Know-how for Horticulture™

Management of downy mildew disease of pea crops and its possible resistance to metalaxyl

Dr. Hoong Pung
Serve-Ag Research Pty Ltd

Project Number: VG00031

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Management of downy mildew disease of pea crops and its possible resistance to metalaxyl

Final Report VG00031

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Horticulture Australia Project

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Industry summary

Although metalaxyl seed treatment had been used successfully for many years for the control of downy mildew on pea seedlings, in recent years, poor crop establishment, severe crop infection and yield loss due to downy mildew on processing pea crops have became more frequent in Australia, and fungicide resistance in the downy mildew pathogen was suspected. This project, therefore, aimed to determine whether *Peronospora viciae* strains in Australia were resistant to metalaxyl; to identify suitable alternative seed treatments for the control of metalaxyl resistant downy mildew; and to develop affordable treatment methods for field downy mildew control. The research in this project was divided into three main areas, with the following major outcomes:

1. Sensitivity of **Peronospora viciae** *to metalaxyl-M*

- A total of 16 collections of *P. viciae* were obtained from pea crops at different sites in northern Tasmania for bioassay tests in 2001. Thirty-eight % of the collections were sensitive to metalaxyl, 31% were resistant and another 31% were partially resistant. This was consistent with similar resistance development in New Zealand. This is the first report of metalaxyl resistance following its use as a pea seed dressing for downy mildew control in Australia.

2. Seed and seedling infection control

- This project demonstrates the importance of seedborne infections and seedling pathogens, as well as the effectiveness of seed dressings with several active ingredients, in controlling several major pathogens and diseases of pea. Fungicide seed dressing is the most cost effective method of controlling seedborne infections and early seedling diseases. The fungicide seed dressings Apron + P-Pickel T, Aliette Super, and Wakil XL, generally gave the best results, consistently increasing the numbers of pea plants and seedling growth. With the establishment of metalaxyl-resistant isolates of *P. viciae* in Tasmania, fungicide resistance management strategies for seed treatment should include alternating metalaxyl or phenylamides with chemicals that have different modes of action, or using metalaxyl in mixtures with non-phenylamide chemicals such as cymoxanil or fosetyl-Al, which can protect seedlings from infection by metalaxyl-resistant isolates. Therefore, Aliette Super and Wakil seed treatments, which contains fosetyl-Al and cymoxanil, respectively, are suitable alternatives to the Apron + P-Pickel T seed treatment. In Apron + P-Pickel T, metalaxyl is the only active ingredient for downy mildew control. Treating seeds with Aliette Super or Wakil XL and storing them for almost 1 year gave no adverse effects on germination or seedling growth.

3. Field downy mildew control

- Downy mildew and *Ascochyta* collar rot are the two most common and important diseases of processing pea crop that impacts on pea yield and quality. Prior to this project, there was no effective or affordable control method for field downy mildew, hence, the impact of downy mildew field infection on pea yield was unknown. In this project, with effective field control, yield increases of 1 to 3 tonnes per hectare were recorded following improved downy mildew control. As for collar rot, yield increases of 1 to 2 tonnes per hectare were recorded following reduced collar rot severity with Bravo applications.

- This project identified three low cost fungicide products, chlorothalonil (Bravo), mancozeb (Penncozeb), and phosphorous acid (Agri-Fos), that provided effective control methods for field downy mildew on processing pea crops. The Agri-Fos + Penncozeb combination gave the best control of downy mildew, but had no effect on collar rot. However, Agri-Fos + Bravo, the second best treatment against downy mildew, was also effective in reducing collar rot severity. The optimum product rate for collar rot control was 1.8 L/ha Bravo. The optimum product rates for downy mildew control were 3.5 L/ha Agri-Fos, and 2.5 L/ha Penncozeb SC or 2.0 kg/ha Penncozeb DF. Agri-Fos or Penncozeb, applied on their own, had little or no effect on downy mildew incidence or severity.

- Two fungicide applications applied to plants at the growth stage of 4 and 8 nodes tended to give better downy mildew control than one application. However, for reducing collar rot severity, one spray application at 4 nodes was adequate. The timing of the fungicide applications was critical.

Early fungicide applications, before diseases occur, only protect plants for a short interval of 10 to 14 days. Therefore, for optimum downy mildew control, the fungicide application must be applied at the first sign of infection in a crop.

- Downy mildew field infections usually occur at the pea growth stage of 6 to 8 nodes, depending on sowing time and weather conditions. As collar rot tends to occur early in the crop, at about 4 nodes, the alternate applications of Agri-Fos + Bravo followed by Agri-Fos + Penncozeb 7-10 days later, could be a suitable program for reducing early collar rot severity as well as controlling downy mildew.

Technical summary

Processing pea seeds used in Australia mainly come from New Zealand, where downy mildew is common. In both countries, metalaxyl seed treatment had been used for many years for the control of downy mildew on seeds and seedlings. However, in recent years, poor downy mildew control in New Zealand was attributed to the development of metalaxyl resistant strains of *Peronospora viciae.* In the late 1990s, poor crop establishment, severe crop infection and yield loss, due to downy mildew on processing pea crops, have became more frequent in Australia. Apart from metalaxyl seed treatment, there has been no cost effective method for controlling the disease in the field.

This project, therefore, aimed to determine whether *P. viciae* strains in Australia were resistant to metalaxyl; to identify suitable alternative seed treatments for the control of metalaxyl resistant downy mildew; and to develop affordable treatment methods for field downy mildew control. The major project outcomes are outlined below.

1. Sensitivity of Peronospora viciae *to metalaxyl-M*

- A total of 16 collections of *P. viciae* were obtained from pea crops at different sites in northern Tasmania for bioassay tests in 2001. Thirty-eight % of the collections were sensitive to metalaxyl, 31% were resistant and another 31% were partially resistant. This was consistent with similar resistance development in New Zealand. This is the first report of metalaxyl resistance following its use as a pea seed dressing for downy mildew control in Australia.

2. Seed and seedling infection control

- This project demonstrates the importance of seedborne infections and damping-off, as well as the effectiveness of seed dressings with several active ingredients in controlling several major pathogens and diseases. Fungicide seed dressing is the most cost-effective method of controlling seedborne infections and early seedling diseases.

- An indication of a good seed treatment is their consistency in performance over many trials, giving excellent crop establishment, with high seedling survival, improved seedling growth and early seedling disease control. Apron + P-Pickel T, Aliette Super, and Wakil XL are commercially formulated seed dressings, which generally gave the best performance, consistently increasing the number of plants and seedling growth. These fungicide seed dressings, each consisting of a mixture of three active ingredients, are all formulated to control downy mildew and *Ascochyta* infections on pea seeds as well as diseases that reduce seedling establishment.

- With the establishment of metalaxyl-resistant isolates of *P. viciae* in Tasmania, fungicide resistance management strategies for seed treatment should include alternating metalaxyl or phenylamides with chemicals that have different modes of action, or using metalaxyl in mixtures with non-phenylamide chemicals such as cymoxinil or fosetyl-Al, which can protect seedlings from infection by metalaxyl-resistant isolates. Therefore, Aliette Super and Wakil seed treatments, which contain fosetyl-Al and cymoxanil, respectively, are suitable alternatives to the Apron + P-Pickel T seed treatment. In Apron + P-Pickel T, metalaxyl is the only active ingredient for downy mildew control. Treating seeds with Aliette Super or Wakil XL and storing them for almost 1 year gave no adverse effects on germination or seedling growth.

3. Field downy mildew control

- Downy mildew and *Ascochyta* collar rot are the two most common and important diseases of processing pea crops that impact on pea yield and quality. Prior to this project, there was no identified effective or affordable control method for field downy mildew, hence, the impact of downy mildew field infection on pea yield was unknown. In this project, with effective field control, yield increases of 1 to 3 tonnes per hectare were recorded following improved downy mildew control. As for collar rot, yield increases of 1 to 2 tonnes per hectare were recorded following reduced collar rot severity with chlorothalonil.

- This project identified three low cost fungicide products, chlorothalonil (Bravo), mancozeb (Penncozeb), and phosphorous acid (Agri-Fos) that provided effective control for against field downy mildew on processing pea crops.

- In trials conducted over 3 years, and at different locations, Penncozeb + Agri-Fos and Bravo + Agri-Fos were the most consistent and effective foliar treatments for field control of downy mildew. Agri-Fos or Penncozeb alone, were shown to have little or no effect on collar rot. Agri-Fos or Penncozeb, applied on their own, also had little or no effects downy mildew incidence and severity. In contrast, Agri-Fos in a mixture with Bravo or Penncozeb reduced downy mildew incidence and severity on plants. This indicates a synergistic effect of the chemical mixture.

- The Agri-Fos + Penncozeb combination was the best treatment against downy mildew, but had no effect on collar rot. However, Agri-Fos + Bravo, the second best treatment against downy mildew, was also effective in reducing collar rot severity. The optimum product rate for collar rot control was 1.8 L/ha Bravo. The optimum product rates for downy mildew control were 3.5 L/ha Agri-Fos, and 2.5 L/ha Penncozeb SC or 2.0 kg/ha Penncozeb DF.

- Two fungicide applications applied to plants at the growth stage of 4 and 8 nodes tended to give better downy mildew control than one application. However, for reducing collar rot severity, one spray application at 4 nodes appeared to be adequate. The timing of the fungicide applications is critical. Early fungicide applications before diseases occur only protect plants for a short interval of 10 to 14 days. Therefore, for optimum downy mildew control, the fungicide application must be applied at the first sign of infection in crops.

- Downy mildew field infections usually occur at the pea growth stage of 6 to 8 nodes, depending on sowing time and weather conditions. As collar rot tended to occur early in crops, at about 4 nodes, the alternate applications of Agri-Fos + Bravo followed by Agri-Fos + Penncozeb at 7-10 days later, could be a suitable program for reducing early collar rot severity as well as controlling downy mildew.

- Among the different types of spray adjuvants examined in the trials, little or no advantage could be found with their addition to the fungicides.

- In two trials, plants treated with metalaxyl only, had similar downy mildew incidence and severity to the untreated plants. This poor control may have been due to the presence of metalaxyl resistant isolates of *P. viciae* in the crops.

Extension to industry

- A poster was presented at the 8th International Congress of Plant Pathology, in Christchurch, New Zealand, on 3-7 February 2003. Copies of the poster were provided to Horticulture Australia and voluntary contributors.

- Results of this project were presented to the meeting of the National Vegetable Pathologists Working Group and State Industry Development Officers, at Adelaide on 21-23 April 2004.

- A poster was presented to South Australian growers at the Virginia Horticultural Centre, South Australia, on 22 April 2004. Printed flyers of the poster were also made available to growers.

- Many of the project's findings have already been adopted by the processing pea industry in Tasmania during the project in 2002 and 2003. The use of alternative seed treatments, as well as Agri-Fos + Penncozeb or Agri-Fos + Bravo, are already industry standards for downy mildew management.

- Project findings will be extended nationally to related pea crop producers (e.g. garden pea, snow pea and sugar snap pea) with the production of a flyer, which will be circulated through the vegetable IDOs' network as well as posted on the web site.

Recommendations

Seed and seedling infection control

- This project established that a significant proportion of isolates of *P. viciae* from pea crops in northern Tasmania had became partially resistant or resistant to metalaxyl. Therefore, the strategies for seed treatment should include alternating metalaxyl or phenylamides with chemicals that have different modes of action, or using metalaxyl in mixtures with non-phenylamide chemicals such as cymoxanil or fosetyl-Al, which can protect seedlings from infection by metalaxyl-resistant isolates.

- Aliette Super and Wakil seed treatments, which contain fosetyl-Al and cymoxanil, respectively, are therefore suitable alternatives to the Apron + P-Pickel T seed treatment. In Apron + P-Pickel T, metalaxyl is the only active ingredient for downy mildew control.

- The active ingredient fosetyl-Al in Aliette Super, is converted into phosphorus acid, which is active against downy mildew. Therefore, if phosphorus acid (Agri-Fos) is also used as foliar spray applications for field downy mildew control, there is a potential for fungal strains to develop resistance from the over-reliance on the use of phosphorus acid.

- The two alternative pea seed dressings, Aliette Super and Wakil, are registered for use in New Zealand, but not in Australia. As almost all processing pea seeds are imported from New Zealand, and seeds could be treated there before shipment to Australia. There is no plan by the seed-dressing manufacturers to register the seed treatment products in Australia. This arrangement, while satisfactory, is not ideal, as some pea seeds produced in Australia could not be treated with these alternative seed dressings.

- Alternative commercial seed dressings already registered for use on broad acre crops such as cereals and canola in Australia should also be evaluated for possible extension of use on pea seeds.

Field downy mildew control

- Agri-Fos + Penncozeb + and Agri-Fos + Bravo, have been shown to be the most consistent and effective foliar treatments for field downy mildew control. Each product on its own had little or no effect on the disease. The product mixture provides growers with a cost effective and affordable method for managing field infections of the two major pea diseases. Chlorothalonil (Bravo™) and mancozeb (Penncozeb™) are already registered for use on peas. Phosphorous acid (Agri-Fos™) is also a fertiliser and therefore chemical residue on plants from its use is not an issue.

- Agri-Fos + Penncozeb gave the best control of downy mildew, but had no effect on collar rot. However, Agri-Fos + Bravo, the second best treatment against downy mildew, was also effective in reducing collar rot severity.

- The optimum product rate for collar rot control was 1.8 L/ha Bravo. The optimum product rates for downy mildew control were 3.5 L/ha Agri-Fos, and 2.5 L/ha Penncozeb SC or 2.0 kg/ha Penncozeb DF. A maximum of two spray applications is recommended at the first sign of infection at the 4 to 8 nodes growth stages to reduce disease severity and improve yield.

- If downy mildew is the only disease, or is the dominant disease, Agri-Fos + Penncozeb should be used for optimum downy mildew control and yield improvement.

- Downy mildew and *Ascochyta* collar rot are the two most common and important diseases of processing pea crops that impact on pea yield and quality. Many crops have both diseases, and therefore the ability to control both is critical for pea disease management. Agri-Fos + Bravo should be used for control of both downy mildew and *Ascochyta* collar rot. Alternatively, an alternate spray program of Agri-Fos + Bravo followed by Agri-Fos + Penncozeb at 7-10 days later, could be used to optimize both collar rot and downy mildew disease management. This is because collar rot tended to occur early in the crop at about the 4 node stage, followed by downy mildew later.

Introduction

Background

In Australia, processing peas (*Pisum sativum*) are mainly produced in northern Tasmania, for processing into frozen vegetables by McCain Foods and Simplot Australia. Small scale processing pea production is also carried out near Kendenup in Western Australia. Downy mildew of pea is caused by the fungus *Peronospora viciae*. The disease is very common in pea crops grown in cool temperate regions of the world, including northern Tasmania and New Zealand (Dixon 1981, Falloon et al 2000). Severe downy mildew infections cause severe stunting of seedlings and may kill them, while less severe infection reduces plant vigour and pea yield (Dixon 1981, Stegmark 1988). The pathogen can infect pods and seeds, which can then transmit downy mildew to subsequent crops. Most processing pea seed used in Tasmania and Western Australia come from New Zealand, where cool and humid conditions are sometimes conducive to downy mildew epidemics. Seed treatment with the systemic fungicide metalaxyl is commonly used in Australia and New Zealand for the control of downy mildew on seeds and seedlings. Apart from downy mildew, metalaxyl also controls seed rot and damping off caused by *Pythium* spp. that are common in soil. In the late 1990's, poor crop establishment, severe crop infection and yield loss due to downy mildew have became more frequent in processing pea crops in northern Tasmania.

Studies conducted in New Zealand in 1996 established that a high proportion of *P. viciae* strains were resistant to metalaxyl as a result of the sole reliance on metalaxyl for downy mildew control on seeds (Falloon et al. 2000). Downy mildew developed in young pea crops from metalaxyl-treated seeds as a result of insensitivity to the fungicide in strains of *P. viciae* after several years of use. In Australia, Apron SD + P-Pickel T, has been the standard fungicide seed dressing used on pea seeds over a number of years. This seed dressing mixture consists of metalaxyl in Apron, plus thiram and thiabendazole in P-Pickel T. Since the mixture relied on metalaxyl for downy mildew control, similar development of fungicide insensitivity may have also occurred in Australia, or gave poor control of resistant strains carried on seeds from New Zealand. It was not known if the current use of metalaxyl seed treatment still provided adequate downy mildew control or whether resistant strains of *P. viciae* had developed or had been introduced into Australia. As the metalaxyl seed treatment constitutes a significant proportion of the pea seed cost, adding about 10% to the total seed cost to the industry, these questions need to be addressed. Research in New Zealand has identified several alternative seed dressing products that could be used successfully to control the downy mildew pathogen, including metalaxyl resistant strains.

Apart from metalaxyl seed treatment, there was also no cost-effective method for controlling the disease in the field. Downy mildew can also survive in soil as oospores, and the pathogen is widespread in major pea-producing regions. Although mancozeb and chlorothalonil are registered for downy mildew control in Australia, disease control by the fungicides is poor. As processing peas are considered to be relatively low value crops compared to other crops such as grapes and opium poppy in Tasmania, there has been little incentive for fungicide manufacturers to optimize control methods or to evaluate new fungicides. Any foliar fungicide applications developed for use on pea crops must also be cost-effective and result in better return for growers.

This project, in collaboration with researchers in New Zealand, would establish whether metalaxyl resistant strains of *P. viciae* are also present in Tasmania, and to identify and help introduce the most suitable pea seed treatments for use in Australia. The project would also evaluate the efficacies of foliar applications of chlorothalonil, mancozeb, and other potential fungicides to pea crops, and to optimize foliar application methods for field downy mildew control.

Aims

This project aimed to determine whether *Peronospora viciae* strains in Australia are resistant to metalaxyl; to identify suitable alternative seed treatments for the control of metalaxyl resistant downy mildew; and to develop affordable treatment methods for field downy mildew control. Therefore, the research studies conducted in this project could be divided into three main areas of studies: 1) Sensitivity of *P. viciae* to metalaxyl-M in order to determine whether there are metalaxyl resistant strains in Australia; 2) Fungicide seed treatments to identify suitable seed dressings for the control of downy mildew on seeds and seedlings; 3) Foliar fungicide applications for field downy mildew control to evaluate the efficacy of foliar fungicide treatments for the control of downy mildew from field inoculum.

1. Sensitivity of *Peronospora viciae* to metalaxyl-M

Assessment of sensitivity to metalaxyl-M in Tasmanian collections of Peronospora viciae

By Dr R Falloon[1], R Lister[1], R Butler[1] & Dr H Pung[2]

Summary

In 2001 and 2002, sixteen collections of *Peronospora viciae*, which causes downy mildew of pea (*Pisum sativum*), were assessed for sensitivity to the phenylamide fungicide metalaxyl-M. These collections were from different field crops of the pea cv. Resal in northern Tasmania, and were supplied for testing as frozen, concentrated conidium suspensions. The sensitivity of each collection to metalaxyl-M was assessed in a laboratory bioassay using excised leaf discs from the pea cv. Bolero (susceptible to downy mildew). The bioassay had previously demonstrated resistance to metalaxyl in New Zealand collections of *P. viciae*. Data from the bioassay were used to determine estimated metalaxyl-M EC_{50}s (concentrations of the chemical to give 50% inhibition of infection of leaf discs) for the *P. viciae* collections.

