of white blister inoculum in UK field trials

10.6.1 Introduction

In European vegetable Brassica production systems, airborne fungal diseases are a common problem (Carisse et al. 2005; Wakeham and Kennedy 2010). Brussels sprout and cauliflower crops are produced throughout the year in UK productions systems. Due to the cosmetic nature of damage by *A. candida,* many opportunities exist for crop loss. Small amounts of disease on sprout buttons and cauliflower leaves can lead to downgrading the value of the product. Currently there are few methods that can detect significant levels of fungal inoculum in air samples rapidly and accurately (Kennedy *et al.* 2000; Kennedy *et al*. 2000; Wakeham and Kennedy 2010). Using an innovative spore trapping system (Mircrotitre immunospore trap (MTIST)) (Kennedy *et al.* 2000) allied to an immunological test (plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA)) we have reported (Section 10.4) on the potential to monitor airborne field inoculum of *A. candida*. In conjunction with a white blister environmental based forecast the likely onset of disease occurrence in crops can be determined.

The results reported in Section 10.4 emphasize the need for rapid methods of detection if measures of target propagules within air samples are to be used in practical decision making for control of plant diseases. The ELISA test is still a laboratory based assay. However the recent development and use of immunochromatographic test strips (lateral flow) to rapidly detect and quantify fungal target inoculum 'in-situ' (Kennedy and Wakeham 2008; Thornton *et al.* 2004) exhibit considerable potential for monitoring airborne spores. The work reported below investigates the potential of using a multi-vial air sampler and a lateral flow test to detect and semi-quantify airborne field disease transmission events '*in-situ'* by non-scientific staff. The system described provides an example where information on inoculum availability can be used directly with environmental disease forecast models to provide information for crop protection regimes.

10.6.2 Material and methods

Monitoring airborne disease transmission events of *Albugo candida* in a commercial Brussels sprout crop. A Burkard multi-vial air cyclone and a MTIST air sampler were operated within a commercial UK Brussels sprout crop at Croppers Farm, Bickerstaff, Lancashire from August to October, 2011. Air temperature, leaf wetness, relative humidity

and rainfall were recorded throughout this period using a Smartlog (Aardware Design, Walton on Thames, UK) and at 30 min. intervals the data was downloaded to provide daily disease risk periods of *A. candida* infection. Each air sampler was loaded weekly, i.e. eight microfuge tubes of the multi-vial cyclone air sampler and 4x8 well microtitre well strips of the MTIST sampler. Prior to field air sampling the microfuge tubes and microtitre wells were each pre-coated with 100µl 0.1mg Poly L Lysine 0.05% sodium azide solution and air-dried (Section 10.4.2). The field air samplers operated between 06:00 and 18:00 h daily. The automated mechanism of the cyclone air sampler provided each tube to a single daily exposure whilst the MTIST remained unchanged to provide a weekly reading. After each eight day period the field exposed tubes and the microtitre wells were collected and stored at -20ºC.

Enumeration of MTIST trapped *A. candida* spores. Visual examination of the base of MTIST trapped field exposed microtitre wells determined that a high concentration of air particulates would prevent accurate enumeration of trapped *A. candida* spores using bright field microscopy. The eight well strips of each field sampling period were then processed as described in Section 10.4.2 by PTA ELISA for immunoquantification of *A. candida* inoculum.

Field exposed microfuge tubes of the multi-vial cyclone air sampler were assessed for *A. candida* inoculum by the addition of 110µl / tube of lateral flow extraction buffer. Each tube was agitated using a Gallenkamp Spin Mix for 5 seconds at high speed. An aliquot of 20ul was removed and if present *A. candida* spore numbers were determined by bright field microscopy (x400). Using the remaining buffer, a lateral flow device developed for field assessment of the white blister pathogen (Section 10.5), was used to semi-quantify *A. candida* inoculum of each field exposed microtube. A 100ul aliquot of each 'field air sample' suspension was applied to the sample pad (Plate 2A) of a lateral flow device and test line development was assessed 15 min. later using an ESE Quant reader.

10.6.3 Results

Monitoring airborne disease transmission events of *A. candida* in a commercial Brussels sprout crop. Weekly field air samples collected using the MTIST sampler and processed by PTA ELISA for *A. candida* inoculum determined that throughout the study the disease risk was low (14th to 21st September, 2011) or there was no risk of white blister infection (Fig 10.8). In the earlier field study (Section 10.4, Fig 10.4) the MTIST microtitre wells were changed daily (24h field exposure) whereas in this study a seven day cumulative air sampling process was recorded.

Microscopic counts of field aerosols suspended in liquid phase proved difficult to identify presence of inoculum of *A. candida*. No counts could be made using this process. Each of the lateral flow devices used to assess white blister disease risk in the collected daily field aerosols provided a low / negative value (Fig 10.9). When the daily optical density readings of the lateral flow devices were combined to provide a weekly value and overlaid with the MTIST weekly PTA ELISA results an association was observed between the two data sets (Fig 10.10).

Throughout the sampling period of August to October 2011 the Brussels sprout crop was assessed visually for white blister. On each occasion no visible symptoms of white blister could be observed.

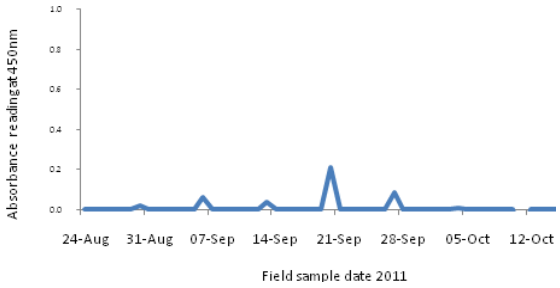


Fig 10.8 Immunoquantification of MTIST field trapped airborne disease inoculum of *A. candida* spores in a UK Brussels sprout commercial crop.

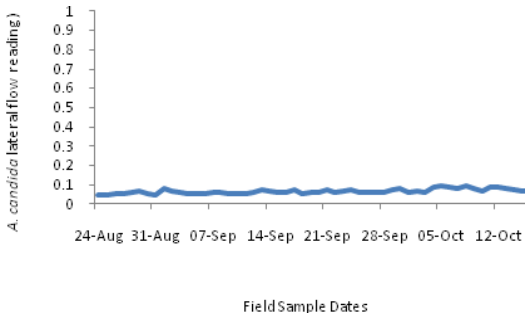


Fig 10.9 *Monitoring A. candida* disease inoculum in field collected aerosols during August to October, 2011 in a UK commercial Brussels sprout crop.

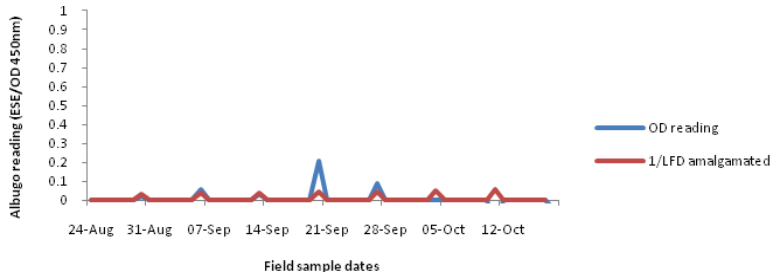


Fig 10.10 Quantification of *A. candida* during seven day periods in collected field aerosols of a UK commercial Brussels sprout crop.

10.6.4 Discussion

In this study, the two immunoassay based sampling systems developed provide corresponding data on field airborne disease transmission events of *A. candida* in a UK Brassica horticultural field cropping system. Crop walking and assessment of white blister disease development within the exposed crop confirmed the results observed. Further work should now investigate whether daily or weekly sampling of airborne disease transmission events is required. The use of weekly estimates of disease inoculum in air samples has been reported for other diseases of field crops Wakeham and Kennedy (2010). Nevertheless, in providing a robust field disease monitoring system it is necessary to determine the inoculum concentration

required for infection and symptom development and, the effect of environmental parameters on this process. This could provide useful information in determining whether daily or cumulative field readings could then be made.

10.7 General Discussion

10.7.1 Development of a competitive lateral flow for *A. candida.*

In this study five MAbs have been raised to zoosporangial material of *A. candida*. Reactivity tests have determined that two of the MAb cell lines may exhibit a level of sensitivity and specifity that could prove useful in a developed field test system. A competitive lateral flow prototype (clfd) has been developed using cell line EMA 256 and this will now be extended to include EMA 258. However, the studies to date have demonstrated that in the present format for the test to be useful, quantitative readings would need to be made using a Quadscan device. In this format a calibration series of *A. candida* zoosporangial concentrations would be a requirement of the test and run concurrently with field samples to determine quantitative information on *A. candida* field trapped zoosporangial numbers. Future work will investigate the optimization of the assay format to enable visual clfd discrimination of trapped *A. candida* airborne inoculum that would give rise to disease development when under optimal environmental conditions. This would preclude the need for an electronic reader and an *A. candida* zoosporangial calibration test series.

10.7.2 Monitoring airborne particles of *A. candida*

Airborne disease inoculum plays an important role in the development of disease epidemics on 'above ground' plant material of horticultural crops (Carisse *et al*. 2005; Wakeham and Kennedy 2010). The two immunological air sampling systems developed for monitoring field aerosols of *A. candida* in commercial Brassica cropping systems demonstrated the potential for determining presence or absence of disease transmission events ahead of symptom development. In the past, air sampling processes have been limited to passive collection of disease inoculum by gravitational deposition (Magarey *et al.* 2004) and /or sampling specific volumes of air by suction air samplers. These techniques have been limited to laboratory analysis as they require considerable amounts of time and expertise if accurate counts are to be obtained. Nevertheless, the Burkard volumetric glass slide air sampler has in this study provided valuable information. Microscopic counts of exposed glass slides in commercial crops of *A. candida* airborne disease concentration has been used to validate two new sampling processes, both of which rely on *A. candida* specific monoclonal antibodies (MAbs) to rapidly immunoquantify *A. albugo* spores.

10.7.3 Preliminary evaluation of air-sampling technology and the lateral flow test strips for *A. candida*

The development of the MTIST air sampling device enables a portable, robust, and inexpensive spore trapping system that incorporates trapping technology alongside an existing well-established immunoassay (ELISA) (Clark *et al.* 1977; Dewey *et al.* 1995; Kennedy *et al.* 2000). The production of two MAbs to zoosporangial material of *A. candida* have both proved equally useful in quantifying airborne disease inoculum of *A. candida* in field crops when used in conjunction with the MTIST PTA-ELISA format. With the addition of sodium-azide to the microtitre wells of the MTIST spore trap, results indicate a test that is able to

detect *A. candida* to race specific level. In an earlier study (Wakeham and Kennedy (2010), field trapped air-spora were inhibited from germinating when wells were pre-coated with sodium azide. The MTIST air sampler provides the potential to detect several target crop pathogens simultaneously providing specific antibody probes are available. A limiting factor of the test system is, however, that although assay time is short (2 hours), a laboratory and specialised staff are still required to process the collected field samples.

The recent development and use of immunochromatographic test strips (lateral flow) to rapidly detect and quantify fungal target inoculum '*in-situ*' (Kennedy and Wakeham 2008; Thornton et al. 2004) exhibit considerable potential for monitoring airborne spores. In this study we have combined an existing air sampling system (cyclone air sampler) and developed a lateral flow assay test which can be used 'in situ' to determine crop exposure to *A. candida* disease inoculum. The test format provides a rapid assay platform with results provided within 15 mins. of the assay start time. The developed test also provides the potential for semi–quantititative measurement of *A. candida* concentration. This data can be recorded using a portable electronic reader. The current lateral flow assay system relies on a single MAb cell line (EMA 256). Results to date show promise for monitoring airborne disease transmission events of *A. candida* and compare favorably with UK MTIST field results.

The current study provides information of airborne disease transmission events of *A. candida* in commercial field crops using three different air sampling systems. The approach described is used as an example where information on inoculum availability can be used directly with environmental disease forecast models (Kennedy and Giles 2003) to provide information for optimal crop protection regimes.

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Chapter 11

Leaf wetness determination for plant disease prediction: getting rid of leaf wetness sensors

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Completed November 2008

Summary
Under laboratory conditions the performance of three different leaf wetness sensors (Model T, Monitor Sensor and Environdata) was compared. Alternative measures of leaf wetness, vapour pressure deficit (VPD) and a fuzzy logic model, were also evaluated. All of the leaf wetness sensors operated within similar tolerance levels, but over time the Environdata sensor showed more variation. VPD provided an excellent estimate of leaf wetness in still air but not in moving air. The fuzzy logic model provided estimates that agreed with actual leaf wetness 75% of the time and had potential for further development.

11.1 Introduction

Leaf Wetness is a key factor in predicting the behaviour of fungal pathogens in most crop disease prediction models (Pitblado 1998; Huber and Gillespie 1992; Latin and Evens 1996; Kushalappa 2001; and Kennedy and Giles 2003). Most fungal pathogens require the presence of water on the plant surface for germination and infection (Yarwood 1978; Lacy 1986; and Rotem *et al.* 1978).

