



Know-how for Horticulture™

**Total crop
management of
clubroot in brassica
vegetables**

Caroline Donald
VIC Department of Primary
Industries

Project Number: VG00044

VG00044

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Clubroot Total crop management

Final report

VG 00044 (June 30th 2003)

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Department of Primary Industries

Horticulture Australia project number VG 00044

Clubroot – Total Crop Management

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This report presents a summary of work conducted in Australia during the period July 2000 – June 2003 by the national clubroot research team. The project, a national initiative, has sought to address the short, medium and long-term needs of the brassica industry to manage clubroot by developing management strategies that encompass whole production systems – seed to transplant to mature crop. Whilst every attempt has been made to present as complete a summary as possible, some sections (ie. section 5, Transplant production and farm hygiene, section 6, Monitoring, prediction and decision making and section 7, Treatment selection, application and crop growth) have been restricted to key findings and research highlights to ensure the report is maintained at a manageable size. Details of individual experiments or trials can be obtained on request from the author.

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1 Media Summary

Clubroot is the most serious disease of the vegetable brassica crops. In Australia it is estimated that the disease is responsible for losses of 5-10% of the national crop, or approximately \$10m annually. This project, a national initiative, has sought to address the short, medium and long-term needs of the brassica industry to manage this disease by developing management strategies that encompass whole production systems – seed to transplant to mature crop. This has been achieved through:

1. Development of best practice protocols for the production of disease free seedlings. This information has been published as a shed poster and accompanying series of 7 fact sheets covering the following aspects of seedling production:
 - ◆ Identifying likely sources of clubroot contamination in the nursery.
 - ◆ Designing the nursery to minimise clubroot risk.
 - ◆ Monitoring and restricting access to the nursery.
 - ◆ Washing, sterilising and disinfecting.
 - ◆ Keeping seeds, water and soil free of contamination.
 - ◆ Monitoring for clubroot in the nursery.
 - ◆ Knowing what to do if clubroot is detected.
2. Demonstration of integrated management strategies, including the development of rates, methods and timing of application of limes, fertilisers and fungicides, that are effective in all states.
3. Development of a transplant mounted machine to simultaneously plant and incorporate treatments to control clubroot into the planting row. Advantages of this simultaneous treatment and planting system include labour and cost savings resulting from a single pass operation and improved treatment efficacy, a result of more accurate planting into the treated rows.
4. Optimisation of a molecular diagnostic protocol to quantify the amount of *P. brassicae* in soil and evaluation of it's predictive ability at 54 sites nationally.

Recommendations contained within this report will virtually eliminate clubroot as a problem in nurseries and provide Australian growers with the most advanced in-field methods to combat this disease. Large-scale use of the quantitative diagnostic test has identified issues of concern in some soils and commercialisation has been placed on hold until these can be rectified. As this test represents the missing link in the delivery of a complete integrated management strategy, further development and commercialisation is high priority for future research.

2 Technical Summary

Clubroot is the most serious disease of the vegetable brassica crops including broccoli, cauliflower, cabbage, Brussels sprouts, Chinese cabbage and other Asian vegetable brassicas. In Australia it is estimated that the disease is responsible for losses of 5-10% of the national crop, or approximately \$10m annually. This project, a national initiative involving researchers from 5 states of Australia, has sought to address the short, medium and long-term needs of the brassica industry to manage this disease by developing management strategies that encompass whole production systems – seed to transplant to mature crop. This has been achieved through:

1. Development of best practice protocols for the production of disease free seedlings. These have been published as a shed poster and accompanying series of 7 fact sheets for nurseries.
2. Demonstration of integrated management strategies that are effective in all states.
3. Development of a transplant mounted machine to simultaneously plant and apply treatments to control clubroot.
4. Ongoing development and evaluation of a quantitative diagnostic protocol to predict crop loss due to clubroot on farms nationally.

The management of clubroot disease is a responsibility of both the nurseryman and vegetable grower. This project has sought to provide a holistic approach to the control of clubroot developing management techniques that address the concerns of both these industries.

In the nursery

- ◆ Plastic trays returned to the nursery from farms were identified as the highest risk for clubroot incursion.
- ◆ High pressure washing was found to be an essential pre-treatment for these plastic trays as the use of biocides or steam sterilisation alone did not eliminate *P. brassicae*.
- ◆ Of the commercially available disinfectant solutions, only sodium hypochlorite significantly reduced the viability of *P. brassicae*.
- ◆ Resting spores remained viable in water for 2 years and caused symptoms of disease at concentrations as low as 10 spores/mL so the use of dam water in nurseries is not recommended. These spores settled if undisturbed, at a rate of 25 cm/day so if water stored in tanks or dams is being used for irrigation on farms it should be sourced from an undisturbed part of the dam, from a pipe mounted on a float to minimise the amount of spores taken into the irrigation system.
- ◆ Hot lime (CaO) added to the planting medium was as effective as the available fungicides at preventing symptoms of clubroot for a fraction of the cost. Such additives however, serve mainly to mask an underlying hygiene problem and are unnecessary if steps are taken to prevent spores of *P. brassicae* entering the nursery production system.
- ◆ Simple changes to nursery design, operational procedures and implementation of a monitoring program that minimise the clubroot risk in nurseries have been detailed in a shed poster and series of 7 accompanying fact sheets.