Three of the Tasmanian collections were non-viable, so their sensitivity to metalaxyl-M could not be assessed. The other 13 collections differed in sensitivity to the fungicide. Five collections were sensitive to metalaxyl-M, with estimated EC_{50} values of less than 0.001 µg/ml. Four of the collections were resistant to the fungicide, with EC_{50} values between 5 and 22 µg/ml, i.e. they were at least 5000 times more resistant to the fungicide than the sensitive collections. Four of the collections showed intermediate sensitivity to metalaxyl-M (EC_{50} values 0.02-0.05 µg/ml metalaxyl-M).

These results demonstrated that Tasmanian populations of *P. viciae* are resistant to metalaxyl, but that the frequency of resistance is slightly less than that previously recorded in New Zealand. This difference may be a reflection of the smaller number of collections assessed from Tasmania, compared with the previous New Zealand assessment. However, the relatively high number of partially resistant collections from northern Tasmania compared with the New Zealand assessment suggests that metalaxyl resistance in *P. viciae* may have been in stages of development in northern Tasmania at the time the field populations were sampled (December 2001 and January 2002).

Recommendations

Metalaxyl (phenylamide) resistance management strategies should be applied in Tasmania to achieve effective control of downy mildew in pea crops. These strategies should include: alternating phenylamides with chemicals with different modes of action, or using phenylamides in mixtures with non-phenylamide chemicals. Appropriate alternative or mixture chemicals include cymoxanil, fosetyl-Al, mancozeb or phosphorous acid. The strategies should be applied both for seed treatment fungicide applications to control downy mildew in young crops and for foliar applications of fungicides to control the disease in more mature crops.

Introduction

Peronospora viciae (Berk.) Casp. causes downy mildew of pea (*Pisum sativum* L.). Phenylamide fungicides have been widely and routinely applied to pea seed prior to sowing to control this disease in young seedlings, to prevent downy mildew epidemics developing at early stages of crop growth. These fungicides have systemic activity in seedlings and prevent infection from seed- or soilborne *P. viciae* inoculum. Seed treatments with phenylamide fungicides, usually in combinations with other chemicals, also protect seedlings from other soilborne seedling pathogens (*Pythium* spp.), which can harm seedling establishment (Falloon et al. 2000). In New Zealand, numerous formulations containing the phenylamide metalaxyl have been used as pea seed treatments for many years.

Recent research demonstrated that some New Zealand field collections of *P. viciae* were resistant to metalaxyl (Falloon et al. 2000). A laboratory bioassay showed that sensitive collections of the pathogen were inhibited by less than 0.01 µg/ml of metalaxyl-M, while metalaxyl-resistant collections were inhibited by 3-11 µg/ml of the chemical. This indicated that the metalaxyl-resistant collections were 300 to >1000 times more resistant to metalaxyl than the sensitive collections.

The present report outlines an assessment of the sensitivity to metalaxyl in collections of *P. viciae* from pea crops in Tasmania, using the bioassay technique described by Falloon et al. (2000).

Materials and methods

Peronospora viciae collections

Sixteen field collections of *P. viciae* (designated T1 – T16), all from the pea cv. Resal, were obtained by taking downy mildew-infected plants from pea crops. Details of the locations from which the collections were obtained are outlined in Table 1. Either the same day or the next, *P. viciae* conidia were washed from leaves of infected plants with water. Conidium suspensions were concentrated by centrifugation, and were then deep frozen in 1.5 ml Ependorf tubes.

Table 1: Details of Peronospora viciae *collections obtained from pea crops (cv. Resal) in northern Tasmania, including collection identification numbers (ID), and the dates, growers and locations from which collections were obtained.*

ID	Collected	Location	Latitude	Longitude
T1	14 Dec 2001	Sassafras	146° 30' E	41° 17' S
T2	17 Dec 2001	Deloraine	146° 37' E	41° 2' S
T3	17 Dec 2001	Cressy	147° 5' E	41° 42' S
T4	17 Dec 2001	Cressy	147° 5' E	41° 42' S
T5	17 Dec 2001	Cressy	147° 5' E	41° 42' S
T6	17 Dec 2001	Cressy	147° 5' E	41° 42' S
T7	17 Dec 2001	Hagley	146° 54' E	41° 32' S
T8	17 Dec 2001	Hagley	146° 54' E	41° 32' S
T9	19 Dec 2001	Sassafras	146° 30' E	41° 17' S
T10	19 Dec 2001	Wesley Vale	146° 29' E	41° 13' S
T11	19 Dec 2001	Wesley Vale	146° 29' E	41° 13' S
T12	21 Dec 2001	Deloraine	146° 37' E	41° 2' S
T13	11 Jan 2002	Forest	145° 15' E	39° 51' S
T14	11 Jan 2002	Forest	145° 15' E	39° 51' S
T15	11 Jan 2002	Forest	145° 15' E	39° 51' S
T16	11 Jan 2002	Forest	146° 30' E	41° 17' S

The collections were despatched from Devonport, Tasmania, on 21 January 2002, and were maintained frozen throughout airfreighting to Christchurch, New Zealand. They arrived at the Crop & Food Research Plant Pathology Laboratory at Lincoln on 25 January 2002, and were immediately placed in a deep freeze (-20°C), and held there for 7 weeks. The collections were then assessed for sensitivity to metalaxyl in a laboratory bioassay.

Bioassay of sensitivity of *P. viciae* collections to metalaxyl

Technical grade metalaxyl-M (96.1% metalaxyl-M) was obtained from Mr G. B. Follas, Syngenta Crop Protection Australasia. The chemical was dissolved in a small amount of acetone, then added to reverse osmosis (RO) purified water to make solutions containing 100, 10, 1.0, 0.1 and 0.01 µg/ml metalaxyl-M. Aliquots (10 ml) of these solutions were added to plastic Petri dishes (55 mm diam.), with 32 dishes prepared containing each solution. Dishes (32) were also each filled with 10 ml RO water as nil experimental standards.

Leaf discs (15 mm diam.) were cut from leaves of 4-week-old, glasshouse-grown pea plants (cv. Bolero: growth stage 110 – 111; vegetative, ten to 11 nodes). The leaf discs were immediately placed in the Petri dishes (five discs per dish) containing the metalaxyl-M solutions or water (experimental standards), with adaxial (upper) leaf surfaces in contact with the solutions and abaxial (lower) surfaces uppermost (Figures 1 and 2).

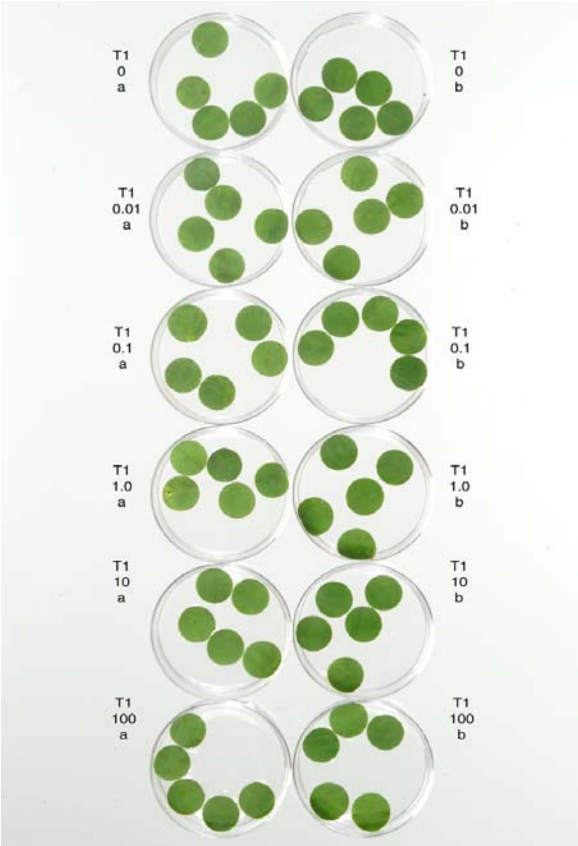


Figure 1: Petri dishes, each with five pea leaf discs, containing different metalaxyl-M solutions (0 – 100 µg/ml), used in the bioassay of sensitivity of Tasmanian collections of Peronospora viciae *to metalaxyl-M. This photo was taken before inoculation with conidia of collection T1 of the pathogen.*

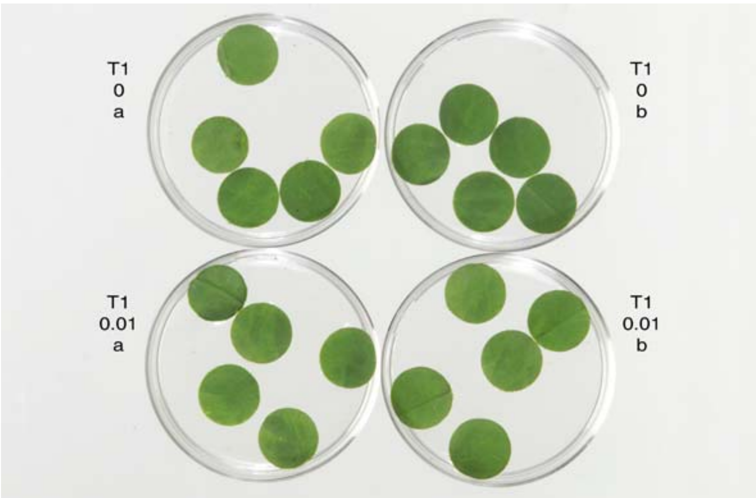


Figure 2: Closeup of Petri dishes in Figure 1 (0 and 0.01 µg/ml solutions of metalaxyl-M).

Frozen conidium suspensions of the 16 *P. viciae* collections were allowed to thaw at room temperature, and were then sprayed on to the leaf discs in the Petri dishes. The inoculum used for each collection was the content of one Ependorf tube (1.5 ml) as received from Tasmania, and no attempt was made to standardise inoculum levels across all of the collections. The Permit to Import Laboratory Specimens issued by the NZ Ministry of Agriculture and Forestry for import of the collections from Tasmania allowed use of the collections only in laboratory assays, precluding the possibility of passing each collection through any infection cycles on glasshouse-grown pea plants to produce fresh inoculum. The conidium concentration for each collection (Table 2) was determined by counting conidia in the suspensions with a microscope slide haemocytometer. Two duplicate Petri dishes (ten leaf discs) of each of the six metalaxyl-M concentrations (including the nil standard) were inoculated with each of the *P. viciae* collections (see Figures 1 and 2).

Table 2: Conidium inoculum concentrations and levels used for inoculation of pea leaf discs with different collections of Peronospora viciae *in a bioassay to determine sensitivity to metalaxyl-M.*

Collection ID	Number of conidia/ml	Number of conidia/leaf disc	Collection ID	Number of conidia/ml	Number of conidia/leaf disc
T1	2.7×10^5	6800	T9	9.3×10^6	232500
T2	5.0×10^6	125000	T10	8.1×10^5	20300
T3	8.3×10^6	207500	T11	9.7×10^5	24300
T4	1.6×10^5	4000	T12	5.2×10^6	130000
T5	3.3×10^6	82500	T13	5.0×10^6	125000
T6	5.4×10^6	135000	T14	4.8×10^6	120000
T7	3.3×10^6	82500	T15	2.3×10^6	57500
T8	6.3×10^6	157500	T16	2.1×10^6	52500

After inoculation, the Petri dishes were placed in an incubator set at 15°C (±0.2°C). The incubator had glass front and rear walls to allow entry of ambient light. After 8 days, when the leaf discs in the nil standards had sporulating *P. viciae* infections on their abaxial surfaces, the severity of downy mildew on each leaf disc was assessed. This was done by comparing them with standard leaf disc area diagrams (Figure 3), to give the proportion of the area of each leaf disc area infected with downy mildew.

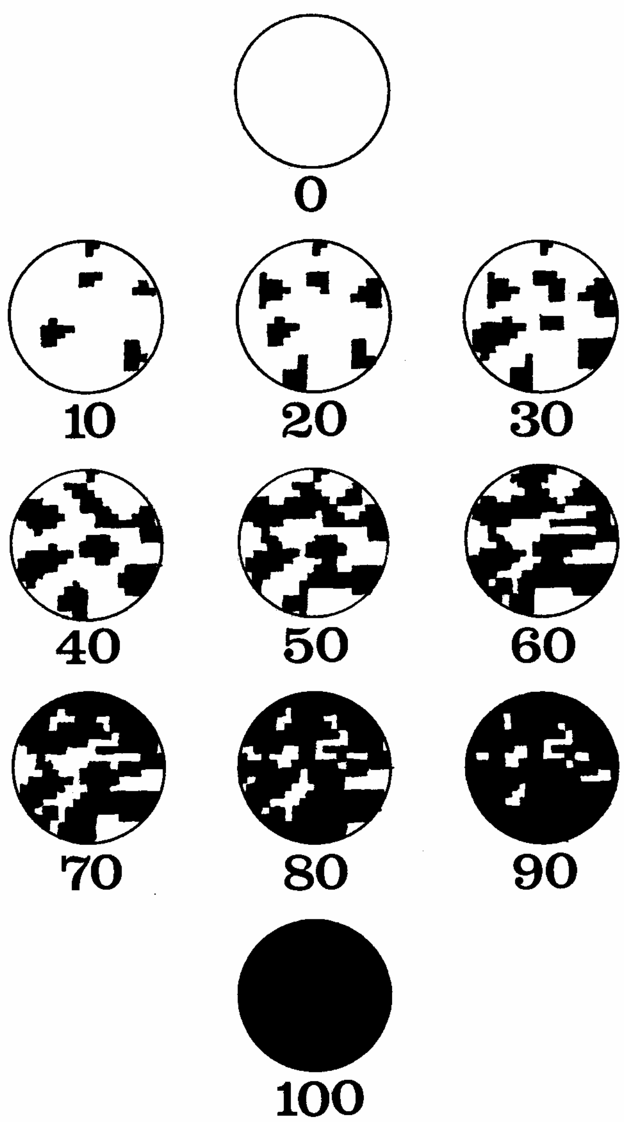


Figure 3: Standard diagrams used for assessment of area of leaf discs infected with Peronospora viciae *in a laboratory bioassay. Proportions (percent) of each disc "infected" are indicated.*

Statistical analyses

Preliminary statistical analyses (analysis of variance and generalised linear mixed model analysis (Schall 1991) were carried out to determine the effects of individual Petri dishes in the bioassay. These analyses indicated that there was no substantial extra variability in the data because of differences between dishes, allowing dishes to be ignored in further analyses. All further statistical analyses carried out were logistic regressions (McCullagh & Nelder 1989) using various models. The main aim of the analyses was to estimate dose/response curves for the effects of metalaxyl-M on the *P. viciae* collections, to compare these, and to assess whether the fitted curves were adequate approximations of the differences between the mean responses to each metalaxyl-M concentration. A logistic dose/response curve of log concentration was used:
where

$$\text{Response} = \frac{C}{1 + \left(\frac{\text{metalaxyl - M concentration}}{EC_{50}} \right)^{b}}$$

C is the percentage of leaf disc area infected for the nil experimental standard (dose = 0),
EC_{50} is the metalaxyl-M dose giving half the leaf disc area infected of the nil standard, and
b is the relative steepness of the curve when plotted against log (dose), at dose = EC_{50}.

Parallel curve analysis (Ross 1984) was used to assess the statistical significance of differences between the response curves for different *P. viciae* collections. Possible relationships between the estimated parameters (C, EC_{50} and b) for the collections were investigated graphically. Goodness of fit of the curves was assessed by comparing the fit of the curves (all data, separate curves for each *P. viciae* collection) with a logistic regression model fitting to the mean for each metalaxyl-M concentration for each collection.

Results

For collections T1, T5, and T13, no infection of leaf discs occurred in the bioassay plates, indicating that the inoculum for these collections was not viable. The data for these collections was excluded from the statistical analyses.

Table 3: Parameters from logistic dose response curves for different Peronospora viciae *collections in the leaf disc bioassay.* C *is the mean percentage of leaf disc area infected in the nil standard,* EC_{50} *is the metalaxyl-M dose giving half the mean leaf disc area infected of the nil standard, and* b *is relative steepness of the curve when plotted against log (dose), at dose* = EC_{50}. *The collections are listed in order of increasing* EC_{50}. *Standard errors (in parentheses) are based on overall dispersion of the data (df = 702).*

Collection	Inoculum (conidia/ml)	C	b	EC_{50} (µg/ml)
T9 (sensitive)	9.3×10^6	58 (2.9)	0.43 (0.14)	0.00002 (0.00005)
T15 (sensitive)	2.3×10^6	63 (2.8)	0.51 (0.17)	0.00004 (0.00009)
T16 (sensitive)	2.1×10^6	63 (2.8)	0.45 (0.10)	0.00007 (0.0001)
T10 (sensitive)	8.1×10^5	29 (2.6)	0.45 (0.14)	0.0003 (0.0004)
T7 (sensitive)	3.3×10^6	21 (2.4)	0.54 (0.16)	0.0009 (0.001)
T2	$5.0 \text{ X } 10^6$	50 (2.9)	2.47 (0.43)	0.024 (0.006)
T11	9.7×10^5	30 (2.6)	1.10 (0.19)	0.031 (0.01)
T3	8.3×10^6	51 (2.9)	4.54 (0.23)	0.042 (0.006)
T14	4.8×10^6	49 (2.8)	5.32 (0.75)	0.048 (0.73)
T6 (resistant)	5.4×10^6	62 (1.6)	2.38 (0.93)	5.2 (1.41)
T8 (resistant)	6.3×10^6	64 (1.6)	1.15 (0.14)	5.5 (0.90)
T4 (resistant)	1.6×10^5	25 (2.4)	8.72 (61.2)	8.1 (*)
T12 (resistant)	5.2×10^6	88 (1.8)	0.31 (0.03)	21.7 (6.54)

* Standard error not available due to failure of asymptotic calculations

Parameters obtained from parallel curve analyses are outlined in Table 3, and the fitted dose/response curves for the viable *P.* viciae collections are illustrated in Figure 4. There was no statistically significant lack of fit of the curves from the treatment means, indicating that the fitted curves were generally good descriptions of the responses of the collections to metalaxyl-M. Collection T10 was an exception with a relatively poor fit because of a slight rise in the mean severity of infection between the mean proportion of leaf disc infected between 0.01 and 0.1 µg/ml (Figure 4). Parallel curve fitting indicated that there were statistically significant ($P \leq 0.05$) differences between some of the strains in all three of the dose response parameters (*C*, EC_{50} and *b*; Table 3).

Pathogenicity of collections

The collections differed in pathogenicity as indicated by the mean disease severities (parameter *C*) where no metalaxyl-M was added to the bioassay plates (nil standards). The most virulent collections were T6, T15, T16, T8 and T12, which all gave greater than 60% of mean leaf disc area infected where no fungicide was applied. Collections T7, T4, T10 and T11 gave less than 30% mean leaf disc area infected. For three of these collections (T4, T10 and T11) the inoculum concentration used was less than 10^6 conidia/ml (Table 2; Figure 4).