Leaf wetness is measured with leaf wetness sensors. There are two types of leaf wetness sensors on the market; (i) a gold plated electrical grid mounted on a plastic circuit board and (2) two electrodes mounted on a canvas leaf replica to measure conductance. A key problem with measuring this parameter is that both of these conventional leaf wetness sensors can lack reliability. A second problem is that manufacturers of leaf wetness sensors do not calibrate them in a standard way so that we can understand what they are truly measuring. Additionally leaf wetness sensors are subject to weathering (Fig 11.1) and can malfunction when coated with fungicide or insecticide sprays.

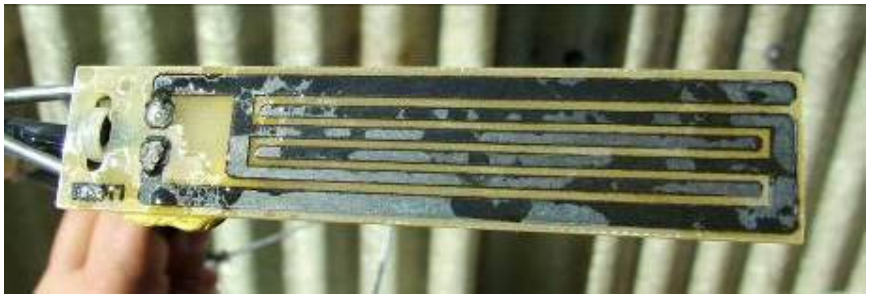

Fig 11.1 Damaged leaf wetness sensor.

Another way of measuring leaf wetness is Vapour Pressure Deficit (VPD). VPD is one measure of the ability air to release moisture. VPD measurement is used regularly as a key

factor for predicting disease potential in greenhouse crops (Prenger and Ling on line). Other researchers are trying to develop models which estimate leaf wetness duration in the field. These models are the fuzzy logic system and the CART/SLD/Wind model (Kim *et al.* 2002, 2006).

This chapter reports on the evaluation three different leaf wetness sensors, under laboratory conditions, to compare their performance.

11.2 Material and Methods

11.2.1 Standard measurement of leaf wetness performance

Each leaf wetness sensor was carefully calibrated by spraying with a mist of distilled water while attached to a sensitive balance to measure the actual wetness (g water cm^{-2} surface area, Fig 11.2). This process allowed for meaningful comparison of performance between difference sensor types. The Model T leaf wetness sensor (Western Electronic Design) was used as the standard for this work. The sensor is the standard type with a gold-plated electrical grid mounted on a plastic circuit board (Fig 11.3). The sensor was tested at a range of temperatures from 4–24°C. The two other leaf wetness sensors tested were the EnvironData leaf wetness sensor (Fig 11.4) and the Monitor leaf wetness sensor, provided courtesy of Monitor Sensors (Fig 11.5).

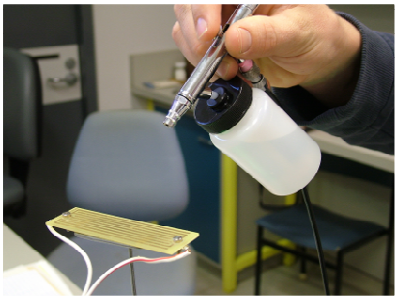

Fig 11.2 Spraying a leaf wetness sensor in the laboratory.

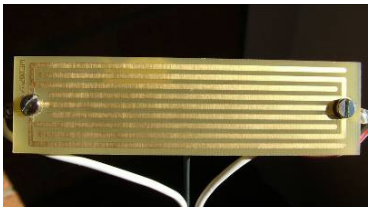

Fig 11.3 Model T leaf wetness sensor.

Fig 11.4 The EnvironData leaf wetness sensor.

Fig 11.5 The Monitor Sensors leaf wetness sensor.

11.2.2 Vapour Pressure Deficit (VPD) as a measure of leaf wetness

A calibrated (Model-T) leaf wetness sensor was used in experiments where leaf wetness (dew deposition), temperature and relative humidity (RH) were measured in a dew chamber under still and moving air flow (Fig 11.6). The chamber was then placed in a refrigerator to allow dew to form on the leaf wetness sensor as it would under field conditions at night. The temperature and relative humidity measurements were used to calculate VPD. VPD was calculated using the equation: $VPD = vp_{sat} - vp_{air}$.

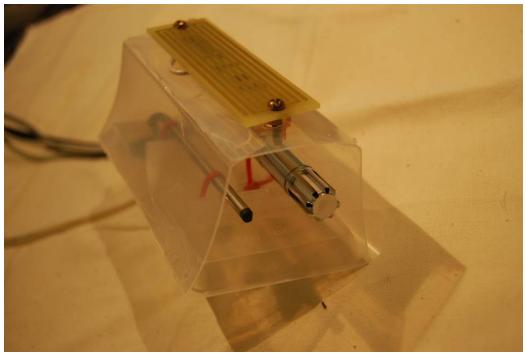

Fig 11.6 Sensor frame with leaf wetness sensor mounted top most, RH sensor (right) and temperature sensor (left).

11.2.3 Fuzzy Logic model

This model predicts leaf wetness based on temperature, RH and wind speed. Temperature and RH are measured in side a Stevenson screen (Fig 11.7), while wind speed is measured by a wind speed anemometer (Fig 11.8). Two weather stations were set up in S.E. Qld at a research site to collect data over the summer period (temperature, humidity, leaf wetness, rainfall, wind speed). The data from the weather stations was used to compare actual leaf wetness measured in the field against the predictive models found in the literature.

Fig 11.7 Stevenson screen containing temperature and RH sensor.

Fig 11.8 Wind speed anemometer.

11.3 Results

11.3.1 Standard measurement of leaf wetness performance

Model T leaf wetness sensor

The calibration curve produced was reliable under laboratory conditions (Fig 11.9). Leaf wetness on the bottom axis represents the units of wetness (electrical conductivity) as measured by the weather station. The sensor was tested at a range of temperatures from 4–24^{o}C with little variation in performance showing that temperature stability was satisfactory.

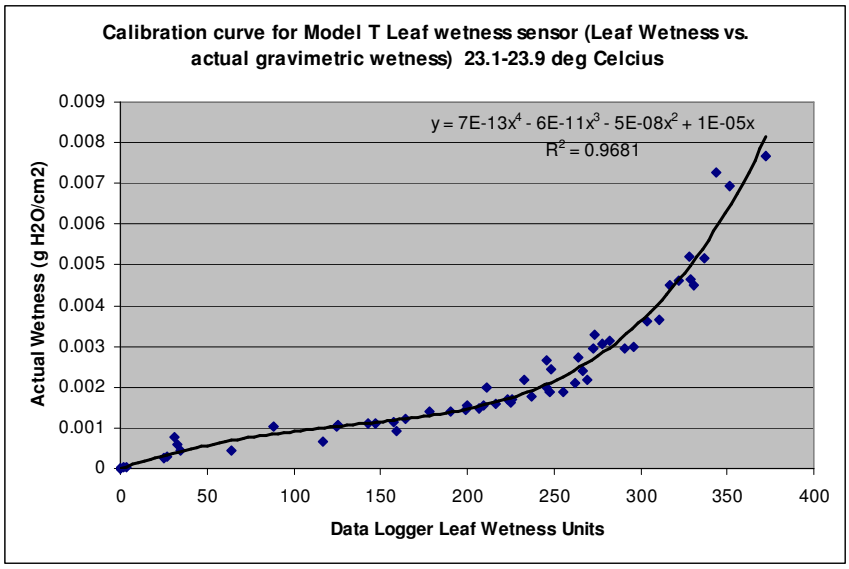

Fig 11.9 Calibration curve for the Model T leaf wetness sensor.

Monitor Sensors leaf wetness sensor

Monitor Sensors leaf wetness sensor provided a reliable calibration curve operating in a similar range to the Model-T sensor (Fig 11.10).

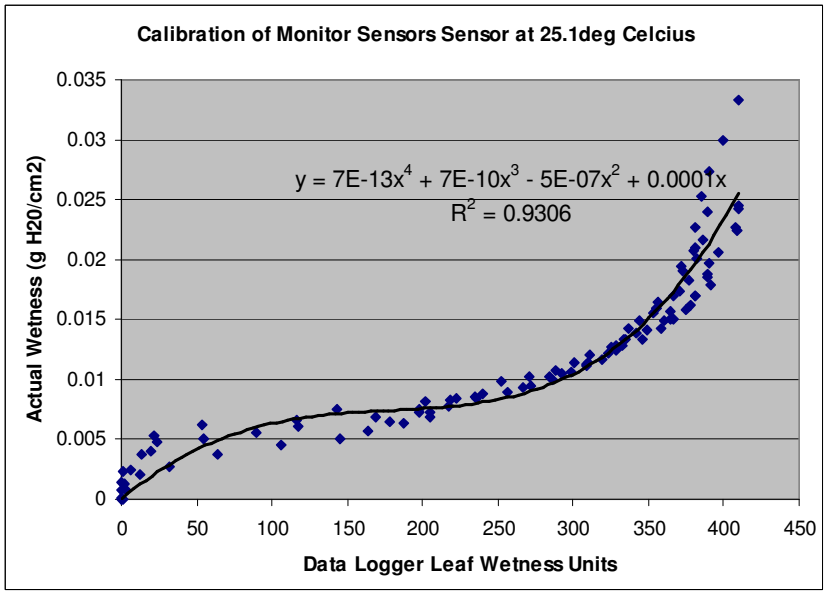

Fig 11.10 Calibration curve for the Monitor Sensors leaf wetness sensor.

EnvironData leaf wetness sensor

This type of unit showed slightly more variation in performance over different test runs. However it was found to be equivalent to the other units tested (Fig 11.11).

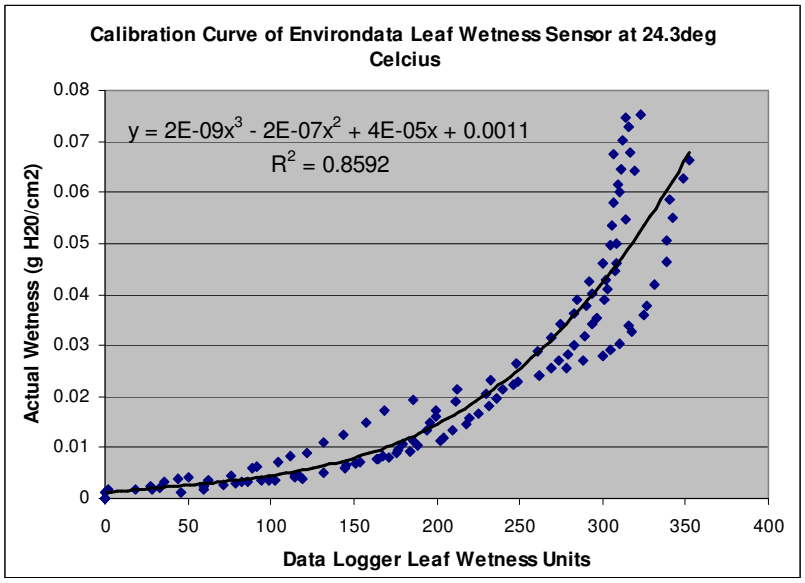

Fig 11.11 Calibration curve for the Environdata leaf wetness sensor.

11.3.2 Vapour Pressure Deficit (VPD) as a measure of leaf wetness

These experiments showed that under still air conditions, VPD provided an excellent estimate of leaf wetness with a correlation of R^2=0.903 (Fig 11.12). The experiments were then repeated using a miniature fan to move the air within the test chamber. Under moving air conditions VPD did not correlate with leaf wetness measured by a leaf wetness sensor as dew does not form.

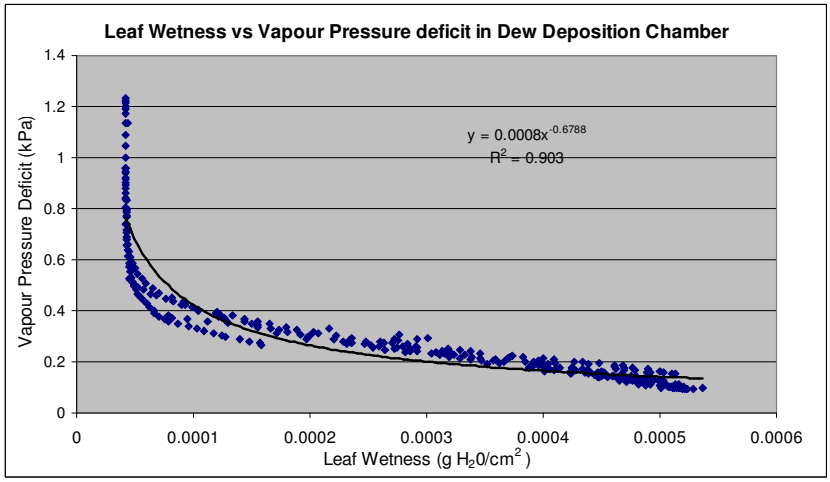

Fig 11.12 Leaf wetness as estimated by VPD.

11.3.3 Fuzzy Logic Model

The Fuzzy Logic Model was evaluated under field conditions by comparing it with actual leaf wetness data collected from a leaf wetness sensor. The Fuzzy Logic model agreed with actual leaf wetness 75% of the time.