On the farm

- ◆ Strategies that include the use of lime, fungicides and plant nutrients (Ca and B) to manage clubroot have been proven and demonstrated nationally.
- ◆ Application of many of these products has been optimised with the development of a planter mounted machine to incorporate treatments into the transplant root zone whilst planting. Advantages of this simultaneous treatment and planting system include labour and cost savings resulting from a single pass operation and improved treatment efficacy a result of more accurate planting into the treated rows.
- ◆ Forty percent of growers in one of Australia's key brassica export regions have now modified existing transplanting machinery to incorporate fertilisers and/or fungicides at planting. These growers report increased profits of \$4000/ha in winter grown cauliflower crops.
- ◆ A molecular diagnostic protocol to quantify the amount of *P. brassicae* in soil has been optimised and it's predictive ability tested at 54 sites nationally. Where a positive test result has been obtained, the test has accurately predicted crop loss in the field. However, there have been a number of soils for which a negative test result has been obtained but severe root galling has been observed in the field. Considerable research effort has been dedicated to determining the cause of these false negative test outcomes, however, to date these remain undetermined and further work is required before the test can be commercialised. This work should include a study of the influence of the timing and method of sampling on the test outcome as it is possible that factors such as repeated tillage may reintroduce spores to the upper surface of the soil.

3 General Introduction

The Australian horticultural brassica industry grows produce valued at \$134 million annually (Australian Horticultural Corporation, 1998). Broccoli, cauliflower and cabbage are the major brassica vegetable crops. Minor crops include Brussels sprouts, Chinese cabbage and other Asian vegetables.

Clubroot is the most serious soilborne disease affecting brassicas world wide. It is caused by *Plasmodiophora brassicae* Woronin, an obligate biotrophic parasite. Currently considered to belong to the Protoctista, it is neither plant, animal nor fungus (Braselton, 1995).

Clubroot was first reported in Australia in 1890. It is likely to have been brought into the country with the early settlers as diseased planting material (Watson and Baker, 1969), although fodder or grazing animals represent an alternative source of contamination. Recent increases in the prevalence of this disease can be associated with the increased use of transplants, narrow rotations, more extensive cropping on the same soil (in some cases, 4 crops per year) and the suspected increased movement of the pathogen on trucks, bulk bins and other farm equipment.

Surveys (Porter *et al.*, 1994) have shown that over 70% of brassica properties in Victoria are affected by clubroot. Crop losses of up to 25 hectares/property have been reported and total national crop loss is estimated at between 5 and 10% of brassica production.

Clubroot is endemic in most of the major production regions of Victoria, New South Wales and Tasmania. Outbreaks have occurred in Stanthorpe (Queensland in 1997), Gatton, (Queensland in 2001) and Manjimup (Western Australia in 1993). Clubroot is now a significant problem in every state of Australia.

Symptoms of disease are restricted to members of the family Cruciferae. Infection can occur at any stage of growth and is restricted to the roots. Infected roots swell forming characteristic galls that may either be large and compact or numerous irregular swellings, depending upon the timing and severity of infection. Infected plants are nutritionally impaired as galled roots have a reduced capacity to assimilate water and nutrients from the soil. The earliest above ground symptom of clubroot is wilting of the leaves of infected plants particularly on warm days. Severely infected plants will be stunted and yield significantly reduced.

Previous research (Porter *et al.*, 1997) identified a number of new fertiliser, nutrient and fungicide treatments that reduced yield losses due to clubroot in Victoria and Western Australia. Long-term management strategies based on good farm hygiene, crop rotation, liming, application of calcium and a preventative fungicide were subsequently developed (Donald *et al.*, 2000). A novel method of application was also developed. More recently, a diagnostic test to detect the presence or absence of *P. brassicae* has been developed (Faggian *et al.*, 1999; Faggian and Parsons, 2002).

The primary aim of the current project was to develop a holistic approach to the management of clubroot that addressed all aspects of production including seedling production, site selection, treatment selection and application. This was achieved by :

- ◆ Development of strategies to eliminate clubroot as a problem *in nurseries*.
- ◆ Development and demonstration of integrated management strategies that are effective *in all states*.
- ◆ Development of a *transplant mounted* machine to simultaneously plant and apply treatments to control clubroot.
- ◆ Optimisation and large scale evaluation of a *quantitative version* of a diagnostic protocol to predict crop loss due to clubroot on farms nationally.

4 General Materials & Methods

Preparation of resting spore suspensions

Clubroot galls were collected from commercial vegetable brassica farms. The galls were washed and stored in a freezer at -20°C for not longer than one year before use. Root galls containing resting spores of *P. brassicae* were homogenised 1:3 (w:v) with distilled water in a mechanical blender and filtered through nylon cloth. Where clean spore suspensions were required, samples were cleaned by repeated (5 times) centrifugation at 2000 g for 5 mins and resuspended in sterile distilled water. The final concentration of resting spores in each suspension was estimated using a haemocytometer.

