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A scoping study for race identification, sources of the epidemic and management of white blister disease on brassica vegetables

Dr. Elizabeth Minchinton
VIC Department of Primary Industries

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DEPARTMENT OF
PRIMARY INDUSTRIES

A scoping study for race identification, source of epidemic and management of white blister (rust) on brassicas

HAL VG02118

(June 2004)

Minchinton *et al*

Primary Industries Research Victoria, Knoxfield Centre

Victoria
The Place To Be

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Purpose of project:
This project details the outcomes of a 12-month scoping study on white blister (rust) on brassicas which investigated the identification of the *Albugo candida* race causing the epidemic, the source of the epidemic and management practices for white blister in the field.

Report completed: June 2004

Funding acknowledgments:
The researchers acknowledge the financial support for this project from Horticulture Australia Limited (HAL), AUSVEG, the Federal Government and the Department of Primary Industries, Primary Industries Research Victoria.

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Chapter 1

Media Release

Exposing white blister

White blister is a fungal disease of broccoli and cauliflower, which devastated Victorian crops during the summer of 2001-02. Within eighteen months it had spread to broccoli and cauliflower crops across the whole of southern Australia. It produces unsightly white blisters and swellings on the leaves and heads. Crops can be totally affected causing up to 100% losses to the grower. Although white blister has previously been reported in Australia on these plants, it has not been associated with epidemics. Researchers at DPI Knoxfield conducted trials to identify the race of the fungus causing the disease, the chemicals required to control it and the possible source of the epidemic. AUSVEG, HAL and the Victorian government supported this work.

Worldwide there are over 10 different races of white blister, which are largely specific to certain plant hosts. The race of the fungus responsible for this epidemic is similar to the established race 9 of white blister found overseas, which infects broccoli, cauliflower, cabbage and Brussels sprouts, but differs in that it does not infect cabbage. A more rapid DNA fingerprinting approach is being evaluated to speed up tests and improve detection.

Fungicide sprays for white blister were evaluated in three field trials. A total of 17 systemic, contact and soft chemicals (those with short or no withholding periods) were identified, which controlled the disease by up to 100%. It is imperative to rotate chemicals from different fungicide groups as white blister can quickly form resistance to fungicides.

The source of the white blister infection is a contentious issue. Seed does not appear to be a consistent source of white blister as no spores were detected in a seed wash of 25,000 seeds from the cultivar Greenbelt. The only evidence of seed-borne infections were the spore-containing galls found on seedlings early in the epidemic.

Implications of this research:

This research has immediate implications for the import and export of *Brassica* crops from Victoria, as we do not have white blister on cabbage. In the future the new DNA test will enable the races to be distinguished rapidly which will assist seed certification, interstate trade and quarantine issues and it may help to identify genes for breeding resistance against white blister.

New research is aimed at:

- determining if other races of white blister can infect broccoli,
- checking a wider range of seeds for contamination using seed washing and a DNA based diagnostic test,
- trialing a predictive model for white blister, in collaboration with Dr Roy Kennedy, Horticulture Research, Warwick University, UK,
- continuing to develop molecular tests for rapid identification of races.

Chapter 2

Technical summary

An outbreak of white blister, caused by the fungus *Albugo candida* (Pers.) Kuntze, devastated broccoli crops in Werribee South, Victoria during the summer of 2001-2002. The disease spread rapidly to other *Brassica* growing regions in Victoria and also to cauliflower crops, in some cases causing up to 100% crop losses. Although *A. candida* has previously been reported in Australia on *Brassica oleracea*, it has not been associated with epidemics.

This 12-month scoping study investigated 1) the means of differentiating races of *A. candida* using both differential hosts and molecular tools, 2) possible sources of the disease, and 3) management strategies for effective control. The major outcomes of the study were:

- Inoculation of a set of the hosts with *A. candida* spores from broccoli indicated that the AC race causing white blister on broccoli is similar to an isolated identified overseas as AC9, but differs in that it did not infect cabbage but was very pathogenic on *B. nigra*.
- A seed-wash assay of 25,000 seeds of broccoli cv. Greenbelt found no contaminating oospores. Seed infested with oospores does not appear to be a consistent source of white blister.
- Systematic surveys conducted during October 2003 on broccoli crops in 10 market gardens east and west of Melbourne, found there was a tendency for a higher incidence of white blister west of Melbourne where crops are in very close proximity to each other.
- Chemical control trials were conducted on seedlings, transplants and the button stage using a total of 17 systemic, contact and soft chemicals (those with no or short withholding periods). The most effective chemicals to control the disease on seedlings by 90-100% were Amistar+contact fungicides, Amistar, or AgriFos 600+Euparen. In the transplant trial Amistar+copper oxychloride, F5160f (sample) were the most effective, achieving 100% disease control on foliage. Whilst Amistar+SDS or Ridomil Gold MZ had the greatest efficacy at the button stage and reduced the disease by 75% or 70%, respectively. Baycor and Plantvax had no efficacy.
- A DNA-based diagnostic test was used to detect *A. candida* in non-symptomatic tissue.
- A DNA fingerprinting technique was developed that may enable races to be distinguished.
- An *A. candida* genomic DNA library was constructed and 192 clones were sequenced. Among the clones are a number of new genes that need to be examined for their usefulness in differentiating races based on genetic polymorphisms.

Implications

This research has immediate implications for the import and export of *Brassica* crops from Victoria, as we do not have white blister on cabbage. In the future it will enable the races to be distinguished rapidly which will assist seed certification, interstate trade and quarantine issues and it may help to identify genes for breeding resistance against white blister.

Recommendations for future work

- Determine if other AC races can infect broccoli to establish potential sources of inoculum.
- Screen other seeds, especially those from niche-market seed companies.
- Investigate other chemical options and combinations. Conduct trials in the field during different seasons to establish the most economical control measures.
- Test the white blister disease predictive model in collaboration with Dr Roy Kennedy (Horticulture Research International, Wellsbourne).
- Investigate whether soil-borne oospores contribute to the incidence of white blister
- Further develop the DNA-based diagnostic test to establish sensitivity for seed and soil detection.
- Continue to examine clones from the genomic library to identify race-differentiating DNA fragments.

Chapter 3

Introduction

White blister (white rust) infected broccoli plants were submitted for diagnosis to the project leader (Dr Liz Minchinton) in January 2002 by a Werribee South crop consultant (Dale O'Conner). The samples included i) etiolated seedlings with galls on the stems, ii) pustules on the undersurface of seedling leaves and iii) broccoli heads with white blisters on swollen florets. The disease was reported to be devastating many broccoli crops and causing up to 100% crop loss. Both seedlings and field crops were affected, resulting in entire crops being ploughed in. Although the causal fungus *Albugo candida* (Pers.) Kuntze has previously been reported in Australia, it has not caused epidemics of disease on broccoli.

After this initial outbreak in the Werribee South market garden region, the disease spread to the market garden areas east of Melbourne and to the Lindenow Valley, in East Gippsland. In December 2002 it was reported in Tasmania, where major eradication measures, which consisted of ploughing in the crop, were immediately implemented. By May 2003 the disease was found on broccoli crops in the Adelaide Hills of SA and broccoli crops in NSW in early June and broccoli in WA in late June. A survey of broccoli and cauliflower crops in Queensland during August and September 2002, by Animal and Plant Health Services, did not detect the disease (Telford, 2002). To-date it has still not been reported on either broccoli or cauliflower crops in Queensland.

The white blister epidemic caused major concern in Victoria, which with 45% of production is the largest Australian producer of broccoli, followed by Queensland with 23% (ABS, 2001). The Victorian outbreak resulted in trade barriers into Queensland and Tasmania, which were later lifted by Tasmania after the disease eradication measures failed. Currently, importation conditions are in place for broccoli exports to Queensland. The following are the current conditions of entry (P. Christudoss, pers. comm.):

(1) Broccoli and cauliflower seedlings are prohibited entry to Queensland.
(2) No certification required when processed as ready to eat such as fresh cuts or frozen.
(3) No certification required when packed free of leaf and stem material (trimmed right up to the florets) for sale through wholesale or retail outlets for human consumption or
(4) If not free of leaf and stem material then inspected at the time of packaging and found free of white blister and certified accordingly by a Plant Health Certificate (PHC) or Plant Health Assurance Certificate (PHAC) or
(5) If produced on a property located more than 20 km from a detection of white blister, the produce has to be certified with a PHC or PHAC.

From a historical perspective, *A. candida* was first reported in Victoria more than a hundred years ago, but the introduction onto *B. oleracea* is a much more recent event. *A. candida* was first reported in Victoria on *Capsella bursa-pastoris* (Shepherd's purse) in 1894, on *Brassica rapa* (Chinese cabbage) in 1895, on *Raphanus sativus* (radish) in 1903 and more recently on *B. oleracea* (cauliflower) in 1980 and *B. oleracea* (broccoli) in 2000. In NSW, *A. candida* was first reported on *B. oleracea* (kale) in 1990. Until this work commenced, the disease had not been reported on *B. oleracea* outside Victoria and NSW. However, in New Zealand white blister was first reported on *B. oleracea* (cabbage) in 1906 and on *B. oleracea* (cauliflower and Brussels sprouts) in 1922 (Pennycook, 1989). *A. candida* has not been reported on *B. oleracea* (cabbage) in Australia.

Internationally, *A. candida* research is occurring at several locations. In the Netherlands, white blister has become a very important disease of cabbage and Brussels sprouts, prompting a recent 3 year study to investigate aspects of epidemiology, host specialisation, resistance and chemical controls of white blister on Brussels sprouts (Gilijamese *et al*., 1998). In the U.K., a forecasting system for foliar diseases of vegetable crops including white blister has reduced fungicide applications by 50% (Humpherson-Jones, 1991; Kennedy and Gilles, 2003). Also in the U.K., Dr Eric Holub and his research group (Horticulture Research International, Wellesbourne) are attempting to identify and genetically characterise sources of resistance in *Brassica* to *A. candida*. In contrast, Dr Rogers Rimmer's group in Canada has looked at the genetic

determinants of virulence in the pathogen. Locally, Phillip Salisbury's group at Melbourne University is conducting race studies and resistance screening of *A. candida* on mustards and Philip Keane's group at LaTrobe University is investigating resistance in commercial and breeding lines of broccoli for a seed company.

This project reports on the results of a 12 month scoping study to investigate 1) means of differentiating races of *Albugo* causing white blister on broccoli, using both differential hosts and molecular tools, 2) possible sources of the disease and 3) management strategies for effective disease control.

3.1 References

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Chapter 4

A literature review of white blister of crucifers

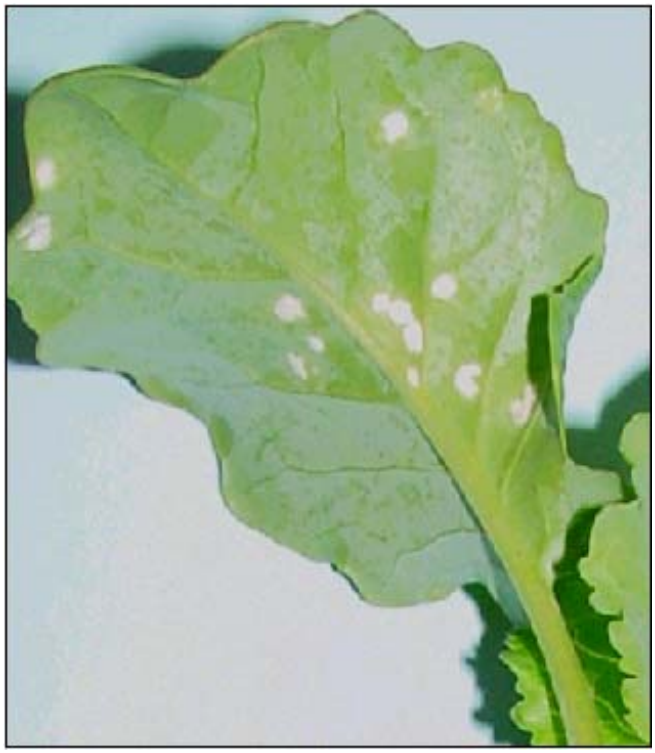
James Cunnington

4.1 Introduction

White blister of crucifers is caused by the fungus *Albugo candida* (Pers.) Kuntze [=*Cystopus candidus* (Pers.) Lev., *A. cruciferarum* (DC.) Gray]. The classical symptoms of the disease are white chalky pustules that can develop over all the aerial parts of the plant, but which are most common on the under surfaces of leaves (Figs 1 and 2). It is a disease recorded worldwide, wherever susceptible hosts are grown. It occurs on a wide range on plants in the Brassicaceae (crucifer family) and Capparidaceae (caper family) (Mukerji, 1975). The presence of host-specialised races of *A. candida* is well-documented (Pound and Williams, 1963). A sound knowledge of these races is of primary importance to understanding and controlling the disease in all its various forms on different hosts.

There is an enormous body of scientific information concerning *A. candida*. This literature review cannot cover all these publications, so has focused on three main areas; races, epidemiology and chemical control. However, it must be realised that even in these areas, the review is not exhaustive. The two most important publications on *A. candida* are a review of economically important *Albugo* species (Saharan & Verma, 1992) and a bibliography and subject index to literature on *A. candida* (Verma & Saharan, 1996). The latter contains almost 600 references and is reported to represent the beginning of a monograph of *A. candida* by the same authors, but we have no knowledge of whether this is still being undertaken. These two publications must be regarded as an essential resource for a research project into *A. candida*. More recently, Singh *et al.* (1998) produced a review of *A. candida* on rapeseed and Indian mustard, however we have not been able to obtain a copy of this document.

Despite being associated with a project on white blister of broccoli, this review discusses white blister on a range on crucifers because of the very limited amount of scientific literature dealing with this disease on broccoli. However all references concerning broccoli, and other members of the species *Brassica oleracea* (e.g. Brussels sprouts and cauliflower) have been included.



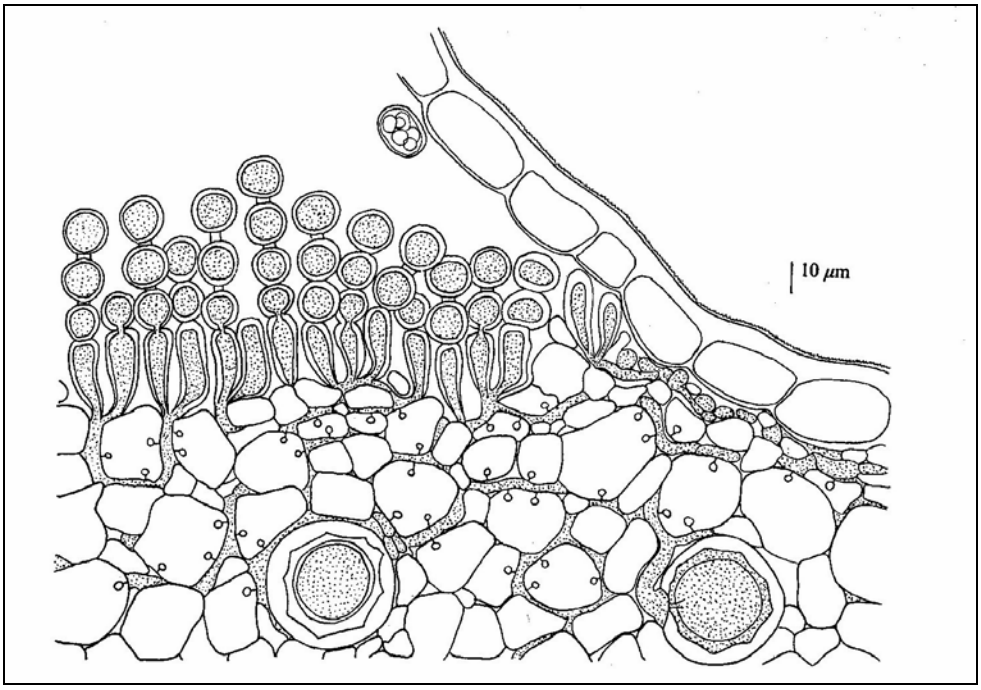


Fig .1 Symptoms of *A*. *candida* on undersurface of a broccoli seedling leaf.
Fig. 2. Section through a pustule showing the production of sporangia, and oospores embedded in the host plant (von Arx, 1987).

4.2 The fungus-life cycle and morphology

Albugo candida is not a true fungus, but is rather a member of the Peronosporales (Oomycota, =oomycetes), which is a group of, usually water dependent, fungi-like organisms (Alexopolous *et al*., 1996). As such, they have a life cycle quite different from that of the true fungi. Recent phylogenetic studies have shown all *Albugo* species to constitute a distinct group within the oomycetes, which includes such plant pathogenic genera as *Pythium*, *Phytophthora* and *Peronospora* (Riethmuller *et al*. 2002). The following lifecycle of *A. candida* (Fig. 3) is based on that given by Saharan and Verma (1992) and Alexopolous *et al*. (1996). While only the microscopic morphology is discussed, there have been several ultrastructural studies on haustoria (Coffey, 1975), sporangia (Khan, 1976; Khan, 1977) and oospores (Tewari and Skoropad, 1977).

4.2.1 Sporangia and asexual reproduction

Most *Albugo* species, including *A. candida*, are first noticed when they form pustules on the leaves of infected plants. These are white or creamy and 1-2 mm in diameter. They form from fungal mycelium underneath the host epidermis, which increases in size and develops short club-shaped sporangiophores that are often characteristically branched (Mukerji, 1975). The sporangiophores produce chains of sporangia, which build up in large amounts along with the mycelium under the host epidermis until the pustule ruptures to release the sporangia. Sporangia are globose-oval, hyaline, thin walled, 12-18 μm in diameter (Mukerji, 1975), and are dispersed by wind and rain. Sporangia absorb water on contact and swell. A pore (papilla) forms on one side of the sporangium. Four to twelve zoospores are exuded through the papilla into a sessile vesicle. The zoospores are concave to convex and possess one long and one short flagellum. The flagella allow the zoospore to swim through water. When a zoospore comes to rest on a susceptible host plant, the flagella are absorbed and the zoospore encysts and germinates by a germ tube. The germ tube penetrates through a stoma to form intercellular mycelium, beginning the infection cycle. Sometimes sporangia germinate directly by a germ tube. In either case, the mycelium produces globose to knob-like haustoria that penetrate the plant cells through minute perforations. Many of these energy and nutrient absorbing haustoria can be formed in each plant cell (Verma *et al*., 1975). The fungus then spreads throughout the intercellular spaces of the infected plant.