11.4 Discussion

Under laboratory conditions, all leaf wetness units operated within similar tolerance levels. Repeated testing of the EnvironData leaf wetness sensor showed that over time, its performance began to change with the calibration curve shifting. This problem has been previously documented by other users of this type of sensor. Although VPD provides a useful way of estimating leaf wetness under still air conditions such as a greenhouse, it is not accurate under the moving air conditions which exist in a field grown crop. The Fuzzy Logic model agreed with actual leaf wetness 75% of the time, but Kim *et al.* 2004 found a 96% similarity. The Fuzzy Logic model showed great potential and further collaboration in underway with the authors.

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Chapter 12

Review of weather stations suitable for monitoring weather conditions relevant to prediction of white blister, powdery mildew and downy mildew

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Completed November 2008

Summary

A range of wireless-enabled weather station systems have been reviewed and costed on the basis of a minimal configuration (MWS - temperature, humidity and leaf wetness) and a conventional weather station with a full set of weather sensors (FWS – temp, humidity, leaf wetness, wind speed and direction, rainfall and solar radiation). Software to manage the stations and weather data, and the ability or otherwise to support IPM disease models has also been noted. Summaries of the features of each system are in the body of the report, but are summarised in the following table.

Minimal and full weather stations compared by capability and cost.

System Name	Approx Cost[1]	Manufacturer	Power	Multihop Capable	Server	Modelling Capability	Temp/ Humidity/ Leaf Wetness	Windspeed & Direction/ Rainfall/ Solar Radiation
Davis MWS Davis FWS	$1,444 $2,403	Davis Instruments	Solar	Yes	PC	No	Yes Yes	No Yes[2]
eKo MWS eKo FWS	$4,304 $5,380	Crossbow	Solar	Yes	Dedicated	Yes [3]	Yes Yes	No Yes
WatchDog MWS WatchDog FWS	$6,832 $8,179	Spectrum Technologies	Battery[4]	No	PC	Available extra cost	Yes Yes	No Yes
WeatherMate MWS WeatherMaster FWS	$6,818 $12,630	Environdata	Solar	Yes	PC	No	Yes Yes	No Yes
Adcon MWS Adcon FWS	$8,300 $15,090	Adcon	Solar	Yes	Dedicated	Yes	Yes Yes	No Yes
Custom MWS Custom FWS	$905 $1,518	Custom	Battery [4]	Yes	PC	No	Yes Yes	No Yes

It is very difficult to compare weather station systems, principally because the cost-effectiveness will vary widely with the nature of the monitoring task. The underlying capability of each system varies widely in terms of the sensors that can be attached, the local and Internet communications options and the storage and manipulation of the data. A system best suited for a single station locally monitoring temperature, humidity and leaf wetness for a single disease will be quite different from one remotely monitoring a full set of weather data, at several sites for a range of disease and production issues. Suitability for use in IPM disease monitoring would require close analysis of the total systems, including software and evaluation of the benefit provided by each system compared to its cost.

[1] Costs are $A, including GST, current as of Dec. 08
[2] Also includes a UV Radiation sensor that adds to cost, but system cannot be purchased without the UV sensor.
[3] Model data can be entered by user. Some models may be outside capability of user programming.
[4] Lasts at least a year

12.1 BACKGROUND

Integrated pest management (IPM) of field grown vegetable crops has the potential to significantly improve the economic and environmental efficiencies of Australian production systems (HAL VG06092). An important aspect of IPM is the use of models to predict the risk of diseases such as downy mildew, powdery mildew and white blister (DM/PM/WB). Better prediction of disease leads to more efficient use of pesticides/fungicides (fewer applications) reducing material and labour costs and reducing the chemical load on the environment and producer.

The disease models are underpinned by measurements of primary weather factors such as temperature, humidity and leaf wetness. These are measured directly by some form of sensor. These readings, in turn are combined with the timing and duration of measurements; combined with each other; and processed by one of a number of disease prediction models to yield an estimate of the risk of disease.

Improvements in disease prediction modelling can therefore contribute directly to IPM. Several significant areas of improvement are under examination.

1) Improving the measurement of weather factors
    a. better sensors
    b. more sensors
    c. more intensive sensing
    d. more convenient sensing
2) Improving disease modelling
    a. improved handling of sensing data
    b. improving specificity for crops and location
    c. modifying current models
3) Improving industry adoption
    a. Increased accessible to non-specialist users
    b. improved cost effectiveness
    c. more support for communities of practice

This review has its impact predominantly in the area of the improvement of measurement of weather factors by assessing the systems available for a range of measurements. As an important consideration is also the cost as this also has an impact on improving industry adoption

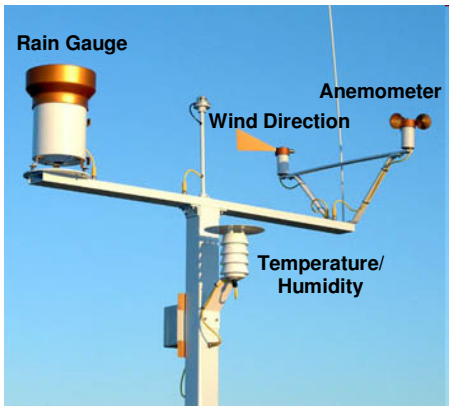
12.2 AIMS

This review seeks to survey the hardware, software and systems that are currently available for their suitability as components of a monitoring system for powdery mildew, downy mildew and white blister.

12.3 INTRODUCTION

The generic name for monitoring systems under review is “weather stations”. Technically, such systems are composed of a broad range of sensors, but typically as a minimum they consist of temperature, humidity, wind speed and direction and rainfall (Fig. 12.1). In the context of horticultural disease monitoring, such a weather station must also include sensors for leaf wetness.

However, in the project commissioning this study – that of providing benchmarking data for modelling disease risk for downy mildew, powdery mildew and white blister (DM/PM/WB) – the "weather station" may only need to provide sensor data on leaf wetness and temperature (Fig. 12.2). This is an important consideration in the scope of this review, as the cheapest, most reliable system will be the one with the fewest components, and the least number of sensors.



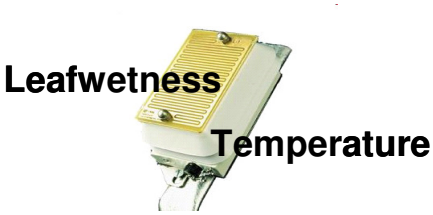


Fig 12.1 "Traditional" Weather Station. Fig 12.2 "Minimal" Weather Station.

We have also noted that current research indicates that leaf wetness is the only sensor that contributes to a reliable prediction of DM/PM/WB disease risk at the local plant level. Temperature and humidity may be a part of models, but can be measured at a more local level. Hence in systems that utilise intensive measurement to improve disease prediction, the important factor is to distribute leaf wetness sensors at the level of plant microclimate, while temperature and humidity may be measured at the plot level.

12.4 WIRELESS WEATHER STATIONS

Virtually all modern weather stations allow some form of wireless connection to the point where the information is analysed. Only wireless-capable systems are considered in this report. Systems that are only capable of logging all data at the station, or that require cabled connections for transmission of the data (including phone modems) are not suited for widespread commercial deployment.

Systems covered in this report are capable of interfacing to leaf wetness, temperature and humidity sensors described above, or have one or more of the sensors integrated into the system. The systems need to have either the capability for unlimited range, or to be capable of multiple hops to give a range of at least one kilometre.

Wireless technologies

The focal part of the weather station is the component to which the sensors are attached and that takes the readings. These measurements may be either stored and used on the node, or, more relevant to the horticultural applications that are under considerations here, to forward these measurements to a central location. At this central location – often denoted as the "server", the results will be collected, stored and analysed.

Two major technologies can be used to achieve this wireless connection, low power, short range radios or a connection using the telecommunications (mobile phone) network. A third technology that is available in some systems, that utilises ultra high frequency (UHF) wireless, will not be covered in detail. It requires special licences, has limited capacity for data and consumes more power. However, such systems may be required where long ranges are needed, and the mobile phone network is unavailable (Table 12.1).

Low Power Wireless

This uses relatively short range, low power wireless signals that are relayed to the central point or "base station" which has a comparable radio receiver that collects the signal. The signal may travel directly (single-hop) or through one or more relay stations (multi-hop) to the base station, depending on the nature of the system. Hence, there is a direct wireless connection from the sensor to the base station. The base station may be either connected directly to or be part of the "server" used to record and analyse the results.

GSM/GPRS/3G

This uses the phone or telecommunications network. In this instance the sensor node has the functional capability of a mobile phone, and makes a connection to the mobile phone network when transferring the data. Established protocols are then used to route the data message to the central point either directly through the phone network, or through the Internet.

These two technologies have various strengths and weaknesses.

Features of Wireless Types

Range

Low power wireless has limited range. Typical single hop distances range up to 500 metres. The range can be extended by better antennas, up to around 2 km. Many systems can be configured to allow multiple hops through repeaters to increase this distance, but practical limits are of the order of less than 5 km unless high power UHF radio systems are used. GSM/GPRS/3G systems have effectively unlimited range, provided the node can connect to the telecommunications network.

Deployment

Low power wireless systems can be deployed anywhere. Generally line of site between nodes is required, and some effort may be required to select the correct placement of nodes and possibly install intermediate relay units. GSM/GPRS/3G is unaffected by terrain or location relative to the destination of the data (which may be thousands of km away), but will only work where there is coverage by one of the telecommunications suppliers. This may be problematic for a particular locality, and even within a deployment, there may be spots with coverage, and some without.

Costs

Low power wireless devices may be slightly cheaper than GSM/GPRS/3G, and the 3G versions are currently significantly more expensive than GSM/GPRS systems. There are no ongoing costs or data charges for local wireless devices, but GSM/GPRS/3G devices incur ongoing network access costs, and often a charge for the amount of data transferred.

Power

Local wireless systems tend to use less power and are more likely to run for prolonged periods on batteries alone. GSM/GPRS/3G systems tend to require larger batteries, more frequent battery maintenance and to have supplementary power provided by solar systems.

Flexibility of Gateway

Most wireless nodes transfer information on specific radio frequencies, using proprietary signal and data formats and require a matched base station to receive the signal. Hence wireless nodes and base stations are almost always available only as part of a system. The link from GSM/GPRS/3G modems to the network uses a generic wireless protocol, and the phone network and Internet use standardised communications format. This allows for the use of a range of gateway hardware and software that can work with different types of modems.

System Configuration

Local wireless systems tend to be always on, and transfer data to the central point virtually as it is read. GSM/GPRS/3G systems may collect and store data locally at the node, and only upload the data at intervals. This can mean that the telephony systems are more complex, in order to deal with scheduling and increased latency in receiving the data.

Table 12.1 Comparison of Low Power Wireless and GSM/GPRS/3G Wireless Technologies

Feature	Local Wireless	GSM/GPRS/3G
Range	0.5 to 5 km	unlimited or None
Deployment	anywhere	depends on coverage
	- requires line of sight	- does not require line of sight
Costs	low running costs	high running costs
Power	low power	higher power
Gateway Flexibility	specific gateway	generic gateway
Configuration	low complexity	higher complexity

Weather monitoring system architecture

The components and linkages that comprise the disease-risk monitoring system defines the "system architecture". At a detailed level, there are a number of different architectures that could be used to build the monitoring system, and this can have an influence on the components that need to be integrated. For example, disease prediction could occur at the level of the sensor, or a gateway local to the farm, or at some distant distributed database server.

The decisions that govern which design to be used are beyond the scope of this report. The principal focus will be on architecture implemented at the local level, although where systems are capable of operating remotely using Internet services will be noted.

Components of System

- *Weather sensors* – collect the weather measurements in a form suitable for transmission.
- *Wireless node* – physically part of, or attached to the weather sensors. This carries the capability of transmitting the data to a point where it can be stored or analysed. We can identify three general types of wireless nodes.
- *Weather Station* – is composed of the Weather Sensors, Wireless node and any power supply devices.
- *Wireless repeaters* – relay the wireless signal from the weather station to its destination where the single hop range of the station is not sufficient to read the base station. Used predominantly with low-power networks.
- *Base Station* – this is the wireless device that receives the signal from the weather stations and converts the radio message into a digital form recognisable by computers.

- *Gateway* – this item captures and stores the data from the weather station. Gateways in a local architecture are generally a PC running some specialised database software. These local gateways may also be connected to the Internet, allowing remote analysis of their data
- *Internet Server* – where data from the weather stations is transmitted over the Internet, a specialised computer running database software will collect and store the data. This server is generally freely accessible across the Internet, to any other computer.
- *Local and Remote Analysis* – Traditionally weather data is gathered at a particular farm, and analysed locally on that farm, generally by a PC running specialised software. Increasingly, the data is being made available via the Internet allowing the analysis to be conducted from an arbitrary location. It may even be more efficient to conduct local analysis, with data that has been sent to the Internet.