Visual assessment of roots for symptoms of clubroot

The roots were shaken free of soil/potting mixture and were visually assessed for galling due to clubroot according to the following scale:

- (1) No visible root galling
- (2) Single gall on lateral roots
- (3) Several small galls on lateral roots (plant healthy)
- (4) Mild galling of the taproot, several small galls on lateral roots
- (5) Moderate galling of the taproot, many small or several large galls on lateral roots
- (6) Severe galling of the taproot, many large galls on laterals
- (7) Severely galled, several healthy roots remaining
- (8) Severely galled, few healthy roots present
- (9) Severely galled, no healthy roots present

Molecular detection of *P. brassicae*

The FastDNA SPIN Kit for soil was used to extract PCR-ready genomic DNA of *P. brassicae* from soil samples. For each soil sample duplicate extraction was performed and for each batch of soil samples a positive control (containing a known amount of spores added to the test soil) was included. Each sample of extracted soil DNA was tested in duplicate with a PCR (polymerase chain reaction) assay to detect DNA of *P. brassicae*, the cause of clubroot (PCR conditions as per Faggian *et al.*, 1999). In each PCR test, negative and positive controls were included. Negative test outcomes were further scrutinised by one or both of the following approaches: (1) re-extraction of DNA from soil with and without addition of various known concentrations of clubroot spores followed by PCR assay, (2) dilution (1/10 and 1/100) of extracted DNA followed by PCR assay.

Statistical Analyses

Analysis of variance was used to study the differences between treatments in trials. Where possible factorial analyses were conducted. Unless otherwise stated the level of significance used was $P=0.05$. Any relationship between treatments was compared by regression analysis. The Genstat 5 (Payne *et al.* 1987) statistical package, release 4.1 (Lawes Agricultural Trust, Rothamsted Experimental Station) was used to conduct these analyses.

5 Transplant production and farm hygiene

5.1 Introduction

Clubroot can and occasionally does manage to get into the nursery production system (Fig. 1). As clubroot is a disease that can persist in the soil for many years, its presence at the seedling stage is of major concern to farm and nursery businesses alike. As relatively small numbers of commercial nurseries now supply seedlings to the majority of the industry, with seedlings often being transported over long distances, the potential for clubroot to be spread in this way is significant. For this reason the development of guidelines containing 'best practice' recommendations that minimise the risk of clubroot entering the nursery production process have been a priority.



Figure 1: Infected seedling

As a result of this project a poster and accompanying series of 7 fact sheets were produced and distributed to brassica nurseries nationally (both commercial and ‘own-farm’). These publications appear as an appendix to this report (Appendix I). Research required for the production of these publications is detailed below.

5.2 Risk Identification (Nursery Audit)

Materials and Method

A systematic sampling and surveying protocol was developed to enable samples and information to be collected in a consistent manner from each of the nursery sites (Appendix II). The following samples were collected where available: water (dam, bore, tank, wash water (from seedling tray washing apparatus)), potting mixture, seed (from opened tins), seedlings, soil scrapings (from nursery floor, benches, shade cloth, unwashed transplant trays). Seven sites were studied in Queensland and Victoria using a molecular diagnostic test for the detection of the DNA of *P. brassicae* (see section 4). Seedlings from a further 3 nurseries in Western Australia have been routinely retained, grown out and monitored for symptom development throughout the course of the project.

Results

Clubroot was not detected in any of the nurseries in Queensland or Western Australia. DNA of *P. brassicae*, the organism causing clubroot disease was detected in some of the samples collected from several of the Victorian nurseries. In general, these were smaller ‘grower owned’, non-commercial nurseries. Positive test results indicating the presence of DNA of *P. brassicae* were obtained from water (dam, bore and wash water) and soil scrapings (shade cloth and unwashed trays).

Among the sites selected for sampling was a small brassica nursery producing transplants for private use. The nursery had experienced a sudden and unexplainable clubroot outbreak where the occasional seedling exhibited clubroot symptoms. Sampling and testing for the presence of the DNA of *P. brassicae* was conducted as described above.

Table 1. Sample results – Victorian nursery

Sample	Replicate	1	2	3	4
Dam water		+	+	+	+
Bore water		+	+	+	+
Tank water		-	-	-	-
Town water		-	-	-	-
Wash water		+	+	+	+
Soil (nearby field)		+	+	+	+
Potting mix		-	-	-	-
Seed		-	-	-	-
Seedlings (symptomless)		-	-	-	-
Seedlings (symptoms)		+	+	+	+
Floor scrapings (soil/dust)		-	-	-	-
Dust (benches)		-	-	-	-
Dust (shade-cloth)		+	+	+	+
Tray scrapings		+	+	+	+

A number of possible sources of clubroot contamination at the nursery were identified (Table 1). Clubroot was detected in bore water, which was used to fill tanks (diluted 1:1 with town water) that provide water for green-house seedlings, and to irrigate field-grown plants. However, contaminated bore water is unlikely to have been the source of the problem in this case, as the expected infection pattern (i.e. evenly distributed low level infection) was not observed. Only isolated plants exhibited symptoms, and galls were prominent. The most likely cause of infection in this case was the presence of PCR positive soil (tray scrapings) on transplant trays. In this case trays were being washed with a biocidal agent before being reused. However, it is possible that viable resting spores can survive the

washing process in the shelter of soil clumps, or the biocidal wash may have become ineffective due to prolonged use or high organic load. Also of concern is that *P. brassicae* was detected in dust scraped from the inside of the shade-cloth wall of the green house. This is the first evidence that *P. brassicae* is dust-borne, and will have implications for the spread of inoculum to new areas and the contamination of water sources.