4.2.2 Oospores and sexual reproduction

Sexual reproduction usually occurs in systemically infected tissues. This usually results in distortion and hypertrophy of the host organ. In the case of the inflorescence, it is often referred to as a “staghead”. Oogonia and antheridia are formed from the mycelium in intercellular spaces. The club-shaped antheridium attaches to the side of the globose oogonium. A nucleus flows through a penetration tube from the antheridium to the oogonium. When nuclei combine, the outer periplasm of the oogonium wall becomes thick and dark, and an oospore is formed. Oospores are chocolate brown, globose, 30 - 55 μm in diameter, have a thick epispore and low blunt irregularly branched ridges that may be smooth or verrucose (Mukerji, 1975). Hypertrophied plant parts may be entirely composed of oospores. The weathering and decay of the host plant subsequently release them. The 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 & Petrie, 1975).

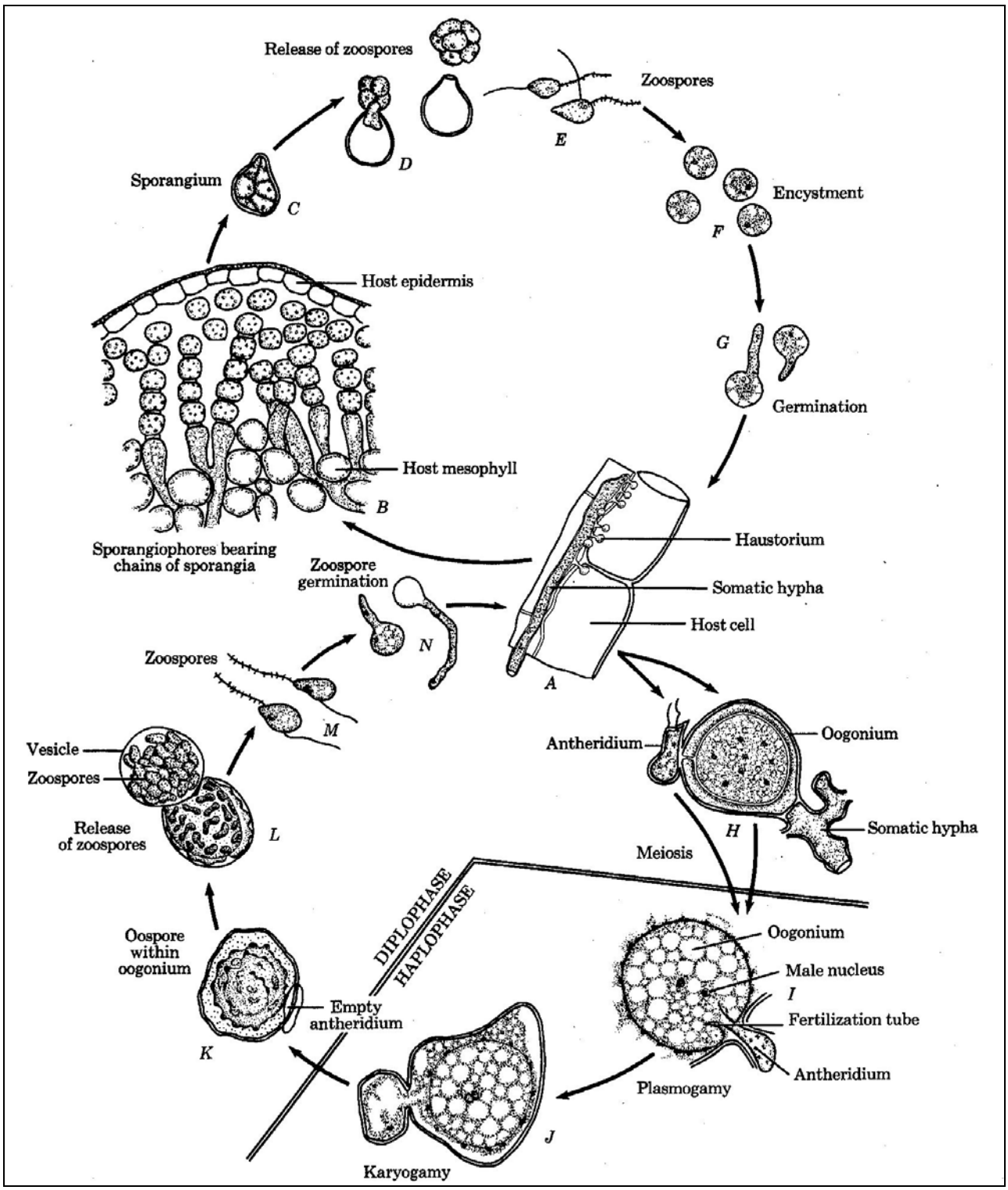


Fig 3. Life cycle of *Albugo candida*. Taken from Alexopoulos *et al*. (1996).

4.3 Host range and specialisation

There has been extensive research undertaken to understand the existence of various host-specialised races within *A. candida*. Numerous authors have reported various host-specialised forms, but the currently accepted classification of races began with host inoculation experiments by Pound & Williams (1963). They recognised six races (Table 1), each occurring on a different species of crucifer; *Raphanus sativus*, *Brassica juncea*, *Armoracia rusticana*, *Capsella bursa-pastoris*, *Sisymbrium officinale* and *Rorippa islandica*. While these results did demonstrate the presence of races of *A. candida* with preferences towards particular species of crucifers, Pound and Williams also noted several unexpected host reactions. For example, all six races were found to infect *Brassica hirta*, and several were pathogenic on *Descurania sophia*. Also race 3 (from *Armoracia rusticana*) was found to infect *Rorippa islandica* and *Erysmium cheiranthioides*. Many races were found to infect *Sisymbrium officiale*, but it was noted that the seedlings used for this cross were very young with immature cotyledons. Despite these problems, this classification has been expanded over the last 40 years.

Race seven was subsequently described by Verma *et al*. (1975) on *Brassica rapa* (*B. campestris*). The naming of races eight to eleven were not reported in mainstream scientific journals, but rather in the Cruciferae Newsletter and also in Williams (1985) (cited in Verma *et al*., 1999). Delwiche and Williams (1977) described race eight from *B. nigra*. Hill *et al*. (1988) described races nine and ten from *B. oleracea* and *B. kaber* respectively. Race eleven was reported on *B. carinata* (Williams, 1985). Two forms of races two and seven were reported by Petrie (1994) and designated 2V and 7V respectively. Verma *et al*. (1999) then added races twelve and thirteen on *B. juncea* and *B. campestris* var. *toria*. The most recent additions by Gupta & Saharan (2002) have moved away from the concept of only one race occurring on each crucifer species, by describing races fourteen to seventeen on *B. juncea*.

There have been several other significant publications on the host specialisation of *A. candida*, published both before and after that by Pound and Williams, which do not fit into their race classification. Napper (1933) described 20 races from England. Singh & Bhardwaj (1984) found nine races on four species of *Brassica*. Bhardwaj & Sud (1988) found nine races on cultivated and wild crucifers. Lakra & Saharan (1988a) found five races on *Brassica* species. Other earlier studies include Novotel'Nova (1968) (cited in Saharan & Verma, 1992), Novotel'Nova & Minasyan (1970) (cited in Saharan & Verma, 1992), Savulescu (1946) (cited in Saharan & Verma, 1992) and Savulescu & Rayss (1930). Additional minor reports are listed in Verma & Saharan (1996).

Given the large number of races that have been, and are currently being described, from individual crucifer species, it is essential that future work adopt an international standardised set of host differentials (Verma *et al*. 1999). Otherwise, there seems little hope that a united classification of *A. candida* races could be recognised.

Table. 1 Races of *A*. *candida*.

Race	Scientific name	Common name	Reference
1	*Raphanus sativus*	Radish	Pound & Williams (1963)
2	*Brassica juncea*	Indian mustard	Pound & Williams (1963)
2V	*B*. *napus*	Canola	Petrie (1994)
3	*Armoracia rusticana*	horseradish	Pound & Williams (1963)
4	*Capsella bursa-pastoris*	shepherd's purse	Pound & Williams (1963)
5	*Sisymbrium officinale*	hedge/tumble mustard	Pound & Williams (1963)
6	*Rorippa islandica*	marsh/yellow watercress	Pound & Williams (1963)
7	*B*. *rapa (campestris)*	Mustard, rape, rapeseed, field mustard, Chinese cabbage, spinach mustard, turnip	Verma *et al*. (1975)
7V	*B*. *rapa* cv. reward	-	Petrie (1994)
8	*B*. *nigra*	black mustard	Delwiche & Williams (1977)
9	*B*. *oleracea*	Broccoli, Brussels sprouts, cauliflower, kale, kohlrabi	Hill *et al*. (1988), Williams (1985)
10	*B*. *kaber* (*Sinapis arvensis*)	Charlock	Hill *et al*. (1988), Williams (1985)
11	*B*. *carinata*	Ethiopian mustard	Williams (1985)
12	*B*. *juncea*	Indian mustard	Verma *et al*. (1999)
13	*B*. *campestris* var. *toria*	-	Verma *et al*. (1999)
14	*B*. *juncea* cv. RL 1359	Indian mustard	Gupta & Saharan (2002)
15	*B*. *juncea* cv. Kranti	Indian mustard	Gupta & Saharan (2002)
16	*B*. *juncea* cv. Kranti	Indian mustard	Gupta & Saharan (2002)
17	*B*. *juncea* cv. RH 30	Indian mustard	Gupta & Saharan (2002)

4.4 *Albugo candida* versus *A. macrospora*

A. candida is considered by some authors to be two species, *A. candida sensu stricto* and *A. macrospora* (Togashi) S.Ito on the basis of the size of the sporangia. These are based around the works of Togashi & Shibashki (1934) (cited in Saharan & Verma, 1992) who established two varieties, *A. candida macrospora* and *A. candida microspora*. This was later modified by Ito and Yokanaga (1935) (cited in Saharan & Verma, 1992), so that the variety with the larger sporangia was named *A. macrospora*, while *A. candida* was retained for the forms with smaller sporangia. In this system, *A. macrospora* has sporangia with size of 18 x 20 µm and occurs on *Armoracia*, *Brassica*, *Erucastrum*, *Raphanus* and *Rapistrum*. *A. candida* has sporangia with a size of 14.5 x 15.5 µm and occurs on a wide range of crucifers. This classification was adopted by Biga (1955) in his monograph on *Albugo*. Thus, the fungus on *Brassica oleracea* would be referable to *A. macrospora*.

Pound & Williams (1963) also examined sporangia sizes during their race testing, and concluded that they did find two forms agreeing with *A. candida macrospora* and *A. candida microspora*. However, the *microspora* forms had sporangia averaging 15.2-15.9 µm in length, while the *macrospora* forms were 16.8-17.5 µm. They also found that the *macrospora* form could infect *microspora* hosts.

However, this classification is not widely used in the current scientific literature. In their key to *Albugo* species, Choi & Priest (1995) accepted both forms under the name *A. candida*. Lakra & Saharan (1988a) measured sporangia from many crucifer species and concluded that the two forms overlapped. In this review, a conservative approach is taken and all forms have been considered to be *A. candida*.

4.5 Symptoms, damage and yield loss

Infection by *A. candida* may be either localised or systemic. Localised infection results in leaf, stem, inflorescence and pod lesions that can coalesce to cover whole plant organs (Saharan & Verma, 1992). This stage is referred to as white blister or white rust (Figs 4a, b, c and 5a). The systemic phase is associated with hypertrophy, distortion and the formation of oospores, particularly of the inflorescence (Fig 4 d), but the stem (Fig 5b, c) and leaves can also be affected. Floral parts may persist and show hyperplasia. Occasionally, roots are affected (Fisher, 1954). Seeds are often aborted in the systemic infection of inflorescence, i.e. staghead. Local or systemic infections are of different economic importance, depending on whether the crucifer crop is grown for its seeds or other organs.

Yield loss varies considerably from year to year on different crops grown in different parts of the world. Losses as high as 60% have been recorded on rapeseed grown in Canada (Petrie & Vanterpool, 1974; Bernier; 1972), while in other parts of Canada, losses of only 1-2% occurred (Berkenkamp, 1972). In Western Australia, losses of 5-10% in rapeseed have been reported (Barbetii, 1981).

Indian mustard can also be severely affected with mixed infections of *A. candida* and *Hyaloperonospora* (*Peronospora*) *parasitica* causing losses of up to 35% (Bains & Jhooty, 1979), while losses from *A. candida* alone can reach 54.5% if the crop is sown late (Saharan *et al*., 1984).

Further high yield losses have been reported in mustard (Petrie, 1973), but canola seems to be rarely heavily affected (Harper & Pittman, 1974). Over the last decade, the disease has become more important in Europe on cabbage and Brussels sprouts (Gilijamse *et al*. 1998).

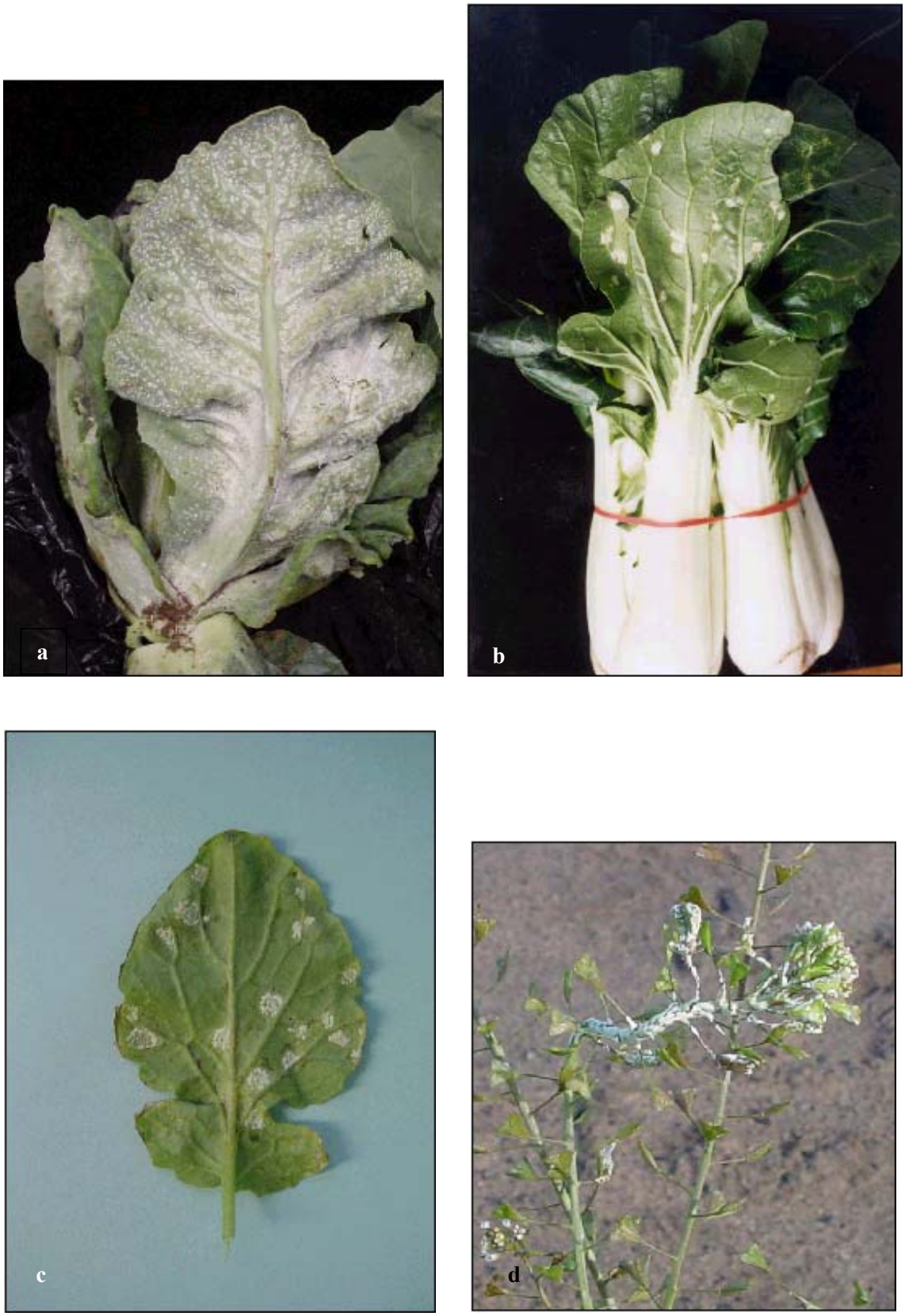


Fig. 4. Downy mildew symptoms associated with localised and systemic symptoms of *A. candida* on cauliflower (a). Localised symptoms of *A. candida* on bok choy (b). Localised symptoms of *A. candida* on radish (c). Staghead infection of *A. candida* on *Capsella bursa-pastoris* (d).

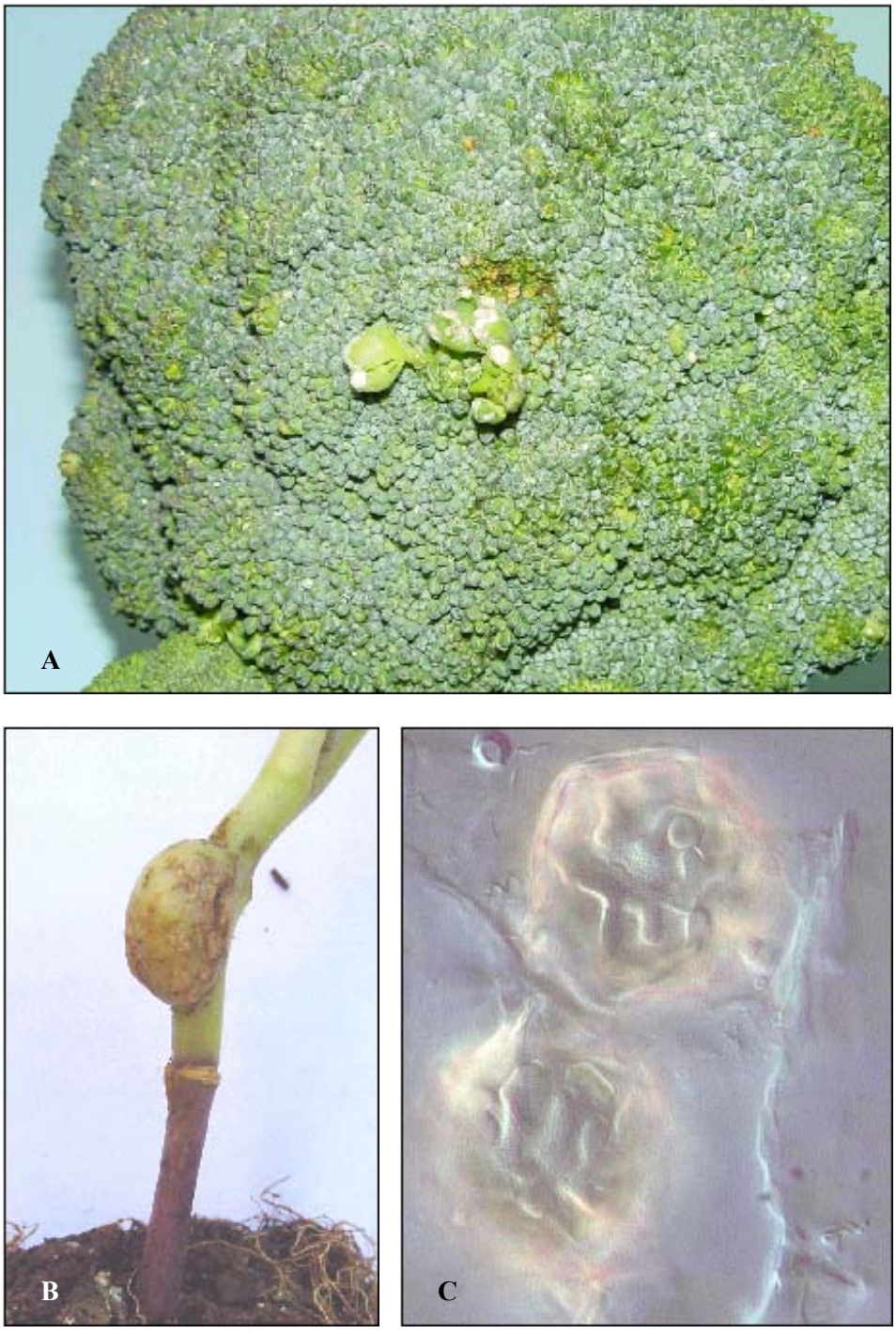


Fig 5. Hypertrophy on broccoli inflorescence, (no oospores present) (A). Gall, containing oospores on stem of broccoli seedling. (B). Oospores in gall (C).