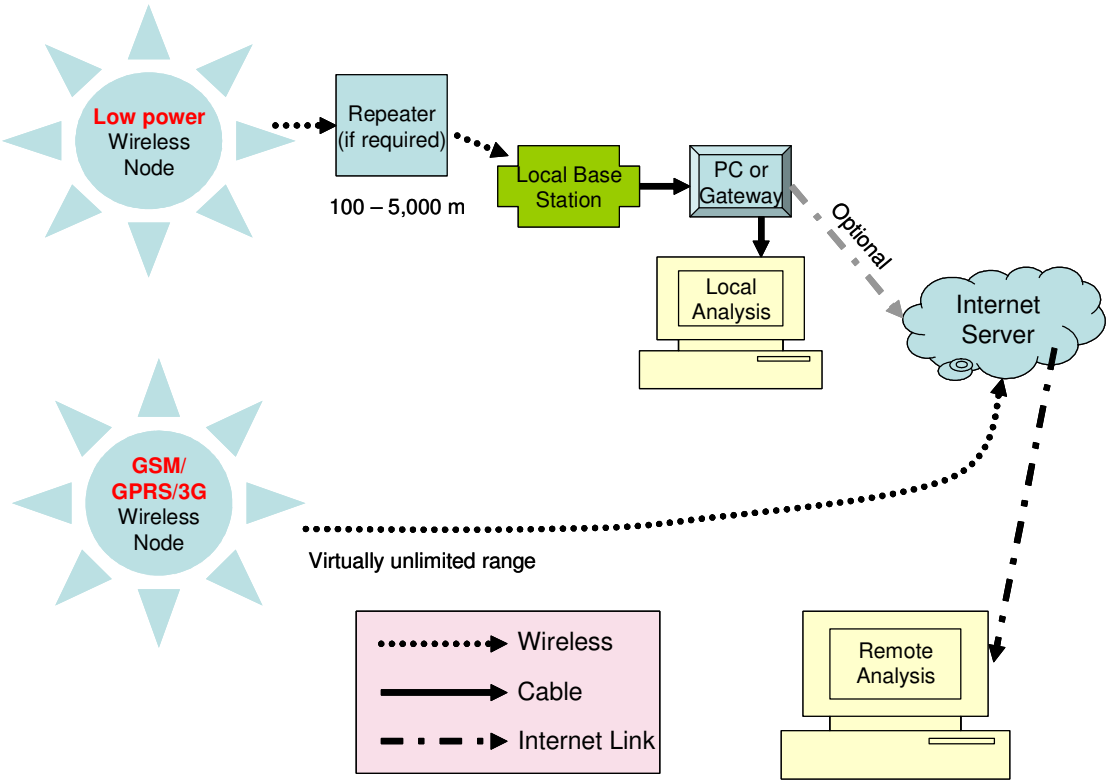


Fig 12.3. Wireless System Architecture options. a) Low Power – Local or Internet; b) High Power – Local or Internet; c) GSM/GPRS/3G – Internet.

12.5 REVIEW OF WEATHER MONITORING SYSTEMS AND COMPONENTS

A number of manufacturers supply components that can be combined to form a wireless weather system. These systems are composed of sensors, attached to a sensor node. The node has wireless capability and this connects to a gateway server where software to store and analyse the data operates.

Davis Instruments

Davis has been supplying weather station systems for over ten years, largely to the hobbyist and research community. A number of companies supply and support Davis products in Australia.

Davis 6345 Leaf Wetness and Soil Moisture station.

Manufacturer: Davis
Type: Local Wireless Node
Reference:
http://www.davisnet.com/weather/products/weather_product.asp?pnum=06345
Approximate base cost: $A324

Minimum Weather Station (MWS)

This Davis 6345 wireless leaf wetness node ($324) can support Davis leaf wetness, temperature and soil moisture sensors. The minimum configuration for this unit would consist of the 6345 Wireless node, leaf wetness sensor ($154) and temperature sensor ($66) for a total cost of $544. A Davis 6382 Wireless Temperature and Humidity station ($350) must be added if humidity sensing is required. A minimum weather station as specified would therefore require two physical stations, one bearing at least one LWS ($478), the other, the Temperature and Humidity station ($350) for a total cost of $828.

Full Weather Station (FWS)

Davis also supply full wireless weather stations. The basic component is the wireless integrated sensor suite (Wireless ISS Plus, Davis 6327 $1,309) which measures temperature, humidity, wind speed and direction, rainfall and solar and UV radiation. This system can be supplied without both of the radiation sensors (Wireless ISS, Davis 6322, for $660). The solar radiation sensor is principally required for evapotranspiration calculations, the UVsensor is not used generally. The modules are wireless enabled and solar powered. It is not possible, however to monitor leaf wetness with this system, and requires that Leaf Wetness Station and sensor ($478) to be co-located with the ISS Plus.

Wireless Connectivity

Both the MWS and FWS link wirelessly to a Davis 6316 Weather Envoy 6316 ($352) as the base station/gateway.

The range of the wireless connection is 300 m maximum line of sight (100-150 m typically), but can be extended using a Davis 7627 Wireless Repeater module ($363) by up to a further 300 m for each repeater that is added. The Davis 7654 module ($440) and Antenna ($440) can extend the range by a further 3 km for each repeater. Antenna options are also available to increase the range.

Data Storage and Analysis

The wireless base station (Envoy) distributes the data to either a PC using the Weather Link product ($264) or to the Internet using an existing broadband connection with Weather Link IP ($418). These products include the data management and weather station control software. There are no Davis-supplied software add-ons for disease modelling although the Davis 6511 Agricultural/Turf management module ($155) is available that provides a report on Leaf Wetness hours in addition to the standard Weather Link reports.

Cost of Wireless Weather Station, Gateway and Software

MWS: Solar Power/Temp/Humidity/Leaf Wetness - $1,444

FWS: Solar Power/Temp/Humidity/Wind Speed & Direction/Rainfall/Solar Radiation/Leaf Wetness - $2,403

Features:

- Well established weather station supply company with tried and tested hardware and software
- Large community of users, good user community support and some third-party developers
- Proprietary leaf-wetness and temperature sensors linked to wireless nodes provides simple configuration
- Options for collecting data locally and remotely
- Relatively low cost hardware

Limitations:

- Maximum of two leaf wetness sensors per system
- Wireless range limited to approximately 120 m without additional repeaters
- Repeaters are specialised nodes, different to sensing nodes
- Third party software required for disease modelling
- Requires separate drivers to use data collected by the weather sensors

Comments

The Davis weather hardware is relatively cheap, but lacks in some flexibility, which can result in higher cost and complexity. In particular, the basic temperature and leaf wetness station (ie no humidity) with simple wireless connections to an existing broadband connection would only cost $1,160, but to add sensing of humidity, requires another station module. Similarly, a standard weather station only costs $1,309 but if the weather station is required to measure leaf wetness, it cannot be configured by just adding a leaf wetness sensor - a full leaf wetness and temperature station must be added as a separate unit for a total cost of $1,787. The other complication is that the software is unable to handle more than one leaf wetness station, so deployments of multiple sensors on the one system, dispersed across plots or farms, cannot be conducted.

The Davis software packages for analysing leaf-wetness and temperature data will not be suitable for particular disease prediction models relevant to every crop, disease and locality. For these purposes, custom software will have to be developed. A key issue is to what extent the data collected by the WeatherLink software is available for third party modelling software. The existence of third party software that can access either Davis station weather hardware directly, or the WeatherLink database, and inbuilt protocols that provide for the automatic export of data from the WeatherLink software, strongly indicate that custom models could access weather data from a Davis system.

In summary, the hardware available from Davis would provide the ability to design a flexible and robust system to measure leaf wetness, temperature and other weather data and to relay this data to an analysis system that could provide a measurement of disease risk. The major limitation of this system is that it can only run as a single system, with only two leaf wetness sensors whose location would be limited by the length of the cable from the sensor node to the sensor.

Crossbow eKo

Crossbow Pty Ltd has been suppling low power wireless sensing technology since 2001. It is the world's largest supplier of such technology. In 2008 it released the ēKo system, a specific focus product line, evolved out of previous generic components, directed to monitoring agricultural systems. The Australian distributor is Davidson Measurements, a subsidiary of Biolab Pty Ltd.

Manufacturer: Crossbow
Type: Local Wireless Node
Reference: http://www.xbow.com/Eko/index.aspx
Approximate base cost: $1,287

Minimum Weather Station

All ēKo stations are currently based on a single type of solar powered wireless node (EN2100, $1,287) to which up to eight sensors can be attached. A Leaf Wetness sensor of the capacitance type (ES1301, $353) and a temperature and humidity sensor (ES1201, $220), would form a minimum weather station. Added to this would be a third party Stevenson Screen, eg from Davis (7714, $132). The total cost of this node would be $1,992.

Local Weather Station

At the time of this review, the sensor suite available from Crossbow, in addition to those above consisted of two types of soil moisture sensors, a soil temperature sensor and a solar radiation sensor. These are sensors produced by other sensor manufacturers, customised for simple installation onto the ēKo nodes. Wind speed and direction, and rainfall sensors are soon to be added to the product line. On the basis of costs for similar sensors, an approximate cost for such a node would be an extra $1,075, for a total of $3,070.

Wireless Connectivity

The ēKo system is based on low power wireless nodes that connect to a gateway by a self-organising and self-repairing mesh network. The maximum, single-hop wireless range is 500 m, lower when obstacles are present in the path between nodes. This range can be easily extended by addition of repeater nodes. This allows for the formation a multiple hop network, which is created without configuration. Nodes communicate with an ēKo base station (EB2110, $596).

Data Storage and Analysis

The ēKo base station is connected to a solid state, low power gateway/server (EG2110, $1,716) that runs a database to collect and store the data. The ēKoView software is supplied with the gateway and provides a web interface for managing the network and is accessed via a browser on a PC connected directly, or via the Internet. The software supports inbuilt models for powdery mildew (Conidial and Ascospore). The software allows the addition of further models, and provision for customised thresholds and alerts sent by email and SMS to mobile phone. The data collected and stored on the gateway is accessible to third party software.

Cost of Wireless Weather Station, Gateway and Software

MWS: Solar Power/Temp/Humidity/Leaf Wetness - $4,304
FWS: Temp/Humidity/Wind Speed & Direction/Rainfall/Solar/Leaf Wetness - $5,380[5]

Features

- Simple robust wireless network with multi-hop capability
- Easy deployment and management, with very little configuration

[5] Estimated as final pricing for Wind Speed and Direction and Rainfall sensors not yet available.

- Simple, robust hardware and software usable by non-specialists
- Moderate cost
- Management software is designed for use over the Internet

Limitations

- Only one type of wireless, not suited to very large area, long range or sparse deployments
- Have to use expensive sensor node as a repeater rather than a "stripped-down" node.

Comments

The Crossbow system has developed out of a technology known as mesh networking, where wireless nodes communicate with each other to form a spread out network that can traverse long distances and wide areas. This has its greatest impact on deployments of multiple sensors at multiple sites. It is competitive in the single node deployments as well, with the proviso that a fully featured node is required to act as a relay if required.

The system is easy to deploy, and in limited testing, robust. It makes use of proven wireless technology and sensors from established companies such as Davis and Decagon. It has a very flexible capacity for different sensors – on one type of node, and a customisable interface that includes disease models and alarms.

Spectrum Technologies WatchDog System

Spectrum Technologies manufacture and supply specialist agricultural monitoring systems. They have a large, global customer base and are well established in the market place. All their current weather monitoring systems can be enabled with wireless, however only one of the three wireless systems is available in Australia. The Australian distributor of Spectrum Technologies systems is John Morris Scientific.

Manufacturer: Spectrum Technologies
Type: Wireless Mini-Weather Station 2450
Reference:
www.specmeters.com/WatchDog_2000_Series_Mini_Stations/WatchDog_2000_Series_Mini_Stations.html
Approximate base cost: $1,875

Minimum Weather Station

Spectrum supply a range of mini-weather stations that can handle up to four sensors. The Mini 2450 station ($1,875) measures temperature and humidity and can be fitted with the leaf wetness sensor ($456) for a total cost of $2,331.

Local Weather Station

The 2000 series of full weather stations handle the full range of sensors. The WatchDog 2700 ($3,080) has the normal weather sensors, to which could be added the Quantum Light sensor ($142), and the leaf wetness sensor (as above) for a node cost of $3,678.

Wireless Connectivity

Wireless capability can be added to each of these weather station types. In the US, three types of modules can be purchased, similar to the Adcon system. One is short range (approx 0.3 km), one is longer range (up to 3 km) the third is a GSM/GPRS modem (range unlimited if within a mobile service zone), However, in Australia it is only possible to purchase the medium range system ($1,934). The system also requires a wireless base station ($1,647) that is connected to a PC gateway. Multi-hop/repeater capability is not available.

Data Storage and Analysis
The Spec 8 software to run the stations, store data and conduct basic analysis costs $920. The system can handle more than a single wireless weather station. The Specware software has a large number of disease model add-ons under the product name of GrapeAlert ($1,144). This appears to include a version of Tomcast.

Cost of Wireless Weather Station, Gateway and Software
MWS: Battery Power/Temp/Humidity/Leaf Wetness - $6,832
FWS: Temp/Humidity/Wind Speed & Direction/Rainfall/Solar Radiation/Leaf Wetness - $8,179

Features
- Flexible wireless options (only one available in Australia)
- Strong range of weather sensors
- Large range of disease models available for extra cost

Limitations
- Only a single wireless type, unable to multi-hop
- Downloads data only on manual command (unless Pro version of software purchased)
- Costly

Comments
The WatchDog systems are well established technology and potentially have a high level of flexibility. It is disappointing that the choices of communication technologies are limited in Australia, but new products are being released in the GSM/GPRS category that might be introduced in the future.