Implications

The example given above highlights an important use for the recently developed molecular diagnostic test. Whilst the results of the nursery survey were specific to each site and required interpretation based on management practices specific to each site, the following generalisations can be made:

1. *Seedling trays returning from farms to nurseries clearly represented the greatest threat for clubroot contamination in the nursery system. Wherever possible, trays leaving the nursery should not come in contact with the ground (field soil). The plugs of trays that are thrown onto the ground after planting frequently become blocked with soil that is difficult to remove simply by dipping the trays in a tank containing a disinfectant solution. This soil shelters viable resting spores which can contaminate subsequent batches of seedlings. One or more of the following management options could be used to reduce the risk of contamination in this case:*
 - *Pull the transplants out of the trays into crates or boxes before they are delivered to the field. In doing so the trays do not leave the nursery and therefore cannot become contaminated with field soil.*
 - *Deliver the trays to the field on a pallet to keep them off the ground. Ensure that field workers return empty trays to the pallet rather than stacking them directly on the ground.*
 - *High pressure wash used trays before dipping in a disinfectant solution to remove soil clumps.*
2. *Dam water should not be used to irrigate nursery stock.*
3. *Nurseries should be located away from affected growing regions or should be adequately screened from the prevailing wind to prevent dust blowing into the nursery.*

5.3 Biocide evaluation

Introduction

Many nurseries currently depend on a range of disinfectant products to clean plastic seedling trays and other surfaces before reuse. Most of these products have never been tested for their ability to kill resting spores of *P. brassicae*. This study was conducted to determine the ability of commercially available disinfectants to kill resting spores of *P. brassicae*. The results have been published in *Australasian Plant Pathology* (Donald *et. al.*, 2002).

5.3.1 The ability of commercial disinfectants to kill resting spores of *P. brassicae*

Materials and Method

Eleven disinfectant solutions from different classes were prepared (Table 2). The actual chlorine or chlorine dioxide concentrations of the halogen based treatments (Table 2) were checked using a HACH DR/2000 direct reading spectrophotometer (N,N-diethyl-p-phenylenediamine: DPD, Hach DR/2000 method 8167 for total chlorine and direct reading Hach DR/2000 method 8138 for chlorine dioxide). A 1 mL aliquot of resting spore suspension (2×10^8 spores/mL) was added to 9 mL of the disinfectant solution. Following exposure of 2.5, 5 or 10 min at room temperature ($22^\circ\text{C} \pm 2^\circ\text{C}$), a 1 mL aliquot was removed and diluted with 9 mL sterile distilled water (phenolics and ethanol treatments) or 9 mL inactivator solution (0.05% sodium thiosulphate plus 10% Tween 80) to halt the disinfection process (Russell, *et. al.*, 1979). There were four replicates of the methylated spirits treatment. All other treatments were replicated six times, however, to minimise delays in the processing of samples (viability assessment using fluorescent technique), the trial was conducted two replicates at a time. Fresh spore suspensions and solutions of each disinfectant were prepared for each group of two replicates (Table 2). Samples were centrifuged once (2500 g, 5 mins) to recover the spores. The pellet was suspended in 1 mL sterile distilled water and had a final resting spore concentration of not less than 10^7 spores/mL.

Table 2: Active ingredients, chemical classes and rates of chemical disinfectants.

Treatment	Active ingredient	Class	Dilution	Inactivator used?
Phytoclean	100 g/L benzalkonium chloride	Quaternary Ammonium	2 %	Yes
Sporekill	120 g/L Didecyltrimethylammonium chloride	Quaternary Ammonium	0.2 %	Yes
Hi-Dab	75 g/L alkyl dimethyl benzyl ammonium chloride 20 g/L chlorhexadine complex 150 g/L ethylene oxide surfactant	Quaternary Ammonium	1.25 ml in 100 ml	Yes
Kendocide	423 g/L dichlorophen present as sodium salt	Phenol	1:100	No
Biogram	18% substituted phenols, contains 16% phenyl phenol	Phenol	1.5% v/v	No
Peratec 5	250 g/L hydrogen peroxide 50 g/L peroxyacetic acid	Peroxygen	1:100	Yes
Oxine	52.6 g/L available chlorine present as 20 g/L chlorine dioxide	Halogen	100 ppm	Yes
Hypochlorite	Sodium hypochlorite (10.5-15% available chlorine)	Halogen	1150 ppm (reps 1&2) 910 ppm (reps 3&4) 1200 ppm (reps 5&6)	Yes
Nylate	920g/kg 3-bromo-1-chloro-5, 5 dimethylhydantoin	Halogen	16.4 mg/L (reps 1&2) 15.5 mg/L (reps 3&4) 20.5 mg/L (reps 5&6)	Yes
Ethanol	ethanol	Alcohol	70 %	No
Methylated Spirits	ethanol	Alcohol	48 % ethanol	No
Water		Control		No
Water & inactivator		Control		Yes

Recovered spore suspensions were stained with equal volumes of 50 µg/ml of ethidium bromide (aq) and 100 µg/ml calcofluor white (Takahashi and Yamaguchi, 1988) and examined under oil immersion (x 1000) using a Nikon optiphot fluorescent microscope. The light source was a super-high pressure mercury lamp (100 W). The epi-fluorescence filter combination consisted of an excitation filter (330-380 nm), a barrier filter (420 nm) and a dichroic mirror (400 nm). The wall layers of all the spores stained by this method exhibited intense blue fluorescence. Active and inactive spores could be differentiated on the basis of the staining reaction of the cytoplasm. The cytoplasm of inactive spores stained by this method displayed red fluorescence, however, the cytoplasm of active spores did not fluoresce or displayed a pale blue fluorescence (Takahashi and Yamaguchi, 1988). The pathogenic activity of each of the spore suspensions was estimated by counting the number of active and inactive spores in a sample of 100 spores.