Changes in the chemical composition of infected plants have been observed. Kumar *et al*. (2002) found that white blister infection resulted in a greater decrease in total sugar and phenol content in susceptible varieties compared to resistant varieties. Nutrient levels are also altered. Khangura & Sokhi (1992) found infected Indian mustard plants to have lower levels of nitrogen, phosphorus, calcium, magnesium, sulfur, iron and manganese, but higher levels of potassium, zinc and copper.

Methods for scoring disease severity have been developed by Fox & Williams (1984), Khangura & Sokhi (1991), and Bhadwaj & Sud (1995).

4.6 Epidemiology and disease development

It is generally accepted that oospores in soil and plant debris are the primary source of inoculum (Saharan & Verma, 1992). However, there is one contradictory report in the literature. Verma *et al*. (1988) surveyed soil from white blister infected areas and soil from which hypertrophied organs had been buried more than 6 months previously, but failed to find any oospores. The most likely site of primary infection is the emerging cotyledon, which may also be a pre-requisite for systemic infection (Verma *et al*., 1975).

Contaminated seed is also known to be primary sources of oospores (Verma *et al*., 1988). Examining naturally infected mustard seed, Sharma *et al*. (1997) found that all of the discoloured seed was infected with *A*. *candida*, as was 4% of symptomless seed. Petrie (1975) also suggested that oospore contaminated seed could be a major source of the survival and spread of white blister. Tracing the occurrence of *A*. *candida* using specific PCR primers, Jacobson *et al*. (1998) suggested that many crucifers may have persistent asymptomatic infections that are vertically transmitted by seed.

Oospores can remain viable for at least 21 years if placed in dry storage and fresh oospores are able to germinate within 2 weeks of collection (Verma & Petrie, 1975). This longevity may be due to the heavily fortified cell wall (Tewari & Skoropad, 1977). Oospores from *B*. *campestris* germinated at temperatures between 10 and 20°C, with an optimum of 13°C (Verma & Petrie, 1975).

Secondary infection by sporangia bearing zoospores has been more widely studied. Gilijamse *et al*. (1998) found that zoospores were released (often called sporangial germination) between 2 and 20°C, with an optimum of 13°C. This agreed with their observation that Brussels sprouts were infected from 5 to 25°C with a latency period from 6 to 37 days. Working with mustard, Lakra *et al*. (1989) found zoospore release between 5 and 22°C, with a maximum release at 12-14°C. However, Napper (1933) did not find any release above 20°C, but reported that it occurred between 1 and 18°C. A film of free water is essential for zoospore release (Lakra *et al*., 1989), but a reduction in water content of the sporangia is not required, despite that suggested by Napper (1933).

Other reports on zoospore release indicate that light may slightly delay germination (Lakra *et al*., 1989), and that a pH around neutral gives best release (Endo & Linn, 1960) (cited in Saharan & Verma, 1992). Zoospore release was reported to stop after 8 hours at any temperature (Lakra *et al*. 1989), but Bartaria & Verma (2001) noted that Indian isolates could remain viable for 4 days at 20°C. However, Canadian isolates did not remain as viable, particularly at 25°C where they lasted only 8 hours.

Disease development has often been evaluated separately from infection studies. In Brussels sprouts, at 20°C it takes only one hour for most zoospores to reach a host stoma, and by 3 hours of leaf wetness, infection had occurred (Gilijamse *et al*. 1998). Disease development was found to be optimal on radish plants between 16 and 18°C, but occurred within a range of 12-21°C (Sempio, 1938; Sempio, 1939; Sempio, 1940) (cited in Saharan & Verma, 1992). Saharan & Verma (1992) reviewed the development of disease on Indian mustard. The fastest rate of pustule development occurred at 11.5-12.5°C and with a mean relative humidity of >75%. Verma & Bhowmik (1989) found similar results, in that a mean temperature of 14.7 degrees and a relative humidity of 73.3 was optimal for disease development. Older leaves of rapeseed are more susceptible to white blister than younger leaves (Kumar *et al*. 1995).

Stagheads, pod deformations and abortions have also been experimentally formed by inoculating flower buds with zoospore suspensions (Bains, 1991; Goyal *et al*., 1996a). Serious staghead production in Indian mustard required a mean minimum temperature of 6-10°C and a mean maximum of 21-25°C, and rainfall up to 160 mm during flower formation (Saharan & Verma (1992).

Oospore production on detached leaves was found to occur over the entire range of incubation temperature of 10-27°C (max 23°C) (Goyal *et al*. 1996b).

4.7 Interaction with downy mildew

The association between *A*. *candida* and the downy mildew *Hyaloperonospora parasitica* (= *Peronospora parasitica*) has been well-documented (Saharan and Verma, 1992). Combined infection has been reported to cause rapeseed-mustard yield losses as high as 37% in India (Kolte, 1985), (cited in Singh *et al*., 2002). The relationship has been best studied on *B*. *juncea* (Indian mustard). Bains & Jhooty (1985) found that a combined inoculation resulted in white blister developing before downy mildew and apparently predisposing the host to infection by downy mildew. However spores of *A*. *candida* and *H*. *parasitica* do not appear to be spread together (Bains & Jhooty, 1985). Singh *et al*. (2002) found that pre inoculation of Indian mustard with compatible *A*. *candida* increased susceptibility to *H*. *parasitica*, while pre-inoculation with an incompatible strain did offered some level of resistance to *H*. *parasitica*.

Petrie & Vanterpool (1974) have listed many other fungi associated with hypertrophy caused by *A*. *candida*.

4.8 Disease control

Control of *A*. *candida* on crucifers is discussed under three broad areas: chemical control, resistance breeding and cultural methods.

4.8.1 Chemical control

Early chemical control of *A*. *candida* focused on copper fungicides then moved to dithiocarbamates, and although multiple applications of these were useful as protectants from local leaf infections, they were not particularly successful against stagheads (Saharan & Verma, 1992). However the acylalanine fungicides (particularly metalaxyl) which are specifically active against the Peronosporales provided good control of stagheads. These successes with metalaxyl will be discussed first.

Both leaf and staghead infections to *B*. *campestris* have been well controlled by foliar, seed and soil applications of metalaxyl (Stone *et al*., 1987a). They found that treating the seed controlled foliar infection up to the 6th leaf stage and when sprayed onto plants up to 4 days after inoculation with zoospores, resulted in at least 95% reduction in leaf infection. Soil drenching controlled foliar infection, but some phytotoxicity occurred, while foliar spraying was found to control both leaf and staghead infections provided it was applied at earlier growth stages. Stone *et. al* (1987a) recommended that seed dressing or soil drenching were good ways to reduce primary infections from germinating oospores. Likewise Stone *et al*. (1987b) found that root absorption was a major route of uptake for metalaxyl, with the largest concentration of the fungicide found to be present in the lower leaves after soil drenching. In addition, metalaxyl was detected in the foliage of seedlings that has had seeds dressed in metalaxyl. Working with mustard, Lakra & Saharan (1988b) found that 6 sprays of 0.2% metalaxyl gave good control of leaf and staghead infections and that the rate of disease development could be kept low if sprays were applied before the onset of the disease.

While metalaxyl is specifically affective against Peronosporales, it may not be as effective against *Albugo candida* as is it against other Peronosporales. Mathur & Bhatnagar (1991) found that twice the concentration of metalaxyl was required to control *A*. *candida*, when compared to *Hyaloperonospora parasitica*. Most modern reports of successful chemical control of *A*. *candida* have involved a combination of chemicals, often centred on metalaxyl.

The combination of azoxystrobin and metalaxyl plus mancozeb was found to be the most efficient foliar spray for *A. candida* on horseradish (Macias & Robak, 1999). Bhargava *et al*. (1997) found that the most efficient and economical combination of chemicals for mustard was to treat seeds with metalaxyl and then use subsequent combinations of sprays of chlorothalonil and mancozeb. However, in some cases the most economical treatments did not involve metalaxyl at all. Trying to control downy mildew and white rust on mustard, Mehta *et al*. (1996) found that the two most economical methods were to either use 4 sprays of mancozeb or an initial seed treatment with metalaxyl followed by 3 sprays of mancozeb.

There are also recent studies comparing fungicide activities that did not include any metalaxyl. Dubey & Mishra (1994) compared carbendazim, copper oxychloride, mancozeb, wettable sulfur, thiophanate-methyl and chlorothalonil, to control white rust of Indian mustard, finding that thiophanate-methyl gave the best control. Dubey (1996) subsequently found good control using thiophanate-methyl + copper oxychloride, and thiophanate-methyl + mancozeb. Azoxystrobin alone has been reported to provide adequate control of white blister on horseradish when applied to the roots (Macias & Robak, 1999).

Chemical control has also been reported for the non fungicidal benzothiadiazole (BTH; S-methyl benzo(1,2,3)thiadiazole-7-carbothioate), which is an activator of plant defence (Kaur & Kolte, 2001). Using this compound on mustard leaves, against *A. candida*, an increase in peroxidase activity and phenol level was observed, resulting in up to 60% protection. Best results were obtained in the field, where staghead formation was reduced up to 82%.

4.8.2 Genetic resistance

There has been considerable research into the genetic basis for resistance to *A. candida*. This review will focus briefly on the occurrence and genetic control of resistance, with some comments on the development of modern molecular markers. The acquisition of such large amounts of information on this subject is in part due to the availability of rapid-cycling populations of many species of *Brassica* species, which allowed rapid research to be conducted on the genetics of the host-pathogen interaction (Williams and Hill, 1986). However there is little genetic information on the fungus (Saharan and Verma, 1992), probably because fungus does not grow in culture, thus making mating and genetic variation experiments quite difficult.

There are few reports of resistant cultivars of *B. oleracea* crops. Meekes *et al*. (2000) reported that the Brussels sprout cultivars Cantante and Niz96-585 were partially resistant to white blister, as are some landraces of Portuguese cole (Santos *et al*. 1996).

However, there is considerable information on resistance in *B. juncea*, which is controlled by a single dominant gene (Saharan *et al*. 1988; Tiwari *et al*. 1988). Interspecific breeding between *B. napus* and *B. juncea* has resulted in the *B. napus* resistance to *A. candida* being transferred to *B. juncea*. The use of molecular markers has allowed this transfer from *B. napus* (Somers *et al*. 2002) and for resistance already in *B. juncea* (Mukherjee *et al*., 2001), to be tracked. Bansal *et al*. (1999) crossed a commercial cultivar of *B. juncea* with a resistant accession, obtaining partially resistant plants that did not develop hypertrophied growth or stagheads under greenhouse and field conditions.

Williams and Pound (1963) found that resistance to *A. candida* on two cultivars of radish was due to a single dominant gene, but that the resistance was manifested in different ways. Bonnet (1981) also found that a single dominant gene controlled resistance to *A. candida* on radish. Through crossing resistant and susceptible cultivars of *B. rapa* Kole *et al*. (1996) concluded that a single dominant gene again controlled resistance. In contrast, resistance in *B. napus* seems to be controlled by several dominant genes (Fan *et al*. 1983; Verma and Bhowmik, 1989)

4.8.3 Cultural control

There have been few recent studies on specific cultural methods that control *A. candida*. Working with cauliflower and horseradish, Savulescu (1960) (cited in Saharan and Verma, 1992), and Glaeser (1973) (cited in Saharan and Verma, 1992) suggested the removal and burning of all diseased plants to stop formation of oospores. Reduction of humidity, avoiding dense sowing, removal of weeds, the use of organic manures, the

avoidance of too much nitrogen, and the addition of more phosphorus and potassium has been recommended (Saharan and Verma, 1992).

However, when purely economic considerations are taken into account, recommendations can differ. Verma and Bhowmik (1996) found that increasing the distance between plants and rows reduced the yield losses from each plant of Indian mustard. However by increasing the number of plants in the same area, individual yield loss was higher, but total yield from the same area was higher.

Timely planting of crops was found to be important for reduction of staghead formation in Western Australian crops of rapeseed (Barbetti, 1981).

4.9 References

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Chapter 5

A survey on incidence of white blister caused by *Albugo candida* in broccoli crops in Victoria

Joanna Petkowski, Elizabeth Minchinton and Fiona Thompson

5.1 Introduction

White blister disease is a relatively new problem for broccoli growers in Victoria and other states in south eastern and Western Australia. Before the summer of 2001/2002 the disease was unknown to the majority of growers and did not pose any threats to the industry. Localised disease outbreaks in 2001/2002 and the rapid spread of white blister in consecutive seasons throughout many cropping regions instigated the need to develop disease management strategies for broccoli crops in Australia.

White blister of cabbage and Brussels sprouts causes yield losses in several European countries including the Netherlands (Gilijamse *et al.* 1998) and UK, where in the latter, a disease forecasting system has been developed for foliar diseases of vegetable brassicas (Kennedy & Gilles 2003). The disease does not seem to be a concern for *Brassica* vegetable growers in the USA (Walker, 1972).

White blister has occurred on radish crops in Victoria for a number of years. Radish growers in cropping areas close to the coast ceased radish production due to high disease pressure from white blister (Minchinton *et al*., 2004). The survey on white blister of radish identified that irrigation timing can have an influence on the incidence of the disease in the crop. Radish crops irrigated at night had higher levels of white blister than those irrigated in the morning (Minchinton *et al.* 2004).

This chapter reports on a survey of the incidence of white blister, that was conducted to determine the scale of disease in cropping areas at various geographic locations in Victoria and to identify agronomic factors contributing to the possible variation in the disease occurrence between cropping regions.

5.2 Method

A questionnaire on disease history, paddock size, timing of irrigation, and crop nutrition and protection was prepared for each grower (Appendix 5.6). It collected information on the crop prior to the disease survey to identify factors that could have an impact on the disease levels in each crop.

Ten 14-16 weeks old broccoli crops, three in Rosebud, one in Cranbourne, one in Dandenong and five in the Werribee South growing areas were surveyed for the incidence of white blister on the 6th, 7th and 10th of October 2003, respectively (Table 5.1). All broccoli crops surveyed east of the Melbourne metropolitan area (Rosebud, Cranbourne and Dandenong) were cv. Marathon and grown from purchased seedlings and all crops west of Melbourne (Werribee South) were cv. Legacy produced from self-grown seedlings. The area of each paddock surveyed was subdivided into ten equal parts and random numbers were used to allocate the bed number and the starting point for assessment from each of ten parts. Ten and fifteen plants were assessed in beds of two rows and three rows respectively. In total, one hundred plants in paddocks with two rows per bed and one hundred and fifty plants in paddocks with three rows per bed were checked for the presence of white blister pustules on leaves and heads. Incidence of white blister was calculated as a percentage of diseased plants per bed. The type of analysis to be carried out would depend on the data collected.

5.3 Results

The survey could not determine any statistically significant influences of management practices on white blister disease incidence in broccoli crops during the scoping study survey. No analysis of the data from the survey was possible due to the confounding of:
(1) cultivar with regions (we are unable to say if the differences in disease were due to the region or due to the cultivar),
(2) the timing of irrigation with region,
(3) the frequency of chemical controls with region and variety,
(4) the source of seedlings with the region and cultivar.

Generally the incidence of white blister was lower in broccoli crops on the eastern side of Melbourne (0 - 38% incidence, except one crop with 94% incidence) than the western side of Melbourne (56 - 94% incidence) (Table 5.1). Weekly fungicide sprays provided total disease control in one crop (0% incidence) and reduced white blister to trace level in two other crops (7.3 – 9% incidence) in the Rosebud area. Fortnightly fungicide applications reduced the incidence of white blister but not to the levels observed in crops with weekly sprays. The disease level, however, was lower (38% incidence) in one crop in Dandenong where only one fungicide spray was applied when the disease was first noticed. On this property the broccoli crop was grown exclusively in the winter months.

The broccoli crops grown in rotation with one or two other vegetable crops (mainly carrot, celery or lettuce) had lower disease incidences than those grown continuously. Broccoli grown in a 1 in 3 rotations had on average 46.8% less disease than broccoli grown in a 1 in 2 rotation and 62.2% less disease than those continuously cropped. There was not enough data to suggest whether timing of irrigation had an influence on disease levels (data not shown).

Table 5.1 Incidence of white blister in Victorian broccoli crops grown in three major broccoli-growing areas (Rosebud, Cranbourne/Dandenong and Werribee South) during October 2003.

Location	Cultivar	Incidence of white blister (%)	Average incidence (%)/region	Seedling origin	Cropping frequency (rotation)	Average Rotation in region	Fungicide application frequency
Rosebud	Marathon	7.3		purchased	1 in 3		weekly
	Marathon	0.0		purchased	1 in 3		weekly
	Marathon	9.0	5.4	purchased	1 in 3	1/3	fortnightly
Dandenong	Marathon	38.0		purchased	1 in 3		once, when noticed
Cranbourne	Marathon	94.0	66.0	purchased	1 in 2	1/2.5	fortnightly
Werribee	Legacy	94.0		self-grown	continuous		none
South	Legacy	75.3		self-grown	continuous		fortnightly
	Legacy	58.0		self-grown	1 in 3		fortnightly
	Legacy	56.0		self-grown	1 in 2		fortnightly
	Legacy	78.0	72.2	self-grown	1 in 2	1/1.8	fortnightly

5.4 Discussion

Despite confounding of data this survey showed that white blister was present in broccoli crops in all regions surveyed east and west of Melbourne. Werribee South (west of Melbourne) generally had a higher incidence of white blister (72%) than Cranbourne and Dandenong (66%) and Rosebud (5.4%). The characteristics of the Rosebud region are the growing of the cultivar Marathon, purchased seedlings, a larger rotation time out of broccoli, weekly spraying and larger distances between crops.