At present, the system works best in the single site situation as there is no built in Internet capability for remote monitoring. Purchasing the Pro version of the software (upgrade of $610) adds some automated download, and Internet upload capability but this is not in a format that allows direct monitoring by a web browser.

Environdata WeatherMaster System

Environdata is an Australian company that have been deploying weather stations for many years. They produce packaged weather stations, or customise stations for particular requirements.

Manufacturer: Environdata
Type: WeatherMate DL40
Reference:
www.environdata.com.au/LiteratureRetrieve.aspx?ID=22848&A=SearchResult&SearchID=434289&ObjectID=22848&ObjectType=6
Approximate base cost: $2,359

Minimum Weather Station
As the Environdata gear is highly customisable, a minimum station can be developed from the base WeatherMate data logger (DL40, $2,359), a temperature (TA10, $280), humidity (RH40 $742) and leaf wetness (LW10, $385), a total cost of $3,766.

Local Weather Station
The WeatherMaster series of stations bear the traditional weather sensors, and the WeatherMaster 2000 ($9,070) bears the standard suite of sensors, and can have the leaf wetness sensor ($385) added as an optional sensor for a total cost of $9,455.

Wireless Connectivity
Environdata have several options for wireless connectivity including short range UHF wireless and NextG modems. Slightly different packages are available for the WeatherMate (UHF-$2,057; NextG - $1,094) and WeatherMaster 2000 (UHF-$2,190; NextG - $1,644). The UHF wireless option also requires a base station ($995). These connections are designed for periodic downloads rather than continuous operation. There are options in the software to allow for scheduled downloading of weather data.

Data Storage and Analysis
Both the WeatherMaster and WeatherMate come with basic data storage and analysis software (EasiAccess). Internet connectivity is available with additional software that allows emailing of text files of the data ($825) and web presentation of the current readings ($572). The software allows for sophisticated graphing, and can be managed at a distance by service technicians, but does not provide for remote access to the data provided by a wireless weather station. There are no disease model add-ons or database server which will allow for development of custom models that can run on live data.

Cost of Wireless Weather Station, Gateway and Software
MWS: Solar Power/Temp/Humidity/Leaf Wetness - $6,818
FWS: Solar Power/Temp/Humidity/Wind Speed & Direction/Rainfall/Solar Radiation/Leaf Wetness - $12,630

Features
- These are rugged weatherstations, manufactured locally
- There are a number of flexible communication options
- The WeatherMaster series are compact, integrated units, easy to set up

Limitations
- Fairly expensive
- Shows a datalogger heritage and is cumbersome to use over the Internet
- No disease modelling software, and no defined interface to add on the capability using third-party software

Comments
Of the systems reviewed, this system most strongly shows that the basic design philosophy of the weather station as a data logger. Data is primarily stored on the weather station, and downloaded on command from a local PC. This has the advantage that collection of the data is not dependent on cable or wireless links to the data storage server. However, this also means that the primary weather data is only available to a single workstation. Only if it is downloaded and placed in another system is it available to distributed workstations across the Internet. Other systems store the weather station data directly to a database, and the analysis is more reflective of a client/server system. The latter is more readily adaptable to remote usage over the Internet, and to the addition of disease modelling and alarm clients that operate on the sensor data.

Adcon Telemetry
Adcon is a German company that has been providing weather station systems for research since 2003. Their systems are deployed widely in the Europe and the US and some deployments have been made in Australia and Adcon have a local office.

Manufacturer: Adcon
Type: A723 Radio Telemetry Unit
Reference: www.adcon.at/english/produkte_rtus_a723_Series4_en.html
Approximate base cost: $1,485

Minimum Weather Station
The basic disease monitoring system would utilise the Adcon leaf wetness sensors ($396) and the Adcon Temperature and humidity sensor (a Vaisala HMP 50 sensor and Stevenson Screen enclosure $1,360). As the sensor interfaces are generic, it may be possible to attach non-Adon leaf wetness and temperature sensors to provide a minimum cost installation. This would be connected to either a low power UHF or GSM/GPRS node ($1,485, $2,780 respectively). A UHF weather station node would cost $3,461.

Local Weather Station
Individual Adcon nodes can handle at least six sensors of the leaf wetness/Temp and humidity type. The addition of sensors for rainfall ($584), windspeed and direction ($1,185) and solar radiation ($1,554) increases the cost of the weather station to $7,231.

Wireless Connectivity
The Adcon system has excellent options for wireless coverage. Three types of wireless nodes are available:

1. Low power UHF with a range of up to one kilometre and multi-hop capability to extend this range ($1350).
2. High power UHF with a range of up to 20 km, multi-hop capability and with the capability to relay data from the low power nodes ($3,877).
3. GSM/GPRS nodes with unlimited range where telephony services are available ($3,058).

Each of these has essentially the same capabilities with respect to the sensors that can be attached. Solar power is an additional $200-$310.

Signals from these nodes are collected at a dedicated, proprietary gateway. This gateway can be configured differently depending on the type (UHF or GSM/GPRS or both) and the number of nodes that are being controlled. The minimal configuration of the A850 Telemetry Gateway connects to five UHF nodes ($3,000). More powerful configurations increase the number of nodes to be managed up to 100 and/or add the ability to connect to GSM/GPRS modems.

Data Storage and Analysis
The Adcon system is managed by addVantage Pro, a sophisticated client/server database management system built upon an Oracle database ($1,400 for five nodes). The software can be accessed directly or via a web browser and offers a high degree of customisability. It provides (at no further cost) for a number of pre-built disease models such as grape powdery and downy mildew, apple powdery mildew, lettuce downy mildew, DSV tomcast and DSV Wisdom tomcast. The software also has documented links to allow third-party developers to build models that utilise the data managed by the addVantage Pro software.

Cost of Wireless Weather Station, Gateway and Software
MWS: Solar Power/Temp/Humidity/Leaf Wetness - $8,300
FWS: Solar Power/Temp/Humidity/Wind Speed & Direction/Rainfall/Solar Radiation/Leaf Wetness - $15,090

Comments
The Adcon system is built around low power UHF radios with multi-hop networking capability. This allows for flexible deployments. Adcon provide a wide range of low power weather sensors designed to interface with their systems. However, the sensor interfaces are generic, and sensors from other manufacturers can be connected.

With the number of options available for connection, and types of sensors, the estimate of costs for a system may vary considerably. For example:

- Nature of the wireless nodes in use (low/medium power or GSM or mixture)
- Type and number of weather sensors
- Need for repeaters for long range
- Number of nodes under management

A simple one sensor node, one gateway system with one leaf wetness sensor, a temperature and humidity sensor and a wireless gateway and minimum software configuration would cost $7,346. One reason for the high cost is the "over engineering" of the gateway which is designed to handle multiple stations, and is underutilised by a single sensor. The Adcon system is much more economical when deployed on a large scale. For example, five systems measuring LW, Temp and Humidity, connected by GSM/GPRS would cost $5,260 per system if the gateway is shared.

The software also provides a wide range in capability. However, the very power, and high cost of the software means that its value is likely to be best utilised by a professional consultant or data bureau that provides a customised service to a community of users.

Features:

- Established wireless weather station company with moderate number of installations
- Range of proprietary and generic sensors can be connected to sensor nodes
- Flexible wireless communication options – short and long range UHF and GSM/GPRS
- All nodes multi-hop reducing complexity of deployment
- Powerful software incorporating customisable disease models, web access and capable of supporting multiple farm installations

Limitations

- High cost
- Complex software requiring specialised service to gain maximum value
- Licence and yearly costs for using long range UHF wireless
- Developing system – unproven weather sensors and capabilities of software

Custom Built

The systems described previously have all been based on equipment currently available at a retail level, by manufacturers of weather monitoring instruments. It may also be possible to construct a basic weather station from a mix of wireless and sensor modules that is cheaper at the component level.

It must be recognised that such systems do not fully reflect the "total cost of ownership". Costs of manufacture and distribution are not included, as is the cost of the support that is needed to set up and maintain these systems - normally provided by the manufacturer or distributor of fully commercial systems. For the purposes of illustration, we examine a system based on short range UHF hardware manufactured by Crossbow, custom-built interface and third party sensors.

IRIS OEM Module

Although Crossbow supplies a custom built weather station solution, it may be "oversized" for certain weather station applications. Crossbow have long manufactured a product line of wireless modules and sensor interfaces that can be integrated with third party sensors to create weather sensors.

IRIS 2.4 GHz OEM module
Manufacturer: Crossbow
Type: Local Wireless Node
Reference: www.xbow.com/Products/productdetails.aspx?sid=263
Approximate base cost: $U75

The starting point for such a system is the IRIS wireless mote OEM chip ($75 approx[6]). This chip essentially has all the electronics of a low power wireless mote in a single chip. This chip would be integrated into a custom designed circuit board to provide the power, antenna and interface connections ($100 approx[2]). Power would be provided by a battery pack of two D cells which will deliver at least a year of operation provided that it was coupled with low power sensors. Solar power could be an additional option. With allowance of $100 for antenna and housing, a battery powered wireless node, capable of carrying multiple sensors, without solar capability, would cost around $275.

Minimal Weather Station

This node could carry most generic sensors available from circuit board and chip manufacturers. A single chip temperature and humidity sensor, as used in several commercial sensors, is available from Sensirion for $35 and a leaf wetness sensor from Environment Information Technology for $15 (described in Appendix 1). These sensors are supported by electronics provided on the custom circuit board constructed to support the IRIS chip. The sensors must be mounted in a Stevenson screen, such as the one available from Davis ($132). In this configuration, the MWS could be constructed for approximately $325.

Local Weather Station

A full weather station would also include sensors for rainfall (Davis, $150), wind speed and direction (Davis, $210) and solar radiation (Davis, $253) sensors would produce a wireless station with a cost around $938.

Wireless Connectivity

The basic node has the low power UHF radio, with a maximum range of 500 m. Adding multiple stations and repeaters is straightforward and allows for multiple sensing points and extended range. Repeater stations would only require the minimal wireless node ($275). The base station could be constructed using commercial Crossbow technology, and, with housing, would cost a further $580. This base station would connect the weather station to a local PC. Other configurations, at higher cost could support direct connection to the Internet.

Data Storage and Analysis

Crossbow supply a free client/server application, MoteView, which runs on any PC. The software also allows for remote monitoring and management of the weather station across the Internet. The software has basic graphing capabilities, threshold alerts and email notification. It does not support disease models, but the client/server structure of the software is well suited to having custom applications running in parallel with the weather network and accessing the data for monitoring and prediction.

[6] Cost is dependent on number purchased, for large scale purchases, the cost is significantly lower.

Costs
MWS: Battery Power/Temp/Humidity/Leaf Wetness - $905
FWS: Battery Power/Temp/Humidity/Wind Speed & Direction/Rainfall/Solar Radiation/ Leaf Wetness - $1,518

Features
- Simple and low cost
- Scalable for multiple local stations and wide areas
- Supports monitoring and use over the Internet

Limitations
- Not commercially supported as a complete system
- Requires some development of electronics
- Low cost components may be lower quality
- Requires periodic replacement of batteries

Comments
The selection of batteries as the source of power runs counter to most designs reviewed above, but the cost and complexity of solar power is not really justifiable where the lifetime of the power supply is in the order of a year or more. Particularly in the vegetable industry where access to weather station nodes is relatively easy, there is no issue of remote, unattended deployments, the cost of occasional service to the unit is trivial. The use of batteries may also lead to less obtrusive deployments of the system as it is not necessary to mount the system in a prominent position to provide solar panels with direct sunlight.

Constructing what is virtually a new wireless sensor node, and weather station offers significant cost savings over commercial systems because of the ability to trim excess capability, utilise niche technology and avoid the costs of commercial production. Of course, a sustainable deployment requires either some form of commercialisation, or some organisation to subsidise the upfront costs of development and overhead costs associated with manufacture, distribution and support.

Other Suppliers

Measurement Engineering Australia

MEA 102 Automatic Weather Station
Manufacturer: MEA
Type: Local Weather Node
Reference: http://www.mea.com.au/files/brochures/B11.pdf
Approximate base cost: $10,439[7]

MEA produce local weather and soil monitoring systems. They have a comprehensive range of sensors and monitoring options. They do not provide a weather system that can be connected by local, low power wireless. Their local system are either based on downloading measurements captured by onboard dataloggers either directly to a laptop or by direct connection by cable to a nearby computer.

MEA do provide sophisticated wireless systems based on the telecommunications network, and these have been used in several regional networks of weather monitoring stations (eg http://myfarm.umag.org.au/station/index.htm). These systems are custom designed. MEA should certainly be considered as a supplier for such systems.

[7] Approximate additional cost for modem link and data transfer is $1,500-$2,000

The software that manages MEA systems does not natively provide for disease prediction models, although custom designed systems may be provided that provide such functions along with notification of events by means such as email and SMS.