Response of collections to metalaxyl-M

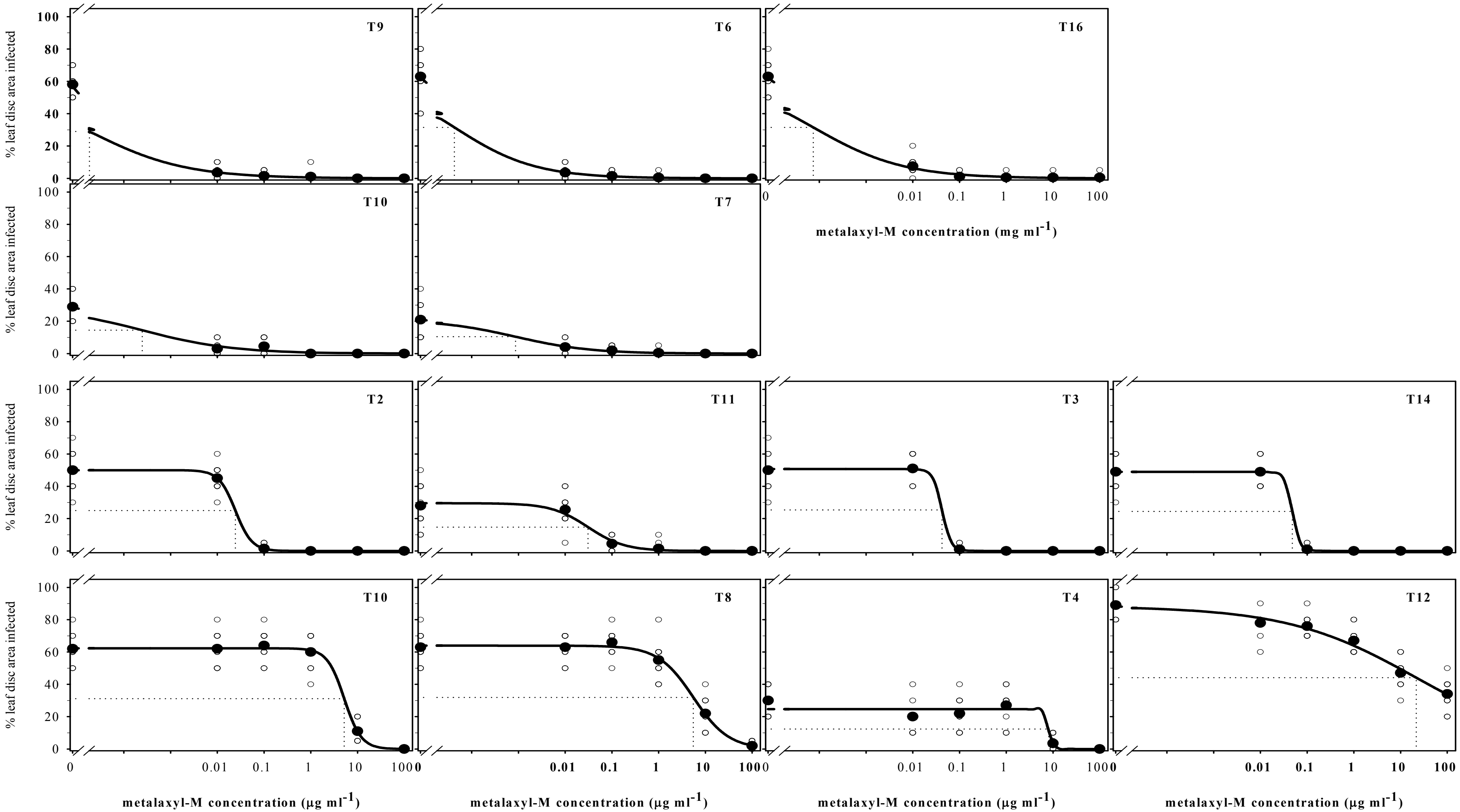
The least effect of increasing metalaxyl-M concentration (parameter b <0.6) occurred with collections T7, T16, T10, T9 and T12 (Table 3; Figure 4). The greatest effect of increasing metalaxyl-M concentration occurred with collection T4, for which the relative slope of the response curve at the EC_{50} was 8.7.

Sensitivity of collections to metalaxyl-M

The lowest EC_{50}s (<0.001 µg/ml metalaxyl-M) were recorded for collections T9, T15, T16, T9 and T7 (Table 3, Figure 4), and these were designated as "sensitive" to the fungicide. Strains T6, T8, T4 and T12 gave EC_{50}s that were greater than 5 µg/ml metalaxyl-M, and these were designated as "resistant" to the fungicide. Four collections (T2, T11, T3 and T14) gave intermediate EC_{50}s (0.2-0.5 µg/ml metalaxyl-M), and these were designated as "partially resistant" to the fungicide. There was no correlation between pathogenicity of collections (parameter *C*) and their sensitivity to metalaxyl-M (EC_{50}).

Susceptibility/resistance to metalaxyl-M in the viable *P. viciae* collections (Table 3) may have been related to the location from which the collections were obtained (Table 1), although the sample size from each location was small (two to four collections). The viable collection from Sassafras (T9) was susceptible to the fungicide, the three viable collections from Forest were susceptible (T15 and T16) or partially resistant (T14), and those from Wesley Vale were susceptible (T10) or partially resistant (T11). On the other hand, the two collections from Deloraine were partially resistant (T2) or resistant (T12) to the fungicide, and the collections from Cressy were partially resistant (T3) or resistant (T4 and T6). The two collections from Hagley were susceptible (T7) or resistant (T8) to the chemical.

Figure 4 (overleaf): Fitted dose response curves of proportions of pea leaf discs infected with different *Peronospora viciae* collections in bioassay plates containing different concentrations of metalaxyl-M (raw data (○) means (●)). Dotted lines indicate estimated EC_{50} values, the metalaxyl-M concentration giving 50% of the leaf disc area infected of the nil experimental standards. Collections are arranged in order of increasing estimated EC_{50}. Collections T9, T6, T16, T10 and T7 were sensitive to metalaxyl-M (EC_{50} <0.001 µg/ml), while collections T10, T8, T4 and T12 were resistant to the fungicide (EC_{50} >5 µg/ml).



Discussion

This study has demonstrated that some field populations of *P. viciae* from Tasmania were resistant to the phenylamide fungicide metalaxyl-M. This chemical makes up either 50 or 100% of the active ingredient in all metalaxyl product formulations, and is the major active ingredient of those products. Of the viable *P. viciae* collections assessed in this study, five (38%) were susceptible to metalaxyl-M, four (31%) were resistant, and four (31%) were partially resistant. These results are similar to those for *P. viciae* collections from New Zealand (Falloon et al. 2000), although the proportion of resistant collections from Tasmania is less than the equivalent proportion for the New Zealand study, where 56% of the collections were resistant. More of the Tasmanian collections were partially resistant to the fungicide than was the case in the New Zealand study. Although the number of collections tested from Tasmania was smaller than the sample size in the New Zealand study, the present results suggest that resistance to metalaxyl in Tasmania was at an earlier stage of development, late in 2001, than in New Zealand in 1995 to 1998.

The level of resistance in some Tasmanian collections of *P. viciae* was more than 5000 times greater than that of the susceptible Tasmanian collections. One collection (T12) had a metalaxyl-M EC_{50} of 21.7 µg/ml, which was at least 24 000 times greater than that of the susceptible collections. These resistance factors are much larger than has been previously recorded. Falloon et al. (2000) recorded metalaxyl resistance factors of 300 to 1000 in New Zealand *P. viciae* collections, and Gisi (1988) made a general observation that "levels of resistance (to phenylamide fungicides) are high; usually higher than 100." The resistant collections in the present study were generally highly pathogenic, as demonstrated by the high mean values of parameter *C* (>60% of leaf disc area infected) that were measured for three of the four resistant collections, and the metalaxyl-resistant collections were as pathogenic as the susceptible ones. This also agrees with the observation that "resistant isolates (of Peronosporales) are as competitive as the sensitive ones" (Gisi 1988).

Populations of *P. viciae* that demonstrated partial (intermediate) resistance to metalaxyl probably contain individuals with differing susceptibilities to the fungicide, rather than having all members with partial resistance. Previous studies (Gisi 1988) have shown that phenylamide resistance in other pathogens was of the monogenic type, so that individuals in a population are likely to be either resistant or susceptible.

Metalaxyl resistance management strategies should be instigated for control of downy mildew of peas in Tasmania. Populations of *P. viciae* with resistance to metalaxyl are likely to be resistant to other phenylamide fungicides, as this has been demonstrated elsewhere (Falloon et al. 2000). Alternating use of phenylamides with non-phenylamides, or use of these chemicals in combinations with non-phenylamides, have been suggested as appropriate strategies to prevent resistance occurring or building up (Gisi 1988; Urech, 1988). Several chemicals with alternative modes of action (e.g. cymoxanil, fosetyl-Al, mancozeb, phosphorous acid) are appropriate alternatives to phenylamides or as candidates for use in mixtures with phenylamides. These approaches should be used in both fungicide seed treatments for control of downy mildew in seedlings in young crops, and in foliar applications of fungicides for control of the disease in more mature plants in older crops. Phenylamides are likely to remain effective for control of soilborne *Pythium* spp., which are harmful in seedling establishment (Falloon et al. 2000). Fungicide combinations (phenylamides with non-phenylamides) should be used as seed treatments to protect seedlings from both soilborne pathogens and downy mildew.

2. Seed and seedling infection control

Summary

Seven field trials and one pot trial were conducted in Tasmania in 2000 and 2001 to further evaluate a range of different fungicide seed treatments for the control of seedborne pathogens (including *P. viciae* and *Ascochyta*) and damping-off pathogens in soil, in order to improve seedling establishment. The evaluations included three fungicide seed dressings, Apron + P-Pickel T, Aliette Super, and Wakil XL, developed commercially in Australia and New Zealand, for use in pea seed dressings for the control of downy mildew as well as other seedborne pathogens.

Aliette Super, Apron + P-Pickel T and Wakil seed treatments generally improved seedling survival, with no adverse effects in the seedling growth. Other fungicide seed treatments tend to delay the initial growth of seedlings, and have little or no effect on downy mildew.

In field trials, Aliette Super seed treatment was the most effective in reducing downy mildew severity on maturing plants, followed by Apron + P-Pickel T. Wakil performed poorly for downy mildew control on maturing plants in comparison to Apron + P-Pickel T. It is possible that this could be due to the lower rate of metalaxyl used in the Wakil seed treatment (350g metalaxyl/tonne seed) compared to that in the Apron + P-Pickel T (525g metalaxyl/tonne seed).

With the presence of metalaxyl-resistant isolates of *P. viciae* in Tasmania, fungicide resistance management strategies for seed treatment should include alternating metalaxyl or phenylamides with chemicals that have different modes of action, or using metalaxyl in mixtures with non-phenylamide chemicals such as cymoxanil or fosetyl-Al, which can protect seedlings from infection by metalaxyl-resistant isolates. Therefore, Aliette Super and Wakil seed treatments, which contains fosetyl-Al and cymoxanil, respectively, are suitable alternatives to the Apron + P-Pickel T seed treatment. In Apron + P-Pickel T, metalaxyl is only active ingredient for downy mildew control.

Poor seedling establishment in the field could also be caused by inherent poor seed quality, as demonstrated by one of the seed lines used in the trials. The relatively low seedling numbers, ranging from 27% to 41%, could not be improved by the various seed treatments in the trials.

In the past, it was suspected that Aliette Super treated pea seed may lose its viability if kept in storage. A pot trial carried out to examine the viability of fungicide treated seed after 1 year of storage, showed that Aliette Super, Apron + P-Pickel T, or Wakil had no adverse effects on seed germination, and seedling emergence and growth.

2.1. Initial field trials on seed treatments in 2000

Trials Summary

	TRIAL 1	TRIAL 2	TRIAL 3
Location	Forth	Wesley Vale	East Sassafras
Soil Type	Red Ferrosol	Red Ferrosol	Grey Sandy Loam
Variety	Small Sieve Freezer	Small Sieve Freezer	Small Sieve Freezer
Trial Design	Randomised complete block	Randomised complete block	Randomised complete block
Replicates	5	5	5
Plot Size	1.2 m x 6 m	3.7 m x 6 m	4 m x 6 m
Sowing density	246 kg/ha	293 kg/ha	254 kg/ha
Seed drill	Oyjord drill	Air-seed drill	Air-seed drill
Row Spacing	15-20 cm	10-12 cm	10-12 cm
Sowing Date	11/08/00	07/08/00	25/09/00
Harvest Date	12/12/00	04/12/00	N/a
Seed treatments	A seed supplier in New Zealand carried out seed treatments with Aliette Super, Wakil XL, and Apron & P-Pickel T. For other seed treatments, 1 kg of untreated seeds from the same seed lot was mixed with the appropriate fungicide in a clean zip-lock plastic bag. In order to facilitate the adherence of powder formulated fungicides on to seed coats, the seeds were first wetted with 5 ml sterile distilled water. Untreated seed was used for the untreated control. The Bion granules were ground into fine powder before use. Maxim was adjusted with sterile distilled water to 5 ml before mixing with the 1kg seed lot. Even seed coating was obtained with all seed treatments.		

Materials and methods

Treatment list for Trial 1

No.	Seed Treatment	Product Rate per tonne seed
1	Agri-Fos + Apron + P-Pickel T	4.0 L + 1.5 kg + 2.0 L
2	Acrobat	1.5 kg
3	Aliette Super	2.9 kg
4	Bavistin	1.5 L
5	Fongarid	2.0 kg
6	Maxim	1.0 L
7	Wakil XL	2.0 kg
8	Apron + P-Pickel T	1.5 kg + 2.0 L
9	Bion + Apron + P-Pickel T	10 kg + 1.5 kg + 2.0 L
10	Untreated control	N/a

Treatment lists for Trials 2 & 3

	Seed Treatment		Foliar Spray		
No.	**Products**	**Product Rate (per tonne seed)**	**Product**	**Product Rate**	**Application Schedule**
1	Apron + P-Pickel T	1.5 kg + 2.0 L	Bravo	1.8 L/ha	2 sprays: at 2 nodes, and 13-14 days after
2	Apron + P-Pickel T	1.5 kg + 2.0 L	Nil	Nil	Nil
3	Aliette Super	2.9 kg	Nil	Nil	Nil
4	Wakil XL	2.0 kg	Nil	Nil	Nil
5	Untreated control	N/a	N/a	N/a	N/a

Assessments and statistical analysis

The number of seedlings per plot was counted for seedling emergence and survival.

Ten plants were collected at random, at half-metre intervals, from the middle of each plot, and assessed for downy mildew incidence. Plant growth was also determined by measuring plant height for the 10 plants sampled in each plot, and the average plant height was tabulated.

Five infected plants were picked at random from the 10-plant sample per plot, and assessed for downy mildew severity. The severity of downy mildew infection was assessed using the disease severity key for downy mildew (Falloon *et al.* 1995), and the percentages of leaves covered by downy mildew on the plants were tabulated).

Collar rot severity was assessed by measuring the length of lower stem that was affected by collar rot and the total stem length of the plant, and then tabulated the percentage of stem affected by the collar rot.

Analysis of variance was conducted on the data set using StatGraphics Plus 2.0. Pairwise comparisons were made of mean values using Least Significant Difference Test. Where data values are not normally distributed and could not be normalized, Kruskall-Wallis Test in a one-way analysis was conducted, and medians were compared instead.

Chronology of events

DATE	DAYS AFTER SOWING	TRIAL 1
26/07/00	-16	Fungicide seed treatments applied.
11/08/00	0	Trial pegged and pea seeds sown.
25/08/00	14	Pre-emergence herbicide applied.
05/09/00	25	Treatment 9 in all replicates was emerging slower than the rest of the treatments. Plant stage ranged from seedling emergence to 1st node.
13/09/00	33	Treatment 9 in all reps was still emerging slower than the rest of the treatments.
18/09/00	38	1st emergence counts of all plots.
21/09/00	41	1st plant height assessment.
09/10/00	59	2nd emergence counts. Downy mildew first noticed on some plants in the trial area.
08/11/00	89	Downy mildew was widespread and affected all plants irrespective of treatments. The disease was mild in severity, affecting only lower leaves. In all treatments, except Treatment 9, the lower leaves affected by downy mildew have started to wilt and desiccate. In Treatment 9 plots, plants were smaller and had fewer flowers compared to those in all other treatments.
14/11/00	95	2nd plant height assessments.
12/12/00	123	Disease assessment.

DATE	DAYS AFTER SOWING	TRIAL 2
07/08/00	0	Trial pegged and pea seeds sown.
29/08/00	22	Seedlings emerging. Treatment differences in seedling density observed, with Treatments 1 and 2 emerging faster.
05/09/00	29	The emergence and growth variation between treatments as described above was still obvious.
07/09/00	31	1st foliar fungicide application of Treatment 1. No disease observed. Crop stage 2-3 nodes.
15/09/00	39	Emergence counts and 1st plant height assessment.
20/09/00	44	2ndt foliar fungicide application of Treatment 1. Plants in Treatments 1 and 2 were bigger than plants in other treatments.
11/10/00	65	Downy mildew widespread.
20/10/00	74	Downy mildew assessments.
29/11/00	114	Collar rot assessment.
30/11/00	115	2nd plant height assessments.
04/12/00	119	Plants collected for yield assessment.

DATE	DAYS AFTER SOWING	TRIAL 3
25/09/00	0	Trial pegged and seeds sown.
23/10/00	28	Seedlings emerged, with growth stage two to three nodes.
23/10/00	28	1st foliar fungicide application of Treatment 1.
03/11/00	39	Emergence counts and plant height assessment.
07/11/00	43	2ndt foliar fungicide application of Treatment 1.
8/11/00	44	Downy mildew disease and height assessments conducted.
18/12/00	84	Almost all plants in trial area completely desiccated due to water stress, no further assessment done. Crop not irrigated due to lack of water.

Results and discussion

Trial 1

In Trial 1, pea seeds were planted using an Oyjord seed drill at the Forthside Research Station. Seedling distribution and numbers in the trial were highly variable between plant rows. This was found to be due to mechanical faults in the drill. As a result of the high variability in seedling distribution, no significant differences could be found on the seedling numbers between treatments at 38 days (p = 0.11) and 59 days (p = 0.28) after sowing (Table 1). However, apart from Bavistin (Treatment 4), all fungicide seed treatments appeared to improve seedling numbers compared to the untreated control. Bion and Apron + P-Pickel T (Treatment 9), delayed seedling emergence and growth. This adverse effect was demonstrated by the significant reduction in the mean plant heights compared to the untreated control at 41 days (p = 0.003) and 95 days (p = 0.01) after sowing (Table 1).