The cultivar Legacy was derived from Marathon and the industry perceives that these cultivars differ in their yield descriptors, but not in susceptibility to white blister (Dale O'Connor, pers. comm.). In this survey Legacy clearly showed more disease than Marathon, but Marathon was only grown east of Melbourne and

Legacy was only grown west of Melbourne. We are unable to say if the differences in disease are due to the region or due to the practices of the growers in the region, as these practices tend to be region-related or in fact due to the particular variety being grown. The only cultivar with tolerance to white blister is Atomic (also called Viper) which is grown almost exclusively in summer.

Rotation time out of broccoli appeared to influence the investigation and severity of white blister. Rosebud had the lowest incidence of white blister, no evidence of systemic infections (swollen tissues containing oospores) and with one in three crops in a paddock planted to broccoli. Werribee South, which had the highest incidence of white blister, had on average every one to 1.8 crops planted to broccoli in a paddock and systemic infections were common. When the infected crop debris is incorporated back into the soil the oospores could survive for 3 to 4 years and be a source of infection for subsequent crops (Goyal *et al.* 1996). Continuous cropping or growing broccoli in short rotations could be enhancing levels of the disease especially in fields with systemic symptoms.

It is possible that a disease forecasting system for white blister would benefit the timing of fungicide applications. The system developed in the UK (Kennedy & Gilles, 2003) helped to reduce the number of sprays applied to a crop by a half. One of the most interesting pieces of information to come out of the survey is the difference between the white blister incidence in Cranbourne (94%) and Dandenong (38%). The lower levels of white blister at Dandenong was associated with 'one spray or a spray when noticed' and only planting in winter. Unfortunately, growers whose main crop is broccoli can not employ the latter practice. It would also be interesting to know if the weekly sprays were more effective at keeping the systemic symptoms at bay.

Growing seedlings in an area of crop production where the majority of crops are infected with white blister may also have contributed to the higher disease incidence in Werribee South area. It is not uncommon for a crop of broccoli to be planted immediately adjacent to the greenhouse where broccoli seedlings were grown. It would be possible for spores of *A. candida* to spread from the field grown crops to seedlings. The project leader has observed high levels of white blister on self-grown seedlings in greenhouses in Werribee South. Growers in the area prefer to produce their own seedling for economic reasons. If another crop other than broccoli was grown immediately outside the greenhouse, greenhouse seedling infections may be reduced. An additional experiment evaluating self-grown and purchased seedlings should be conducted in Werribee South to determine the impact of this factor on white blister.

The Werribee South cropping region is characterised by the immediate proximity of crops, whereas growers in Rosebud, Dandenong and Cranbourne are either not completely surrounded by other growers and broccoli crops or are separated by some kilometres. Once disease appeared in Werribee South it could easily spread to neighbouring crops and potentially increase the levels of airborne inoculum. Further, broccoli crops grown in Rosebud, Cranbourne and Dandenong are often separated from other vegetable crops on the same farm with tall hedges, which may hinder the spread of spores. In the white blister survey on radish, bunch line growers close to the coast stated they had stopped growing radish due to high disease pressure (Minchinton *et al.* 2004). Similarly Werribee South with the highest incidence of white blister is also right on the coast whereas the other production areas are further inland.

This scoping study survey has highlighted the problems of confounded agronomic factors. We have learned a lot about these confounding factors and any future surveys could be more successfully designed with this knowledge.

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5.6 Appendix

Survey of Victorian broccoli crops for the presence of white blister (*Albugo candida*)

Date	
Grower name	
Location	
Cropping area	
Variety	
Speedlings-Selfgrown or Purchased	
Winter/Summer cropping	
Harvest time?	
Rotation/cropping frequency	
No. of rows in a bed	
Distance between heads	
Irrigation type and timing	
Fertilisers/date and rate application records	
Weed control/application records	
Pest control/application records	
Disease control/application records	
Type of spray unit	
QA	
Monitoring for pests and diseases	
IPM Practices	
Nutrient analysis - foliage	
Nutrient analysis - soil	
Residue tests	
Best time to contact	
Overseas/Interstate/Local work visits?	
Comments	

Crop development in the winter and summer growing seasons

Plant	Emerge	Seedlings	Field (total)	Field (Prehead)	Field (Post head)	Harvest
Summer	4days	6-7 weeks	16 weeks	5-6 weeks	2-3weeks	after 18 weeks
	7days	8 weeks				
Winter	10-12 days	10 weeks	16-22 weeks	9-10 weeks		after 26 weeks
		12 weeks				

Chapter 6

Detection of *Albugo candida* oospores in broccoli seed cv. Green Belt

Joanna Petkowski and Elizabeth Minchinton

6.1 Introduction

An epidemic of white blister, caused by *Albugo candida* (Pers. Ex. Lev.) Kuntze devastated broccoli and cauliflowers crops in the summer of 2001/02 in south eastern Australia. In addition to yield losses, market access was restricted for produce from the disease-affected areas. Several broccoli seedlings with pronounced stem galls entirely filled with oospores were found on plants produced in a grower's nursery in Werribee South in January 2002. This symptom is consistent with seed infestation by oospores.

Seed-borne oospores have the potential to play a critical role in the initiation of field infections. For instance, Petrie (1975, 1978) identified high levels of seed contamination with 28 oospores/g seed of turnip rape (*B. rapa*). Also, 80% of commercial turnip rape seed lots were contaminated with oospores in Canada. Verma and Petrie (1980) demonstrated that inoculation of turnip rape seed with oospores resulted in a significant increase in local and systemic symptoms of white blister on plants in field plots. Circumstantial evidence also pointed to oospore contamination of radish seed as a source of primary infection in Canada, as over 60% of seed lots contained at least 1.8 oospores/gm seed (Petrie, 1986).

Oospores are the survival form of the pathogen and the primary source of inoculum in all *Albugo* species (Verma *et al.,* 1975; Saharan and Verma, 1992). *A. candida* oospores are thick-walled, chocolate brown, globose, 35-50 µm in diameter (Mukerji, 1975) and they release 40-60 biflagellate zoospores that cause infection in the same way as zoospores liberated from sporangia. Oospores are also present in hypertrophied, disease-affected plant parts and can stay dormant in organic debris in soils for 3-4 years (Goyal *et al.,* 1996) and up to 20 years in dry storage (Verma and Petrie, 1980).

The source of the recent Australian epidemic of white blister in broccoli and cauliflower crops remains unknown. The first reports of white blister on broccoli come from Victoria in 1980 and New South Wales in 1990 (Australian Plant Disease Database), but until now, the disease has not been economically important.

These chapter reports on the examination of a seed sample of the most commonly grown broccoli cultivar in Australia to determine whether infested seed could be a source of white blister in broccoli crops.

6.2 Materials and Methods

A sample of 25,000 seeds (125 g) of the commercial broccoli cultivar Green Belt (SPS Lot No. 333 1119 MMM) was selected on the recommendation of the advisory committees and screened for the presence of *A. candida* oospores using a seed washing technique (Petrie, 1975; De Tempe and Binnerts, 1979). The seed, which was coated with the fungicide Thiram®, was divided into 5 g samples. Each sample was agitated on an orbital lab mixer for 2 hours in a solution of 10 ml of deionised water and one drop of Tween 20. The seeds in solution were then transferred to 50 ml Falcon tubes and agitated at high speed on a Vortex mixer for a further 30 sec. Seed samples were separated from wash water on a sieve and gently rinsed with 10 ml of sterile deionised water. All wash water was filtered through millipore Swinnex 25 using 2.5 cm Whatman No.1 filter discs. Filter discs were air dried, cut in half and mounted in mineral oil on microscope slides. Cleared filter discs were observed under a light microscope for the presence of oospores, using 100x and 400x magnifications.

6.3 Results

No oospores of *A. candida* were found in the 25,000 seeds of Broccoli cv Greenbelt from SPS Lot No. 333 1119 MMM.

6.4 Discussion

No *A. candida* oospores were detected in the 25,000 seeds of broccoli tested using the seed wash technique. Petrie (1975, 1978) found large numbers of spores per gram of turnip rape seed and considered the technique to be reliable for screening large numbers of *Brassica* seed samples for the presence of oospores. Therefore, the failure to detect oospores of *A. candida* in the batch of broccoli seed cv. Greenbelt is likely to be due to their absence rather than a flaw in the screening technique and this seed lot was not a source of white blister inoculum. The major disadvantage of this technique was however that it took 5 days to complete. It is therefore time consuming and not a practical method to regularly screen seed lots.

This scoping study examined one batch of 25,000 seeds from a commonly grown cultivar and did not find any oospores. However, it is impossible to rule out the possibility that contaminated seed may have entered the vegetable production areas some time in the past. The strongest evidence for contaminated seed is the discovery of galled seedlings in the early stages of the epidemic. No further galled seedlings have been found and it has not been possible to trace the source of this seed or recover samples. Seed therefore does not appear to be a consistent source of infection, although it may have been associated with the recent outbreak of *A. candida*.

More commercial seed samples of various broccoli and cauliflower varieties as well as small packets of imported garden Brassica vegetable seeds need to be examined, to rule out seed as a source of inoculum. In addition artificial inoculation of seed with varying levels of oospores would determine the threshold levels of infected seed required to initiate an epidemic in the field.

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Chapter 7

Host studies to differentiate races of *Albugo candida*

Joanna Petkowski and Fiona Thomson

7.1 Introduction

White blister, caused by the oomycete *Albugo candida* (Pers. ex. Lev.) Kuntze, is an important disease that affects a wide range of cruciferous hosts, including vegetable Brassicas. The pathogen's high level of biological specialisation is well established. Pound and Williams (1963) described six races of *A. candida*, each occurring on a different species of crucifer. Their host set, which has since been supplemented by a number of authors, forms the basis for present-day race classification of *A. candida* (Table 1). Race seven (AC7) was identified by Verma *et al.* (1975) on *Brassica rapa* (*B. campestris*), AC8 by Delwiche and Williams (1977) on *B. nigra*, AC9 and AC10 by Hill *et al.* (1988) on *B. oleracea* and *B. kaber* respectively and AC11 on *B. carinata* by Williams (1985). The virulence of races AC1 to AC11 is based on American pathotypes (Verma *et al*., 1999). Unfortunately, *A. candida* races have not been classified on a set of internationally standardised host differentials, for which there is a great need (Saharan and Verma, 1992; Verma *et al.,* 1999).

Table 7.1 List of *A. candida* races (AC) and their hosts.

AC Race	Host scientific name	Host common name
1	*Raphanus sativus L.*	radish
2	*Brassica juncea (L.) Coss.*	Indian mustard
3	*Armoracia rusticana Gaertn., Mey., & Scherb.*	horseradish
4	*Capsella bursa-pastoris (L.) Medik.*	Shepherd's purse
5	*Sisymbrium officinale (L.) Scop.*	hedge/tumble mustard
6	*Rorippa islandica (L.) Bess.*	marsh/yellow watercress
7	*B.rapa L. (campestris, Pekinensis, Chinensis)*	field mustard, Chinese mustard, Chinese cabbage, pak-choi
8	*B.nigra (L.) Koch*	black mustard
9	*B.oleracea L.*	broccoli, Brussels sprouts, cauliflower, kale, kohlrabi, cabbage
10	*B.kaber (DC.) Wheeler (Sinapis arvensis)*	charlock

Canola is *B. napus* and appears to be immune to *A. candida* (A. Gurung pers. comm., Petrie, 1980)

This chapter reports on the preliminary identification of the *A. candida* race causing white blister of broccoli in Australia.

7.2 Materials and methods

7.2.1 Trial design

Two differential host experiments were established. The first experiment was set up in two growth cabinets and the second experiment was set up in a glasshouse. The controlled environment in the growth cabinets consisted of day/night temperature set at 17/22°C and 85% relative humidity. In the glasshouse the temperature ranged from 17°C at night to 25°C during the day. Seed was sown on the 25/3/04 for the growth cabinet trial and on the 5/4/04 for the glasshouse trial.

Twelve Brassicaceae species or varieties (Table 1), some corresponding to species used by Pound and Williams (1963), Verma *et al.* (1975), Delwiche and Williams (1977), and Hill *et al.* (1988), were used in both trials. The only exceptions were that pak choi (*B. rapa*) was used in the glasshouse trial while fodder rape (*B. napus*) was used in the growth cabinet experiment. Seeds were sown in potting mix in Lannan trays, consisting of 144 cells in a 12x12 matrix. Each column in the Lannan tray was sown with a seed of the same differential host so that the 12 differential hosts were represented in each of one column. In the trials each Lannon tray represented a block, which was replicated 6 times, in a randomised complete block design. In the growth cabinet trial there were 3 Lannon trays in each growth cabinet, whilst in the glasshouse trial 3 Lannon trays were arranged on each side of a double bench. Seedlings were irrigated twice per day for 1 min. by overhead sprinklers. Seedlings were fertilised weekly using Aquasol™ solution at 5g/10L.

7.2.2 Preparation and application of the spore suspension

A spore suspension was prepared by collecting zoosporangia with a cyclone spore collector from sori on fresh broccoli leaves. A single plant was used as a source of inoculum. Zoosporangia were suspended in distilled water and the concentration adjusted to $1x10^4$ zoosporangia/ml. Seedlings were inoculation at the fully developed cotyledon stage. Seedlings in the growth cabinet trial were inoculated on the 5/4/04 and the 10/4/04 whilst those in the glasshouse were inoculated on the 19/4/04 and the 27/4/04 using a hand held atomiser. After each inoculation, trays with seedlings were covered with plastic sheets for 48 hours to ensure sufficient leaf wetness for infection.

7.2.3 Assessment

The incidence of white blister (percent of plants infected) on the host seedlings was assessed on both cotyledons and seedling leaves on 30/4/04 (growth cabinet trial) and on the 3/5/04 (glasshouse trial). Data was analysed using analysis of variance within GenStat 7.1, Lawes Agricultural trust (Rothamsted Experimental Station).

7.3 Results

7.3.1 Trials and tribulations (Table 7.2)

Seedlings in the growth cabinets grew poorly and were etiolated. Generally, the incidence of white blister on seedlings was lower and more variable in the growth cabinet trial than in the glasshouse trial. The disease incidence data collected from fodder rape (*B. napus*) was excluded from the analysis due to a poor germination ratio of only 4% (3 out of 72). There were significant differences between the incidence of white blister on *B. oleracea* (cauliflower and broccoli) in the cabinet trial but not in the glasshouse trial. Similarly, there was a significant difference between the incidence of white blister on *B. oleracea* (broccoli) and *B. nigra* in the cabinets, but not in the glasshouse trial. *B. juncea* was not susceptible in the cabinet but was in the glasshouse trial. *B. oleracea* (cabbage) was consistently resistant in both trials. The other 7 host differentials (*R. sativus, C. bursa-pastoris, R. islandica*), three of *Brassica rapa* (pak choi, Chinese cabbage, and turnip) and *B. alba* did not show any white blister symptoms in either experiment.

Table 7.2 Incidence of white blister on differential host seedlings in growth cabinets and glasshouse experiments

Host scientific name	Host common name	Host cultivar name	Incidence of white blister (%)		Severity of white blister (scale 0-5)	Hill *et al* (1988)[d]
			Growth cabinets	Glasshouse		
Raphanus sativus	radish	Radar	0	0	0	0
Brassica juncea	Indian mustard	Giant Curl	0	6.9	1	1
Capsella bursa-pastoris	shepherds purse	-	0	0	0	-
Rorippa islandica	water cress	True Water	0	0	0	-
B. napus [a]	fodder rape	Hobson	66.7 [c]	-	-	5
B. rapa [b]	pak choi	Mei Quing Choi	-	0	0	1
B. rapa	turnip	Tap	0	0	0	1
B. rapa	Chinese cabbage	Matilda	0	0	0	1
B. nigra	Black mustard	-	7.1	73.7	5	1
B. oleracea	cabbage	Atlas	0	0	0	9
B. oleracea	cauliflower	Prestige NS	14.2	79.7	1	9
B. oleracea	broccoli	Green Belt	50.8	78.4	5	9
B. hirta (B. alba)	white mustard	White/Yellow	0	0	0	100%[e]
l.s.d (5%)			19.4	13.6		-

[a] Seedlings of fodder rape were grown in growth cabinets
[b] Seedlings of pak choi were grown in the glasshouse
[c] Poor germination of seedlings
[d] Early differential host work on a scale where 9 = fully susceptible and 0 = no infection
[e] Pound and Williams (1963), not included in Hill *et al.* (1988)

7.4 Discussion

The results of this preliminary investigation into the host specialisation of the *A. candida* race causing white blister in Victorian broccoli crops are inconclusive. The results do however partially correspond with the assumption that the Victorian isolate belongs to AC9, based on the classification system of Hill *et al*. (1988). The high incidence of white blister on broccoli and cauliflower is consistent with that reported by Hill *et al*. (1988), who observed a 'Disease Index' score of 9 for rapid-cycling populations of *B. oleracea*. In contrast the results appear to differ as (1) cabbage (*B. oleracea*) was immune, (2) cauliflower (*B. oleracea*) had a low severity and (3) *B. nigra* had a higher than expected severity and disease incidence of white blister.

The difficulties in conducting and interpreting this trial were:
1. The original description of the race 9 of *A. candida* was based on the disease index in 'rapid cycling ' populations of *B. oleracea* with known genetic make-up (Hill *et al*., 1988).
2. The rating system used to define susceptibility to white blister varies between authors.

It was not possible to obtain the exact set of host genera and species used by Pound and Williams (1963) and Hill *et al*. (1988) to describe AC1, 2, 3, 4, 5 and 6 or AC1, 2, 7, 8, 9, and 10, respectively. In fact it was impossible to obtain seeds of *S. officinale* and *A. rusticana*. The host set used was substituted with lines as similar as possible to the original as advised by local plant breeders and resellers. Work in this area needs a defined, readily available set of hosts, with known genetic profiles. Also Pound and Williams (1963) recorded +/- sporulation and disease incidence, whilst Hill *et al*., (1988) recorded infection on a scale of 0-9, based on Williams (1985). It has been impossible to locate a complete description of this scale.

The lack of any symptom development on cabbage is inconsistent with its susceptibility to *A. candida* isolates in Europe (Gilijamse *et al.* 1998) and the Disease Index score of 9 on rapid-cycling populations of *B. oleracea* reported by Hill *et al*., (1988). Our isolate did not cause disease on cabbage, which may indicate a specialisation towards broccoli and cauliflower or variation within AC9. Petrie (1994) reported two forms of AC2 and AC7. In fact, more recent race nomenclature has moved away from only one race occurring on each *Brassicaceae* species, as AC14-17 have been described from various cultivars of *B. juncea* (Gupta and Saharan (2002).