Following the chemical disinfectant treatments, a small subsample of each spore suspension was pipetted drop-wise onto Thermanox® 13 mm round plastic coverslips (Nalge Nunc International, USA) that had been coated in a 2% (w/v sdH₂O) solution of gelatin and air-dried for 10 minutes. The cover slips were placed on a level surface for 10 minutes to allow the spores to adhere. The excess was then removed and the coverslips immersed in a 4% (v/v) glutaraldehyde fixative solution (ProSciTech, Thuringowa, Qld) for 2 hours. Samples were removed from fixative and washed 3 times with sdH₂O, 15 minutes per wash. Samples were dehydrated using an ethanol series: 10%, 25%, 50%, 75%, 90%, 95%, 100% for 15 minutes per solution. After the final dehydration step, samples were critical point

dried using a LADD critical point drier (LADD Research Industries), attached to aluminium stubs (ProSciTech, Thuringowa, Qld) and coated with gold using a sputter coater (SPI Supplies) (R. Faggian, pers. comm.). Each specimen was examined using a scanning electron microscope (Philips XL-30) to examine damage to the spore wall.

Broccoli seed (cv. Marathon) was planted, one seed per pot, into pasteurised soilless potting mixture in 7 cm diameter plastic pots. Seedlings were maintained for the duration of the experiment under mist irrigation (applied for 3 mins every 12 hrs) at 20 ± 2 °C in a glasshouse. Three seedlings were inoculated per sample at the two leaf stage by pipetting 200 µL of recovered spore suspension into a small depression in the soil at the base of the plant.

Six weeks after inoculation the plants were removed from their pots. The roots were shaken free of potting mixture and were visually assessed for root galling due to clubroot on the 1-9 scale previously described (section 4).

Results

With the exception of ethanol (70%), none of the disinfectant treatments resulted in a significant decrease in the viability of the inoculum measured by fluorescence. The plant bait results confirmed this finding with ethanol resulting in almost complete kill, hypochlorite significantly ($p < 0.05$) reducing disease and all of the other treatments having no effect on the development of disease (Fig. 2). Increasing the time of exposure to the disinfectants from 2.5 to 5 to 10 minutes did not consistently reduce either viability (measured by fluorescence) or disease (measured by plant baits). In this experiment there was a strong positive correlation ($r=0.81$) between plant bait and fluorescence results.