Table 1: Seed treatment effects on plant density and height in Trial 1

		Average plant numbers/m^2		Average plant height (cm)	
No.	**Seed Treatment**	**38 DAS**	**59 DAS**	**41 DAS***	**95 DAS***
1	Agri-Fos + Apron + P-Pickel T	155 a	154 a	4.6 b	82 ab
2	Acrobat	148 a	151 a	5.3 b	90 c
3	Aliette Super	167 a	166 a	4.8 b	89 bc
4	Bavistin	142 a	128 a	4.7 b	89 bc
5	Fongarid	161 a	153 a	5.2 b	90 bc
6	Maxim	147 a	148 a	4.9 b	91 c
7	Wakil XL	157 a	158 a	4.9 b	84 abc
8	Apron + P-Pickel T	174 a	159 a	4.7 b	86 bc
9	Bion + Apron + P-Pickel T	144 a	147 a	3.0 a	77 a
10	Untreated control	138 a	138 a	4.9 b	85 bc

* Means followed by the same letter are not significantly different at the 5% level according to LSD Test.
DAS = Days after sowing

Plants in Treatment 9 also had a significantly lower downy mildew incidence (p = 0.001) and collar rot severity (p = 0.05) compared to the untreated control and other treatments (Table 2). However, these effects are likely to be due to the slower plant growth in Treatment 9. At the time of the disease assessment, plants in Treatment 9 plots were still small and sparse. This allowed rapid drying and reduces the wet conditions that encourage downy mildew and collar rot.

Table 2: Seed treatment effects on downy mildew incidence and collar rot severity in Trial 1 at 123 days after sowing

No.	Seed Treatment	% Downy mildew incidence^	% Collar rot severity
1	Agri-Fos + Apron + P-Pickel T	100 b	10 b
2	Acrobat	100 b	9 b
3	Aliette Super	98 b	11 b
4	Bavistin	100 b	10 b
5	Fongarid	100 b	10 b
6	Maxim	100 b	12 b
7	Wakil XL	98 b	10 b
8	Standard	100 b	10 b
9	Bion + Apron + P-Pickel T	64 a	5 a
10	Untreated Control	100 b	9 b

^ Statistical analysis based on Kruskall-Wallis test.
Means followed by the same letter in each column are not significantly different at the 5% level according to analysis of variance and LSD Test.

Trial 2

All fungicide seed treatments in this trial gave substantial increases in seedling survival and numbers ($p = 0.0001$) compared to the untreated seeds. Increases due to the seed treatments ranged from 57 to 72% (Table 3).

All the fungicide seed treatments also increased plant growth. At 39 days after sowing, the greatest mean plant heights were on plants from seeds treated with the standard Apron + P-Pickel T, followed by Aliette Super and Wakil XL (Table 3). At 115 days after sowing, the increased plant height due to Apron + P-Pickel T was still obvious.

Table 3: Treatment effects on seedling density and plant height in Trial 2

No.	Seed Treatment	Seedling survival at 39 DAS		Mean plant height (cm/plant)	
		No. seedlings/m^2	% Increase in seedlings/m^2	39 DAS	115 DAS
1	Apron + P-Pickel T & 2 x Bravo sprays	111 b	63	5.8 c	101.7 c
2	Apron + P-Pickel T	118 b	72	5.9 c	100.3 c
3	Aliette Super	107 b	57	5.2 b	92.3 a
4	Wakil XL	111 b	63	5.2 b	96.6 bc
5	Untreated Control	68 a	0	4.3 a	90.4 a

Means followed by the same letter in each column are not significantly different at the 5% level according to LSD Test.

Downy mildew was evident on plants at 65 days after sowing. Seed treatments did not reduce downy mildew incidence on young plants at 74 days (Table 4). The downy mildew severity was not significantly different between treatments ($p > 0.05$) and was considered low, with percentage of leaf area infected ranging from 3.5 to 4.6%.

The percentage of nodes with dead, dry leaves was a measure of the proportion of lower nodes where attached leaves were dead. In this study, although the ratings for percentage nodes with dead leaves was conducted as part of the downy mildew severity rating, the dead leaves may also have been caused by other factors such as collar rot and *Septoria* infections. The Bravo foliar sprays in Treatment 1 significantly reduced the percentage of nodes with dead leaves when compared to the untreated control and fungicide seed treatments (Table 4). The Bravo foliar sprays also reduced collar rot severity on the trial plants at 114 days after sowing.

Table 4: Treatment effects on downy mildew and collar rot diseases in Trial 2

No.	Treatment	Downy mildew disease assessment at 74 DAS			% Collar rot severity at 114 DAS
		% Disease incidence	% Disease severity	% Nodes with dead leaves	
1	Apron + P-Pickel T + 2 Bravo foliar sprays	100	4.6 a	11 a	10 a
2	Apron + P-Pickel T	100	3.8 a	31 c	15 b
3	Aliette Super	100	3.8 a	31 c	15 b
4	Wakil XL	100	3.9 a	30 bc	15 b
5	Untreated Control	100	3.5 a	22 b	15 b

Means followed by the same letter in each column are not significantly different at the 5% level according to LSD Test.

Trial 3

In contrast to Trial 2, there were no differences in the number of seedlings that emerged from Apron + P-Pickel T, Aliette Super and Wakil XL treated seeds in comparison to the untreated seeds in Trial 3 (Table 5). Seedlings from seeds treated with Aliette Super (Treatment 3) and Wakil XL (Treatment 4) were bigger, as shown by their greater plant heights in comparison those in the untreated control. In the disease assessment at 44 days after sowing, there were no significant differences between treatments in downy mildew incidence and severity, or the percentage nodes with dead leaves. This trial was terminated early, as almost all plants in the trial area were desiccated before they reached maturity, due to lack of rainfall and irrigation.

Table 5: Treatment effects on plant numbers, growth, and downy mildew severity and incidence

No.	Seed Treatment	Seedling assessment at 39 DAS		Disease assessments at 44 DAS		
		Plant numbers/m^2	Plant height (cm)	% Downy mildew incidence	% Downy mildew severity	% Nodes with dead leaves
1	Apron + P-Pickel T + 2 Bravo foliar sprays	89 a	13.1 abc	100 a	6.1 a	4.3 a
2	Apron + P-Pickel T	95 a	12.3 ab	100 a	6.6 a	4.8 a
3	Aliette Super	94 a	13.9 bc	98 a	5.5 a	4.9 a
4	Wakil XL	101 a	14.3 c	96 a	5.4 a	0.7 a
5	Untreated	91 a	11.7 a	100 a	5.3 a	0.0 a

Means followed by the same letter in each column are not significantly different at the 5% level according to LSD Test.

2.2. Trials on seed treatments in 2001

Trials Summary

	POT TRIAL	FIELD TRIALS			
		4	5	6	7
Location	Bellfield, Tas.	Wesley Vale	Wesley Vale	Wesley Vale	Wesley Vale
Pea cultivar	Resal	Resal	Small Sieve Freezer	Resal	Small Sieve Freezer
Soil Type	Potting mix + field soil (7:3 ratio)	Sandy loam	Sandy loam	Ferrosol	Ferrosol
Pot or Plot Size	5 L pot	1.5 m x 1.1 m	1.5 m x 1.1 m	1.5 m x 1.6 m	1.5 m x 1.6 m
Sowing Date	27/07/01	20/09/01	20/09/01	10/10/01	10/10/01
Sowing Density	20 seed/pot	100 seeds/m^2	100 seeds/m^2	100 seeds/m^2	100 seeds/m^2
Sowing Depth	2 cm	6 cm	6 cm	6 cm	6 cm
Sowing Method	Hand Sown				
Trial Design	Randomised complete block				
Replicates	5				

Materials and methods

Untreated seeds (1 kg samples) from the same seed lot were mixed with the appropriate fungicide as described in previous trials in 2000. The numbers of seedlings per meter square in each replicate plot were counted, to determine the percentage of seedling emergence and survival. Plants were assessed for growth and downy mildew as described in 2.1. Collar rot and leaf spot, due to *Phoma medicarginis* were also present in the field trials. It was not possible to distinguish the cause of dead and desiccated leaves of plant nodes close to the ground, which may have been caused by collar rot, downy mildew or water stress. Therefore, the proportion (%) of plant nodes with dead leaves was assessed separately.

Treatment list for pot trial

No.	Product	Product rate (per tonne seed)
1	Untreated control	N/a
2	Aliette Super	2.9 kg
3	Wakil XL	2.0 kg
4	Serenade	2.0 kg
5	Amistar	0.5 kg
6	Bion	0.25 kg
7	Bion	0.5 kg
8	Captan	0.5 kg
9	Fongarid	0.5 kg
10	Fongarid	1.0 kg
11	Bion + Wakil	0.25 kg + 2.0 kg
12	Bion + Fongarid	0.25 kg + 1.0 kg
13	Amistar + Fongarid	0.5 kg + 1.0 kg
14	Maxim + Fongarid	0.5L + 1.0 kg
15	Maxim + Apron	0.5 kg + 0.5 kg
16	Amistar + Apron	0.5 kg + 0.5 kg
17	Maxim + Tecto + Apron	0.5 L + 1.0 L + 0.5 kg
18	Amistar + Tecto + Apron	0.5 kg + 1.0 L + 0.5 kg
19	Amistar + Tecto + Fongarid	0.5 kg + 1.0 L + 1.0 kg
20	Raxil + Agri-Fos Supa 400	1 kg + 3 kg

Treatment list for Field Trials 1 - 4

No.	Product	Product rate/tonne seed
1	Untreated control	N/a
2	Aliette Super	2.9 kg
3	Wakil	2.0 kg
4	Apron + P-Pickel T	1.5 kg + 2.0 L
5	Amistar + Apron	0.5 kg + 0.5 kg
6	Amistar + P-Pickel T	0.5 kg + 2.0 L
7	Maxim + P-Pickel T	0.5 kg + 2.0 L kg
8	Amistar + Fongarid	0.5 kg + 0.5 kg
9	Bion + Fongarid	0.25 kg + 0.5 kg
10	Bion + Maxim + Fongarid	0.25 kg + 0.5 kg + 0.5 kg
11	Bion + Amistar + Fongarid	0.25 kg + 0.5 kg + 0.5 kg
12	Amistar + Tecto + Apron	0.25 kg + 1.0 L + 0.5 kg
13	Amistar + Tecto + Fongarid	0.25 kg + 1.0 L + 0.5 kg
14	Amistar + Fongarid + Serenade	0.5 L + 0.5 kg + 1.0 kg
15	Elexa + Amistar + Fongarid	0.25 kg + 0.5 kg + 0.5 kg

Chronology of events

Date	Days after sowing (DAS)	Pot Trial
17/07/01	-3	Fungicide seed treatments applied.
20/07/01	0	Pea seeds sown.
23/07/01	3	Pots randomised.
06/08/01	17	Seedlings started emerging.
13/08/01	24	1st seedling density assessment.
05/09/01	47	2nd seedling density assessment.

Date	DAS	Field Trials 4 & 5
07-08/09/01	-12 & -13	Pea seeds treated and air-dried.
20/09/01	0	Seeds sown.
11/10/01	21	Assessment for plant density and height of Resal seedlings in Trial 4. Slow and poor seedling emergence of SSF treated seeds noted in Trial 5.
19/10/01	29	Assessment for plant density and height of SSF seedlings in Trial 2.
28/11/01	69	Fresh shoot weight assessment of Trial 1.
30/11/01	71	Disease assessment of Trial 4.
27/12/01	98	Disease assessment of Trial 5.

Date	DAS	Field Trials 6 & 7
7/09/01	-33	Pea seeds treated and air-dried.
10/10/01	0	Seeds sown
01-02/11/01	22-23	Assessment for plant density and height of Resal seedlings in Trial 6. Slow and poor seedling emergence of SSF treated seeds noted in Trial 7.
12/11/01	33	Assessment for plant density and height of SSF seedlings in Trial 7.
27/11/01	48	Disease assessment of Trial 6.
27/12/01	78	Disease assessment of Trial 7.

Results

Table 1: Seed treatment effects on plant density and growth of Resal treated seed in the Pot trial

No.	Treatment	% Surviving seedlings/pot		Average plant height (cm)
		24 DAS	47 DAS	47 DAS
18	Amistar + Tecto + Apron	83 h *	87 g *	17 g *
16	Amistar + Apron	82 h *	85 fg *	17 g *
11	Bion + Wakil	71 gh *	83 fg *	13 de *
17	Maxim + Tecto + Apron	78 gh *	83 fg *	18 g *
13	Amistar + Fongarid	78 gh *	82 fg *	16 efg *
14	Maxim + Fongarid	80 gh *	82 fg *	18 g *
19	Amistar + Tecto + Fongarid	79 gh *	80 fg *	17 g *
12	Bion + Fongarid	73 gh *	76 fg *	12 cd *
15	Maxim + Apron	67 fg *	73 fg *	17 g *
3	Wakil XL	54 ef *	71 ef *	16 efg *
9	Fongarid	52 e *	57 de *	14 def *
10	Fongarid	42 de	51 cd *	14 def *
20	Raxil + Agri-Fos Supa 400	41 de	37 bc	14 def *
2	Aliette Super	29 bcd	34 b	13 de *
4	Serenade (WP)	32 cd	33 b	12 cd *
7	Bion	25 abc	30 ab	8 ab
1	**Untreated control**	**28 bcd**	**29 ab**	**9 ab**
5	Amistar	17 ab	17 a	9 bc
6	Bion	15 ab	17 a	6 a
8	Captan	13 a	17 a	9 ab

Treatment sorted according to plant densities at 24 and 47 days after sowing (DAS), in a descending order.
Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.
* Treatment significantly better than the untreated control.

Pot trial

In the pot trial, with the exception of seed treated with Aliette Super, and Raxil + Agri-Fos Supa 400, treatment of seed with a combination of two or more fungicides resulted in improved seedling establishment compared to the untreated seed (Table 1). Treatments with only a single active ingredient gave relatively low percentage of surviving seedlings that were similar to those from the untreated seed. This indicates that a range of fungal pathogens were involved in reducing seedling survival in the soil. Therefore, in subsequent field trials, two or more active ingredients were evaluated for seed treatments under field conditions. Although Aliette Super contained three fungicides (fosetyl-Al, thiram and thiabendazole) and Raxil + Agri-Fos Supa 400 contained two fungicides (tebuconazole and phosphoric acid), these fungicides may not be effective against the spectrum of seedling pathogens encountered in the soil mix used in this pot trial.

Resal treated seed in Field Trials 4 & 6

In the field trials, the most obvious differences in seedling establishment were between the two seed varieties. In Trials 4 & 6, the cv. Resal seed lot used gave high seedling numbers and excellent growth. In contrast, the Small Sieve Freezer (SSF) seed lot used in Trials 5 & 7 gave poor germination and low seedling establishment. The poor SSF seed quality is believed to have resulted from fumigation of the seed by quarantine for insect control. With these differences in seed qualities, the outcomes of Resal treated seed in Field Trials 4 & 6 are discussed first, followed by those for SSF treated seed in Field Trials 5 & 7.

Table 2: The effects of seed treatments of Resal seed on seedling numbers and growth, and downy mildew incidence and severity, in Trial 4, on sandy loam soil

		21 DAS		69 DAS	71 DAS		
No.	**Treatment**	**Seedling density (per m²)**	**Seedling height (cm)**	**Total fresh shoot weight (g/10 plants)**	**Downy mildew incidence (%)**	**Downy mildew severity**	**% Plant nodes with dead leaves**
2	Aliette Super	92 bc	4.2 ef	215 a	100	3.9 a	41.9 a
4	Apron + P-Pickel T	95 cd	4.4 f	168 a	100	4.4 ab	43.6 a
9	Bion + Fongarid	96 cd	3.2 a	222 a	100	4.4 ab	47.1 a
8	Amistar + Fongarid	95 cd	3.6 bc	174 a	100	4.6 ab	48.4 a
5	Amistar + Apron	96 cd	3.8 cd	195 a	100	4.8 b	51.4 a
1	**Untreated control**	**88 a**	**4.3 f**	**201 a**	**100**	**4.8 bc**	**46.2 a**
13	Amistar + Tecto + Fongarid	95 cd	3.4 ab	189 a	100	4.8 bc	41.6 a
10	Bion + Maxim + Fongarid	96 cd	3.5 ab	209 a	100	5.0 bc	44.4 a
3	Wakil	97 d	4.0 def	224 a	100	5.1 bc	46.6 a
7	Maxim + P-Pickel T	86 a	3.8 cde	236 a	100	5.1 bc	49.1 a
11	Bion + Amistar + Fongarid	92 bc	3.2 a	180 a	100	5.1 bc	48.9 a
15	Elexa + Amistar + Fongarid	95 cd	3.7 bc	184 a	100	5.1 bc	47.4 a
12	Amistar + Tecto + Apron	95 cd	3.5 abc	197 a	100	5.2 bc	46.0 a
6	Amistar + P-Pickel T	89 ab	3.7 bc	212 a	100	5.7 c	45.7 a
14	Amistar + Fongarid + Serenade	93 cd	3.7 bc	208 a	100	5.8 c	44.7 a

Treatment sorted according to downy mildew severity at 71 days after sowing (DAS), in a descending order.
Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

In Trial 4, with the exception of lower plant numbers from seed treated with Amistar + P-Pickel T and Maxim + P-Pickel T, all other seed treatments significantly increased plant numbers compared with that from untreated seed (Table 2).

In Trial 6, except for two treatments, all other treatments resulted in similar plant numbers to those from the untreated seed (Table 3). Plant numberswere increased by Treatment 4 (Apron + P-Pickel T) and Treatment 14 (Amistar + Fongarid + Serenade), when compared to those from the untreated control. In general, the differences in the plant numbers between all the seed treatments in Trials 4 & 6 were small.

The fungicide mixtures developed for pea seed treatments, Aliette Super, Apron + P-Pickel T and Wakil, had no adverse effects on seedling growth (Tables 2 & 3). In contrast, many of the other seed treatments initially reduced seedling growth.

As the plant size of cv. Resal increased, creating a moist environment in the dense plant canopy, downy mildew became evident on the leaves of all plants in Trials 4 & 6, inside the trial area as well as throughout the commercial crop. All maturing plants in the trial area had downy mildew (Tables 2 and 3).

However, in Trials 4 & 6, plants from Aliette Super treated seed gave the greatest reduction in downy mildew disease severity, followed by Apron + P-Pickel T (Tables 2 & 3). Although not significantly different to the untreated control, plants from seed treated with Apron + P-Pickel T also tended to have lower downy mildew severity. These results indicate that treating seed with Aliette Super or Apron P-Pickel T probably had a prolonged effect on downy mildew, even at approximately 10 weeks after sowing.