The high disease incidence on broccoli is unlikely to be due to oospore contamination of seed of cv. Greenbelt as no oospores were detected in the seed wash (Chapter 6) and the same batch and packet of seeds were used in the host studies trial. To avoid the possibility of oospore contamination of seed influencing the outcome of trials in the future, trays of seedlings of the various hosts need to be grown a the same time but maintained in a different glasshouse to the trial, to avoid cross contamination.

The variation in incidence of white blister on the *B. oleracea* hosts cauliflower and broccoli within the growth cabinets and between the growth cabinets and glasshouse, may be attributable to less than optimal growth conditions in the former, such as lower light intensity than that of sunlight. It is reasonable to expect that higher scores would be achieved with optimised glasshouse growth conditions. Pound and Williams (1963) also found that symptom development was better when plants were growing more 'luxuriantly' in the glasshouse.

The high incidence and severity of white blister on *B. nigra* in the glasshouse experiment is not typical of AC9. Hill *et al*., (1988) reported a Disease Index score of 1 on *B. nigra* using rapid-cycling populations of these *Brassica* species. The possibility of seed contamination with oospores cannot be discounted in the case of *B. nigra*. In-fact when 4 samples of 5 gm each of the *B. nigra* seed was checked for oospores following the method described in Chapter 6, on average 11 oospores/5g seed were detected. It is unknown at present if this would be enough to produce 80% incidence of white blister in seedlings. Verma and Petrie (1980) reported that seed contaminated with oospores could lead to localised or systemic infection on seedlings of turnip rape.

Future work should focus on cross-inoculation experiments to determine possible sources of inoculum for *B. oleracea* hosts such as broccoli or cauliflower. For instance, white blister commonly occurs on radish (*R. sativus*), shepherd's purse (*C. bursa-pastoris)*, and Chinese cabbage and pak choi (*B. rapa*) in market garden areas around Melbourne. It would be interesting to know if spores from *R. sativus* AC1, *C. bursa-pastoris* AC4, *B. rapa* AC7 and possibly *B. nigra* AC8 are as pathogenic as AC9 on a range of *B. oleracea* cultivars (e.g. broccoli, cauliflower and Brussels sprouts). Such cross inoculation could determine if other races are a source of inoculum for broccoli in Australia. Some populations of *B. oleracea var. italica* (broccoli) cv. Green Mountain were found susceptible to AC1 from radish (Pound and Williams, 1963). European isolate of *A.candida* from *C. bursa-pastoris* caused disease in seedlings of Brussels sprouts and cauliflower (Gilijamse *et al.* 1998). In addition, *B. alba* formally *B. hirta* (white mustard) is susceptible to AC1, AC2, AC3, AC4, AC5 and AC6 (Pound and Williams, 1963), while *S. officinale* is susceptible to AC2, AC3, AC5 and to a lesser degree to AC1, AC4 and AC6 (Pound and Williams 1963). Both can therefore be considered universal hosts for several races and potential reservoirs of inoculum. Fortunately neither *B. alba* nor *S. officinale* are seen in Victorian market gardens.

7.5 References

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Chapter 8

Molecular differentiation and detection of *Albugo candida*.

Robert Faggian and Joanna Petkowski

8.1 Introduction

The sudden outbreak of severe white blister infection in broccoli led to interstate bans on Victorian fresh produce and seedlings. This was in part because of suspicion that the outbreak represented a new race of the pathogen not previously encountered in other states.

A. candida has been divided into 17 races using differential host experiments (see Chapter 7). Race differentiation using hosts is a technically cumbersome and time-consuming method that is difficult to replicate because 1) the original host varieties are not readily available and 2) disease expression varies widely depending on plant growth conditions and the heterogeneous nature of the differential hosts. It also relies on having large amounts of fresh inoculum, which does not allow the differential host method to be used as a rapid or pre-emptive screening test to ascertain race status.

The goals of this component of the scoping study were therefore 1) to investigate a new means to differentiate races of *A. candida* using molecular biology tools and 2) to develop a detection assay for seed and other sources of contamination.

There has been no published work done in the area of molecular race differentiation, probably because the pathogen is an obligate biotroph and it is therefore difficult to obtain pure DNA. Eric Holub's group at Horticulture Research International (HRI) in the U.K. have sequenced the ribosomal RNA genes from the eleven main races (unpublished data) but found that they only divided into two groups, not eleven as hoped. Alternatively, a diagnostic DNA-based test has already been developed for the detection of *A. candida* and was available in the public domain prior to the commencement of this project.

8.1.1 Race Differentiation

The challenge posed was to investigate the use of molecular biology tools for the differentiation of *A. candida* races, within the framework of a twelve-month scoping study. This necessitated striking a balance between high-risk approaches that may or may not yield successful protocols (assuming the research would not be supported beyond the scoping study) and longer-term approaches with a greater chance of success (assuming the research would be supported beyond the scoping study). As a result, two different lines of investigation were followed: 1) sequencing of phylogenetically important genes in *A. candida* and discovery of new genes or other genomic DNA fragments for identification of race-specific polymorphisms and 2) development of DNA fingerprinting techniques (Fig 8.1).

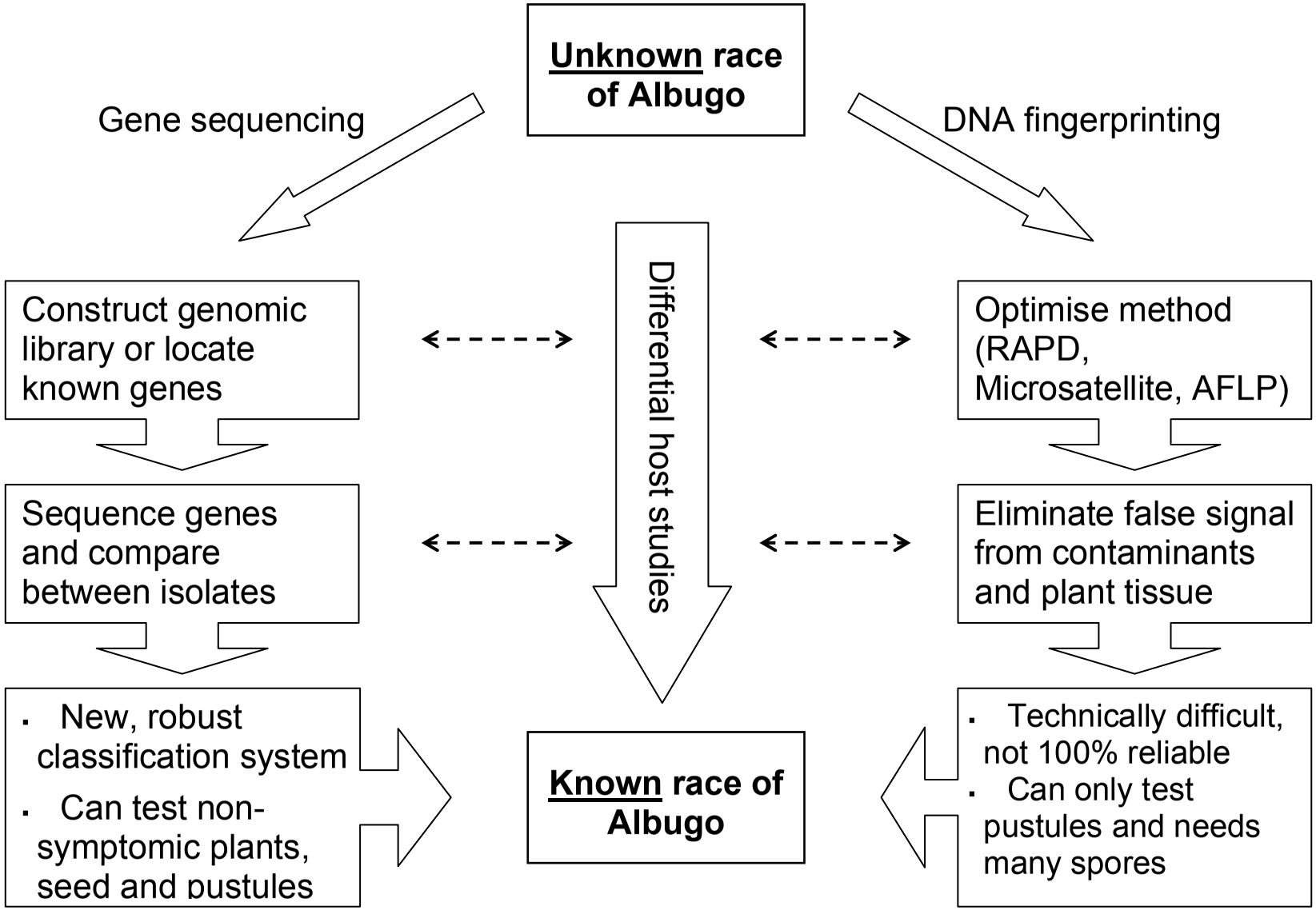

Fig. 8.1 Flowchart illustrating methods to achieve race differentiation of *A. candida*

The advantage of following the gene-sequencing/gene library route is that success would facilitate the development of a technically simple and specific test that could be used on infected tissue, non-symptomatic tissue, seed and fungal material. The major disadvantage of this approach is that it requires a methodical experimental search for informative genes or DNA fragments, which could take anywhere up to three years.

Alternatively, the DNA fingerprinting approach is quick to develop and in theory can be used to immediately determine whether two or more isolates differ. However, because *A. candida* cannot be cultured and therefore pure inoculum is not available, the technique is prone to error from contaminating organisms, is often difficult to reproduce and can only be used when large amounts of fungal material are available.

8.2 Methods

8.2.1 Degenerate primer design

Degenerate primers were designed for two genes, actin and β-tubulin. Complete coding region sequences or messenger RNA sequences from a number of oomycetes, true fungi and other eukaryotes were obtained from GeneBank and aligned using bioinformatics software (for example, Fig. 8.2). The multiple sequence alignments were used to identify suitably conserved regions for PCR primer design. Primers were designed to span one or more genetically variable regions of the target gene. A third gene was also targeted, histone, but the constraints imposed on the design process resulted in no viable primer pairs being identified.

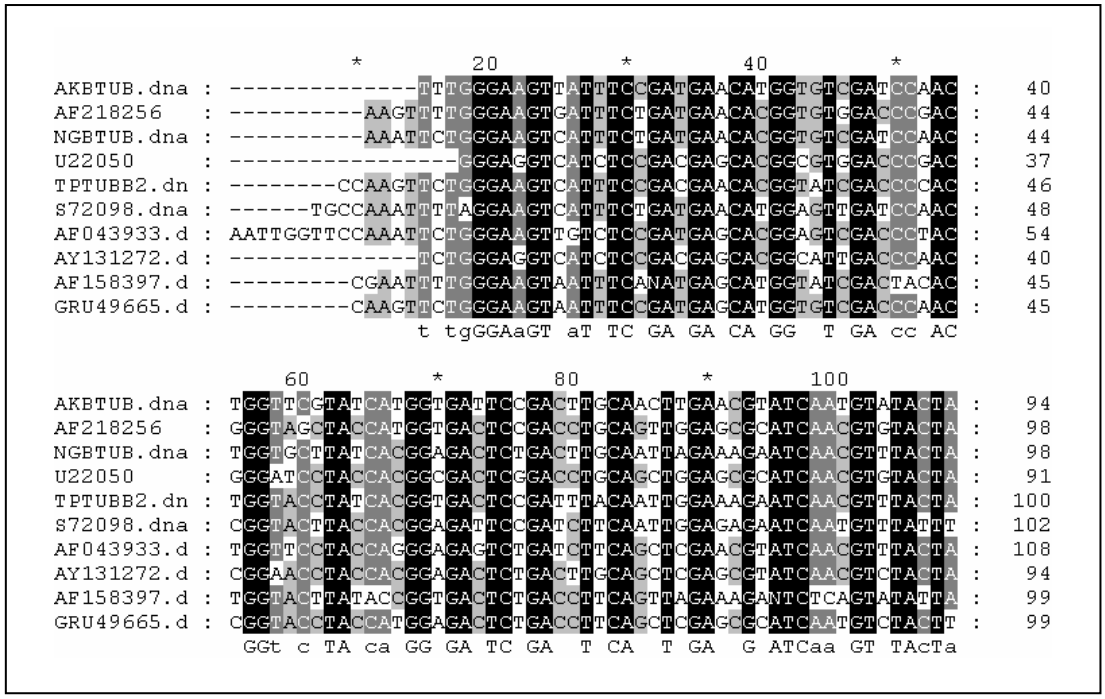

Fig. 8.2 Partial multiple sequence alignment showing tubulin gene homology between species. At each nucleotide position, black shading represents 100% homology, dark grey represents 80-99% homology and light grey represents 50-80% homology. Sequence codes: AKTUB = *Achlya klebsiana*, AF218256 = *Pythium ultimum*, NGBTUB = *Naegleria gruberi*, U22050 = *Phytophthora cinnamomi*, TPTUBB2 = *Tetrahymena pyriformis*, S72098 = *Euplotes focardii*, AF043933 = *Bigelowiella natans*, AY131272 = *Chytriomyces confervae*, AF158397 = *Glomus geosporum*, GRU49665 = *Gigaspora rosea*

8.2.2 DNA extraction procedures

A. candida zoosporangia were collected from pustules on infected broccoli (*Brassica oleracea* var. *italica*), Chinese cabbage (*B rapa* var. *chinensis*), radish (R*aphanus sativus*), wild radish (*Raphanus raphanistrum*) and Shepherd's purse (*Capsella bursa-pastoris*) using 1.5 ml Eppendorf tubes attached to a circular spore collector and stored at –20°C for no longer than 3 months. DNA was extracted from zoosporangia using four procedures; 1) the FastPrep Soil extraction kit (Q-Biogene), 2) the DNeasy mini kit (Qiagen), 3) the MoBio Soil kit (MoBio) and the method of Dellaporta *et al*., (1983).

DNA was extracted from plant tissue and whole diamondback moths (*Plutella xylostella*) moths and using the FastPrep Soil extraction kit (Q-Biogene).

8.2.3 Rapid Diagnostic Test for *A. candida*

PCR primer pair AC13 and AC28 (Table 1) for the detection of *A. candida*, developed by Jacobson *et al*. (1998), were tested for their ability to detect Australian *A. candida* isolates, as well as *A. candida* in symptomatic and non-symptomatic plant tissue, and *A. candida* on artificially inoculated diamondback moths.

DNA was extracted from lesion-containing broccoli leaf tissue, non-symptomatic tissue on the same leaf, leaf tissue from healthy plants and broccoli seeds. Also, the heads of individual diamondback moths were gently rubbed in leaf lesions and whole moths were then subjected to DNA extraction procedures, as well as 30 samples of moths trapped within an infected broccoli crop (which were not artificially contaminated with zoosporangia).

8.2.4 Genomic Library

Approximately 100mg of *A. candida* zoosporangia were collected from pustules on axillary shoots of an infected broccoli plant. DNA was extracted from the zoosporangia using the FastPrep Soil extraction kit (Q-Biogene). Whole genome DNA was amplified using GenomiPhi and cloned into plasmid vectors using the TOPO shotgun subcloning kit (Invitrogen). The presence of insert-containing plasmids in *E.coli* hosts was tested by PCR, and successful clones were selected for downstream processing and sequencing. A total of 192 clones were sequenced from both directions and analysed by comparison with existing entries in GeneBank.

8.2.5 DNA fingerprinting

A Randomly Amplified Polymorphic DNA (RAPD) PCR protocol was optimised in terms of primer sequence as well as concentration of primer, template DNA and $MgCl_2$. The protocol was used to generate a DNA fingerprint profile for the isolate that was tentatively assigned to race 9, based on host differential studies.

8.3 Results & Discussion

8.3.1 Gene sequencing

This approach relies on genes that code for informative macromolecules that are ubiquitous and whose structure and function have not changed greatly through the evolutionary process. Macromolecules that have proven suitable for this purpose include ribosomal RNA (Sogin, 1989; Forster *et al*., 1990), actin (Jimenez-Gasco *et al*., 2002) and tubulin (Keeling & Doolittle, 1996), among others.

The task can be straightforward if the gene has already been sequenced in the target species, as it is simply a matter of designing PCR primers to amplify the gene segment of interest. However, in the case of *A. candida*, the only existing sequence data available was for the ribosomal RNA gene, which has already been shown to be uninformative. It was therefore necessary to infer the likely sequence of other genes in *A. candida* (i.e. actin, tubulin and histone) using the gene sequences from different organisms.

Three pairs of degenerate primers were designed (Table 8.1). The actin primer-pair successfully directed PCR amplification from two *A. candida* isolate (broccoli and wild radish), but not from a third (Chinese cabbage) (Fig. 8.3). Attempts to sequence the amplification products directly resulted in poor data from which further primers could not be designed. Given that there seem to be differences in the efficiency of amplification of the actin gene, it is reasonable to assume that sequence differences exist between the isolates. These sequence differences may prove useful for differentiating *A. candida* races. The amplification products generated with the actin primers will therefore need to be cloned to enable successful sequencing to occur.

PCR amplification using the other primer pairs did not produce amplicons with *A. candida* DNA.

Table 8.1 Primers used for gene sequencing and detection of *A. candida*.

Primer	**Sequence**	**Length**	**Melting Temperature (°C)**
Ac13	5′ CCA CTG CTG AAA GTT TGT GGA 3′	21	61.1
Ac28	5′ CTA TCC ACG TGA ACC ACT TTG 3′	21	57.9
ACT01	5′ ACC AAC TGG GAC GA(T/C) ATG GA 3′	20	~62
ACT02	5′ CCA CC(G/A) ATC CAG ACI GAG TA 3′	20	~58
BTUB01	5′ TCA ACG TIT ACT ACA ACG AG 3′	20	~50
BTUB02	5′ CGA AAC C(G/A)A TCA TGA AGA A 3′	19	~55
BTUB03	5′ TAC CAG TGC AAG AAI GC 3′	17	~52
BTUB04	5′ GGI AAC AAI TGG GCC AAG GG	20	~60

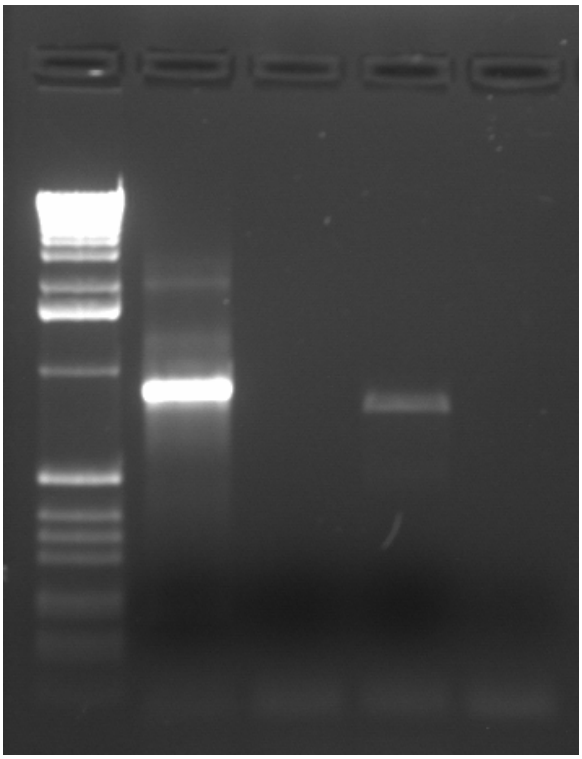

Fig. 8.3 Amplification of actin gene fragments from *A. candida*: broccoli (*B. oleracea*) isolate (lane 1), Chinese cabbage (*B. rapa)* isolate (lane 2), wild radish (*R. raphanistrum*) isolate (lane 3) and negative control (lane 4).