Observant

Observant C2
Manufacturer: Observant
Type: Wireless Node
Reference: http://www.observant.com.au/ObservantHTML/Observant_C2_features.html
Approximate base cost: $ 2,722

Observant is an Australian company that has developed a wireless sensing product that uses a multi-hop UHF radio system to achieve 20-50 km single hop range. The solar powered nodes are extremely rugged, designed for outback use, and are capable of both sensing and control. The range of sensors that can be attached is very flexible and includes cameras, pump monitoring and control, rain gauge and RFID tag readers. It is not primarily designed for weather station monitoring – only rain measurement is natively supported by the system – but can be modified to read various weather sensors.

The system is also moderately expensive, a single node system with base station and software costing approximately $4,200 without interfaces and sensors.

This system would be attractive for remote locations where telecommunications coverage was poor or absent and long range connections were required.

12.6 SUMMARY OF SYSTEMS REVIEWED

Minimal and full weather stations compared by capability and cost.

System Name	Approx Cost[1]	Manufacturer	Power	Multihop Capable	Server[2]	Modelling Capability	Temp/ Humidity/ Leaf Wetness	Windspeed & Direction/ Rainfall/ Solar Radiation
Davis MWS Davis FWS	$1,444 $2,403	Davis Instruments	Solar	Yes	PC	No	Yes Yes	No Yes[3]
eKo MWS eKo FWS	$4,304 $5,380	Crossbow	Solar	Yes	Dedicated	Yes [4]	Yes Yes	No Yes
WatchDog MWS WatchDog FWS	$6,832 $8,179	Spectrum Technologies	Battery[5]	No	PC	Available-extra cost	Yes Yes	No Yes
WeatherMate MWS WeatherMaster FWS	$6,818 $12,630	Environdata	Solar	Yes	PC	No	Yes Yes	No Yes
Adcon MWS Adcon FWS	$8,300 $15,090	Adcon	Solar	Yes	Dedicated	Yes	Yes Yes	No Yes
Custom MWS Custom FWS	$905 $1,518	Custom	Battery [4]	Yes	PC	No	Yes Yes	No Yes

[1] Costs are $A, including GST, current as of Dec. 08
[2] Dedicated servers are designed for connection to another computer or the Internet for monitoring and analysis. Where the server is a PC, this has not been costed as part of the system
[3] Also includes a UV Radiation sensor that adds to cost, but system cannot be purchased without the UV sensor.
[4] Model data can be entered by user. Some models may be outside capability of user programming.
[5] Lasts at least a year

Issues

Costs

The prospect of deploying a system which costs less than $1,000 – particularly when the infrastructure costs (relays, gateways, software, data server) are included – on at a single site is remote. It is possible to design such system, for limited sensor coverage, but the cost of a commercial version would still likely be over this base cost.

A possible scenario is to provide an unobtrusive weather station of restricted functionality, such as one measuring temperature, humidity and leaf wetness and perhaps soil moisture which has a sufficiently large market, and which provides the end user with access to management tools that provide the value to justify the cost. That such a system could be designed is certain, but ultimately requires a commercial partner to support the device, and probably an Internet-based service to support the management of data and provision of the decision support tools such as monitoring and disease prediction. This paradigm will be discussed more fully later.

The vegetable industry is one possible group where such a system may have the combination of ability to use a restricted sensor suite, a requirement for detailed monitoring and the ability to manage intensively and finally, a large number of potential end users.

System Comparison

It is very difficult to compare weather station systems, principally because the cost-effectiveness will vary widely with the nature of the monitoring task. A system best suited for a single station locally monitoring temperature, humidity and leaf wetness for a single disease will be quite different from one remotely monitoring a full set of weather data, at several sites for a range of disease and production issues.

The comparisons are further complicated by the variability in the provision of weather data to disease prediction models, and management tools based on those models. This is despite the fact that the underlying data reported by systems is largely equivalent.

Internet Integration

While management is confined to systems operating locally, this may be of lesser importance. However, there may be considerable gains to be made by collecting regional data, or by providing systems that are agnostic to the weather station, and hence allow consistency and cost sharing across a number of installations.

An attractive option is to collect the data in a standard form, and then to collect this in a central location, using the Internet as the communication conduit. This standardised data can be combined with other regional data, stored in a consistent format – independent of the type of weather station – and then standard models can be applied to the farm, local or regional area to allow for management decisions to be made.

There are at least two further benefits to this system. As the data is centralised on the Internet, it is possible to design remote access software to allow management from any location, not just on a PC local to the weather station. The centralisation of the data also provides a resource for other users and researchers to design additional management and research tools to make use of combined data, which would not be possible with isolated local systems.

Recognising this, many manufacturers now support such Internet-based services, or their systems can be integrated into customised central data stores. What is not available is the ability to integrate information from different systems.

It should be a priority to investigate the feasibility of establishing such a service architecture – to capture data streams from different sensing systems and provide query and management tools that run centrally on this aggregated information. This system would be designed to function, using web clients on local computers, but provide management for any location for which data is being collected.

12.7 APPENDIX 1: WEATHER SENSORS

Leaf Wetness

Introduction

Leaf wetness sensors are designed to measure the extent to which the surface of a leaf is covered by moisture, generally water, but it can also be frost. There are a number of practical aspects that influence how leaf wetness is measured, its accuracy, precision and cost. There are a number of different types of sensors available, but only the conductive grid type of sensors are practical in agricultural applications. Within this category, there are some important factors that influence the utility of the leaf wetness sensor.

Nature of the sensor

Two types of conductive grid sensors are in practical use. Both use a grid of conductive material on a rectangular or leaf shaped matrix.

One type measures a decrease in *resistance* – generally reflected in an increased voltage signal at the sensor interface. The conductive material is in two sets of interdigitated strips, each one attached to a different polarity. In the dry state, the resistance across the sensing terminals is very large as there is no connection between the conductors. When moisture is applied, the small droplets bridge the conductors and reduce the resistance.

The other measures an increase in *capacitance* – this is affected by the dielectric properties of the sensor. Air has a low dielectric constant (1) while frost and water have much higher dielectric constants (5 and 80 respectively). These sensors do not require direct contact between the moisture and the conductive grid.

In the context of DM/PM/WB disease-risk monitoring, there is no established preference for either of these types. Capacitive sensors may be more resistant to effects of corrosion as the moisture and any contaminants it contains, does not come into direct contact with the conductive grid.

The key issue is that the responses of the sensor types must be compared, and if they differ, the models used must be validated for each sensor type separately.

Sensor Coating

Both the response of the sensor to moisture, and the resistance to fouling are affected by the coating that covers the conductive material that forms the primary sensor. A number of studies have shown that the nature of the coating can have a very significant effect on the response and performance. Most sensors are supplied pre-coated and the performance of the coating has to be evaluated under operating conditions. Some sensors are supplied uncoated, and can be used in this fashion, but consideration would have to be given to applying a standard coating that may improve the characteristics of the sensor. A latex paint is often suggested as a coating, but would require characterisation for type, density, colour etc for the particular application.

Placement of the Sensor

The condensation, and persistence of moisture is affected by the shape, orientation and local environment on the sensor. LW sensors are generally rectangular in shape, but some (eg Decagon) are shaped like a leaf. Sensors are supplied with flexible mounting options. This also impacts on placement in the local environment, where factors such as height, placement in the canopy and exposure to contaminants (dust, sprays etc) can affect the response and performance of the sensor. There are no unambiguous general rules to govern placement. An implication of this variability is that more robust estimates of leaf wetness may be provided if multiple sensors are used, and an aggregated measurement be constructed from the individual measurements.

Commercial Leaf Wetness Sensors

Davis 6420 Leaf Wetness Sensor

Manufacturer: Davis Instruments
Type: Resistive, three wire half-bridge
Reference: www.davisnet.com/weather/products/weather_product.asp?pnum=06420
Approximate cost: $154

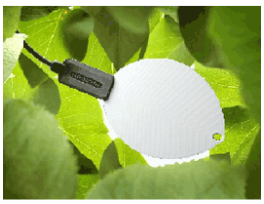
In a Davis Vantage system it provides measurements that range from 0 (completely dry) to 15 (saturated). Designed for use with the Davis DAV-6343 Leaf & Soil/Temp Station. Includes 40' (12 m) cable. Cable connections are available so this sensor can be used with other sensor nodes. This sensor requires and excitation voltage to produce a reading.

Campbell 237 Leaf Wetness Sensor

Manufacturer: Campbell Scientific
Type: Resistive, three wire half bridge
Reference: www.campbellsci.com/237-l
Approximate cost: $210

The 237 is shipped unpainted; the customer determines the appropriate paint to apply to the sensor's surface. Supplied with cable to connect to generic data loggers.

Decagon LWS Leaf Wetness Sensor



Manufacturer: Decagon Devices
Type: Dielectric, three wire half bridge
Reference: www.decagon.com/ag_research/environ/wetness.php
Approximate cost: $250

The Decagon LWS can detect small amounts of water or ice on the sensor surface for leaf wetness applications. Because the LWS measures the dielectric constant of the sensor's upper surface, it can detect the presence of water or ice anywhere on the sensor's surface. This sensor does not require painting or calibration of individual sensors. This sensor is designed to be used generic dataloggers.

A number of weather systems incorporate this sensor, notably the Crossbow eKo monitoring system has a customised version of this sensor incorporating a smart interface.

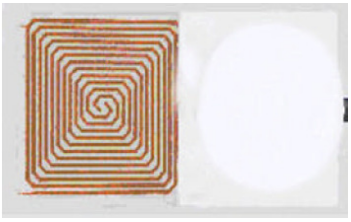
MEA 2040 Leaf Wetness Sensor

Manufacturer: Measurement Engineering Australia
Type: Dielectric
Reference: www.mea.com.au/products/mea2040-leaf-wetness-sensor/2/
Approximate cost: $165

The MEA2040 leaf wetness sensor uses a capacitive detection method, based on the alteration of the dielectric field by droplets of water. This sensor has a longer-lasting surface than conductive grids.

ICT LW2 Leaf Wetness Sensor

Manufacturer: ICT International
Type: Resistance
Reference: www.ictinternational.com.au/leafwetness.htm
Approximate cost



ICT International's LW2 leaf wetness sensor measures the resistive effects of moisture on the sensor. The circuit board incorporates the sensing element, but also a microcontroller which governs the sensing events, recording measurements, storing threshold values and providing control outputs that allow the sensor to be used "stand alone" in certain applications.

EME Systems LWET Leaf Wetness Sensor

Manufacturer: EME Systems
Type: Resistive
Reference: http://www.emesystems.com/lwet_dat.htm
Approximate cost: $US65

The LWET from EME Systems is a resistive sensor mounted on a fibreglass circuit board. This sensor is energised by an AC current, and the sensor responds to increases in moisture level by increasing the frequency of the output from an oscillator circuit integrated on the sensor. A voltage output option is also available from the same sensor. The sensor has grids on both sides and can be painted if required.

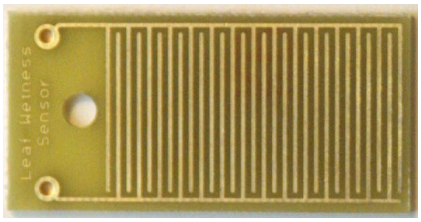
HOBO Leaf Wetness Sensor for HOBO Weather Station Data Logger

Manufacturer: Onset Computer Corporation
Type: Capacitive
Reference: www.onsetcomp.com/products/sensors/s-lwa-m003
Approximate cost: $US105

The leaf-wetness smart sensor for the HOBO Micro Station Data Logger or HOBO Weather Station is a capacitive grid type. A mounting bracket is included, so the sensor can be easily positioned to mimic the wet-dry characteristics of the plants being studied.

Hobby-Boards Leaf Wetness Sensor

Manufacturer:
Type: Resistive
Reference: www.hobby-boards.com/catalog/product_info.php?products_id=1544)
Approximate cost: $US2



This is a board designed for hobbyist use, and comes without wires, mounting or any coatings. It is however, fundamentally the same sensor as all the resistive sensors. A similarly uncomplicated sensor is available from Environment Information Technology (www.eitechnology.com.au/sensors.htm) for approximately $15

Adcon Telemetry Leaf Wetness Sensor

Manufacturer: Adcon Gmbh
Type: Resistive
Reference:
http://www.Adcon.at/english/produkte_sensoren_blattnaesse_en.html
Approximate cost: $360

The Adcon leaf wetness sensor works on the principle of electric conductivity. The sensor element is mounted on a flexible holder to ease installation. The sensor is Teflon-coated and maintained by periodic wiping to clean off residues.

Temperature and Humidity Sensors

Introduction

Temperature and humidity are usually measured by a single instrument, although these are two separate sensors combined. Temperature and humidity at the level of the farm is generally fairly uniform across plots. Hence measuring temperature and humidity at a single point is generally sufficient for disease models. This can be at the point where leaf wetness measurements are made, or at some central location.

Temperature and humidity sensors must be placed in a Stevenson screen and provided with adequate, representative airflow. Where this is not provided as part of the sensor, inexpensive screens are available from a number of weather station suppliers, and the Davis 7714 is representative:

Davis 7714 Radiation Shield

Manufacturer: Davis Instruments
Reference: www.davisinstruments.com.au/products/installation/7714_radiation_shield.html
Approximate cost: $143

For a minimally configured system, temperature sensors alone may be sufficient. Robust accurate sensors are available from a number of sources, the Davis 6470 is representative:

Davis 6470 Temperature Probe

Manufacturer: Davis Instruments
Reference:
www.davisinstruments.com.au/products/sensors/6470_stainless_steel_temp_probe.html
Approximate cost: $68

If a "bare-bones" system that is to be constructed out of components, a well-regarded temperature and humidity, single chip component is the Sensirion SHT75. This is the chip used by Crossbow in its eKo temperature and humidity sensor (see below).