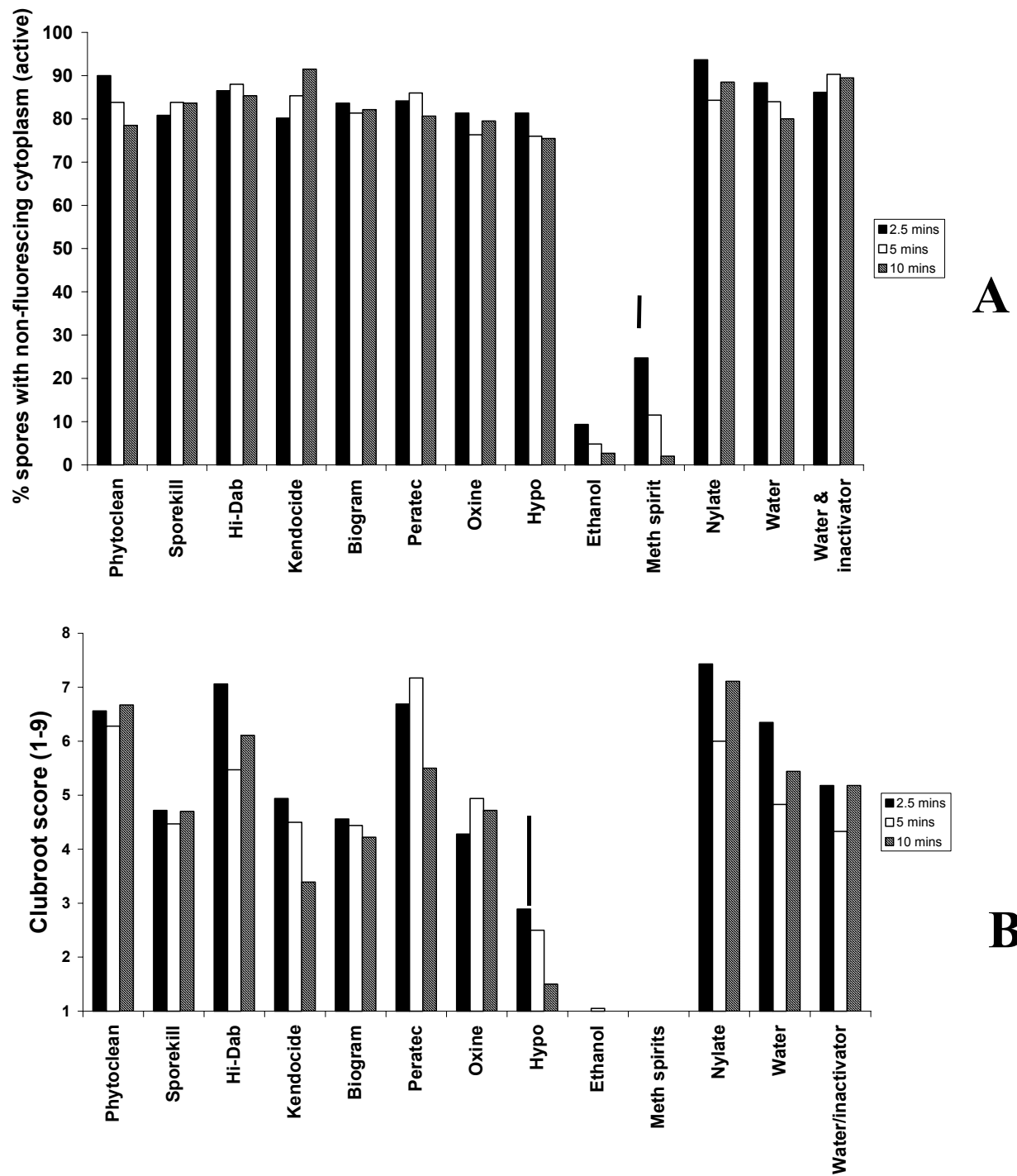


Figure 2: The effect of disinfectant treatment on (A) activity of resting spores of *Plasmodiophora brassicae* (predicted by fluorescent staining reaction) l.s.d. ($P=0.05$) = 8.2 and (B) clubroot severity on broccoli l.s.d. ($P=0.05$) = 1.6. l.s.d.s refer to treatment differences (time did not produce a significant effect).

Scanning electron microscope studies revealed that with the exception of the alcohol based treatments, there was no physical damage to the resting spores (Fig. 3).

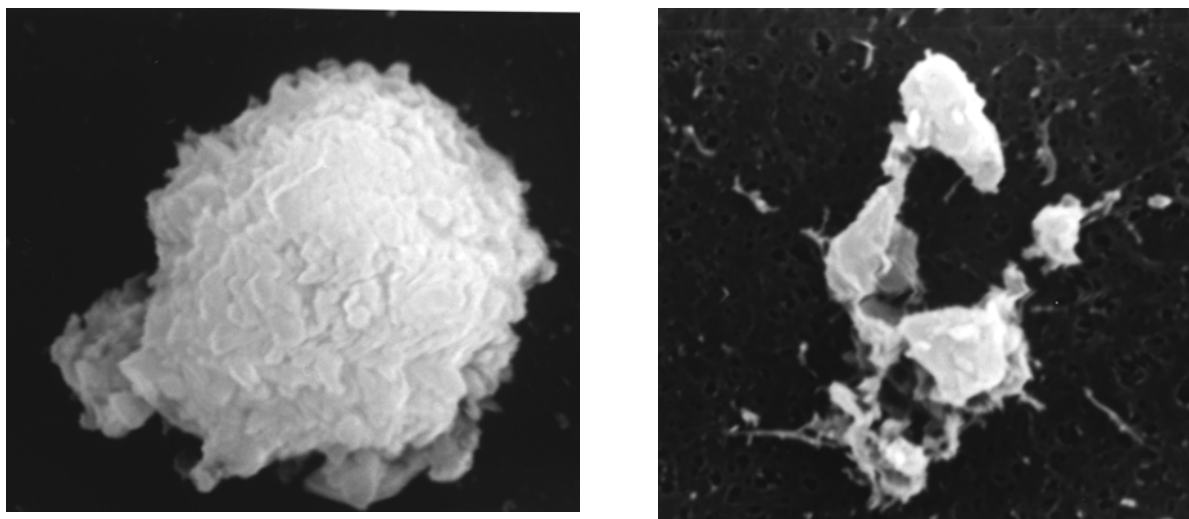


Figure 3: Scanning electron micrographs comparing untreated (left) resting spores of *P. brassicae* with those that had been treated with 70% ethanol for 10 mins (right).

Implications: *Clearly growers cannot rely solely upon many of the commercially available disinfectant products to clean plastic trays before reuse. All trays should be high-pressure washed before disinfection to remove as much of the inoculum as possible.*

5.3.2 Determining the minimum effective rate and contact time for ethanol

Materials and Method

Resting spore suspensions were challenged for 2.5, 5 or 10 minutes with ethanol at 70%, 60%, 50%, 40%, 30%, 20%, 10% 0%. Recovered spores were cleaned, stained (Takahashi and Yamaguchi, 1988) and used to inoculate bait plants (3 plants per treatment/time combination) as previously described.

Results

At concentrations of ethanol greater than 50%, less than 10% of spores were deemed to be viable by the fluorescent staining technique (Fig. 4), however, concentrations in excess of 30% were sufficient to eliminate symptoms of disease (Fig. 5).