Given that the most likely gene candidates did not produce immediate and satisfactory results, this approach was abandoned. Instead, a gene library was constructed to maximise the data that could be analysed in the time available.

8.3.2 Gene Library

The gene library yielded 192 cloned and sequenced fragments of DNA that was extracted from zoosporangia. Initial analysis indicates a range of genes, some with no homologous relatives in the public sequence databases, others known and others still that are of bacterial or plant origin.

The diverse set of DNA fragments contained within the genomic library is as expected. *A. candida* is an obligate biotroph meaning it is impossible to culture the organism to obtain pure starting material for DNA extraction. *A. candida* material must therefore be collected from leaf-surface pustules, which are likely to contain dozens of species of phyllosphere and contaminating bacteria and fungi. Therefore DNA extracted from pustule material will consist of a mixed population that is largely *A. candida* DNA but also other contaminating DNA.

The clones need to be analysed further to identify all known genes contained within the library. These will complement existing publicly available sequence data and allow the development of new primer pairs that are specific for *A. candida*. It is hoped that a combination of several polymorphism-containing genes can be assembled to create a classification matrix, using the ITS region (which divides *A. candida* into two distinct groups) as a starting point.

8.3.3 DNA Fingerprinting

The RAPD PCR technique is a random and non-specific process that does not require any prior knowledge of the DNA sequence of the target organism. Any DNA that is present in the sample has the potential to contribute to the eventual fingerprint pattern that is produced. Therefore, a sample that potentially contains DNA from more than one species, such as zoosporangia collected from infected leaves, can give misleading results. In this case, only a single isolate was used due to time constraints, but the results were consistent after repeated tests .

However, the inability to discount spurious PCR products from contaminating DNA means that any pathotype-differentiation using this technique will be difficult to defend should it be subjected to scientific scrutiny.

8.3.4 Rapid Diagnostic Test

The diagnostic PCR primers, which were developed using only a single Canadian isolate, successfully detected all Australian isolates.

The sensitivity of the assay was not assessed, but it was used to detect the presence of *A. candida* in non-symptomatic leaf tissue (Fig. 8.4), indicating a level of sensitivity that may prove useful for applications such as seed and seedling tests.

A. candida was not detected on diamondback moths that were trapped within an infected crop. However, *A. candida* was detected on artificially inoculated moths that were rubbed in lesions (which resulted in few to several zoosporangia adhering to the moth surface). This gave another indication that the level of sensitivity of the test is high and will have many potential applications if it can be enhanced to discriminate between races.

Several commercial broccoli seed lots were also tested but *A. candida* was not detected. Again, controls that were artificially contaminated tested positive.

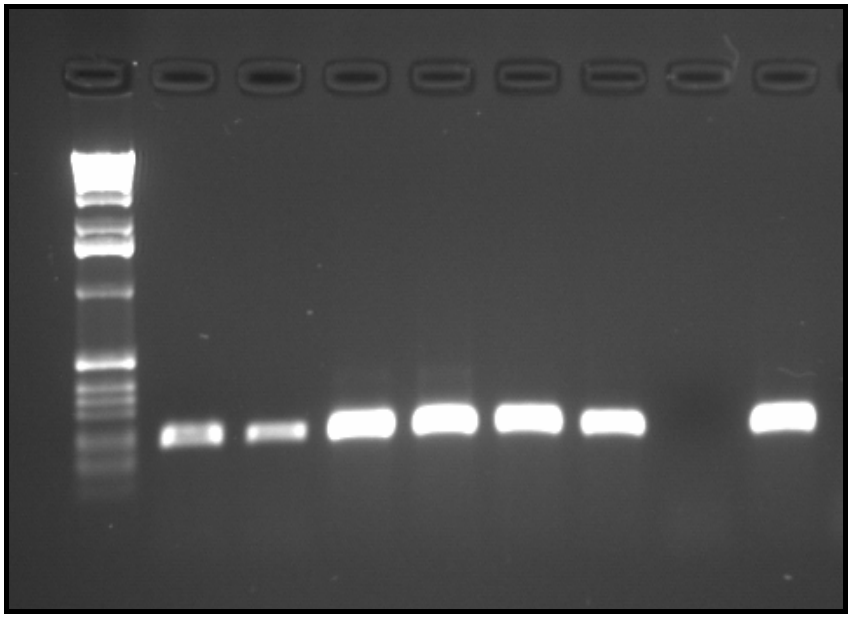

Fig. 8.4 Detection of *A. candida* in: non-symptomatic leaf tissue from an infected plant (lanes 1 & 2), leaf-lesion tissue (lanes 3 & 4), scrapings from leaf-lesions (lanes 5 & 6), negative control (lane 7) and positive control (lane 8).

8.4 References

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Chapter 9

Efficacy of chemicals to control white blister

Elizabeth Minchinton, Joanna Petkowski, Fiona Thomson and Savitri Nadesan

9.1 Introduction

Since the summer of 2002, white blister has become a devastating disease of broccoli and cauliflower, causing up to 100% crop losses and exhibiting both localised and systemic infections. When this project was conceived no chemicals were registered for white blister on brassicas in Australia. Most work on chemical control of *Albugo candida* in the literature has been on seed, mustards, *Eruca sativa* (rocket), radish and horseradish. There are few reports of chemical control work in broccoli or other brassicas in the *Brassica oleracea* group in recent times.

Chemicals trialed for *A. candida* control of turnip, radish, rocket, mustards and spinach were a number of systemic and contact fungicides, surfactants and an activator of plant defence systems. The systemic and contact fungicides trialed have come from Fungicide Activity Groups A, C, D, E, K, X and Y (Avcare). These fungicides include metalaxyl, metalaxyl+mancozeb, oxadixyl, cymoxanil, dimethomoph, benalaxyl, copper oxychloride, thiram, ziram, mancozeb, iprodione, triadimefon, captan, triadimefon, propineb, sulphur, zineb+copper, captafol, calixin, benomyl, carbendazim, thiophanate-methyl, azoxystrobin, fluazinan, chlorothalonil, dichlofluanid (Arvinder Kaur and Kolte, 2001; Dubey 1996; Dueck and Stone, 1979; Glaeser, 1973; Godika *et al*., 2001; Godika and Pathak 2002; Gupta *et al*., 1977; Khangura and Sokhi 2000; Khunti *et al*., 2001; Kumar, 1996; Macias and Robak, 1999; Pandya *et al*., 2000; Stone *et al*., 1987a). All except benomyl (Gupta *et al*., 1977) have been reported to have a degree of efficacy against *A. candida* on the previously mentioned hosts.

Consecutive foliage application of contact fungicides controlled localised foliage symptoms on turnip rape and radish (Dueck and Stone, 1979; Glaeser, 1973) but not systemic infections (Dueck and Stone, 1979). The most effective controls were metalaxyl-based fungicides, azoxystrobin or thiophanate-methyl, often used in combination with contact fungicides (Khunti *et al*., 2001; Godika and Pathak, 2002; Godika *et al*., 2001; Kumar, 1996; Macias and Robak, 1999; Dubey, 1996). The activator of plant defence systems, benzothiadiazole, gave good control of systemic and foliage symptoms on mustards and radish, respectively (Arvinder Kaur and Kolte, 2001; Laun, 1998). Unfortunately its availability is limited and its registration is uncertain. The surfactants Naiad and sodium dodecyl sulphate were as effective as fungicides in controlling white blister on spinach (Irish *et al*., 2002). The application times for most of the fungicide spray programs were at 15 to 20 day intervals (Khangura and Sokhi, 2000; Gupta *et al*., 1977; Kumar 1996; Dubey, 1996; Macias and Robak, 1999). The economics of these spray programs, which were developed for mustards, have been well documented (Godika and Pathak, 2002; Kumar, 1996; Dubey, 1996; Bharagava *et al*., 1996).

Although seed dressings with fungicides have efficacy for white blister control they do not provide sufficient reduction in disease levels and need to be followed up with foliage fungicide applications to maximise disease control. Stone *et al*. (1987a) demonstrated that metalaxyl (Apron) had potential as a seed treatment as it could be found in seedling leaves. However, when metalaxyl 50WP or metalaxyl SD-35 (Apron) was used as seed dressings they gave only up to 12% or 40% control of white blister on foliage of turnip rape. But additional sprays of either Ridomil MZ, chlorothalonil or mancozeb reduced white blister incidence by a further 60% to 100% in field trials (Stone *et al*., 1987b; Bharagava *et al*., 1997). Mancozeb behaved similarly to metalaxyl SD-35 (Apron) when trialed as a seed treatment (Bharagava *et al*., 1997). Seed dressings of metalaxyl (Apron Combi FS) were not effective for control white blister on spring oil seed rape in Scotland (Coll *et al*., 1998) and soil drenching of seedlings with metalaxyl 50WP was phytotoxic to seedlings of mustard (Stone *et al*., 1987b).

This chapter reports on evaluation of the efficacy of systemic, contact and soft chemicals (those with short or no withholding periods), for control of white blister on seedlings, transplants and heads of broccoli during 2003-2004.

9.2 Material and Methods

9.2.1 Seedling trial

Broccoli seedlings of cv Greenbelt grown in Lannan trays were supplied, courtesy of Boomaroo Nurseries Lara Vic, at 10 days of age (cotyledon stage). The Lannan trays, which contained 144 seedlings, were laid out in a randomised block design in a glasshouse at DPI Knoxfield on the 1/9/03. There were 6 blocks each containing 10 trays representing each of 10 treatments (plots). Plants were fertilised weekly with Aquasol™ (5g/10L) and irrigated twice per day for 1min by overhead sprinklers. The temperature in the glasshouse ranged from 17^{o}C at night to 25^{o}C during the day.

9.2.1.1 Chemicals and their application

There were 9 fungicide treatments and a water control (Table 9.1). Seedlings were sprayed with fungicides starting at 4/9/03 (week 1) and weekly thereafter till 2/10/2003 (week 5) (Table 9.2). Chemicals were applied with a single hollow cone nozzle SPX brown No 12, at 30psi by a Silvan Selectra 12v knapsack (Silvan pumps and Sprayers (Aus) Pty. Ltd.). Fungicides were applied at a volume of 1000L/ha.

Table 9.1 Chemicals and rates of application for the seedling, transplant and head trial

Trade name	Active ingredient	Company	Rate
Acrobat®	dimethomorph	BASF	2 kg/ha
Agri-Fos® 600	phosphonic acid	AgriChem	3L/100L
Amistar®	azoxystrobin	CropCare (Syngenta)	60g/100L – 1000L/ha
Barrack®	chlorothalonil	CropCare(Syngenta)	3L/ha
Baycor 300EC®	bitertanol	Bayer	100ml/100L
Vibrex™	chlorine dioxide	Grayson	50ppm head trial
Vibrex™	chlorine dioxide	Grayson	100ppm transplant trial
Dithane DF®	mancozeb	Dow AgroScience	1.7-2.2kg/ha
Dithane DF®	mancozeb with acrobat	Dow AgroScience	1.6kg/ha _mancozeb 750
Euparen®	dichlofuranid	Bayer	2 kg/ha
F51601f	boscalid & pyraclostrobin	BASF	100ml/100L
Fruvit®	propioneb & oxadixyl	Bayer	250g/100L
Kocide®	copper oxychloride	Brycop	150g/100L
Li700™	soyal phospholypids & propionic acid	Nufarm	250ml/100L
Plantvax®	oxycarboxin	Crompton Specialties	20g in 15L knapsack
Ridomil Gold MZ®	mancozeb & metalaxyl	Novartis	2.5kg/ha/1000L
Thiovit®	sulphur	Novartis	200g/100L
SDS	sodium dodecyl sulphate	Sigma	2g/L
Zineb®	zineb	Barmac	2kg/ha

Table 9.2 Treatment schedule for the seedling trial

Chemical treatments	Week of application (date) 1 (4/09/2003)	2 (10/09/2003)	3 (17/09/2003)	4 (24/09/2003)	5 (1/10/2003)
Control	water	water	water	water	water
Amistar	Amistar	Amistar	water	water	water
Amistar/Acroba+Dithane	Amistar	Amistar	Acrobat+Dithane	Acrobat+Dithane	Amistar
Amistar/Bravo	Amistar	Amistar	Bravo	Bravo	Bravo
Amistar/Euparen	Amistar	Amistar	Euparen	Euparen	Euparen
Amistar/Zineb	Amistar	Amistar	Zineb	Zineb	Zineb
AgriFos600 + Euparen	AgriFos600 + Euparen	AgriFos600 + Euparen	AgriFos600 + Euparen	AgriFos600 + Euparen	AgriFos600 + Euparen
Plantvax	Plantvax	Plantvax	Plantvax	Plantvax	Plantvax
Sporekill	Sporekill	Sporekill	Sporekill	Sporekill	Sporekill
Sulphur	Sulphur	Sulphur	Sulphur	Sulphur	Sulphur

9.2.1.2 Preparation and application of A. candida inoculum

Seedlings were inoculated with a sporangial suspension (10^4 zoosporangia/ml) 24 hours after the fungicide application. The sporangial suspension was prepared by sucking zoosporangia from blisters with a cyclone spore collector, suspending them in distilled water, adjusting the suspension to $1x10^4$ zoosporangia/ml and applying to plants with a hand-held atomiser. Plants were covered with plastic sheets for 48 hours to ensure infection.

9.2.1.3 Assessments

Seedlings were examined for the presence of white blister from week 4 (25/9/03) to week 7 (16/9/03). White blister was determined as the percentage of plants with white blister symptoms on foliage. The data were analysed by ANOVA.

9.2.2 Transplant trial

The transplant trial was preformed in two stages, transplant to button stage and button stage to harvest. The trial site was located at Whites Road, Werribee South, Vic. Speedlings of cv Greenbelt produced by the grower were transplanted at 3 rows per bed with an outside spacing of 33cm and an inside spacing of 38cm on 17/11/2003. The trial was laid out in a randomised block design and covered 6 beds with each bed representing a block or replicate. Along each bed there were 9 plots (10.2m x 1.68m) representing each of 9 treatments.

9.2.2.1 Chemicals and application

There were 8 chemical treatments and a water control (Table 9.3). Treatments (Table 9.1) for the first stage of the trial (transplant to button stage) commenced on 21/11/03 and were applied weekly for 4 weeks. Treatments were applied with 3 hollow cone nozzles mounted on a boom as previously described. Fungicides were applied at a volume of 1000L/ha. A barrier of black plastic 1m high by 3m long and reinforced with aluminium stakes was constructed and placed at the end of plots to prevent drift. Treatments for the second stage of the trial (button stage to harvest) commenced on 16/12/03 and were applied weekly thereafter for 11 weeks, as previously described (Table 9.3).

Table 9.3 Treatment schedule for the transplant trial

Treatment	Transplant to button stage (weeks 1-4)				Button to head stage (weeks 5-11)						
No	1	2	3	4	5	6	7	8	9	10	11
	21/11/03	27/11/03	3/12/03	11/12/03	16/12/03	22/12/03	30/12/03	7/1/04	14/1/04	21/1/04	28/1/04
1	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water	Water
2	Amistar	Water	Water	Water	Amistar	Water	Water	Water	Water	Water	Water
3	Amistar	Water	Water	CuOxCl	Amistar	SDS	SDS	SDS	SDS	SDS	SDS
4	Amistar	Water	CuOxCl	CuOxCl	Amistar	ClO_2	ClO_2	ClO_2	ClO_2	ClO_2	ClO_2
5	Amistar	CuOxCl	CuOxCl	CuOxCl	Amistar	Li700	Li700	Li700	Li701	Li702	Li703
6	CuOxCl	CuOxCl	CuOxCl	CuOxCl	CuOxCl	CuOxCl	CuOxCl	CuOxCl	CuOxCl	CuOxCl	CuOxCl
7	F5160f	-	F500	-	F500	Water	Water	Water	Water	Water	Water
8	Fruvit	-	Fruvit	-	Fruvit	Water	Water	Water	Water	Water	Water
9	AF+B	AF+B	AF+B	AF+B	AF+B	AF+B	AF+B	AF+B	AF+B	AF+B	AF+B

CuOxCl, Copper oxychloride: AF, AgriFos 600, B, Baycor, ClO_2, Chlorine dioxide

9.2.2.2 Assessments

The incidence of white blister on foliage was assessed at week 5 (16/12/03) on 10 randomly selected plants in the middle row and measured as previously described. A total of 7 plants at either end of each plot were treated as guard plants and not included in the assessment. Due to 100% incidence of the disease across all treatments, only the severity of white blister on foliage was measured at 7 weeks (30/12/03), on plants in the middle row, by scoring foliage on a scale of 0 to 5, where 0 = <15% of leaves with small pustules; 1 = 15 < 30% of leaves with small pustules; 2 = 30 < 60% of leaves with small pustules; 3 = 60 < 90 % of leaves with large pustules; 4 = 90% and more of leaves with large pustules and no more than one hypertrophied leaf and 5 = 90% and more of leaves with large pustules and more than one hypertrophied leaf. The incidence (%) of white blister on broccoli heads was measured before harvest (27/1/04) on plants in the middle row, as previously described and after harvest (3/2/04) on all plants with heads in the plot, except guard plants. The data at week 4 were analysed by generalised linear mixed model analysis. The severity of white blister at week 7 and the incidence of white blister on broccoli heads were analysed by ANOVA.

9.2.3 Head trial

The head trial located in Edwards Lane, Werribee South, Vic., commenced at button stage when plants of cv Legacy were 5 to 6 weeks old and continued till harvest. Plant spacing and bed width were the same as previously described for the transplant trial. The trial was laid out in a randomised block design and covered 6 beds with each bed representing one block or replicate. Along each bed there were 7 plots of 12m long by 1.68m wide representing each of 7 treatments.