Table 3: The effects of seed treatment of cv. Resal on seedling numbers and growth, and downy mildew incidence and severity, in Field Trial 6 on a ferrosol soil

		23 DAS		48 DAS			
No.	**Treatment**	**Seedling density (per m2)**	**Seedling height (cm)**	**Total fresh shoot weight (g/10 plants)**	**Downy mildew incidence (%)**	**Downy mildew severity**	**% Plant nodes with dead leaves**
2	Aliette Super	94 bcde	4.3 ij	116 e	100	3.7 a	26.3 cdef
4	Apron + P-Pickel T	97 e	4.6 j	69 abc	100	4.1 ab	28.1 def
11	Bion + Amistar + Fongarid	90 ab	2.7 a	81 cd	100	5.0 bc	17.8 ab
10	Bion + Maxim + Fongarid	90 ab	3.1 ab	97 d *	100	5.1 bc	15.8 a
1	**Untreated control**	**91 abc**	**4.1 ghi**	**68 abc**	**100**	**5.3 bcd**	**31.4 ef**
5	Amistar + Apron	94 bcde	3.7 efg	62 ab	100	5.9 cde	25.0 bcdef
6	Amistar + P-Pickel T	89 a	3.6 def	68 abc	100	6.1 cde	28.7 def
12	Amistar + Tecto + Apron	93 abcde	3.4 cde	62 a	100	6.4 def	21.4 abcde
7	Maxim + P-Pickel T	94 abcde	3.9 fgh	67 abc	100	6.5 def	32.3 f
13	Amistar + Tecto + Fongarid	94 abcde	3.2 bc	63 ab	100	6.5 def	24.2 abcdef
3	Wakil	96 cde	4.1 hi	78 bc	100	6.6 ef	18.2 abc
8	Amistar + Fongarid	93 abcde	3.3 bcd	66 abc	100	6.6 ef	17.6 ab
9	Bion + Fongarid	93 abcde	3.1 ab	95 d	100	6.7 ef	19.4 abc
14	Amistar + Fongarid + Serenade	96 de	3.3 bcd	60 a	100	7.2 ef	28.1 def
15	Elexa + Amistar + Fongarid	92 abcd	3.3 bc	62 ab	100	7.4 f	20.6 abcd

Treatment sorted according to downy mildew severity at 48 days after sowing (DAS), in a descending order.
Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

Bigger plants were also noted in the Aliette Super treatment compared to all other treatments in Trial 6, as shown in the total fresh shoot weight (Table 3). This increase in plant growth may be associated with the reduced downy mildew severity resulting from Aliette Super seed treatment.

In Trial 4, there were no differences in the proportion (%) of plant nodes with dead leaves between all the treatments (Table 2). In Trial 6, seed treated with Wakil or fungicide mixtures containing Bion (Treatments 9, 10, and 11) and Fongarid (Treatments 8, 9, 10, 11, and 15), had lower % plant nodes with dead leaves compared to the untreated control (Table 3). The causes for these reductions were not obvious.

SSF treated seed in Field Trials 5 & 7

In Trials 5 & 7, poor seedling establishment was obtained from the SSF seed used, with seedling numbers ranging from 27 to 41 per square metre (Tables 4 & 5). Seed treatments did not improve the seedling numbers, indicating that inherent poor quality of the seed lot used was the cause of poor germination and emergence.

As a result of poor seedling establishment, subsequent growth and disease assessments for Trials 5 & 7 were only carried out on Treatments 1 to 4, which were the untreated control and the three commercial pea seed fungicide mixtures.

In Trial 5, on a sandy loam soil, plants from seed treated with fungicide mixes containing Bion tended to have smaller plants, which were indicated by lower plant heights (Table 4), although this effect was not statistically significant. In Trial 4, on a ferrosol soil, plants from seed treated with fungicide mixes containing Amistar were significantly shorter compared to plants from untreated seed (Table 5).

In Trial 7, plants from seed treated with Wakil, Apron + P-Pickel T, and Aliette Super, had significantly lower downy mildew severity than those from the untreated control (Table 5). As in Trial 6, the Wakil seed treatment in Trial 7 gave significantly lower proportion (%) of plant nodes with dead leaves than the untreated control (Table 5).

Table 4: The effects of SSF treated seed on seedling density and growth, and downy mildew incidence and severity, in Trial 5, on a sandy loam soil

No.	Treatment	29 DAS		98 DAS			
		Plant density (per m2)	Seedling height (cm)	Total fresh shoot weight (g/10 plants)	Downy mildew incidence (%)	Downy mildew severity	% Plant nodes with dead leaves
4	Apron + P-Pickel T	33 a	5.2 a	1041.9 a	90 a	2.1 a	29.4 a
2	Aliette Super	35 a	5.3 a	1052.1 a	95 a	2.4 a	35.3 a
3	Wakil	31 a	5.4 a	1133.6 a	100 a	2.6 a	37.2 a
1	**Untreated control**	**33 a**	**5.4 a**	**979.6 a**	**100 a**	**3.1 a**	**32.8 a**
6	Amistar + P-Pickel T	34 a	5.5 a	N/a			
7	Maxim + P-Pickel T	32 a	5.3 a				
13	Amistar + Tecto + Fongarid	33 a	5.3 a				
12	Amistar + Tecto + Apron	36 a	5.2 a				
8	Amistar + Fongarid	35 a	5.1 a				
5	Amistar + Apron	31 a	5.0 a				
14	Amistar + Fongarid + Serenade	32 a	4.9 a				
15	Elexa + Amistar + Fongarid	36 a	4.8 a				
9	Bion + Fongarid	34 a	4.7 a				
10	Bion + Maxim + Fongarid	27 a	4.7 a				
11	Bion + Amistar + Fongarid	34 a	4.4 a				

Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

Table 5: The effects of SSF treated seed on plant density and growth, and downy mildew incidence and severity, in Field Trial 7, on a ferrosol soil

No.	Treatment	33 DAS		78 DAS			
		Plant density (per m2)	Average plant height (cm)	Total fresh shoot weight (g/10 plants)	Downy mildew incidence (%)	Downy mildew severity	% Plant nodes with dead leaves
3	Wakil	34 a	7.9 f	1094.9 a	96 a	2.4 a	15.7 a
4	Apron + P-Pickel T	36 a	7.6 ef	1023.5 a	98 a	3.0 ab	26.9 b
2	Aliette Super	33 a	8.0 f	998.6 a	98 a	3.0 b	23.1 b
1	**Untreated control**	**36 a**	**7.6 ef**	**1067.3 a**	**100 a**	**3.8 c**	**26.5 b**
10	Bion + Maxim + Fongarid	35 a	7.6 ef	N/a			
8	Amistar + Fongarid	30 a	7.2 def				
7	Maxim + P-Pickel T	35 a	7.2 cde				
11	Bion + Amistar + Fongarid	34 a	7.1 bcde				
9	Bion + Fongarid	32 a	7.0 bcde				
14	Amistar + Fongarid + Serenade	34 a	6.9 abcd				
15	Elexa + Amistar + Fongarid	41 a	6.8 abcd				
6	Amistar + P-Pickel T	34 a	6.7 abcd				
12	Amistar + Tecto + Apron	33 a	6.6 abc				
13	Amistar + Tecto + Fongarid	32 a	6.5 ab				
5	Amistar + Apron	37 a	6.3 a				

Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

2.3. Storage effect of fungicide treated seeds

Materials & Methods

Untreated and fungicide treated seeds in Trial 1 in 2000 were kept for approximately 1 year, and a pot trial was conducted to determine their viability in 2001.

Soil mixture containing potting mix and field soil was prepared at 7:3 ratio by weight. Pots (19 cm diameter) were each filled with 4 litres of the soil mixture. Twenty treated seeds were sown in each pot, at a depth of 2 cm. The trial design was randomised complete block, with five replicates for each treatment.

The number of seedlings in each pot was counted at 23 and 47 days after sowing to determine the percentage of seedling survival, as well as the rate of emergence. Analysis of variance was conducted on the data set using StatGraphics Plus 2.0. Pairwise comparisons were made of mean values using Least Significant Difference Test.

Treatment list

No.	Seed treatment	Product rate (per tonne seed)
1	Agri-Fos + Apron + P-Pickel T	4.0 L + 1.5 kg & 2.0 L
2	Acrobat	1.5 kg
3	Aliette Super	2.9 kg
4	Bavistin	1.5L
5	Fongarid	2.0 kg
6	Maxim	1.0 L
7	Wakil XL	2.0 kg
8	Apron + P-Pickel T	1.5 kg & 2.0 L
9	Bion + Apron + P-Pickel T	10 kg + 1.5 kg & 2.0 L
10	Untreated control	N/a

Chronology of events

Date	Days after sowing	Events
26/07/00	-308	Seed treatment.
28/05/01	-2	Potting mix prepared and pots filled.
30/05/01	0	Seed sown.
22/06/01	23	1st assessment for seedling density.
28/06/01	29	2nd assessment for seedling density.
09/07/01	40	3rd assessment for seedling density.

Results and discussion

Table 1: The effects of storage on the seedling emergence of fungicide treated seed

No.	Seed Treatment	% Seedling emergence		
		23 DAS	29 DAS	40 DAS
7	Wakil XL	88 f	88 d	89 c
3	Aliette Super	81 ef	84 d	85 c
8	Apron + P-Pickel T	71 de	74 d	81 c
5	Fongarid	75 ef	82 d	80 c
1	Agri-Fos + Apron + P-Pickel T	65 de	72 cd	76 c
10	**Untreated control**	**56 cd**	**56 bc**	**53 b**
6	Maxim	56 cd	57 bc	51 b
2	Acrobat	46 bc	46 b	43 b
9	Bion + Apron + P-Pickel T	35 ab	41 ab	43 b
4	Bavistin	25 a	26 a	21 a

Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.
Treatments sorted according to % seedling emergence at 40 DAS, in a descending order.
DAS = days after sowing

At the beginning of the study, the initial proportion of germinated seed for the untreated seed, Aliette Super, Apron + P-Pickel T and Wakil treated seed was 88%, 85%, 88% and 88%, respectively. After approximately 1 year, seed treated with Aliette Super, Apron + P-Pickel T, and Wakil, showed no adverse effects on seedling emergence (Table 1), with seedling emergence ranging from 80% to 89%. Seed treatment with Wakil, Aliette Super, Apron + P-Pickel T, Fongarid, and Agri-Fos + Apron + P-Pickel T, gave significantly higher seedling emergence at 40 days after sowing, than resulted from the untreated seed.

General discussion

Most fungicide seed treatments increased seedling numbers and growth when compared to untreated experimental controls. The three commercial seed dressings, Apron + P-Pickel T, Aliette Super, and Wakil XL, developed for pea seeds, were shown to be effective in improving seedling establishment. Apron + P-Pickel T is registered in Australia for use on pea seeds, while the latter two are only registered for use in New Zealand. Trials conducted in this study showed that there were no consistent differences in plant density or growth between the three seed dressings.

Various fungicide mixtures that included Fongarid, Amistar, and Bion, also showed potential for improving seedling survival. However, many of these alternative seed treatment mixtures also reduced initial growth of seedlings, and had little or no effect on downy mildew.

A desirable seed treatment is expected to provide effective control both of targeted seedborne pathogens and a wide range of damping-off pathogens in soil, in order to maximise seedling survival without adversely affecting seedling growth. Among the three commercial seed treatments, Aliette Super, Apron + P-Pickel T and Wakil fulfilled these criteria, giving high levels of seedling survival, and no adverse effects on seedling growth.

Apron + P-Pickel T treatment improved seedling growth, but this increase did not endure as the plants matured. In contrast, the Aliette Super treatment consistently reduced downy mildew severity on mature plants in the two field trials, and resulted in bigger flowering plants. Aliette Super seed treatment was the most effective in reducing downy mildew severity on maturing plants, followed by Apron + P-Pickel T. Wakil performed poorly for downy mildew control on maturing plants in comparison to Apron + P-Pickel T. This could be due to the lower rate of metalaxyl used in the Wakil seed treatment (350 g metalaxyl/tonne seed) compared to that in the Apron + P-Pickel T treatment (525 g metalaxyl/tonne seed).

In view of the development of metalaxyl-resistant isolates of *P. viciae* in Tasmania, fungicide resistance management strategies should be considered in the control downy mildew in processing pea crops in Tasmania. The strategies for seed treatment should include alternating metalaxyl or phenylamides with chemicals that have different modes of action, or using metalaxyl in mixtures with non-phenylamide chemicals such as cymoxanil or fosetyl-Al. Glasshouse and field studies conducted by Falloon et al (2000) showed that seed treatments containing cymoxanil or fosetyl-Al protected seedlings from infection by metalaxyl-resistant isolates. Therefore, Aliette Super or Wakil seed treatments are suitable alternatives to the Apron + P-Pickel T seed treatment. In Apron + P-Pickel T, metalaxyl is the only active ingredient for downy mildew control.

3. Field control of downy mildew

Summary

Nine field trials were conducted from 2000 to 2004, within commercial pea crops in Tasmania, to evaluate and optimize foliar fungicide application methods to control downy mildew from the field inoculum of *Peronospora viciae*. As peas are considered to be a low value crop, low cost fungicide products such as chlorothalonil (Bravo), mancozeb (Penncozeb), and phosphorous acid (Agri-Fos) were mainly used in the trials.

Plants treated with Apron or metalaxyl only had similar downy mildew incidence and severity to the untreated plants. This poor control might be due to the presence of metalaxyl resistant isolates of *P. viciae* in the crop.

Collar rot and downy mildew are the two most common and important diseases of processing pea crops that impact on pea yield and quality. In some trials, even when downy mildew or collar rot ranged from mild to moderate, they could still adversely affect pea yield when the crops were subjected to water stress. In the trials, yield increases of 1 to 3 tonnes per hectare were recorded following improved downy mildew control. As for collar rot, yield increases of 1 to 2 tonnes per hectare were recorded following reduced collar rot severity with Bravo applications.

The trials demonstrated that Penncozeb SC + Agri-Fos and Bravo + Agri-Fos were the most consistent and effective foliar treatments for field downy mildew control. Agri-Fos or Penncozeb alone, were shown to have little or no effect on collar rot. Agri-Fos or Penncozeb, applied on their own, also had little or no effect on downy mildew incidence and severity. In contrast, Agri-Fos, in a mixture with Bravo or Penncozeb, significantly reduced downy mildew incidence and severity on plants. This indicates a synergistic effect from the spray mixture. The Agri-Fos + Penncozeb combination was the best treatment against downy mildew, but had no effect on collar rot. Agri-Fos + Bravo, the second best treatment against downy mildew, was also effective in reducing collar rot severity. Therefore, where the two major pea diseases were present, and collar rot was the dominant disease, Agri-Fos + Bravo treatment was more suitable. However, when downy mildew was the only or dominant disease, Agri-Fos + Penncozeb tended to give better downy mildew control and yield improvement. The optimum product rate for collar rot control was 1.8 L/ha Bravo. The optimum product rates for downy mildew control were 3.5 L/ha Agri-Fos, and 2.5 L/ha Penncozeb SC or 2.0 kg/ha Penncozeb DF.

Two fungicide applications applied to plants at the growth stage of 4 and 8 nodes gave better downy mildew control than one application. However, for reducing collar rot severity, one spray application at 4 nodes appeared to be adequate. The timing of the fungicide applications is critical. Early fungicide applications before disease occurs only protect plants for a short interval of 10 to 14 days. Therefore, for optimum downy mildew control, the fungicide application must be applied at the first sign of infection in the crop. Field infections usually occur at the pea growth stage of 4 to 8 nodes, depending on sowing time and weather conditions. As collar rot tended to occur early in the crop at about 4 nodes, the alternate applications of Agri-Fos + Bravo followed by Agri-Fos + Penncozeb at 7-10 days later, could be a suitable program for reducing early collar rot severity as well as controlling downy mildew.

The spray mixture containing the dry flowable formulation of Penncozeb (Penncozeb DF) also reduced collar rot severity compared to the untreated experimental controls, or the mixture containing the suspension concentrate formulation (Penncozeb SC). The differences between the two Penncozeb formulations may be related to the lower rates of mancozeb used in the Penncozeb SC formulation (1400 g and 1050 g/ha of mancozeb in Penncozeb DF and Penncozeb SC, respectively).

3.1. Preliminary field trials in 2000

Trials Summary

	Trial 1	Trial 2
Location	Moriarty	Wesley Vale
Soil Type	Ferrosol	Ferrosol
Variety	Small Sieve Freezer	Small Sieve Freezer
Trial Design	Randomised complete block	Randomised complete block
Replicates	5	5
Plot Size	1.6 m x 8 m	1.6 m x 8 m
Sowing Date	02/07/00	03/07/00
Harvest Date	28/11/00	21/11/00

Materials and methods

In 2000, two preliminary trials were conducted to evaluate a range of fungicides for use in foliar sprays to control field downy mildew. The trials were conducted within commercial pea crops, which were sown in July, 2000, with seeds treated with Apron + P-Pickel T, the standard seed treatment. Fungicide products used in the trials included Bravo, which is registered for downy mildew control on peas, and Acrobat and Ridomil Gold MZ, which are used for control of downy mildew in poppy.

All fungicide treatments were applied using a knapsack air-pressurised sprayer, fitted with a 1.5 m boom and TX8 hollow cone nozzles, at 500 kPa pressure and applying 200 L water/ha. Product formulations and rates of active ingredients are listed in Appendix i.

Ten plants were collected at random at half-metre intervals from the middle of each plot, and assessed for downy mildew incidence. Plant growth was also determined by measuring the fresh shoot weight of the 10 plants sampled in each plot. Five of the 10 plants sampled in each plot were assessed for downy mildew severity. The downy mildew severity was based on the percentage leaf cover by downy mildew, and the assessment was made using the disease severity key for downy mildew (Falloon *et al.* 1995). It was not possible to distinguish the cause of dead and desiccated leaves of plant nodes close to the ground, which may have been caused by collar rot, downy mildew or *Septoria*. Therefore, the proportion (%) plant nodes with dead leaves were assessed separately.

At crop maturity, ten plants were collected at random at half-metre intervals from the middle of each plot, and assessed for collar rot incidence and severity. The collar rot severity was based on the percentage of stem length affected by the collar rot. Mature pods were then removed from the ten plants for yield assessment. Analysis of variance was conducted on all the data sets using StatGraphics Plus 2.0.

Treatment lists for Trial 1

	Foliar spray application		
No.	**Treatments**	**Product Rate/ha**	**Application schedule**
1	Acrobat	2 kg	2 foliar sprays: 1st spray at 2 nodes; 2nd spray at 5-6 nodes 14 days later.
2	Amistar	75 g	
3	Ridomil Gold MZ	2.5 kg	
4	Bravo	1.8 L	
5	Agri-Fos 400 + Bravo*	3.0 L + 1.8 L	
6	Elexa + Bravo*	19.4 L + 1.8 L	
7	Bion + Bravo*	100 g + 1.8 L	
8	Nil	Nil	

* Products were applied as a mixture

Treatment lists for Trial 2

	Foliar spray application		
No.	Treatment	Product Rate/ha	Application schedule
1	Acrobat	2 kg	2 foliar sprays: 1st spray at 2 nodes; 2nd spray at 6-7 nodes 14 days later.
2	Amistar	75 g	
3	Ridomil Gold MZ	2.5 kg	
4	Bravo	1.8 L	
5	Agri-Fos 400 + Bravo*	3.0 L + 1.8 L	
6	Elexa + Bravo*	19.4 L + 1.8 L	
7	Bion + Bravo*	100 g + 1.8 L	
8	Mancozeb & Bion**	2.2 kg & 100 g	
9	Nil	Nil	

* Products were applied as a mixture
** Mancozeb spray applied in alternation with Bion

Chronology of events

DATE	DAYS AFTER SOWING	TRIAL 1
02/07/00	0	Seeds sown.
02/08/00	31	Pegged trial plots.
12/08/00	41	1st foliar fungicide application of Treatments 1-7. No downy mildew disease observed on plants inside or outside trial area.
27/08/00	56	2nd foliar fungicide application of Treatments 1-7. Some stunted plants observed, but no obvious downy mildew.
05/09/00	65	Plant stage at 7-8 nodes. *Septoria* leaf infections observed throughout the trial area, but no downy mildew. Initial symptoms of collar rot observed on a few plants.
18/09/00	79	Increased incidence of collar rot inside and outside the trial area, with low disease severity. Low incidence of downy mildew noticed in trial area.
27/09/00	87	Low incidence of downy mildew and collar rot in trial area. Top soil dry and hard.
11/10/00	101	Downy mildew present, but at low severity.
16-18/10/00	106-108	Downy mildew and plant height assessments.
28-29/11/00	149-150	Collar rot and yield assessments.