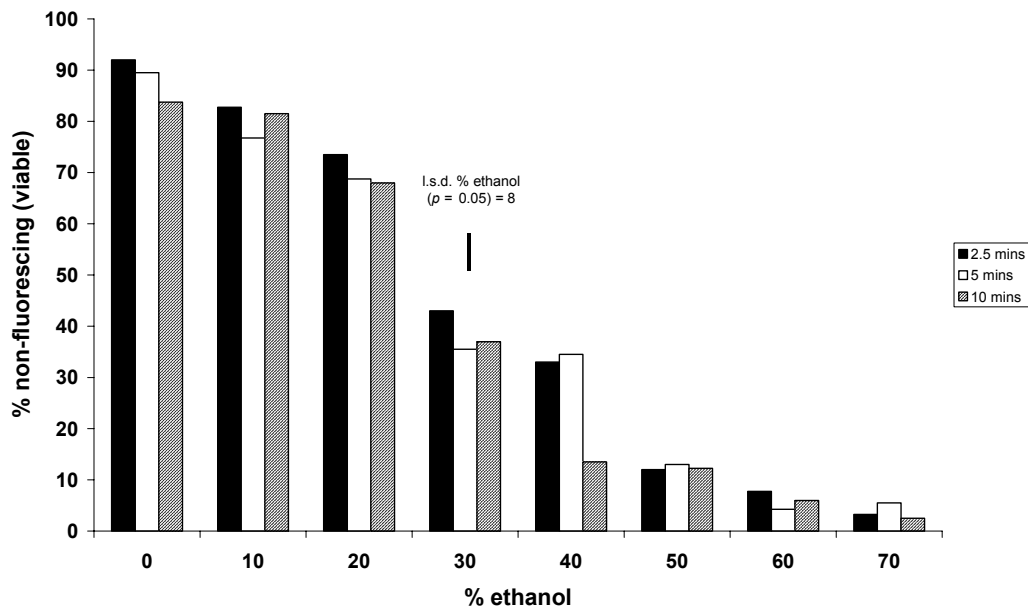


Figure 4: The effect of ethanol on the pathogenic activity of resting spores predicted by fluorescent stain. LSDs refer to treatment differences as there was no significant effect of time.

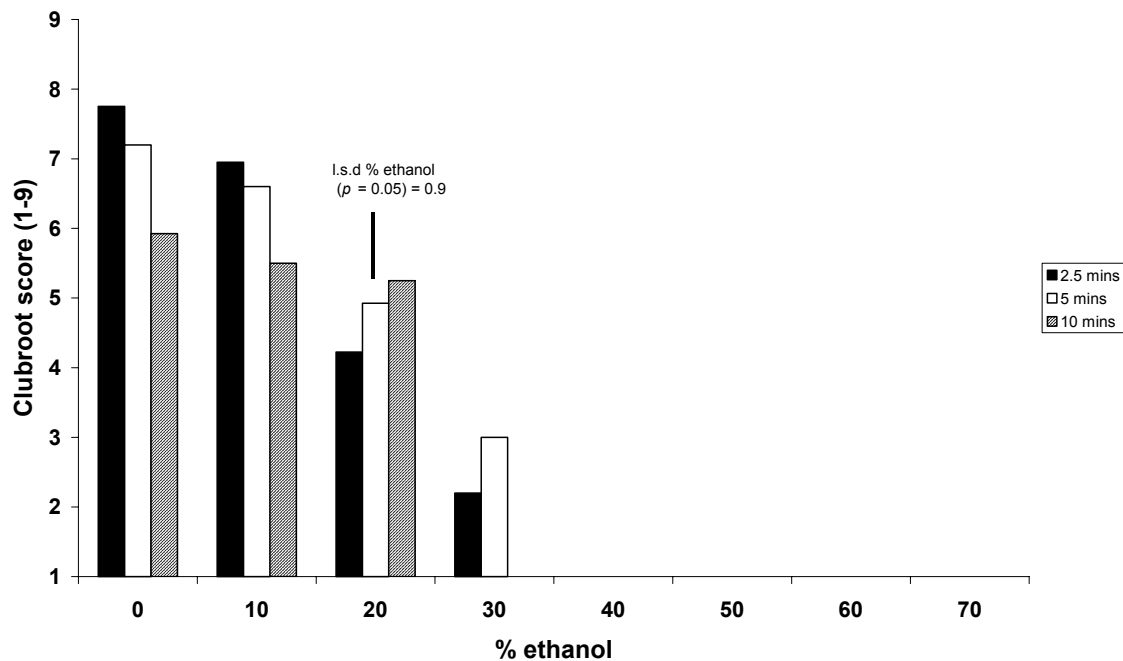


Figure 5: The effect of ethanol on root galling due to clubroot on broccoli. LSDs refer to treatment differences as there was no significant effect of time.

5.4 Cleaning of reusable plastic seedling trays

Introduction

Plastic seedling trays returned to the nursery from farms for reuse were previously identified as a high risk means of entry of spores of *P. brassicae* into the nursery (5.2). As the majority of the industry uses these trays in the production process, an experiment was conducted to determine the most effective means of decontaminating the trays before reuse.

Materials and Method

Plastic seedling trays were filled with field soil artificially inoculated to contain 10^6 spores of *Plasmodiophora brassicae* per gram. These trays were watered and left to stand overnight. Soil was then tapped out of the trays and the following treatments replicated 3 times were administered:

- Control (Unwashed)
- High pressure wash
- Soak in hypochlorite solution (1000 mg/L) for 5 minutes
- High pressure wash, followed by soak in hypochlorite solution (1000 mg/L) for 5 mins.
- Steam trays in steriliser (70°C for 1hr)
- High pressure wash, then steam (70°C for 1hr)

Following treatment, scrapings were made of any soil residues remaining on the trays. These scrapings were tested using a molecular diagnostic test developed to detect *P. brassicae* (section 4).

Results

Wherever soil residue existed in sufficient quantity to collect, *P. brassicae* was detected. Trays that had been thoroughly washed using the high-pressure hose had little or no soil residue. The most effective treatment combination was the high-pressure wash followed by steam sterilisation.

Implications: *Regardless of the disinfection treatment chosen, it is critical that all trays be thoroughly washed (before treatment) using a high pressure washer.*

5.5 Water and irrigation

Introduction

Water (in particular dam water) was previously identified as a high-risk means of entry for spores of *P. brassicae* into the nursery (5.2). Laboratory and glasshouse studies have been directed towards developing best practice recommendations for irrigation in nurseries. Trials have investigated:

5.5.1 How long can spores remain viable in water?

Materials and Method

Resting spore suspensions (10^8 spores/mL) were prepared by macerating root galls 1:3 W/V in distilled water (section 4). These were stored at 3°C for periods of up to 2 years. The stored suspensions were then used to inoculate broccoli and Chinese cabbage bait plants.