9.2.3.1 Chemical application

No chemicals had previously been applied to the crop. There were 6 chemical treatments and a water control (Table 9.4). Treatments (Table 9.1) commenced on 18/11/2004 (week 5) and were applied weekly for 2 weeks, as previously described for the transplant trial.

9.2.3.2 Assessments

The incidence of white blister on foliage was assessed at week 5 prior to commencement of treatments. Guard plants were employed as previously described. Ten broccoli heads in the middle row of each plot were assessed for disease incidence (percentage of head with white blister symptoms) at week 9 (18/12/2003). The data were analysed by ANOVA.

Table 9.4 Treatment schedule for the head trial

Week 5 (18/11/2003) Button	Week 6 (27/11/2003)	Week 7 (3/12/2003) Heading
Water (control)	Water	Water
Ridomil Gold MZ	Water	Water
Ridomil Gold MZ	Copper oxychloride	Copper oxychloride
Ridomil Gold MZ	AgriFos 600 + Baycor	AfriFos 600 + Baycor
Ridomil Gold MZ	SDS	SDS
Ridomil Gold MZ	Li700	Li700
Ridomil Gold MZ	Chlorine dioxide	Chlorine dioxide

9.3 Results

9.3.1 Seedling Trial

The first symptoms of disease appeared at week 4, but only a low incidence of the disease had developed by week 6 (Table 9.5). Fungicide treatments containing the systemic fungicide Amistar or AgriFos 600 plus Euparen were the most effective and controlled the disease by 90% to 100%. In fact two consecutive application of Amistar gave 4 weeks control of white blister symptoms. The addition of contact fungicides to the Amistar treatments had no additional significant benefits in disease control. Sulphur and Sporekill, which have a one-day and nil withholding period respectively, were less effective than the systemic fungicides but significantly controlled the disease by 50%. Plantvax, a fungicide designed for control of diseases caused by true rust fungi (basidiomycetes) had no efficacy in white blister control.

Table 9.5 Incidence of white blister on seedlings at 6 weeks in the glasshouse

Treatment	Incidence of white blister (%) at week 6
Control (water)	6.3 a
Plantvax	4.2 ab
Sulphur	3.4 b
Sporekill	3.4 b
Amistar	0.6 c
AgriFos 600 + Euparen	0.6 c
Amistar / Acrobat+Dithane	0.3 c
Amistar / Zineb	0.1 c
Amistar / Barrack	0.0 c
Amistar / Euparen	0.0 c
l.s.d. (5%)	2.2

Numbers followed by a different letter are significantly different.

9.3.2 Transplant Trial

In the first stage of this trial, transplant to button stage, the natural incidence of white blister was 43% (Table 9.6). Applications of Amistar followed by one, 2 or 3 sprays of copper oxychloride or the systemic fungicide F5160f provided almost 100% control of the disease. One spray of Amistar provided 88% control of the disease. Fruvit, AgriFos 600+Baycor and 4 sprays of copper oxychloride provided no significant control of the disease on foliage (P<0.001).

Table 9.6 Incidence of white blister on foliage at 4 weeks after transplanting into the field (Transplant trial)

Treatment	Treatment No	Incidence of white blister (%) at week 5[A]
Control (water)	1	43.3
Fruvit	8	48.3
AgriFos 600 + Baycor	9	43.3
Copper oxychloride	6	35.0
Amistar	2	5.01
Amistar / Copper oxychloride *3	5	0.01
Amistar / Copper oxychloride *2	4	0.01
Amistar / Copper oxychloride *1	3	0.01
F5160f	7	0.01
P<0.001		

[A], Incidence was calculated from the number of plants with white blister symptoms out of the 10 plants assessed for each of the 6 replicates.

In the second stage of the transplant trial, button to head, the severity of the disease varied significantly but the incidence of white blister on foliage across all treatments was 100% (week 7) (Table 9.7). Natural severity of symptoms was high, about 4.0 (90% and more of leaves with large pustules and no more than one hypertrophied leaf) on unsprayed treatments. All treatments except AgriFos 600+Baycor significantly reduced the severity of the disease. Treatments containing Amistar or F5160f had the greatest efficacy reducing it to a severity or 1.0 (< 30% of leaves with small pustles), whilst copper oxichloride and Fruvit reduced it to a severity of about 2.0 (30 < 60% of leaves with small pustles). The addition of Li700, chlorine dioxide or SDS to the Amistar treatment had no additional benefits in reducing the severity of symptoms.

Table 9.7 Severity rating of white blister on foliage in the transplant trial at week 7

Treatment	Treatment No	Severity of white blister on leaves at week 7 (scale 0-5)[A]
Control (water)	1	3.83 a
AgriFos 600 + Baycor	9	4.17 a
Copper oxychloride	6	2.17 b
Fruvit	8	2.00 b
Amistar	2	1.50 bc
Amistar / Li700	5	1.00 c
Amistar / Chlorine dioxide	4	1.00 c
Amistar / SDS	3	1.00 c
F5160f	7	1.00 c
l.s.d. (5%)		0.62

[A], scale of 0 to 5, where 0 = <15% of leaves with small pustules; 1 = 15 < 30% of leaves with small pustules; 2 = 30 < 60% of leaves with small pustules; 3 = 60 < 90 % of leaves with large pustules; 4 = 90% and more of leaves with large pustules and no more than one hypertrophied leaf and 5 = 90% and more of leaves with large pustules and more than one hypertrophied leaf. Numbers followed by a different letter are significantly different.

In the transplant trial formation of heads was delayed due to cool weather and drought in January 2004. The effect of the Amistar, Fruvit and F5160f treatments were confounded by the 6 weeks of no fungicide applications due to weather conditions prolonging the trial. Consequently they are not reported here. The incidence of white blister was low on heads before the first harvest date and there were no significant differences between treatments (Table 9.8). By the second harvest copper oxychloride, Amistar/Li700 and Amistar/SDS were the most effective in controlling the disease and significantly reduced it by up to 48% to 75%. When treatments of Amistar followed by 6 weeks of water and Amistar followed by 6 weeks of SDS sprays are compared the addition of SDS reduced the incidence of white blister by 64%.

Table 9.8 Incidence of white blister (%) on broccoli heads before and after the first pick in the transplant trial (January to February 2004)

Treatment	Treatment No	Incidence of white blister on broccoli heads (%)	
		Before 1st harvest (27/1/04)	After first harvest (3/2/04)
Fruvit / water	8	8.5	na
Amistar / water	2	7.3	17.4 ab
Copper oxychloride	6	7.2	10.8 bc
AgriFos 600 + Baycor	9	3.4	15.8 ab
Amistar / Li700™	5	3.4	12.6 bc
Control (water)	1	2.2	24.3 a
Amistar / SDS	3	2.2	6.2 c
F5160f / water	7	2.2	na
Amistar / Chlorine dioxide	4	0	18.1 ab
l.s.d. (5%)		ns	8.6

na, Not applicable; Numbers followed by a different letter are significantly different.
ns, Not sigificantly different.

9.3.3 Head Trial

All the Ridomil Gold MZ treatments significantly reduced the incidence of white blister on broccoli heads (Table 9.9). There was no additional benefit in disease control by adding copper oxychloride, AgriFos 600 + Baycor, Li700, SDS, or chlorine dioxide to the Ridomil Gold MZ treatment. In this trial the incidence of white blister was reduced by 48% to 80%.

Table 9.9 Incidence of white blister on broccoli heads in the head trial (November to December 2003)

Treatment	Incidence (%) of white blister on heads at week 9
Control (water)	12.2a
Ridomil Gold MZ / Copper oxychloride	6.4 b
Ridomil Gold MZ / AgriFos 600 + Baycor	6.2 b
Ridomil Gold MZ / Li700	5.9 b
Ridomil Gold MZ / SDS	4.7 b
Ridomil Gold MZ / water	3.5 b
Ridomil Gold MZ / Chlorine dioxide	2.4 b
l.s.d. (5%)	5.78

Numbers, which are followed by a different letter, differ significantly.

9.4 Discussion

These trials have identified systemic (Amistar, Ridomil Gold MZ), contact (copper oxychloride) and soft chemicals (sulphur, Sporekill, LI700 and SDS) for control of white blister at various stages of broccoli crop development. The systemic fungicides had greater efficacy than the contact fungicides and soft chemicals, which is consistent with the literature. It is possible that the soft chemicals may be useful for spraying broccoli heads between harvests when there is not sufficient time to use fungicides which have with-holding periods of a week or more. Data generated by this work has been passed onto AgAware and ServAg to aid in minor chemical use registration. Currently, Amistar has a minor use permit for white blister on radish. Copper oxychloride has a minor use permit for white blister on radish, swede, turnip, broccoli, Brussells sprouts and cauliflower. Ridomil Gold MZ has a minor use permit for leafy herbs, rocket, mizuma and tatsoi and also broccoli and cauliflower in WA (expires June 2004) and Tasmania (expires March 2005). Ridomil Gold 25G has a minor use permit for broccoli, cauliflower and cabbage in NSW, which expires in August 2004 (Peter Dal Santo pers. comm.).

The genera *Albugo*, *Phytophthora* and *Peronospora* belong to the order Peronosporales and are closely related (Riethmuller *et al*., 2002). Consequently fungicides which control diseases caused by one of the genera could be expected to control diseases caused by the others, such as the phenylamides, dithiocarbamates and coppers. In our trial sulphur had efficacy but the systemic fungicides produced greater control of the disease. The efficacy of SDS is interesting and useful as its mode of action is in lysing spores (Irish *et al*., 2002). Despite rumours in the industry that Plantvax and Baycor had efficacy we were not able to find data to support their use for white blister control.

The systemics Ridomil Gold MZ and Amistar appeared to have efficacy, which lasted longer than 7 days. Although this may be related to weather conditions during the trials, it is possible that broccoli crops may not need to be sprayed on a weekly basis to control white blister.

Persistent and large-scale use of metalaxyl has lead to the development of resistant strains of *Phytophthora* (Ferrin and Rhode, 1992) and downy mildews (O'Brien, 1992; Klein, 1994). *A. candida* is known to develop resistance to fungicides after five consecutive sprays of a systemic fungicide (R. Rimmer, pers. comm). Fortunately Ridomil Gold MZ and Amistar are from separate Fungicide Activity Groups so they may be used in rotation to reduce development of fungicide resistance in *A. candida* in broccoli crops.

9.4.1 Future directions

- Evaluate the predictive model to develop effective and economical spray programs.
- Several chemicals are still to be evaluated under field conditions, such as Acrobate and Euparen + AgriFos 600.
- The use of surfactants should be examined in more detail as they show potential in reducing disease levels.
- Seed treatment is an option that should be further explored for growers producing their own seedlings in an area of crop production as it would give an initial degree of disease control during emergence.

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Chapter 10

Relationships with industry

Craig Murdoch – Vegetable Extension Officer

10.1 Background

Industry advisory groups and project steering committees have proved to be an excellent means of accelerating the impact of R&D projects. These groups provide an opportunity for researchers to present their work plans and results to both vegetable growers and representatives from allied support businesses including crop advisers, nurserymen, seed suppliers and chemical manufacturers. The ensuing discussions have given everyone cause to improve their approach. We have come to appreciate each others contribution to our greater understanding of the many issues concerning white blister on broccoli. The group member's diverse experience and their special industry networks have encouraged each of us to contribute more towards achieving a successful project outcome.

Some of the unique benefits of the project advisory group have been:

1. Putting a human face to the white blister problem, its impact, consequences and shared celebration of progress towards its control.
2. A deeper understanding of the impact of white blister on broccoli crops that could only come through several discussions with group members throughout the course of the project.
3. The opportunity to demonstrate how a combination of research approaches has combined to provide a richer understanding of the nature of this disease and its control.
4. Researchers have the opportunity to deliver progress reports on their work to a supportive industry audience. This has better prepared us to deliver presentations to interstate growers.
5. The reputation of our researchers as creditable contributors to the vegetable industry has been spread nationally through "word-of-mouth" by advisory group members in the course of their daily business.
6. All group members are strong advocates of the value of the R&D levy and have give personal examples to critics & sceptics of this system.
7. Our researchers have been invited to several grower properties to inspect problems including leeks, parsley, parsnips and broccoli, which may lead to future R&D proposals.
8. Participating growers have developed better relationships with researchers and those working in the nursery, chemical and seed industries which has given them even more confidence to raise questions on recurring problems.

The advisory group approach works very well and is our preferred approach to group involvement with the Vegetable industry. The advisory group model has been successfully applied to other vegetable projects including Bunching Vegetables VG01045, Onion White Rot VG01096, and the Lettuce Aphid advisory group under Lettuce Best Practice VG01038.

10.2 Advisory group members

Not all growers are willing or able to be involved in activities such as advisory groups. Growing and marketing vegetables demands a great deal of a growers time and for the most part growers are advised by vegetable agronomists and other providers on improving vegetable production. The approach we have adopted at DPI-Vic has been to invite these crop agronomists and similar "information retailers" to join with researchers and those growers prepared to plan and discuss the white blister problem first-hand. As discussed earlier, the resulting advisory group has proved to be a huge success.

The individual members who have contributed to the success of the White Blister Industry Advisory Group are:

James Kelly – Operations Manager - Kelly Bros. Market Gardeners - Dandenong

Mark Milligan – Operations Manager - A&G Lamattina & Sons - Rosebud

Kon Koroneos – Victorian HAL brassica representative - K Koroneos & Co - Werrribee

Rob Nave – VGA committee and Pres. Werribee growers group - Nave Produce - Werribee

Karl Riedel – Vegetable crop agronomist - E.E. Muir & Sons - Cranbourne

Dale O'Connell – Vegetable Agronomist - E.E. Muir & Sons - Werribee

Ian Willert & Matt Newland – Nursery Managers – Boomaroo Nurseries - Lara

David McDonald – Technical Manager- Brassicas - South Pacific Seeds - Dandenong

Daniel Gleeson – Technical Manager - Broccoli - Henderson Seeds - Bulleen

Dr Elizabeth Minchinton – Project leader- DPI-Knoxfield

Joanna Petkowski –- White Blister Project Officer- DPI-Knoxfield

Dr Robert Faggian – Molecular Biologist - DPI-Knoxfield

Craig Murdoch –Vegetable Extension Officer - DPI-Knoxfield

10.3 Appendix

Minutes of advisory committee meetings

Minutes of 1st White Blister Advisory Group Meetings

Monday 4th August - Amstel Golf Club, Cranbourne
Tuesday 5th August - Bridge Hotel, Werribee

Present:

Cranbourne:

Karl Riedel – E.E. Muir & Sons
James Kelly – Kelly Bros. Market Gardeners
Mark Milligan – A&G Lamattina & Sons
David McDonald – South Pacific Seeds
Richard Mapson – DPI Plant Standards

Werribee:

Dale O'Connell – E.E. Muir & Sons
Kon Koroneos – K Koroneos & Co
Rob Nave – Nave Produce
Ian Willert – Boomaroo Nurseries, Lara

DPI-Knoxfield:

Liz Minchinton – Project leader- Mycology
Joanna Petkowski –- White Blister Project Officer
Rob Faggian – Molecular Biologist
Craig Murdoch –Vegetable Extension Officer

The Project:

"A scoping study for race identification, source of the epidemic and management of white blister/rust on brassicas" (HAL project VG 02118)

Expected Outcomes:

This 12 month project will:

1. determine which race of *Albugo candida* is causing white blister on broccoli in Victoria,
2. look for sources of the disease,
3. develop a test to screen seeds for fungal contamination,
4. use molecular tools to distinguish 10 races of the fungus,
5. produce a literature review,
6. conduct preliminary fungicide trials to identify best management practices.

Lines of Inquiry:

Given we only have 12 months available for this scoping study, eight distinct lines of inquiry will be investigated to get a better understanding of controlling White Blister on broccoli.

1. Literature review

James Cunnington - Taxonomic Mycologist at DPI-Knoxfield will locate, interpret and summarise the scientific literature published on white blister research from Europe, America and Asia.

2. Differentiation of races - Molecular approach

Rob Faggian – Molecular Biologist at DPI-Knoxfield recently met with white blister researchers at the Horticultural Research Institute in the UK. Rob will be working to identify host specific genes to quickly discriminate up to 10 races of white blister. The project team will assist Rob by providing samples of different host plants affected with white blister.

3. Differentiation of races – classic differential host approach

Joanna Petkowski is sourcing the varieties of host plants originally used to describe the different races of white blister. Trials will commence in September to establish whether or not the white blister on Australian broccoli is a new race. If original varieties cannot be sourced, commonly available local host varieties will be substituted.

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4. Chemical trials – on seedlings in greenhouse at Knoxfield

Joanna Petkowski will also establish & maintain a white blister culture on broccoli seedlings at Knoxfield. This isolate will be used to infect other seedlings treated the day before with one of several fungicides. Seedlings of the susceptible variety GREENBELT have been generously provided by Ian Willert-BOOMAROO nurseries. The information from the seedling studies will be used to develop better control programs for field testing. BOOMAROO have offered a skilled nurseryman ½ day each week to help with the glasshouse studies at Knoxfield.

5. Chemical trials – on broccoli in the field

Field trials will establish the relative effectiveness of different fungicide treatments under commercial conditions. The major risk with field trials is the uncertainty of natural infection. This uncertainty can be reduced by running the trials on a property already showing white blister. The advisory committee believed that a cooperative grower will be found. Field trials are planned for one planting of cv.GREENBELT that will be harvested early in December.

6. Seed testing

White blister can be transmitted on seed. A 3 week study of white blister on radish found only 3 oospores in one batch of 30,000 seeds. As this work demands so much time, the study will be restricted to1 batch of cv. GREENBELT.

7. Grower Survey

Surveys of grower practices have proved useful in identifying best management practices for pest & disease control in spring onions & radish. A simplified interview will be prepared to identify the perceived causes of the outbreak, the varieties grown, soil type, irrigation & spray practices, seedling source and a scout of the crops for white blister. It may be possible for field officers from BOOMAROO & E.E. MUIR & Sons to assist in gathering this information.

8. Predicting disease from weather data – predictive modelling

Liz Minchinton & Rob Faggian described the latest developments in microsensor weather stations and the success of computer models to predict white blister outbreaks from local weather data. Robs contacts in the UK can provide us with the computer model they use. Assuming the model can be sourced from the UK within a few months, we can test the model on real weather data collected at Werribee since Oct 1999. More accurate prediction will require weather sensors actually in the crop canopy.

*** NEXT MEETING: A progress review meeting will be arranged for late September .***

Other Issues:

- Need to assist Rob Faggian in molecular differentiation of white blister races by providing samples of different host plants affected with white blister
- Need to source original host plant varieties to discriminate between the 10+ races of white blister. Substitute local varieties if original hosts cannot be found.
- The broccoli cultivar GREENBELT will be used throughout this trial as it is widely supplied & grown and is very susceptible to white blister.
- All seedling work with white blister will be done in glasshouses at DPI-Knoxfield.
- Significant international contacts have been made with researchers in the UK.
- Opportunity to host an international forum with leading white blister experts.
- DNA sequencing of white blister races will be a world class achievement.
- Media release to be prepared for national grower press.