DATE	DAYS AFTER SOWING	TRIAL 2
03/07/00	0	Seeds sown.
02/08/00	30	Pegged trial plots.
12/08/00	40	1st foliar fungicide application of Treatments 1-8. No downy mildew observed on plants inside or outside trial area.
27/08/00	55	2nd foliar fungicide application of Treatments 1-8. Some stunted plants observed, but no obvious downy disease.
05/09/00	64	Plant stage at 8-9 nodes. A few plants with *Septoria* leaf spot observed in the trial area.
11/10/00	100	Downy mildew present, but at low severity.
16-18/10/00	105-107	Downy mildew and plant height assessments.
21-23/11/00	141-142	Collar rot and yield assessments.

Results and discussion

The foliar fungicide applications did not control downy mildew incidence or severity in Trials 1 and 2. There were no significant differences (p>0.05) in downy mildew assessments between Trial 1 (Table 1) and Trial 2 (Table 2). In both trials, the fungicide applications were applied very early, at 2-3 node growth stage. Downy mildew and collar rot developed much later than expected in the crops where the trials were sited. Downy mildew infected plants were only evident at about 30-40 days after the second fungicide applications. This late disease development could be due to dry weather conditions between July to September 2000.

In the final disease assessments, all plants that were assessed had collar rot. Although not significantly different, spray applications of Bravo alone, appeared to reduce collar rot severity compared to all other treatments in both trials. This is consistent with previous trial findings on collar rot disease management, where Bravo was shown to reduce collar rot severity (Pung & Cross 2000).

Table 1: Treatment effects on downy mildew, collar rot and yield in Trial 1

		Downy mildew at 106 DAS			Collar rot & yield at 149 DAS	
No.	**Treatments**	**% Disease incidence**	**% Disease severity**	**% Nodes with dead leaves**	**% Collar rot severity#**	**No. of pods (10 plants)**
1	Acrobat	100	3.2	36	45.	93
2	Amistar	100	3.1	37	40	101
3	Ridomil Gold MZ	98	4.7	31	43	90
4	Bravo	100	3.4	25	39	107
5	Agri-Fos 400 + Bravo	100	3.4	31	42	98
6	Elexa + Bravo	94	3.4	28	66	98
7	Bion + Bravo	98	4.2	28	41	102
8	Nil	100	3.7	31	45	90

* All plants have collar rot; DAS = days after sowing.
All data were not significantly different at the 5% level according to analysis of variance.

Table 2: Treatment effects on downy mildew, collar rot and yield in Trial 2

		Downy mildew at 105 DAS			Collar rot & yield at 141 DAS	
No.	**Treatment**	**% Incidence**	**% Disease severity**	**% Nodes with dead leaves**	**% Collar rot severity***	**No. of pods (10 plants)**
1	Acrobat	100	3.3	27	23	87
2	Amistar	94	3.3	22	22	92
3	Ridomil Gold MZ	100	3.3	28	26	89
4	Bravo	96	2.7	26	13	104
5	Agri-Fos 400 + Bravo	98	3.0	24	21	101
6	Elexa + Bravo	96	3.2	29	18	90
7	Bion + Bravo	98	2.9	24	19	103
8	Mancozeb & Bion	90	2.4	26	18	105
9	Nil	94	3.0	28	27	96

* All plants have collar rot; DAS = days after sowing.
All data were not significantly different at the 5% level according to analysis of variance.

3.2. Field trials on potential foliar fungicide treatments in 2001

Trials Summary

	TRIAL 1	TRIAL 2	TRIAL 3
Location	Sassafras	Sassafras	Sassafras
Soil Type	Ferrosol	Ferrosol	Ferrosol
Variety	Small Sieve Freezer	Small Sieve Freezer	Resal
Trial Design	Randomised complete block	Randomised complete block	Randomised complete block
Replicates	5	5	3
Plot Size	2 m x 6 m	2 m x 6 m	4 m x 5 m
Sowing Date	24/08/01	19/09/01	09/10/01
Harvest Date	06/12/01	31/12/01	17/01/02
Others	Crop not irrigated		Crop irrigated using centre pivot

Materials and methods

In 2001/02, three trials were conducted within commercial crops to evaluate the effectiveness of foliar spray applications for field downy mildew control. In considering the crop value, only relatively low cost products such as Bravo and Penncozeb were used in the trials. These include products that may work by stimulating or inducing the development of systemic acquired resistance in plants, such as Agri-Fos, Bion, and Micro-Gyp (calcium sulphate). Generally, such products are believed to be more effective when applied in combination with with protectant fungicides. Therefore, in the field trials these products were examined in combination with either Bravo or Penncozeb. Apron was used instead of Ridomil Gold MZ (metalaxyl + mancozeb) in the trials, in order to enable evaluation of the effects of metalaxyl without additional active ingredient on field downy mildew.

Trials 1 and 2 were conducted mainly to evaluate the effectiveness of Bravo and Penncozeb, applied alone or in combinations with Agri-Fos, Bion and Micro-Gyp, for field downy mildew control. Trial 3 was set up after the first two trials, to determine whether Agri-Fos alone was effective against downy mildew, and to determine its optimum rate.

All the trials were set up in crops that showed early signs of downy mildew infections at 4-5 nodes, when the first spray was applied. All fungicide treatments were applied using a knapsack air-pressurised sprayer, fitted with a 2.0 m boom. Spray applications in Trial 1 were applied with TX8 hollow cone nozzles, at 500 kPa and in 200 L water/ha, while Trials 2 & 3 were applied with TX12 hollow cone nozzles at 400 kPa and in 320 L/ha. Product formulations and rates of active ingredients are listed in Appendix i.

Ten plants were collected at random at half-metre intervals from the middle of each plot, and assessed for downy mildew incidence and severity as described in Section 3.1. Plant growth was also determined by measuring the fresh shoot weight of the 10 plants sampled in each plot.

At crop maturity, plants from a 2 x 1 metre area from the middle of each treatment plot were harvested for pea yield assessment. Peas were removed from plants and pods using a mechanical viner at the Forthside Research Station. Peas were also assessed for maturity or firmness with a maturometer, and the pea yields were then adjusted to a standard pea maturity index of 235 to enable comparison between treatments. In Trial 2, pea yield measurements were only carried out selectively on Treatments 1, 3, 4, 6, 8 and 10 due to time constraints and harvesting just before the commercial harvest started.

Analysis of variance was conducted on the data set using StatGraphics Plus 2.0, and pairwise comparisons were made of mean values using Least Significant Difference Test.

Treatment list for Field Trial 1

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated Control	Nil	N/a
2	Bravo	1.8 L	1 spray at 4 - 5 nodes
3	Penncozeb SC	2.5 L	4 sprays; 1st spray at 4 - 5 nodes, then at 10 - 14 day intervals
4	Apron	286 g	2 sprays: 1st spray applied at 4-5 nodes; 2nd spray at 6-7 nodes.
5	Bravo	1.8 L	
6	Bravo + Bion	2.5 L + 100 g	
7	Bravo + Agri-Fos 400	1.8 L + 2.5 kg	
8	Bravo + MicroGyp	1.8 L + 2.5 kg	
9	Penncozeb SC	2.5 L	
10	Penncozeb SC + Bion	2.5 L + 100 g	
11	Penncozeb SC + Agri-Fos	2.5 L + 5.0 L	
12	Penncozeb SC + MicroGyp	2.5 L + 2.5 kg	

Treatment list for Field Trial 2

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated Control	Nil	N/a
2	Bravo + Chitosan	1.8 L +	2 sprays: 1st spray at 4-5 nodes; 2nd spray at 7-8 nodes.
3	Apron	286 g	
4	Bravo	1.8 L	
5	Bravo + Bion	2.5 L + 100 g	
6	Bravo + Agri-Fos 400	1.8 L + 2.5 kg	
7	Bravo + MicroGyp	1.8 L + 2.5 kg	
8	Penncozeb SC	2.5 L	
9	Penncozeb SC + Bion	2.5 L + 100 g	
10	Penncozeb SC + Agri-Fos	2.5 L + 5.0 L	
11	Penncozeb SC + MicroGyp	2.5 L + 2.5 kg	

Treatment list for Field Trial 3

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated Control	Nil	2 Sprays 1st spray applied at 6-7 nodes; 2nd spray at 8-9 nodes.
2	Agri-Fos 400	2.5 L	
3	Agri-Fos 400	5.0 L	
4	Agri-Fos + Bravo	2.5 L + 1.8 L	
5	Agri-Fos + Penncozeb SC	2.5 L + 2.5 L	
6	Agri-Fos + Dithane DF	2.5 L + 1.46 kg	

Chronology of events

Date	Days after sowing	Trial 1
24/08/01	0	Peas sown.
01/10/01	38	Trial pegged. Low level of downy mildew noticed on plants.
02/10/01	39	1st foliar fungicide application of Treatments 2 - 12.
16/10/01	53	2nd foliar fungicide application of Treatments 3 - 12, downy mildew to the 6th node.
26/10/01	63	Severe collar rot causing yellowing of lower half of plants. Downy mildew widespread.
30/10/01	67	3rd foliar fungicide application of Treatment 3. Plants have *Ascochyta* rot from seed level on upper taproot to the 1st node. Lower leaves are yellow and dead to the 5th node. Downy mildew on leaves at the 6th node and above.
8/11/01	76	Downy mildew and fresh shoot weight assessments.
13/11/01	81	4th foliar fungicide application of Treatment 3.
11/12/01	109	Yield assessment.

Date	Days after sowing	Trial 2
19/09/01	0	Peas sown.
01/11/01	43	Trial pegged, low level of downy mildew noticed on plants.
02/11/01	44	1st foliar fungicide application of Treatments 2 - 12.
13/11/01	55	2nd foliar fungicide application of Treatments 2 - 12. Plants have downy mildew to the 7th node, with no collar rot.
06/12/01	78	Disease assessment.
03/01/02	106	Yield assessment.

Date	Days after sowing	Trial 3
09/10/01	0	Peas sown.
21/11/01	43	Trial pegged. Low level of downy mildew on plants.
22/11/01	44	1st foliar fungicide application of Treatments 2 - 6.
29/11/01	51	2nd foliar fungicide application of Treatments 2 - 6. Temperature and leaf wetness sensor set-up.
04/01/02	87	Downy mildew assessment.
17/01/02	100	Yield assessment.

Results and discussion

Trial 1

In Trial 1, at the first foliar spray application, 39 days after sowing, a relatively low level of downy mildew was already evident on lower plant leaves. Later, at flowering, the crop had severe downy mildew and *Ascochyta* collar rot, with many stunted plants. Even though the downy mildew severity based on proportion of leaf area affected ranged from only 0.2% to 3.4%, the disease was considered to be severe in the crop, when other effects of the disease such as plant desiccation and reduced growth were also taken into account.

Table 1: The effects of foliar spray treatments on plant growth, and downy mildew incidence and severity in Trial 1, at 76 days after sowing (DAS)

No.	Treatment*	76 DAS			
		Fresh shoot weight (g/10 plants)	Downy mildew incidence (%)	Downy mildew severity (% leaf cover)	% Plant nodes with dead leaves
11	Penncozeb SC + Agri-Fos 400	81.3 b	14 a	0.2 a	41.2 a
7	Bravo + Agri-Fos 400	79.8 b	44 a	0.7 a	42.3 a
10	Penncozeb SC + Bion	61.7 a	94 b	1.9 b	45.2 a
6	Bravo + Bion	63.4 a	94 b	2.1 bc	46.4 a
4	Apron	66.6 ab	94 b	2.4 bcd	49.8 a
9	Penncozeb SC	60.5 a	98 b	2.6 bcde	46.1 a
1	**Untreated control**	**54.3 a**	**98 b**	**2.8 cde**	**42.8 a**
3	Penncozeb SC (4 sprays)	62.7 a	98 b	3.0 cde	43.1 a
2	Bravo (1 early spray)	66.3 ab	100 b	3.1 de	44.9 a
5	Bravo	68.3 ab	100 b	3.2 de	42.8 a
8	Bravo + MicroGyp	60.5 a	98 b	3.3 e	42.1 a
12	Penncozeb SC + MicroGyp	60.4 a	98 b	3.4 e	48.0 a

Treatment sorted according to downy mildew severity in a descending order.
* Except for Treatments 2 & 3, all treatments have 2 spray applications.
Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

Plants treated with Penncozeb SC + Agri-Fos, and Bravo + Agri-Fos had significantly lower downy mildew incidence and severity compared to all other treatments (Table 1). Penncozeb SC + Bion also reduced downy mildew severity compared to the untreated control. However, the plants treated with Apron or metalaxyl alone had similar downy mildew incidence and severity to the untreated plants. This poor control may be due to the presence of metalaxyl resistant isolates of *P. viciae* in the trial.

Table 2: The effects of foliar spray treatments on pea yield in Field Trial 1, at harvest, 109 DAS

No.	Treatment*	Weight of harvested peas ($g/2m^2$)	Pea maturity index (MI)	Yield adjusted to standard MI 235 ($g/2m^2$)	Yield at MI 235 and adjusted to tonne/ha
11	Penncozeb SC + Agri-Fos 400	741.4	184.2	1011.9	5.1 d
7	Bravo + Agri-Fos 400	598.6	205.2	703.6	3.5 c
10	Penncozeb SC + Bion	467.4	202.4	539.4	2.7 bc
6	Bravo + Bion	457.8	213.1	527.8	2.6 bc
5	Bravo	420.3	223.5	484.2	2.4 abc
2	Bravo (1 spray)	396.0	247.3	420.5	2.1 ab
3	Penncozeb SC (4 sprays)	381.3	226.8	394.7	2.0 ab
8	Bravo + MicroGyp	381.6	252.2	388.9	1.9 ab
4	Apron	366.3	242.7	369.2	1.9 ab
9	Penncozeb SC	360.2	243.9	365.5	1.8 ab
1	**Untreated control**	**338.7**	**247.7**	**336.5**	**1.7 ab**
12	Penncozeb SC + MicroGyp	320.1	256.3	299.9	1.5 a

Treatment sorted according to adjusted yield to std MI in a descending order.
Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

At flowering stage, 81 days after sowing (DAS), consistently bigger plants were found in plots that had been treated with Penncozeb SC + Agri-Fos and Bravo + Agri-Fos, compared to all other treatments. These differences were reflected in the greater total fresh shoot weight at 76 DAS (Table 1), and pea yield at 109 DAS (Table 2). Plants treated with Penncozeb SC + Agri-Fos gave the highest pea yield, followed by those treated with Bravo + Agri-Fos (Table 2). Penncozeb SC + Bion and Bravo + Bion treatments also tended to result in higher yields. The increase in plant growth, vigour and yield are likely to be due to improved downy mildew control by the two foliar treatments.

Trial 2
At commencement of this trial, the level of downy mildew was lower than that in Trial 1. Later, at flowering, even though the crop in Trial 2 had similar levels of downy mildew severity as in Trial 1, there was no collar rot. The absence of collar rot may have contributed to the vigorous crop growth, where at 78 days after sowing, the fresh shoot weight of plants in Trial 2 was approximately seven times higher than those in Trial 1 (Tables 1 & 3). These differences in plant size were also reflected in the much higher yield of peas in Trial 2 (Table 4). This suggests that the two major diseases of peas, downy mildew and collar rot, may interact and together have an even greater impact on crop vigour and yield than when each occurs alone.

Table 3: The effects of foliar spray treatments on plant growth, and downy mildew incidence and severity in Trial 2 at 78 DAS

No.	Treatment*	Fresh shoot weight (g/10 plants)	Downy mildew incidence (%)	Downy mildew severity (% leaf cover)	% Plant nodes with dead leaves
10	Penncozeb SC + Agri-Fos 400	376 a	50 a	1.1 a	52 b
6	Bravo + Agri-Fos 400	427 a	88 b	1.8 ab	48 ab
9	Penncozeb SC + Bion	408 a	94 b	2.0 b	46 a
7	Bravo + MicroGyp	446 a	98 b	2.3 bc	45 a
8	Penncozeb SC	383 a	90 b	2.4 bc	43 a
5	Bravo + Bion	478 a	96 b	2.4 bc	45 a
2	Bravo + Chitosan	463 a	96 b	2.4 bc	45 a
11	Penncozeb SC + MicroGyp	396 a	100 b	2.6 bcd	51 b
3	Apron	405 a	98 b	2.8 cd	48 ab
1	**Untreated control**	**438 a**	**96 b**	**3.1 cd**	**46 a**
4	Bravo	471 a	94 b	3.3 d	44 a

Treatment sorted according to downy mildew severity in a descending order.
* All treatments have 2 spray applications.
Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

In Trial 2, plants treated with Penncozeb SC + Agri-Fos had the lowest downy mildew severity (Table 3). Bravo + Agri-Fos and Penncozeb + Bion also reduced downy mildew severity compared to the untreated control. In this trial, Apron also had little or no effect in reducing downy mildew incidence and severity (Table 3). Although not statistically significant, Penncozeb SC + Agri-Fos treatments gave the highest pea yields in Trial 2 (Table 4). Since there was no collar rot in Trial 2, the yield improvement with Penncozeb + Agri-Fos is likely to be due to downy mildew control.

Table 4: The effects of foliar spray treatments pea on yield in Field Trial 2, at 109 DAS

No.	Treatment	Weight of harvested peas (g/2m^2)	Pea maturity index (MI)	Yield adjusted to standard MI 235 (g/2m^2)	Yield at MI 235 and adjusted to tonne/ha
10	Penncozeb SC + Agri-Fos 400	1823.0	205.0	2053.6	10.3 a
4	Bravo	1757.9	227.0	1942.3	9.3 a
3	Apron	1815.1	232.0	1851.1	9.3 a
6	Bravo + Agri-Fos 400	1719.3	236.0	1791.1	9.0 a
8	Penncozeb SC	1691.5	253.0	1726.8	8.6 a
1	**Untreated control**	**1626.1**	**231.0**	**1672.5**	**8.4 a**

Treatment sorted according to adjusted yield to std MI in a descending order.
Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

Trial 3
As for Trial 2, the crop in Trial 3 had mild downy mildew and collar rot severity, with vigorous plant growth, resulting in relatively large, healthy plants. There was no obvious difference in the downy mildew incidence or severity between the different treatments (Table 5). However, there was a trend for better downy mildew control with fungicide treatments compared to the untreated control, and also improved disease control with 5.0 L compared to 2.5 L of Agri-Fos 400. This suggests that the rates of Agri-Fos 400 could be increased to 5.0 L of product (2.0 L of active ingredient) for best downy mildew control in the fungicide mixtures.