Sensirion SHT 75 Temperature and humidity sensor

Manufacturer: Sensirion
Reference: www.sensirion.com/en/01_humidity_sensors/06_humidity_sensor_sht75.htm
Approximate cost: $US25

There is a very large range of commercial temperature and humidity sensors designed for weather stations use but only low power consumption designs are suitable for use in unattended weather stations. Low power temperature sensors are readily available, but low power humidity sensors are less common.

Combined Temperature/Humidity Sensors

Campbell CS215 Temperature and Humidity Sensor

Manufacturer: Campbell Scientific
Type: SHT 75
Reference: www.campbellsci.com/cs215
Approximate cost: Only available in USA at $350

Crossbow ES1201 Temperature and Humidity Sensor

Manufacturer: Crossbow Technology
Type: SHT 75
Reference: www.xbow.com/eko/eko_product2.aspx#sensors
Approximate cost: $220

Vaisala HMP50 Temp/Humidity Sensor

Manufacturer: Vaisala
Type: Proprietary
Reference: http://www.vaisala.com/instruments/products/hm-hmp50.html
Approximate cost: $350

This sensor is also marketed by Campbell Scientific as their HMP50-L sensor, and by Adcon in their Combisensor. These items include extra cables or Stevenson screens in addition to the sensor element.

12.8 APPENDIX 2 – AUSTRALIAN DISTRIBUTORS

Listed are some contacts for the reviewed systems, that act as Australian distributors. Other secondary outlets may also distribute all or part of these systems. In many instances more details, and a larger product line is available in the country of parent company or manufacturer, where they are located overseas.

Davis Instruments

Davis weather station items are available from a large number of distributors. The following is a sample selection. A Google search for "davis weather stations" for Australian pages will provide a comprehensive listing

Ecowatch:
http://www.davisinstruments.com.au/
Contact:
EcoWatch
Unit 5 / 17 Southfork Drive
Kilsyth Vic 3137
Telephone: (03) 9761 7040

Davis Net Shop:
http://shop.davisnet.com.au/index.php?main_page=index&zenid=09e957981d1825f4c2ffe77163c02849

Weather Downunder:
http://www.weatherdownunder.net.au/index.php?main_page=index&cPath=46_25

Crossbow Technology

Davidson Measurements
http://www.davidson.com.au/products/wireless/crossbow/
Contact:
Colm Kinsella
Sales Engineer
Biolab (Aust) Pty Ltd
Industrial Technologies Division
5 Caribbean Drive
Scoresby 3179, Victoria
Telephone: 1300 736 767

Spectrum Technologies

John Morris Scientific
http://www.johnmorris.com.au/ssl/store/zcust_shopdispcatproductlist.asp?id=16907&trail=2169&cat=Spectrum+Technologies
Contact:
Andre Wyzenbeek
John Morris Scientific Pty Ltd
61-63 Victoria Ave
Chatswood, NSW 2067
Australia
Telephone: 61 2 9417 8877

Environdata

Environdata
http://www.environdata.com.au/
Contact:
Sandra Wilson
Environdata Weather Stations Pty Ltd
42-44 Percy Street
Warwick Queensland
Telephone: +617 4661 4699

Adcon

Adcon Telemetry
http://adcon.at/index.htm
Contact:
Peter Toome
Managing Director
Adcon Telemetry Australia Pty Ltd
1/184 Prospect Rd
PROSPECT SA 5082
Telephone: 08 8342 5343

Measurement Engineering Australia

MEA
http://www.mea.com.au/products/weather-stations-index/2/
Contact:
Tanya Liddell
Marketing Manager
Measurement Engineering Australia Pty Ltd
41 Vine Street
MAGILL SA 5072
Telephone: 08 8332 9044

Observant

Observant
http://www.observant.com.au/ObservantHTML/index.html
Observant Pty Ltd
Level 1/106 Victoria Street
Fitzroy Victoria 3065
Telephone: 1300 224 688

Chapter 13

Technology transfer and recommendations

Summary

The results of this research were reported nationally through field days, workshops and industry publications. Project management through the Steering Committee and the Industry Advisory Committee ensured that the project remained focused on the task, lead to a better mutual understanding between the researchers and industry and enhanced the impact of project outputs within the industry. This chapter reports on the project's steering committees and the various methods used to deliver information to industry, such as meetings, field days, conferences, and articles in industry and technical publications. Recommendations for future research are listed at the end of the chapter.

13.1 Introduction

This project was conceived from the IPM Gap Analysis Project and was one of the projects commissioned under the IPM Plant Pathology Program. The project Steering Committees were an excellent means of ensuring research directions remained consistent with industry needs. The committees also enhanced grower involvement and accelerated industry uptake of R&D outputs. The opportunity to report research nationally at seminars in conjunction with reports from other commissioned projects in the IPM Plant Pathology Program ensured that scientists from the public and private sector worked together and evaluations involved a holistic approach to IPM.

The steering committee for this project consisted of vegetable growers and representatives from allied support businesses including crop advisers, nurserymen, seed suppliers, chemical manufacturers and chemical resellers. These groups provided an opportunity for researchers to present their work plans and results whilst the ensuing discussions gave everyone a chance to participate in the project. The group member's had diverse experience and their industry networks, both local and overseas, enhanced the project outcomes. This approach ensured an appreciation for the perspectives of the sector in relation to *Brassica*, lettuce and cucurbit production. The scientists involved were able to ensure the research was relevant for industry, whilst the industry representatives involved developed a greater understanding of the scientific rigor and quality assurance behind the research.

The steering committee model has been applied successfully to other vegetable research projects including 'A scoping study for race identification, source of epidemic and management of white blister on brassicas' VG02118, the 'Evaluation of a disease forecasting model to manage late blight (*Septoria*) in celery' VG04016, the 'Scoping study to investigate management of root-rot diseases in parsley' VG04025, 'Bunching Vegetables' VG01045, 'Onion White Rot' VG01096, and the Lettuce Aphid Advisory Group under 'Lettuce Best Practice' VG01038.

13.2 Steering committee members

Growing and marketing vegetables places high demands on growers and consequently, many are unable to participate in steering committees. Growers often receive advice from vegetable agronomists and other service providers and hence the involvement of these "information retailers" with growers and researchers in meetings discussing the resolution of disease

problems (e.g. white blister on brassicas), has been very successful because the meetings have provided benefits for everyone.

The individual members who have contributed to the success of the White Blister Industry Steering Committee are:

- Mark Milligan – Operations Manager, A&G Lamattina & Sons, Rosebud
- Peter Cochrane – Co-owner, PJ & J and vegetable grower, Devon Meadows
- Jo Kelly – Co-owner, Tullamore Gardens, Cranbourne
- Luis and Paul Gazzola – VGA President/Director and Marketing Manager, respectively, L&G Gazzola & Sons, Somerville
- Harry Velisha – Co-owner, Velisha Brothers, Werribee South
- Anthony Mason – Vegetable grower, Werribee South
- Crain Arnott – Vegetable grower, Clyde
- Rocky Lamattina – Vegetable grower, Clyde
- Karl Riedel – Vegetable crop agronomist, E. E. Muir & Sons, Cranbourne
- Brian Brewer – Vegetable crop agronomist, Elders, Packenham
- Stephen Moore – Vegetable agronomist, E.E. Muir & Sons, Werribee
- Jerome Thompson – Werribee South Farm Supplies, Werribee South
- Ian Willert and Matt Newland – Nursery Managers, Boomaroo Nurseries, Lara
- David McDonald – Technical Manager - Brassicas, South Pacific Seeds, Dandenong
- Dr Elizabeth Minchinton – Project leader, DPI, Knoxfield
- Desmond Auer - White Blister Project Officer, DPI, Knoxfield
- Joanna Petkowski – White Blister Project Officer, DPI, Knoxfield
- Slobodan Vujovic –Industry Development Officer East, VGA/DPI

13.3 Dissemination of information to industry

Adults acquire information in different ways such as through reading, talking and visual cues. Some forms of information distribution are more useful or accessible than others. There are many ways to distribute information to growers, such as through field days, industry publications, workshop meetings and steering committees. During this project we used a wide range of information delivery methods and took every opportunity to report to industry. The Appendix lists the steering committee meetings, field days, workshops, and industry and technical publications.

13.4 Recommendations

The major outcomes of this project were benchmarking the efficacy and economics of various management strategies: timing of irrigation, resistant cultivars, nutrients, weekly preventative fungicide sprays, systemic fungicide spray regimes, and the use of a limited selection of alternative chemicals and biologicals for the control of white blister on brassicas and downy mildew on lettuce. An in field detection kit for airborne spores of white blister was developed with a view to using it in conjunction with the disease predictive model. Additionally a presumptive disease predictive model for powdery mildew of cucurbits was developed. Benchmarking efficacy of the various treatments was undertaken in field trials conducted in Victoria, Tasmania and Queensland and in glasshouse trials conducted in South Australia. Benchmarking economic benefits of the various treatments was undertaken by a professional economist, Mr Lindsay Trapnell.

Recommendations from the project

White blister

- Under conditions of low disease pressure, the Brassica$_{spot}$™ model can be used to control white blister on broccoli but the model needs to be modified for use when disease pressure is high.
- Under conditions of high disease pressure, the most economical ways to control white blister are either: (i) a weekly spray of a copper based fungicide or a spray program consisting of a rotation of systemic fungicides and a copper based fungicide. An additional advantage of copper sprays is that they have a one day withholding period. The disadvantage of copper sprays is that: (i) a spray cannot be missed; (ii) sprays can be phytotoxic in cold weather; (iii) copper can build up in the soil and (iv) copper is detrimental to earth worms.
- Good control of white blister on the wrap leaves of Chinese cabbage can be achieved with a single application of a registered systemic fungicide 14 days before harvest. An additional spray 21 days before harvest may improve control.
- Of the three fungicide alternatives compared with weekly sprays and use of the disease predictive models for efficacy against white blister, none had a similar efficacy or the economic advantages of weekly copper sprays. Only one had a similar efficacy to the disease predictive model. Often the economics of the alternatives was prohibitive.

Downy mildew

- Use of the BREMCAST™ disease predictive model to time fungicide sprays provided better control of downy mildew on lettuce than a grower-timed reduced fungicide spray program. Efficacy against weekly spray programs was variable and largely depended on the timing and use of contact fungicides early and systemic fungicides later in the life of the crop.
- Of the three fungicide alternatives compared with the weekly sprays and use of the disease predictive models for efficacy against downy mildew, only Bion™ had some efficacy and favourable economics, but its performance was highly variable.

Powdery mildew

- The recently developed disease predictive model for powdery mildew on cucurbits showed promise. In the field, use of the model reduced fungicide spray programs by one application whilst maintaining yields. Further field and glasshouse trials to evaluate and improve the model are warranted.

Nutrients

- High rates of calcium nitrate should be applied to lettuce seedlings in preference to potassium or ammonium nitrate. The application of calcium nitrate (9.35 gm/L anhydrous and 13.46 g/L tetrahydrate) reduced the susceptibility of lettuce seedlings to downy mildew and anthracnose. However, substituting calcium applications (FoliCal Plus™) for some fungicide sprays in one field trial, did not improve downy mildew control.

Irrigation

- Broccoli plants should be irrigated in the morning, from 04:00 h. In comparison with evening irrigation, morning irrigation reduced leaf wetness periods and consequently incidence of white blister by 50% and increased profits by 5%.

Varieties

- Resistant varieties of broccoli should be grown. However, resistant varieties will still need to be sprayed. The use of resistant varieties reduced incidence of white blister disease by 90% and increased profits by 22%.

Areas of future research which would benefit the industry

White blister

- Improve the Brassica$_{spot}$™ model by reducing the spray threshold from a "Disease Index" of 1.0 to a "Disease Index" of 0.5 or 0.75 so that application of a spray is triggered before sporulation of lesions commences instead of at "maximum sporulation" when the Disease Index is 1. If this is successful, preventative fungicides could be used instead of systemic fungicides in the spray program.
- In the long term, produce and use an Australian disease predictive model for white blister. This will be more efficient because the cost of the Brassica$_{spot}$™ model has already become prohibitive due to circumstances in the UK. In Australia, consultants have also experienced difficulties in downloading the model from the UK website.
- Extensively evaluate the white blister spore detection kit under Australian conditions for use in conjunction with the Brassica$_{spot}$™ disease predictive model or as a stand alone decision support tool.
- Identify new cultivars of cauliflower, Brussels sprouts and especially cabbage that are less susceptible to white blister. This will prepare the industry for future introductions of exotic races of the blister pathogen (*A. candida*) with virulence on cauliflower, Brussels sprouts and cabbage. The introduction of new Brassica lines would allow the industry to reduce its reliance on systemic fungicides and increase the use of more cost effective preventative fungicides. Additional benefits could be retarding the development of fungicide resistance and extending the life of systemic fungicides.
- Determine the physiological stage when broccoli heads are susceptible to white blister. The application of fungicide sprays at this time will increase the efficacy of disease control.
- Determine the role of oospores in the epidemiology of white blister on broccoli in Australia. This will increase understanding of disease development and management.
- Develop a molecular test for variants of Race 9 of *A. candida*. The introduction of other variants of Race 9 is a biosecurity threat for the Australian *Brassica* industry. In Australia, Race 9 is very aggressive on broccoli but not on cabbage, whilst in Europe, Race 9 is very aggressive on cabbage and Brussels sprouts. Use of the molecular test may prevent the introduction of aggressive variants of Race 9 and avoid epidemics of white blister on cabbage and Brussels sprouts, similar to the epidemic on broccoli in 2001/02.