Minutes of 2nd White Blister Advisory Group Meetings

Thursday 23rd October - Bridge Hotel, Werribee
Monday 27th October - Amstel Golf Club, Cranbourne

Members:

Cranbourne:

Karl Riedel – E.E. Muir & Sons
James Kelly – Kelly Bros. Market Gardeners
Mark Milligan – A&G Lamattina & Sons
David McDonald – South Pacific Seeds
Richard Mapson – DPI Plant Standards

Werribee:

Dale O'Connell – E.E. Muir & Sons
Kon Koroneos – K Koroneos & Co
Rob Nave – Nave Produce
Ian Willert – Boomaroo Nurseries, Lara

DPI-Knoxfield:

Liz Minchinton – Project leader- Mycology
Joanna Petkowski –- White Blister Project Officer
Rob Faggian – Molecular Biologist
Craig Murdoch –Vegetable Extension Officer

The Project:

"A scoping study for race identification, source of the epidemic and management of white blister/rust on brassicas" (HAL project VG 02118)

Expected Outcomes:

This 12 month project will:

1. determine which race of *Albugo candida* is causing white blister on broccoli in Victoria,
2. look for sources of the disease,
3. develop a test to screen seeds for fungal contamination,
4. use molecular tools to distinguish 10 races of the fungus,
5. produce a literature review,
6. conduct preliminary fungicide trials to identify best management practices.

Apologies:

James Kelly – Kelly Bros. Market Gardeners
Mark Milligan – A&G Lamattina & Sons
Richard Mapson – DPI Plant Standards

Guests:

Matt Newland – Boomaroo Nurseries, Lara

Project Update Summary

(details provided in workshop notes)

1. Literature review

James Cunnington - Taxonomic Mycologist at DPI-Knoxfield has completed the world-wide review of scientific literature on white blister and its control.

2. Differentiation of races - Molecular approach

Rob Faggian – Molecular Biologist at DPI-Knoxfield is testing a molecular probe to diagnose white blister in plant material not presently showing symptoms. The search for race specific genetic markers has already checked 4 of several genes that have been used to identify races of other plant pathogens.

3. **Differentiation of races – classic differential host approach**
   Joanna Petkowski– Project Scientist will commence cross infection studies in November to establish the race of white blister affecting Australian broccoli and its ability to cross to other brassica hosts. Commonly available local varieties will be used as hosts where "original" varieties are unavailable.

4. **Chemical trials – on seedlings in greenhouse at Knoxfield**
   Dramatic differences in control were observed between treatments and the most promising treatments will now be evaluated in field trials.

5. **Chemical trials – on broccoli in the field**
   Field trials will establish the relative effectiveness of different fungicide treatments under commercial conditions. Trials will be conducted in November to evaluate options that protect broccoli heads from expressing symptoms of white blister. A second study will evaluate options for protecting leaves before heading.

6. **Seed testing**
   White blister can be transmitted on seed. A study of 1 batch of cv. GREENBELT will be undertaken after the field trials have been completed, early in 2004.

7. **Grower Survey**
   A pre-harvest survey of 13 broccoli crops at Werribee, Cranbourne, Rosebud and Bairnsdale identified a range of practices to control white blister. Untreated crops showed high levels of diseased leaves. Weekly spray programs provided virtual total control. Fortnightly spray programs were nearly as effective.

8. **Predicting disease from weather data – predictive modelling**
   UK scientists will be collaborating on predictive disease modelling studies in Victorian broccoli crops.

*** NEXT MEETING: A field inspection of chemical trials on broccoli heads will be held at Werribee, late in November - date to be advised.**

Other Issues:

- Media update to be prepared after field trials.
- Why do we see White blister on broccoli but not on Victorian sprout or cabbage crops ?
  The literature suggests that race 9 affects these crops as well as Broccoli & Cauliflower. (Perhaps we use European sprout varieties that are resistant to White Blister. Cabbage crops may be infected but not showing symptoms)
- How much leaf disease can we tolerate before we risk head failure ?
- Some chemical treatments are very pH sensitive, need to buffer spray mix if water pH too high.
- AMISTAR effective for 3 weeks, why spray weekly?
- Search for tolerant varieties in Europe, trial in Australia.
- 2nd cut often shows more problems, need a "no-residue" protectant.
- Grower workshops to follow outcomes of field trials.
- Brief update to be sent to Veg IDO's and brassica reps. in all states.

DEPARTMENT OF
PRIMARY INDUSTRIES

White Blister Project Advisory Group

UPDATE: DECEMBER 2003

Purpose: To update White Blister Advisory Group members on recent progress with preliminary field trials at Werribee.

Aim of field trials:
Two field trials were established to evaluate the efficacy of chemical treatments for control of white blister under commercial field conditions.

Field trial #1, Head trial – late control
Establish the most effective chemical treatments for protecting broccoli heads from developing symptoms of white blister as they begin to form in the field under conditions of high disease pressure.

Field trial #2. Transplant trial – early prevention
Establish the most effective chemical treatments for control of white blister on field broccoli from planting till harvest.

Head trial – completed 9 Dec 2003

Background:

- Trial conducted on a 6-7 week old broccoli crop at Werribee which had no prior treatment for white blister.
- At the beginning of the trial, disease incidence on leaves was close to 100%.
- Crop had been water stressed due to severe water restrictions at Werribee. This could have enhanced the expression of white blister symptoms including leaf distortion and small & variable head size at maturity.
- The effectiveness of six treatments were evaluated based on an initial application of a **systemic** fungicide 21 days before harvest, followed with **non-systemic** treatments applied at 14 days & 7 days before harvest.

Findings – Head trial

- Of those plants left untreated, 12% of broccoli heads developed white blister defects.
- A single application of **systemic** fungicide 21 days before harvest reduced blister defects on heads to 8%.
- Followup weekly applications of some **non-systemic** fungicides reduced defects to 3% or less.

Transplant trial – in progress, due to finish mid Jan 2004

Background:

- Broccoli transplanted 17 Nov 2003, first treatment applied on 21 Nov (4 days after planting).
- The effectiveness of eight treatments have been evaluated at the 5th week of the crop, (before head formation)
- In the absence of broccoli heads, disease incidence has been assessed on leaves.

Findings – transplant trial

- 43% of untreated transplants developed symptoms of white blister within 5 weeks.
- A single application of **systemic** fungicide at 4 days reduced defects on leaves to 5%.
- weekly followup sprays of **non-systemic** fungicides reduced defects on leaves to 0%.

Implications

An effective, economic and sustainable White blister management strategy must minimise chemical applications to reduce the risk of the disease developing resistance to existing fungicides.

These preliminary results suggest that reduced application of **systemic** fungicides will be effective in controlling white blister in the field when supplemented with **non-systemic** fungicides.

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SPS South Pacific Seeds
E.E. Muir & Sons INNOVATORS IN AGRIBUSINESS
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Minutes of 3rd White Blister Advisory Group Meetings

Wednesday 5th May - Bridge Hotel, Werribee
Thursday 6th May - Amstel Golf Club, Cranbourne

Present:

Cranbourne:

James Kelly – Kelly Bros. Market Gardeners
Mark Milligan – A&G Lamattina & Sons
David McDonald – South Pacific Seeds
Kon Koroneos – K Koroneos & Co
Ian Willert – Boomaroo Nurseries, Lara

Werribee:

Kon Koroneos – K Koroneos & Co
Rob Nave – Nave Produce
Dale O'Connell – E.E. Muir & Sons
Ian Willert & Matt Newland– Boomaroo, Lara
Daniel Gleeson – Henderson Seeds, Bulleen

Apologies:

Karl Riedel – E.E. Muir & Sons

DPI-Knoxfield:

Liz Minchinton – Project leader- Mycology
Joanna Petkowski –- White Blister Project Officer
Rob Faggian – Molecular Biologist
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The Project:

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Expected Outcomes:

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Project Update Summary

(details in advisory group notes. Only a brief summary & issues arising are shown here)

Ian Willert – Boomaroo Nurseries, Lara and Kon Koroneos – K Koroneos & Co, Werribee South attended both meetings. <u>All agreed to hold joint meetings in future</u>

Liz Minchinton recently attended the National Australian Vegetable Pathology Working Group in Adelaide and made useful contacts with researchers in other states.

- WA export cauliflower markets have been largely lost to China.
- WA growers are looking to the Eastern states as an alternate market.
- Brassica virus in WA ? not yet a problem to Victorian growers.

Liz has reported our progress with White Blister to growers in Tasmania, WA, NSW, QLD

- Visit by Roy Kennedy (UK) in early August. UK scientists will be collaborating on predictive disease modelling studies in Victorian broccoli crops.
- White blister workshop for Victorian growers planned for growers in Werribee, Cranbourne and Bairnsdale.

White Blister Race Identification:

Joanna Petkowski reported on her differential host studies.

- Initial results suggest we are dealing with **Albugo race 9**
- Difficult to raise seedlings in growth cabinet, lighting unnatural?
- Recommend all future work be done in glasshouse.
- Recommended that we also plant some other brassica hosts with broccoli in the field to help demonstrate host specificity of Albugo races to growers. This years field trials showed the occasional cabbage seedlings in affected broccoli plantings were not affected by WB.
- Joanna will undertake a PhD study of White Blister on Brassicas which will give us access to high tech university equipment and academics.

Rob Faggian reported on progress with molecular differentiation of races.

- A diagnostic test is now working. The test identifies the presence of WB DNA but does not discriminate between different races.
- The Plant Biotechnology Centre (Bundoora) have been contracted to construct a WB gene library. The gene library be screened to identify new/unknown genes that may enable race differentiation.
- A DNA fingerprinting protocol has been developed but will not be useful until host differentials are working consistently, since a sound system for comparing fingerprinting results is essential.

General discussion:

Broccoli Cultivars

VIPER is generally considered to be more tolerant to White Blister, infection is not seen on true leaves only auxiliary shoots. As the last summer plantings of VIPER are harvested we can expect to see an increase in WB with more susceptible varieties. Industry mainstay cultivars MARATHON & GREENBELT have lost sales to a several new cultivars including LEGACY, GRAVELIA, MAMBA, ENDURANCE and TRIATHALON. Many of these newer varieties have better tolerance to downy mildew as well. There is a lot more preventative spraying of broccoli crops especially at Cranbourne & Rosebud. Indeed, white blister is seen by these growing areas to be a problem only in Werribee.

Certified Broccoli Seed ?

International seed testing of broccoli seed for WB freedom? Similar to black rot free seed in cauliflower will incur additional costs.
Heat treated seed is not often used because of reduced germination.
It may be possible to use Robs diagnostic PCR test for Albugo to assess batches of seed.

The use of certified WB free seed will not reduce the incidence of WB in the field as WB is widely spread in throughout broccoli growing areas.

Future work

The progress and success of our 12 month scoping study has been recognised by Horticulture Australia who have agreed to support a further 3 years work . All advisory group members were happy to continue and it was recommended that SYNGENTA be approached to provide chemical expertise to our group. RIDOMIL® & AMISTAR® are both SYNGENTA products.

NEXT MEETING: - White Blister Workshops (Werribee, Cranbourne & Bairnsdale)– Mid August 2004
Dates, venues and co-presenters are yet to be finalised

Chapter 11

Publication summary

Publications

Anon (2004). Steps towards white blister control. *Southern Farmer*. March 2004, p13.

Kita, N., Minchinton, E., Murdoch, C. and Nguyen, N. K. (2003). White blister/rust on Vegetables. Poster. Werribee Vegetable Expo, Glenormiston College Werribee, Vic.

Minchinton, E. (2002). White blister on the burst? *Vegetable Matters* Issue 6:3.

Minchinton, E. (2002). White blister on the broccoli burst? *VegeLink SA*. Issue 2: 2.

Minchinton, E. and Kita, N. (2002). Management of white blister. *Access to Asian Vegetables.* June 2002, Issue 50: 2.

Minchinton, E. and Kita, N. (2003). White blister/rust update. *Brassica IPM*, Issue 2:2.

Minchinton, E., Kita, N., Nguyen, N. and Murdoch, C. (2002). White blister/rust. In 'Improving interaction and communication between RIRDC Asian foods program leaders and industry (DNT-28a)'. Minutes of meetings held in Melbourne and Geelong, on June 19-21, 2002. Edt. Melinda Gosbee, Northern Trerritory Department of Business, Industry and Resource Development.

Minchinton, E., Faggian, R., Kita, N. and Murdoch, C. (2003). Management of White Blister. Handout. DPI, IHD Knoxfield, 2pp.

Petkowski, J.E., Faggian R., Cunnington J., Murdoch C., Minchinton E., (2003). White blister threatens Australian broccoli. Abstract. DPI Horticulture Conference 2003, Tatura

Petkowski, J.E., Faggian, R., and Minchinton E. (2004). Latest findings from white blister research. Vegetable Platter, Vegetable Industry Development Officers – February 2004 Issue of Good Fruit and Vegetables.

Petkowski, J. and Minchinton, E. (2004). Towards better ontrol of white blister in broccoli. *Good Fruit and Vegetables*. March 2004, p17.

Petkowski, J. and Minchinton, E. (2004). Steps towards white blister control strategies. *Victorian Vegetable Grower, VegeLink* 18:7

Petkowski, J. Faggian, R. and Minchinton, E. (2004). Research confirms control methods for white blister on broccoli seedlings. *Victorian Vegetable Grower, VegeLink* 17: 7.

Petkowski, J. Faggian, R. and Minchinton, E. Cahill, D. (2004). Studies on host specialisation of *Albugo candida* causing white blister on broccoli in Victoria. Abstract. ComBio Conference, Perth 2004

Newspaper reports

Fungus hurts broccoli exports. Kathleen Cuthbertson, Herald Sun, May 22, 2002; p28.

Crop Crisis, virulent disease cripples local broccoli trade. Melissa Iaria. Werribee Banner, May 1, 2002: p1.

Fungus causes havoc. Broccoli fungus woe. Katie Fisher. Weekly Times May 15, 2002;p 19, 65
.
Minchinton, E. (2003). Countering a threat to broccoli. Southern Grower. October, p8

Meetings field days and conferences

Seminars:

White blister. Asian Vegetable Conference, Geelong 21 June 2002.
White blister. Muirs growers, Cranbourne 7 June 2002.
White blister. Muirs young growers, Werribee 11 April 2002.
White blister. Patrick Ulloa's Group, Werribee May 2003
White blister. SA Brassica Forum, Virginia, SA, 27 May 2003.
White blister. Bairnsdale vegetable growers, Lindenow, 14 October 2003.
White blister: SA Vegetable Growers 3 February 2004
White blister: Tasmanian vegetable growers, 26-27 February 2004.
White blister: WA vegetable growers 23-25 August 2004
White blister: NSW and QLD vegetable growers TBA.
White blister: Victorian vegetable growers, 13, 16,17 August 2004.

Poster: White blister threatens Australian broccoli. DPI Horticulture Conference 2003, Tatura. J.E. Petkowski, R. Faggian, J. Cunnington, C. Murdoch, E. Minchinton.

Field Day: White blister trial. Werribee South, 3/2/04.

Advisory committee meetings

Cranbourne and Werribee 4, 5 August 2003
Cranbourne and Werribee 23, 27 October 2003
Cranbourne and Werribee 5, 6 May 2004

Poster

DEPARTMENT OF
PRIMARY INDUSTRIES

WHITE BLISTER THREATENS AUSTRALIAN BROCCOLI

J.E. Petkowski, R. Faggian, J. Cunnington, C. Murdoch, E. Minchinton
DPI Knoxfield, PO BOX 15 Ferntree Gully Delivery Centre 3156 Victoria, Australia
Email joanna.petkowski@dpi.vic.gov.au

Introduction

Victorian broccoli industry, which produces 45% of Australia's crop, has recently been threatened by outbreaks of white blister (rust).

The disease, caused by the oomycete *Albugo candida* (Pers.) Kuntze, attacks the heads of broccoli (Fig. 1), spoiling their appearance and downgrading marketability. It also affects seedlings (Fig. 2), leaves (Fig. 3) and axillary shoots. White blister is not commonly found on broccoli in Australia, but causes damage on radish, Chinese brassicas and cruciferous weeds. However, in summer 2001/2002, epidemics of white blister occurred on broccoli in Victoria, while crops in Tasmania, South Australia, southern New South Wales and Western Australia were affected in summer and autumn 2003. The disease has not been reported on broccoli in Queensland.

Fig.1 Broccoli head with white blister pustules

Fig. 2 Seedling grown from infected seed or/and in infected media

The project

A new research project for white blister on brassicas has been developed by the vegetable industry, DPI and Horticulture Australia Ltd.

The project aims to:

- Determine which race of *Albugo candida* causes white blister of broccoli in Victoria (Table 1)
- Identify sources of the disease
- Develop a test for detection of fungal contamination of seeds
- Use molecular tools for pathogen race differentiation
- Conduct a literature review
- Evaluate the use of fungicides for the management of this disease

Fig. 3 White blister pustules on cauliflower leaf

Results to date

The ITS1 region within the rRNA gene is useful for the development of a generic diagnostic test for A. candida, (Fig.4), but not suitable for pathogen race differentiation.
Currently other genes, such as actin and ß-tubulin are being screened for the presence of race specific polymorphisms.

Table 1 Currently there are 17 races of *Albugo candida* described in the literature. The list below shows 10 races that will be included in studies on differential host differentiation of races under this project.

A.candida race	Host (botanical name)	(common name)
1	*Rhaphanus sativus*	radish
2	*Brassica juncea*	Indian mustard
3	*Armoratia rusticana*	horseradish
4	*Capsella bursa-pastoris*	shepherds purse
5	*Sisymbrium officinale*	hedge/tumble mustard
6	*Rorippa islandica*	marsh/watercress
7	*Brassica rapa (campestris)*	Chinese cabbage and mustard, Bok Choi
8	*Brassica nigra*	black mustard
9	*Brassica oleracea*	Broccoli, B. sprouts, cauliflower
10	*Brassica kaber*	charlock

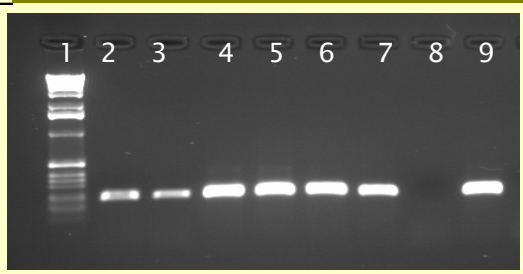

Fig. 4 Lanes:1- molecular weight marker; 2,3 - non-symptomatic infections; 4,5 - leaf pustules; 6,7 - sporangia; 8 - blank; 9 - positive control

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