Table 5: The effects of foliar spray treatments on plant growth, and downy mildew incidence and severity in Field Trial 3, at 87 DAS

No.	Treatment	Fresh shoot weight (g/10 plants)	Downy mildew incidence (%)	Downy mildew severity (% leaf cover)	% Plant nodes with dead leaves
1	Untreated Control	510 a	100	5.2 a	32.7 a
2	2.5 L Agri-Fos 400	727 a	100	5.0 a	31.9 a
3	5.0 L Agri-Fos 400	570 a	100	4.3 a	23.5 a
4	2.5 L Agri-Fos + 1.8 L Bravo	682 a	100	4.4 a	25.3 a
5	2.5 L Agri-Fos + 2.5 L Penncozeb SC	649 a	100	4.7 a	33.6 a
6	2.5 L Agri-Fos + 1.4 kg Dithane DF	661 a	100	4.9 a	33.7 a

Within each column, means followed by the same letter are not significantly different at the 5% level according to LSD Test.

There was high variability in shoot weight and pea yield of plants from the replicate plots of each treatment (Tables 5 & 6). Although not statistically significant, in Trial 3, Agri-Fos + Penncozeb gave the highest yield (Table 6), consistent with the yield increases recorded from this treatment in Trials 1 & 2, as a result of good downy mildew control.

The peas in the untreated plots had high maturity indices and there was low plot variability or standard error in the pea yield from these treatments (Table 6). The fungicide treatments, however, tended to have lower pea maturities and high variability in the pea yield. The low maturity indices indicate that the plants in the treated plots were still growing and many of the pea pods were less mature. Many immature peas are usually lost due to crushing damage and sieving during the mechanical pea separation process, hence accounting for the high variability in the pea yield between plots. As a result, pea yield on plants that had not reach full maturity may have been underestimated.

Table 6: The effects of foliar spray treatments on pea yield in Field Trial 3, at 97 DAS

No.	Treatment	Weight of peas ($g/2m^2$)	MI # of harvested peas	Yield adjusted to standard MI 235 ($g/2m^2$)	Yield at MI 235 and adjusted to tonne/ha** ± std error
1	Nil	1887.5	195.0	2219.1	11.1 ± 0.2
2	5.0 L Agri-Fos 400	1881.7	188.3	2307.9	11.5 ± 1.0
3	2.5 L Agri-Fos 400	2001.7	193.3	2357.2	11.8 ± 1.3
4	2.5 L Agri-Fos + 1.8 L Bravo	1562.3	166.7	2167.1	10.8 ± 1.8
5	2.5 L Agri-Fos + 2.5 L Penncozeb SC	1724.5	167.5	2418.9	12.1 ± 1.9
6	2.5 L Agri-Fos + 1.4 kg Dithane DF	1720.0	195.0	2060.4	10.3 ± 1.0

** Not significantly different at the 5% level in an analysis of variance.

3.3. Field trials to optimize control methods in 2002 and 2003/04

Trials summary

	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4
Location	Wesley Vale	Sassafras	Sassafras	Sassafras
Soil Type	Sandy loam	Ferrosol	Ferrosol	Ferrosol
Variety	Small Sieve Freezer	Resal	Resal	Resal
Trial Design	Randomised complete block	Randomised complete block	Randomised complete block	Randomised complete block
Replicates	5	5	5	5
Plot Size	2 m x 6 m	2 m x 6 m	2 m x 6 m	1.8 m x 6 m
Sowing Date	25/07/02	04/10/03	07/11/03	07/11/03
Trial completed	26/11/02	30/12/03	9/01/04	18/12/03

Materials and methods

Four field trials were conducted within commercial crops, to identify and refine the optimum application methods for field downy mildew control. Trial 1 was conducted in 2002, while the other trials were postponed to the 2003/04 season, as a result of dry weather conditions. All fungicide treatments were applied using a knapsack air-pressurised sprayer at 500 kPa pressure, with 200 L water/ha.

Trials 1 & 2 were to determine the number of sprays required for significant downy mildew control in either a mildly or a severely infected crop. A mixture of three fungicides (Agri-Fos, Bravo and Penncozeb) was also tested for downy mildew control.

Trial 3 was to examine the optimum rates of Agri-Fos and Penncozeb, as well as to compare the two formulations of Penncozeb for both collar rot and downy mildew control. Two relatively new fungicide products, BAS 518 and Acrobat were also included in the trial evaluation.

Trial 4 was to investigate the use of spray adjuvants for improved disease control from Agri-Fos and two formulations of mancozeb (a dry flowable, and a liquid suspension concentrate).

Instead of Agri-Fos 400, the new Agri-Fos 600 g/L formulation was used in all these trials. Product formulations and rates of active ingredients are listed in Appendix i.

Treatment lists

Trials 1 & 2

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated Control	Nil	Nil
2	Bravo	1.8 L	1 spray at 4 - 5 nodes
3	Agri-Fos 600 + Bravo	3.0 L + 1.8 L	
4	Agri-Fos 600 + Penncozeb SC	3.0 L + 2.5 L	
5	Agri-Fos 600 + Bravo + Penncozeb SC	3.0 L + 1.8 L + 2.5 L	
6	Bravo	1.8 L	2 sprays at 8 nodes
7	Agri-Fos 600 + Bravo	3.0 L + 1.8 L	
8	Agri-Fos 600 + Penncozeb SC	3.0 L + 2.5 L	
9	Agri-Fos 600 + Bravo + Penncozeb SC	3.0 L + 1.8 L + 2.5 L	
10	Agri-Fos 600 + Bravo + Nufilm	3.0 L + 1.8 L + 0.6 L	

Trial 3

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated Control	Nil	Nil
2	Untreated Control	Nil	2 sprays at 9 days interval: 1st spray at 6 nodes; and 2nd spray at 8 nodes.
3	Agri-Fos 600	3.5 L	
4	Agri-Fos 600 + Bravo	3.5 L + 1.1 L	
5	Agri-Fos 600 + Bravo	3.5 L + 1.8 L	
6	Agri-Fos 600 + Penncozeb SC	3.5 L + 1.8 L	
7	Agri-Fos 600 + Penncozeb SC	2.0 L + 2.5 L	
8	Agri-Fos 600 + Penncozeb SC	3.5 L + 2.5 L	
9	BAS 518	2.0 kg	
10	Agri-Fos 600 + Acrobat	3.5 L + 0.36 kg	
11	Agri-Fos + Bravo / Agri-Fos + Penncozeb SC	3.5 L + 1.8 L / 3.5 L + 2.5 L	
12	Agri-Fos + Penncozeb SC / Agri-Fos + Bravo	3.5 L + 2.5 L / 3.5 L + 1.8 L	

Trial 4

No.	Treatment	Product Rate/ha	Application Schedule
1	Untreated Control	Nil	Nil
2	Untreated Control	Nil	
3	Agri-Fos 600	3.5 L	2 sprays at 7 days interval: 1st spray at 4-6 nodes, 2nd spray at 6-7 nodes.
4	Penncozeb SC	2.5 L	
5	Agri-Fos 600 + Penncozeb DF	3.5 L + 2.0 kg	
6	Agri-Fos 600 + Penncozeb DF + Activator (ad)	3.5 L + 2.0 kg + 30 ml/100 L	
7	Agri-Fos 600 + Penncozeb DF + Tactic (ad)	3.5 L + 2.0 kg + 125 ml/100 L	
8	Agri-Fos 600 + Penncozeb DF + Sporekill	3.5 L + 2.0 kg + 100 ml/100 L	
9	Agri-Fos 600 + Penncozeb SC	3.5 L + 2.5 L	
10	Agri-Fos 600 + Penncozeb SC + Activator (ad)	3.5 L + 2.5 L + 30 ml/100 L	
11	Agri-Fos 600 + Penncozeb SC + Bond (ad)	3.5 L + 2.5 L + 100 ml/100 L	
12	Agri-Fos 600 + Penncozeb SC + Nufilm (ad)	3.5 L + 2.5 L + 0.6 L	

Assessment and statistical analyses

Assessments for downy mildew and yield were carried out as described in previous sections. Plants were also assessed for collar rot incidence and severity. The collar rot severity was based on the percentage plant stem with collar rot. Analysis of variance was conducted on the data set using StatGraphics Plus 2.0. Pairwise comparisons were made of mean values using Least Significant Difference Test. Where data values were not normally distributed, a square root transformation was applied prior to analysis.

Chronology of events

Date	Days after sowing	Trial 1
25/07/02	0	Peas sown.
30/09/02	67	Trial pegged, low level of downy mildew noticed on plants.
01/10/02	68	1st foliar fungicide application of treatments 2-10 at 4 nodes. No downy mildew.
17/10/02	84	2nd foliar fungicide application of treatments 6-10 at 8 nodes. Onset of downy mildew infection on lower leaves.
30/10/02	97	Downy mildew assessment.
20/11/02	118	Collar rot assessment.
26/11/02	124	Yield assessment.

Date	Days after sowing	Trial 2
04/10/03	0	Peas sown.
04/11/03	31	Trial pegged.
13/11/03	40	1st foliar fungicide application of treatments 2-10 at 5 nodes. No downy mildew.
23/11/03	50	2nd foliar fungicide application of treatments 6-10 at 8 nodes; showers later in the day. Onset of downy mildew infection on lower leaves.
08/12/03	65	Downy mildew disease assessment. No collar rot.
30/12/03	87	Yield assessment.

Date	Days after sowing	Trial 3
07/10/03	0	Peas sown.
13/11/03	37	Trial pegged.
14/11/03	38	1st foliar fungicide application of treatments 3-12 at 6 nodes. No downy mildew
23/11/03	47	2nd foliar fungicide application of treatments 3-12 at 8 nodes; showers later in the day. Patchy downy mildew on lower leaves. Low incidence of collar rot.
11/12/03	65	1st disease assessment of replicates 1 & 2.
15/12/03	69	1st disease assessment of replicates 3 to 5.
09/01/04	94	Yield assessment.

Date	Days after sowing	Trial 4
07/10/03	0	Peas sown.
29/10/03	22	Trial pegged.
22/11/03	46	1st foliar fungicide application of treatments 3-12 at 4-6 nodes.
29/11/03	53	2nd foliar fungicide application of treatments 3-12 at 6-7 nodes. Downy mildew found on lower leaves. Collar rot on stems but low in severity.
17-18/12/03	71-72	Downy mildew and collar rot disease assessments.
31/12/03	85	Commercial harvest but peas still relatively immature.
06/01/04	90	Yield assessment.

Results and discussion

Trials 1 & 2

In Trial 1, plants were affected by both collar rot and downy mildew, with collar rot being the dominant disease (Table 1). All plants had collar rot in Trial 1. Although the downy mildew incidence on untreated plants was high, severity of the disease was mild to moderate. In Trial 2, there was little or no collar rot in the trial area; hence no collar rot assessment was conducted. Downy mildew was the main disease in Trial 2. Treatments in both trials showed similar levels of disease control (Table 1). The effects of the same fungicide treatments or the number of spray applications were subsequently analyzed and these results are summarized in Tables 2 & 3.

Table 1: Treatment effects at one and two spray applications on collar rot and downy mildew incidence and severity in Trials 1 & 2

No.	Treatment	Application Schedule	Trial 1			Trial 2	
			Stem with collar rot (%)	Downy mildew		Downy mildew	
				Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
1	Untreated Control	Nil	14.9 c	98 c	3.1 c	100 b	3.9 d
2	Bravo	1 spray at the growth stage of 4 nodes	10.3 ab	98 c	3.0 c	98 b	3.0 cd
3	Agri-Fos 600 + Bravo		10.3 ab	60 b	1.4 b	88 b	1.7 bc
4	Agri-Fos 600 + Penncozeb SC		14.6 c	28 a	0.8 ab	82 bc	2.3 cd
5	Agri-Fos + Bravo + Penncozeb SC		10.7 ab	44 ab	1.4 b	94 b	2.3 cd
6	Bravo	2 sprays at the growth stage of 4 nodes & 8 nodes	12.2 abc	96 c	3.2 c	96 b	2.6 cd
7	Agri-Fos 600 + Bravo		9.6 a	38 ab	0.7 ab	94 b	1.6 bc
8	Agri-Fos 600 + Penncozeb SC		13.0 bc	28 a	0.5 a	50 a	0.6 a
9	Agri-Fos + Bravo + Penncozeb SC		11.6 abc	28 a	0.5 a	46 a	0.9 a
10	Agri-Fos + Bravo + Nufilm		9.4 a	38 ab	0.9 ab	60 ab	1.0 ab

Within each column, means followed by the same letter in each column are not significantly different at the 5% level according to LSD Test. *

These trials demonstrate that Agri-Fos and Penncozeb had no effect on collar rot, while Bravo, alone, had no effect on downy mildew. All treatments containing Bravo, alone or in a mixture with Agri-Fos or Penncozeb, reduced collar rot severity (Table 2). Agri-Fos + Bravo and Agri-Fos + Penncozeb reduced downy mildew incidence and severity in both Trials 1 & 2. Agr-Fos + Penncozeb gave the best control of downy mildew, but had no effect on collar rot, while Agri-Fos + Bravo was effective against both downy mildew and collar rot.

Table 2: A summary of fungicide applications on collar rot and downy mildew incidence and severity in Trials 1 & 2

No.	Treatment	Trial 1			Trial 2	
		Stem with collar rot (%)	Downy mildew (%)		Downy mildew (%)	
			Incidence	Severity	Incidence	Severity
1	Untreated Control	14.9 b	98 c	3.1 b	100 b	3.9 c
2 & 6	Bravo	11.2 a	97 c	3.1 b	100 b	2.8 bc
3 & 7	Agri-Fos 600 + Bravo	9.9 a	49 b	1.0 a	91 b	1.7 ab
4 & 8	Agri-Fos 600 + Penncozeb SC	13.8 b	28 a	0.7 a	66 a	1.4 a
5 & 9	Agri-Fos + Bravo + Penncozeb SC	11.2 a	36 ab	0.9 a	70 a	1.6 a

Within each column, means followed by the same letter in each column are not significantly different at the 5% level according to LSD Test. *

Two fungicide applications applied on to plants at the growth stage of 4 and 8 nodes gave better downy mildew control than one application (Table 3). However, for reducing collar rot severity, one spray application appeared to be adequate.

Table 3: A summary on the effects of the number of fungicide spray applications on collar rot and downy mildew incidence and severity in Trials 1 & 2

No.	Treatment	Trial 1			Trial 2	
		Stem with collar rot (%)	Downy mildew (%)		Downy mildew (%)	
			Incidence	Severity	Incidence	Severity
1	Untreated Control	14.9 a	98 b	3.1 b	100 b	3.9 c
2, 3, 4, 5	1 spray	11.6 b	58 a	1.6 a	91 b	2.4 b
6, 7, 8, 9	2 sprays	11.5 b	48 a	1.2 a	77 ab	1.4 a

Within each column, means followed by the same letter in each column are not significantly different at the 5% level according to LSD Test. *

The pea crop in Trial 2 was much more vigorous in growth than the crop in Trial 1, due to better growing weather conditions, and hence gave a much higher pea yield (Table 4). In Trial 1, the collar rot and downy mildew appeared to have no obvious impact on the crop yield, probably because of the unusually dry and hot weather conditions that occurred later in the trial period (Tables 4 & 5).

Table 4: Foliar spray treatments effects on pea yield in Trials 1 & 2

No.	Treatment	Application Schedule	Pea yield (adj. to tonne/ha ± std error)		Maturity index of peas	
			Trial 1	Trial 2	Trial 1	Trial 2
1	Untreated Control	Nil	5.2 ± 0.2	7.5 ± 0.4	219	184
2	Bravo	1 spray at the growth stage of 4 nodes	4.3 ± 0.1	6.7 ± 0.3	259	193
3	Agri-Fos 600 + Bravo		5.8 ± 0.6	8.6 ± 1.3	211	171
4	Agri-Fos 600 + Penncozeb SC		5.5 ± 0.9	8.7 ± 0.9	202	162
5	Agri-Fos + Bravo + Penncozeb SC		4.9 ± 0.5	8.2 ± 0.9	232	169
6	Bravo	2 sprays at the growth stage of 4 nodes, and 8 nodes	5.0 ± 0.6	9.0 ± 0.7	220	164
7	Agri-Fos 600 + Bravo		5.8 ± 0.3	8.6 ± 0.5	213	150
8	Agri-Fos 600 + Penncozeb SC		5.6 ± 0.6	8.6 ± 1.1	207	151
9	Agri-Fos + Bravo + Penncozeb SC		5.4 ± 0.5	9.0 ± 0.8	221	146
10	Agri-Fos + Bravo + Nufilm		5.3 ± 0.6	9.3 ± 0.6	216	156

In Trial 2, generally, there was a yield improvement as a result of downy mildew control in treatments containing Agri-Fos (Table 5). Peas from these treatments also tended to have lower maturity indices compared to the untreated control or Bravo treatments (Table 5). Although the yield was adjusted to a standard maturity index of 235, many immature peas were also crushed or lost through the sieve on the mechanical harvester used to extract peas from plants and pods. Therefore, the increase in pea yield as a result of better downy mildew control was likely to have been higher than recorded. There was a trend towards slight improvement in yield by two spray applications compared to one (Table 6).

Table 5: A summary of fungicide applications on pea yield and maturity in Trials 1 & 2

No.	Treatment	Pea yield (adj. to tonne/ha ± std error)		Maturity index of peas	
		Trial 1	Trial 2	Trial 1	Trial 2
1	Untreated Control	5.2 ± 0.2	7.5 ± 0.4	219	184
2 & 6	Bravo	4.6 ± 0.3	7.8 ± 0.5	240	179
3 & 7	Agri-Fos 600 + Bravo	5.8 ± 0.3	8.6 ± 0.6	212	161
4 & 8	Agri-Fos 600 + Penncozeb SC	5.5 ± 0.5	8.7 ± 0.7	205	157
5 & 9	Agri-Fos + Bravo + Penncozeb SC	5.2 ± 0.3	8.6 ± 0.6	227	158

The three fungicides mixture, Agri-Fos + Bravo + Penncozeb, reduced collar rot severity and also gave excellent control of downy mildew (Table 2). In Trials 1 and 2, there was no obvious benefit in the pea yield with the three fungicides mixture compared to only two (Table 5). The control of downy mildew and collar rot by Agri-Fos + Bravo + Nufilm was similar to Agri-Fos + Bravo (Table 1). This indicates that the spray adjuvant, Nufilm, has little or no effect on the disease control.