Downy mildew of lettuce

- Format the BREMCAST™ model into to more user-friendly software, validate it against weekly sprays and reduced grower spray programs, and enable crop consultants and leading growers to evaluate it in the field.

Anthracnose of lettuce

- Develop a rapid molecular test to track *Microdochium panattonianum* during epidemiology and control studies.
- Develop a "spray warning" model to indicate the time for first sprays in spring.
- Evaluate local and overseas cultivars for resistance to the disease.
- Evaluate new generation chemistry with improved efficacy against anthracnose.

Powdery mildew of cucurbits

- Evaluate and improve the disease predictive model for *P. fusca* over several seasons in field and glasshouse situations in states where cucurbits are produced.

Disease predictive models

- Link with private providers to deliver a website where industry can access disease predictive models.
- Investigate the use of VPD, Fuzzy Logic models and CART models to replace the use of leaf wetness data.

Nutrients

- Determine if nutrient excess or deficiency increases the susceptibility of lettuce plants to downy mildew or anthracnose and broccoli plants to white blister.

Chemicals

- Investigate the use of alternative chemistries for disease control on lettuce, broccoli and cucurbits, especially under conditions of low disease pressure and on organic crops.
- Develop a rapid molecular test to identify *B. lactucae* and *A. candida* isolates that are resistant to the main systemic fungicides, e.g. metalaxyl and azoxystrobin.

13.5 Appendix

Publications - Technical

Minchinton EJ, Auer DPF, Trapnell LN, Kennedy R, Petkowski JE, Holmes R and Thomson F. (2011). Benchmarking the Brassica disease predictive models against weekly sprays and fungicide alternatives. ACPP APPS Darwin 2011 New frontiers in plant pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT. p 43. Oral presentation and abstract.

Minchinton EJ, Trapnell LN, Galea VJ, Kushalappa A, Auer DPF, Petkowski JE and Thomson F (2011). Benchmarking the BREMCAST disease predictive model for control of downy mildew in lettuce. ACPP APPS Darwin 2011 New frontiers in plant pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT. p 43. Oral presentation and abstract.

Petkowski JE, Thomson FM, de Boer RF and Minchinton EJ (2011). Management strategies for root rot of continental parsley. ACPP APPS Darwin 2011 New frontiers in plant pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT. p 109. Abstract and poster.

Petkowski JE, Minchinton E, Thomson F, Faggian R and Cahill D (2009). Races of *Albugo candida* causing white blister rust on *Brassica* vegetables in Australia. Accepted for publication in Acta Horticulture Brassica 2009.

Rawnsley B, Bartlett L and Hall BH (2011). Nitrogen affects lettuce susceptibility to downy mildew (*Bremia lactucae*). ACPP APPS Darwin 2011 New frontiers in plant pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT. p 111-112. Abstract and poster.

Rawnsley B, Bartlett L and Hall BH (2011). The influence of variety and nitrogen fertiliser on susceptibility of lettuce to anthracnose (*Microdochium panattonianum).* ACPP APPS Darwin 2011 New frontiers in plant pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT. p 112. Abstract and poster.

Sapak Z, Galea V, Joyce D and Minchinton E (2009). Uniform distribution of powdery mildew conidia using an improved spore settling tower. APPS 2009 Plant Health Management: An Integrated Approach. 29 September – 1 October 2009, Newcastle, NSW Australia. Abstract. p 200.

Sapak Z, Galea VJ, Joyce D. and Minchinton EJ (2011). The effect of temperature and vapor pressure deficit on in vitro germination of *Podosphaera fusca*. ACPP APPS Darwin

2011 New frontiers in plant pathology for Asia and Oceania, 26-29 April 2011, Darwin Convention Centre, Darwin, NT. p 112. Abstract and poster.

Publications - Industry

Anon (2011). Benchmarking predictive models, nutrients and irrigation for management of downy and powdery mildews and white blister. Project no: VG07070. Vegenotes 25: 1-3.

Minchinton E (2008). An article was published in 3 grower newsletters, the VegeLink (Vic), Lettuce Leaf (NSW) and Brassica IPM (SA), to promote the project.

Fischer K (2009). Remember TWO key management strategies in the fight against white blister. Vegetables Victoria Vegelink Issue 36. p 6.

Minchinton E (2009). Remember TWO key management strategies in the fight against white blister. Good Fruit and Vegetables Volume 80, No. 5. p 15.

Hinchsman M (2009). Benchmarking predictive models, irrigation for downy mildews and white blister. Swan Hill Regional Premium Pickings Volume 17, No. 4. p 15

Minchinton E (2009). Brassica$_{spot}$™ disease predictive model: A new option for control of white blister on Chinese cabbage. Brassica IPM National Newsletter Issue 13, October 2009. p 2, 4.

Minchinton E (2009). Lettuce downy mildew model. *Lettuce Leaf*, Issue No. 36, August 2009. p 1-2.

Minchinton E, Trapnell LN, Petkowski JE, Auer DPF, Faggian RF and Thomson FM (2010). Early-bird irrigation reduces disease. *Vegetables Australia* **5.4**: 32-33

Minchinton EJ *et al.* (2010). Early bird irrigation reduces disease. *Vegetables Australia* **5.4** (Jan/Feb): 32-33.

Minchinton EJ (2010). Development of an in-field spore test kit for white blister. Brassica IPM National Newsletter, Issue 14, October 2010.

Minchinton EJ (2010). Tailor nitrogen to manage vegetable diseases. Vegetable Industry Annual Report 2009/10, HAL, AUSVEG. p 50.

Rawnsley B (2010). Disease susceptibility in lettuce. *Vegetables Australia* (July/August). p 48-49.

Sapak Z, Galea V, Joyce J, and Minchinton E (2011). Developing a disease forecasting system for powdery mildew in cucurbits. Article submitted to GrowCom QLD.

Posters

Three posters on lettuce downy mildew, white blister and irrigation were prepared for the DPI tent at the National Vegetable Expo at Werribee, Victoria, 7-8 May 2009.

Sapak Z, Galea V, Joyce D and Minchinton E (2009). Uniform distribution of powdery mildew conidia using an improved spore settling tower. APPS 2009 Plant Health Management: An Integrated Approach. 29 September – 1 October 2009, Newcastle, NSW Australia. Poster. p 200.

Minchinton EJ, Trapnell LN, Petkowski JE, Auer DPF, Faggian RF and Thomson FM (2009). Evaluation of the efficacy of Brassica$_{spot}$™ models for control of white blister on broccoli. APPS Conference 2009 Plant Health Management: An Integrated Approach. 29 September – 1 October 2009, Newcastle, NSW Australia. Abstract.

Auer DPF, Minchinton EJ, Kennedy R, Petkowski JE, Faggian RF, Holmes RJ and Thomson FM (2009). Evaluation of the efficacy of Brassica$_{spot}$™ models for control of white

blister in Chinese cabbage. APPS Conference 2009 Plant Health Management: An Integrated Approach. 29 September – 1 October 2009, Newcastle, NSW Australia. Presentation and abstract. p 58.

Minchinton EJ, Auer DPF, Petkowski JE, Faggian RF, Galea V and Thomson F (2009). Management of white blister on vegetable brassicas with irrigation and varieties. APPS Conference 2009 Plant Health Management: An Integrated Approach. 29 September – 1 October 2009, Newcastle, NSW Australia. Presentation and abstract. p 60.

Auer DPF, Minchinton EJ, Galea VJ, Kushalappa A, Thomson FM, Petkowski EJ and de Boer RF (2010). Evaluation of BremCast™ and DownCast™ disease predictive models for control of downy mildew on lettuce. AgriBio Science Conference 2010, La Trobe University, 29 November 2010.

Minchinton EJ, Auer DPF, Trapnell LN, Kennedy R, Petkowski JE and Thomson FM (2010). Benchmarking the Brassica$_{spot}$™ disease predictive models, weekly sprays and fungicide alternatives for white blister control. AgriBio Science Conference 2010, La Trobe University, 29 November 2010.

Minchinton EJ, Auer DPF, Trapnell LN, Thomson F, Petkowski JE and Galea V (2010). Benchmarking disease predictive models for lettuce downy mildew against weekly sprays and fungicide alternatives. AgriBio Science Conference 2010, La Trobe University, 29 November 2010.

Other

- Project notes were published in September 2008 for distribution by Elders, Muirs, Werribee South Farm Supplies and Boomaroo Nursery.
- An article was prepared for the HAL booklet on the Vegetable Pathology Program and a poster was prepared for the Vegetable Conference, Melbourne Convention Centre, in Melbourne, Victoria, 5-6 May 2009 and the National Vegetable Expo at Werribee, Victoria 7-8 May 2009.
- Handouts of the three Werribee field day posters were produced for the VGA tent at the National Vegetable Expo, Werribee, Victoria 7-8 May 2009.
- Three articles were produced for the new Victoria "IDO Update" on "Downy mildew trial on lettuce in Werribee South", "White blister trial in Werribee South" and "White blister spore detection kit update".
- Minchinton, E. (2010). Pest control methods examined. Bairnsdale Advertiser, 23 August 2010.
- VIDP report on line.

Conferences

- AUSVEG Conference Melbourne, May 2009.
- APPS Conference Newcastle, October 2009.
- AUSVEG Conference Gold Coast, April 2010.
- AgriBio Science Conference November 2010, La Trobe University.
- AUSVEG Conference Brisbane, April 2011.
- ACPP APPS Conference Darwin April 2011.

Workshops/meetings

To date, 16 workshops and meetings covering project results have been presented to industry. Six workshops are still pending. These will be presented in WA, SA and Vic.

- The project was launched at a meeting in Cranbourne, Vic. on 14 March 2008 that was attended by growers and scientists from QLD, SA, WA and TAS.
- North Queensland Pest and Disease Seminars were presented on 25-28 March 2008 at Bowen, Gumlu, Ayr and Bundaberg as part of VG07127 project updates.
- A workshop was held at Cranbourne on 21 November 2008.
- A report was presented to the Vegetable Pathology Program in Melbourne on 27-28 November 2008.
- North Queensland Pest and Disease Seminars were presented from 29 March to 1 April, 2009 at Bowen, Ayr and Mareeba, as part of VG07127 project updates.
- A meeting was held at Cranbourne on 19 June 2009 in conjunction with the Soil-borne, Soil Health, Onion White Rot, Pythium, Fungicide Alternatives and Benchmarking projects.
- A meeting (workshop) was held with growers from the IPM Technologies Group to brief them on white blister at Werribee South on 27 October 2009.
- A presentation was given to organic growers at Koo Wee Rup on 11 May 2010.
- A report on the powdery mildew module was presented at meetings held in Bowen on 30 March 2010 and in Ayr on 31 March 2010.
- North Queensland Pest and Disease Seminars were presented on 30-31 March 2010 at Bowen and Ayr as part of VG07127 project updates.
- A workshop and steering committee meeting was held at Amstel Golf Course on 9 February 2010. Meeting notes and power point presentations were placed on the VGA website.
- A report was presented on 11 August 2010 to growers at Gympie, QLD.
- A report was presented on 4 August 2010 to growers in Devonport, Tas.
- A report was presented on 12 August 2010 to growers in Gatton, QLD.
- A report was presented on 8 December 2010 to growers in Bathurst, NSW.
- A report and workshop notes were presented on 14 December 2010 to growers in Cranbourne Vic. Meeting notes and power point presentations were placed on the VGA website.

Steering committee meetings

- Two steering committee meetings were held on 29 April 2008 and 16 May 2008 at Werribee and Cranbourne Vic., respectively.
- A steering committee meeting was held at Cranbourne on 6 February 2009 and at Werribee on 19 February 2009.
- Steering committee meetings were held for lettuce at Five Ways on 12 June 2009 and for lettuce and brassicas at Werribee South on 24 July 2009.
- A steering committee meeting was held at Amstel Golf Course on 9 February 2010.
- A steering committee meeting was held at Ranfurley Golf Course on 14 December 2010.
- Meeting notes and power point presentations were placed on the VGA website.

Field days

- A field day to view the irrigation trial was held at Werribee on 12 November 2008.

Visiting scientists

- A visit by Emma Garrod, a Nuffield Scholarship holder from the UK, was hosted in 2010.
- A visit by Dr Alison Wakeham and Dr Roy Kennedy, collaborators on the project, was hosted in 2010.
- Dr Roy Kennedy gave a presentation to industry consultants on 9 April 2010 at DPI, Snydes Rd, Werribee.