Table 6: A summary on the effects of the number of fungicide spray applications on collar rot and downy mildew incidence and severity in Trials 1 & 2

No.	Treatment	Pea yield (adjusted to tonne/ha ± standard error)	
		Trial 1	Trial 2
1	Untreated Control	5.2 ± 0.2	7.5 ± 0.4
2, 3, 4, 5	1 spray	5.1 ± 0.3	8.0 ± 0.5
6, 7, 8, 9	2 sprays	5.4 ± 0.2	8.8 ± 0.4

Trial 3

Trial 3 was conducted within a vigorously growing commercial crop. Collar rot incidence was very low (Table 7), and disease severity ranged from 0.1 to 0.3 % stem infection, and therefore, no treatment effect on collar rot was evident in the trial.

Downy mildew was the main disease in the trial, with high incidence on untreated plants, and mild to moderate disease severity (Table 7). Among all the treatments, Treatment 11 gave the best downy mildew control, followed by Treatment 8. These findings suggest that the optimum product rates were 3.5 L/ha for Agri-Fos and 2.5 L/ha for Penncozeb SC, and that the 2nd spray application was only required for downy mildew control, due to the late onset of the disease. In the trial area, there were no downy mildew infected plants at the 1st spray application, while first sign of infected plants was noted at the 2nd spray application.

Table 7: Treatment effects on collar rot and downy mildew incidence and severity in Trial 4

No.	Treatment	Collar rot (%)		Downy mildew (%)	
		Incidence	Severity	Incidence	Severity
1 & 2	Untreated Control combined	20	0.2	99 e	2.9 d
3	3.5L Agri-Fos 600	10	0.1	86 de	2.0 c
4	3.5L Agri-Fos + 1.1L Bravo	10	0.2	62 bcd	1.1 b
5	3.5L Agri-Fos + 1.8L Bravo	6	0.1	82 cde	1.7 c
6	3.5L Agri-Fos + 1.8L Penncozeb SC	16	0.2	68 cd	1.0 b
7	2.0L Agri-Fos + 2.5L Penncozeb SC	20	0.2	60 bc	0.9 b
8	**3.5L Agri-Fos + 2.5L Penncozeb SC**	**12**	**0.2**	**44 b**	**0.7 ab**
9	2 kg BAS 518	10	0.1	96 e	2.2 cd
10	3.5L Agri-Fos + 0.36 kg Acrobat	10	0.1	80 cde	1.7 c
11	**3.5L Agri-Fos + 1.8L Bravo *fb* 3.5L Agri-Fos + 2.5L Penncozeb SC**	**10**	**0.1**	**30 a**	**0.4 a**
12	3.5L Agri-Fos + 2.5L Penncozeb SC *fb* 3.5L Agri-Fos + 1.8L Bravo	12	0.2	76 cde	1.7 c

Apart from Treatment 9, all treatments containing Agri-Fos, either on its own or in combinations with Bravo and Penncozeb, improved yield compared to the experimental control (Table 8). In Treatment 9, the fungicide BAS 518 had little or no effect on pea yield compared to the untreated control. The pea yields for Treatments 11 and 12, with alternate applications of Agri-Fos + Bravo and Agri-Fos + Penncozeb, were among the highest in the trial. This indicates that these alternate treatments are likely to provide a safe choice of application methods, especially when there is uncertainty as to whether collar rot, or downy mildew disease, will be the dominant disease.

Table 8: Treatment effects on pea yield and maturity in Trial 4

No.	Treatment	Pea yield (adj. to tonne/ha ± std error)	Maturity index of peas
1 & 2	Untreated Control	8.8 ± 0.3	259
3	3.5L Agri-Fos 600	10.7 ± 0.8	230
4	3.5L Agri-Fos + 1.1L Bravo	10.0 ± 0.9	246
5	3.5L Agri-Fos + 1.8L Bravo	9.5 ± 0.6	249
6	3.5L Agri-Fos + 1.8L Penncozeb SC	10.1 ± 0.5	202
7	2.0L Agri-Fos + 2.5L Penncozeb SC	9.2 ± 1.0	210
8	3.5L Agri-Fos + 2.5L Penncozeb SC	10.2 ± 0.6	204
9	2 kg BAS 518	8.8 ± 0.7	258
10	3.5L Agri-Fos + 0.36 kg Acrobat	9.8 ± 0.2	245
11	3.5L Agri-Fos + 1.8L Bravo *fb* 3.5L Agri-Fos + 2.5L Penncozeb SC	10.4 ± 0.6	243
12	3.5L Agri-Fos + 2.5L Penncozeb SC *fb* 3.5L Agri-Fos + 1.8L Bravo	10.9 ± 0.9	206

Trial 4

In Trial 4, plants were affected by both collar rot and downy mildew, with downy mildew being the dominant disease (Table 9). The downy mildew severity on the pea crop ranged from mild to moderate. At close to maturity, the crop was probably water stressed, with poor crop vigour and uneven plant maturity resulting.

Table 9: Treatment effects on collar rot and downy mildew incidence and severity in Trial 4

No.	Treatment	Stem with collar rot (%)	Downy mildew (%)	
			Incidence	Severity
1	Untreated Control	2.3 b	96 e	2.5 d
3	Agri-Fos 600	2.0 ab	70 de	1.3 c
4	Penncozeb SC	2.6 b	82 e	1.1 bc
5	Agri-Fos + Penncozeb DF	1.9 ab	48 cd	0.6 ab
6	Agri-Fos + Penncozeb DF + Activator (ad)	1.6 a	34 abc	0.5 ab
7	Agri-Fos + Penncozeb DF + Tactic (ad)	1.6 a	20 a	0.2 a
8	Agri-Fos + Penncozeb DF + Sporekill	1.6 a	28 ab	0.2 a
9	Agri-Fos + Penncozeb SC	2.4 b	44 bcd	0.6 ab
10	Agri-Fos + Penncozeb SC + Activator (ad)	2.4 b	18 a	0.2 a
11	Agri-Fos + Penncozeb SC + Bond (ad)	2.3 ab	26 abc	0.7 ab
12	Agri-Fos + Penncozeb SC + Nufilm (ad)	2.0 ab	40 bcd	0.4 a

Within each column, means followed by the same letter in each column are not significantly different at the 5% level according to LSD Test. * Downy mildew severity equivalence: 1 = mild; 3 = moderate & 5 = severe.

The effects of the types of fungicide treatments, with and without spray adjuvants, were subsequently analysed and summarized in Table 10. Agri-Fos or Penncozeb, applied on their own, had little or no effects in the collar rot severity, and downy mildew incidence and severity (Tables 9 & 10). Mixture of the two products, however, significantly reduced downy mildew incidence and severity. This indicates a synergistic effect from the chemical mixture.

Table 10: A summary of fungicide types on collar rot and downy mildew incidence and severity in Trial 3

No.	Treatment	Stem with collar rot (%)	Downy mildew (%)	
			Incidence	Severity
2	Untreated Control	2.3 b	96 b	2.5 c
3	Agri-Fos 600	2.0 ab	82 b	1.3 b
4	Penncozeb SC	2.6 b	70 b	1.1 b
5, 6, 7 & 8	Agri-Fos + Penncozeb DF ± spray adjuvant	1.7 a	32 a	0.4 a
9, 10, 11& 12	Agri-Fos + Penncozeb SC ± spray adjuvant	2.3 b	32 a	0.4 a

Within each column, means followed by the same letter in each column are not significantly different at the 5% level according to LSD Test. * Downy mildew severity equivalence: 1 = mild; 3 = moderate; 5 = severe.

The spray mixture containing the dry flowable formulation of Penncozeb (Penncozeb DF) also reduced collar rot severity compared to untreated control (Table 10). The suspension concentrate formulation (Penncozeb SC), alone or in mixture has no effect on collar rot. The differences between the two Penncozeb treatments may also be related to the lower rates of mancozeb in the Penncozeb SC formulation than in Penncozeb DF (1050 and 1400 g/ha of mancozeb respectively).

Table 11: Treatment effects on pea yield and maturity in Trial 3

No.	Treatment	Pea yield (adj. to tonne/ha ± std error)	Maturity index of peas
1	Untreated Control	7.6 ± 0.4	278
3	Agri-Fos 600	8.6 ± 0.6	229
4	Penncozeb SC	6.5 ± 1.0	267
5	Agri-Fos + Penncozeb DF	9.0 ± 0.4	242
6	Agri-Fos + Penncozeb DF + Activator (ad)	8.6 ± 0.4	192
7	Agri-Fos + Penncozeb DF + Tactic (ad)	7.4 ± 0.4	243
8	Agri-Fos + Penncozeb DF + Sporekill	8.1 ± 1.0	235
9	Agri-Fos + Penncozeb SC	5.4 ± 0.6	221
10	Agri-Fos + Penncozeb SC + Activator (ad)	6.0 ± 0.9	205
11	Agri-Fos + Penncozeb SC + Bond (ad)	8.4 ± 0.5	277
12	Agri-Fos + Penncozeb SC + Nufilm (ad)	7.0 ± 0.7	229

Pea yields were related to the reduction in collar rot severity (Tables 9 & 11). A similar pattern was also observed in the summary of the types of fungicide treatments (Tables 10 & 12). Poor collar rot control by treatments containing Penncozeb SC probably resulted in lower pea yield. This indicates that even though the collar rot was considered to be relatively mild, the disease could still adversely affect pea yield in the presence of water stress.

Table 12: A summary of fungicide types on pea yield and maturity in Trial 3

No.	Treatment	Pea yield (adj. to tonne/ha ± std error)	Maturity index of peas
2	Untreated Control	7.6 ± 0.4	278
3	Agri-Fos 600	8.6 ± 1.0	229
4	Penncozeb SC	6.5 ± 0.5	267
5, 6, 7 & 8	Agri-Fos + Penncozeb DF ± spray adjuvant	8.3 ± 0.3	228
9, 10, 11& 12	Agri-Fos + Penncozeb SC ± spray adjuvant	6.7 ± 0.4	233

General Discussion

Nine field trials were conducted from 2000 to 2004, within commercial pea crops, to evaluate and optimize foliar fungicide application methods to control downy mildew from field inoculum of *P. viciae*. As peas are considered to be low value crop, mainly low cost fungicide products such as chlorothalonil (Bravo), mancozeb (Penncozeb), and phosphorous acid (Agri-Fos) were used in the trials. The fungicides were also applied in combinations with products (Agri-Fos, Bion and Micro-Gyp (calcium sulfate) that may stimulate or induce systemic acquired resistance in plants.

2000 trials

In 2000, in two preliminary field trials with two spray applications were carried out. The first spray was applied at 2-3 node growth stage before downy mildew occurred in the crops. These fungicide treatments did not reduce downy mildew incidence or severity, where the disease occurred at approximately 1 month after the second spray application. This indicates the importance of spray timing and the crop protection period by the fungicides for disease control.

2001/02 trials

In two trials carried out in 2001, the first spray was applied at the onset of downy mildew on lower leaves of plants. These trials demonstrated that Penncozeb SC + Agri-Fos, was the most effective treatment for field downy mildew control. Two foliar sprays of Penncozeb SC + Agri-Fos at the 4-5 node plant stage, consistently reduced downy mildew and increased pea yields. Among the other treatments evaluated, Bravo + Agri-Fos, followed by Penncozeb + Bion, were the second and third most effective treatments for downy mildew control. Penncozeb + Bion was more effective, if applied early, before most infection occurred and under low disease pressure. Penncozeb alone, applied in four sprays had no effect on downy mildew. As Bion is unlikely to be commercially available, subsequent trials conducted in 2002 to 2004 were focused on treatments with Agri-Fos, Bravo, and Penncozeb.

Plants treated with Apron or metalaxyl alone had similar downy mildew incidence and severity to the untreated plants. This poor control may have been due to the presence of metalaxyl resistant isolates of *P. viciae* in the crop.

A third trial in 2001/02 indicated that 5.0 L/ha of Agri-Fos 400 is likely to be more effective than 2.5 L/ha. Therefore, an equivalent of 5.0 L/ha Agri-Fos 400 (i.e. 2 kg active/ha) were used in trials conducted in 2002 to 2004, at 3.0 L/ha and 3.5 L/ha Agri-Fos 600, a new formulation (i.e. 1.8 kg and 2.1 kg active/ha).

2002 – 2003/04 trials

Four trials were conducted in 2002 to 2004, in order to optimize fungicide application methods for both downy mildew and collar rot control. These are the two most common and important diseases impacting on processing pea crops yield and quality. In some trials, even when downy mildew or collar rot was considered to be relatively mild, they could still adversely affect pea yield when crops are subjected to water stress. Most crops have both diseases, and therefore the ability to control both is critical for pea disease management. Even though one disease is usually dominant in a crop, it can be difficult to predict which will become the major disease. Where it is possible to determine which disease is of major concern in a crop, it is important that the effective fungicide application is used. Bravo was consistently shown to be effective in reducing collar rot severity in this project and in a previous study on collar rot (Pung & Cross 2000).

Agri-Fos or Penncozeb alone, were shown to have little or no effect on collar rot. Agri-Fos or Penncozeb, applied alone, also had little or no effects on downy mildew incidence and severity. In contrast, Agri-Fos, in a mixture with Bravo or Penncozeb, reduced downy mildew incidence and severity on plants. This indicates a synergistic effect by the spray mixture of these two products.

The Agri-Fos + Penncozeb in combination gave the best control of downy mildew, but had no effect on collar rot. However, Agri-Fos + Bravo, the second best treatment against downy mildew, was also effective in reducing collar rot severity.

Two fungicide applications applied on to plants at the growth stage of 4 and 8 nodes gave better downy mildew control than one application. However, for reducing collar rot severity, one spray application at 4 nodes appeared to be adequate.

Where the two major pea diseases were present, and collar rot was the dominant disease, Agri-Fos + Bravo treatment reduced severity of both collar rot and downy mildew. The optimum product rate of

Bravo for collar rot control was 1.8 L/ha. However, if downy mildew was the only disease or was the dominant disease, Agri-Fos + Penncozeb tended to give better downy mildew control and yield improvement. The optimum product rates for downy mildew control were 3.5 L/ha Agri-Fos, and 2.5 L/ha Penncozeb SC or 2.0 kg/ha Penncozeb DF.

Early fungicide applications before diseases occurr only protect plants for a short interval of 10 to 14 days. Therefore, for optimum downy mildew control, the fungicide application must be applied at the first sign of infection in the crop. As collar rot tends to occur early in the crop, the alternate applications of Agri-Fos + Bravo followed by Agri-Fos + Penncozeb could be a suitable program for optimum collar rot and downy mildew control. In many crops, the onset of field downy mildew infection tended to occur later. Pea fungicide seed treatments could also protect seedlings from early infection.

The spray mixture containing the dry flowable formulation of Penncozeb (Penncozeb DF) also reduced collar rot severity compared to untreated experimental controls or the mixture containing the suspension concentrate formulation (Penncozeb SC). Apart from formulation, the differences between the two Penncozeb products may also be related to the lower rates of mancozeb in the Penncozeb SC formulation compared with Penncozeb DF.

Among other fungicides also examined, Apron and BAS 518 had little or no effect on downy mildew, while Agri-Fos + Acrobat was only as effective as Agri-Fos + Bravo.

References

Dixon, G.R. (1981): Downy mildews in peas and beans. In 'The Downy mildews', Spencer, D.M. (Ed), Academic Press, London, pp 487-514.

Stegmark, R. (1988): Downy mildew resistance of various pea genotypes. Acta Agri. Scand. 38:373-379.

Falloon, R.E., Viljanen-Rollinson, S.L.H., Coles, G.D. & Poff, J.D. (1995): Disease severity keys for powdery and downy mildews of peas and powdery scab of potato. New Zealand Journal of Crop and Horticultural Science 23: 31-37.

Falloon, R.E., Follas, G.B., Butler, R.C. & Goulden, D.S. (2000): Resistance in *Peronospora viciae* to phenylamide fungicides: reduced efficacy of seed treatments of pea (*Pisum sativum*) and assessment of alternatives. Crop Protection 19: 313-325.

Gisi, U. (1988): Population dynamics in Peronosporales treated with phenylamide fungicides. In Delp, C.J. (ed.), *Fungicide resistance in North America*. American Phytophathological Society, St Paul, USA: pp. 66-71.

McCullagh, P., Nelder, J.A. (1989): *Generalized Linear Models*, 2nd Edition. Chapman & Hall, London: xix + 515 p.

Pung, H., Cross, S. (2000): Ascochyta rot on peas and its control. HRDC Project VG97051, Final Report.

Ross, G.J.S. (1984): Parallel model analysis: fitting non-linear models to several sets of data. COMSTAT 1984 – 6th Symposium on Computational Statistics: 458-463

Schall, R. (1991): Estimation in generalized linear models with random effects. *Biometrika* **76**: 719-727.

Urech, P.A. (1988): Phenylamide resistance management strategies. In Delp, C.J. (ed.), *Fungicide resistance in North America*. American Phytophathological Society, St Paul, USA: pp. 74-77.

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Appendix i - Product Formulations

Product	Active Ingredient (a.i.)	Concentration of a. i.	Formulation
Acrobat MZ690	Mancozeb + Dimethomorph	600 g/kg + 90 g/kg	Wettable powder
Agri-Fos Supa 400	Phosphoric acid	400 g/L	Liquid
Agri-Fos Supa 600	Phosphoric acid	600 g/L	Liquid
Aliette Super	Fosetyl-Al + thiram + thiabendazole	528 g/kg + 172 g/kg + 129 g/kg	Wettable powder
Amistar	Azoxystrobin	500 g/kg	Water dispersible micro-granules
Apron 350SD	Metalaxyl	350 g/kg	Wettable powder
Bavistin	Carbendazim	500 g/L	Soluble concentrate
Bion 50WG	Acibenzolar-S-methyl	500 g/kg	Water dispersible granules
Bravo 720	Chlorothalonil	720 g/L	Suspension concentrate
Captan	Captan	800 g/kg	Water dispersible micro-granules
Elexa	Chitosan	4 %	Suspension liquid
Fongarid 250WP	Furalaxyl	250 g/kg	Wettable powder
Maxim	Fludioxinil	100 g/L	Suspension concentrate
Penncozeb 750DF	Mancozeb	750 g/kg	Dry flowable
Penncozeb 420SC	Mancozeb	420 g/L	Suspension concentrate
P-Pickel T	Thiram + thiabendazole	360 g/L + 200 g/L	Soluble concentrate
Raxil	Tebuconazole + cypermethrin	25 g/kg + 4 g/kg	Suspension concentrate
Serenade	Bacillus subtilis	5×10^9 cfu/g	Wettable powder
Wakil XL	Cymoxinil + metalaxyl + fludioxinil	100 g/kg + 175 g/kg + 50 g/kg	Water dispersible granules

Photographs

Downy mildew infected leaves (Photograph 1), and constrictions of lower stems due to the black Ascochyta *collar rot (Photograph 2)*

Photograph 1

Photograph 2

Patchy and unthrifty pea plants due to poor downy mildew control (Photograph 3), and dense and healthy pea plants with effective downy mildew control by chlorothalonil + phosphorous acid spray application (Photograph 4)

Photograph 3

Photograph 4