Results

Some of the inoculum that had been stored for up to 2 years remained viable and produced root galls on bait plants. It should be noted however, that the spore suspensions used were stored under ‘ideal’ conditions and were of high initial concentration of *P. brassicae*. It is anticipated that the concentration of spores in a dam for example, would be much lower and would also be subject to biological degradation.

Implications: *It is theoretically possible for resting spores of P. brassicae to remain viable in dam water for several years. Dam water should not be used for irrigation purposes in commercial nurseries, particularly if the nurseries are located in a brassica-growing region.*

5.5.2 What level of contamination of the water is required to cause disease?

Materials and method

Large volumes of inoculum were prepared at 10^6 , 10^4 , 10^3 , 10^2 and 10 spores/mL. Mains water was used as an untreated control. Broccoli seedlings (cv. Greenbelt) were grown in pots (1 plant per pot, 10 replicate pots per treatment) containing uninfected field soil and were watered daily with 300mL of the appropriate inoculum solution. The pots were left to stand in trays in the glasshouse to maintain high soil moisture (conditions ideal for the development of disease). After 6 weeks the plants were removed from their pots and assessed for root galling symptoms on a scale 1-9 as described (section 4).

Results

Even at the lowest inoculum concentration (10 spores/mL), a small amount of root galling was evident on several plants indicating that it is theoretically possible for infected water to result in field infection (Fig. 6). However, the actual concentration of viable inoculum likely to be present in a dam is not known (but is likely to be lower than 10 spores/mL).

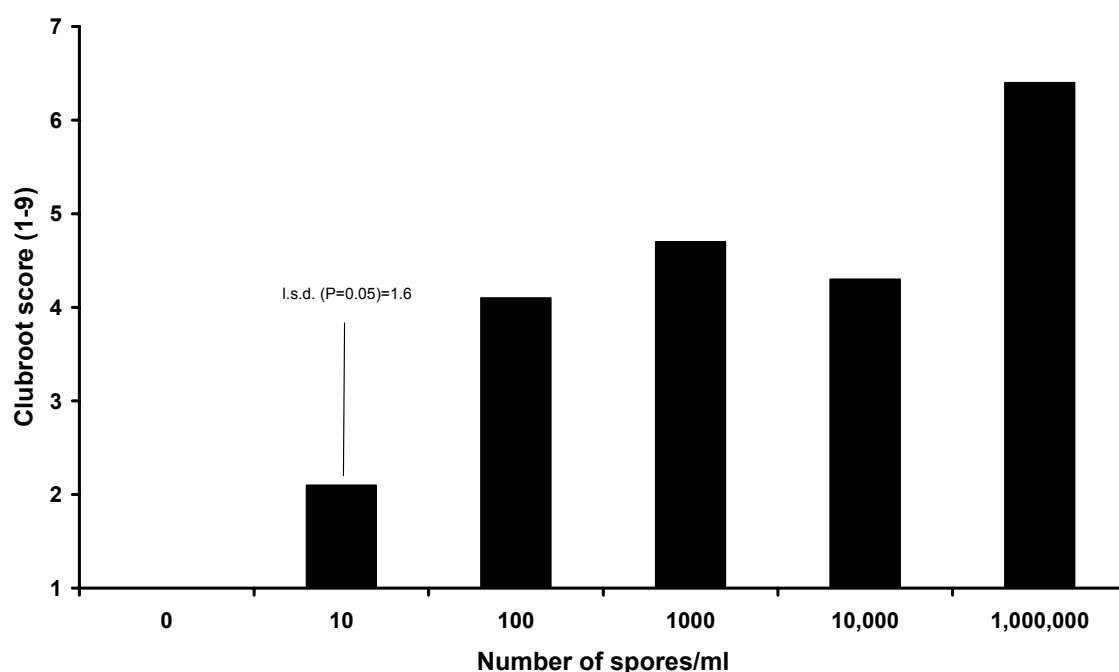


Figure 6: The theoretical risk associated with irrigation using water containing resting spores of *P. brassicae*.

Implications: Even at low concentration (10 spores/ mL), it is theoretically possible for symptoms of clubroot in nursery grown transplants to have been caused by irrigation with dam water containing resting spores of *P. brassicae*.

5.5.3 How quickly can resting spores settle to the bottom of a dam?

Materials and Method

Three 1L measuring cylinders (9 cm diam, 50 cm high) were used as settling columns. Each cylinder was filled with 1 L of a *P. brassicae* resting spore suspension containing 10^6 spores/ mL. Specialised sampling equipment was created by connecting thin copper pipe to a 60 mL syringe by an intermediate piece of rubber tube. Samples were collected from the surface, 200 (10 cm), 400 (20 cm), 600 (30 cm) and 800 (40 cm) mL and the bottom (50 cm) of each cylinder at 1, 2, 5, 24, 48 and 72 hours. The number of resting spores in each sample was counted using a haemocytometer.

Results

During first five hours there was a slow downwards movement of spores. After 24 hours most spores settled at the very bottom of cylinders (the 1000 mL mark, see Fig. 7). After 48 and 72 hours only single spores were observed at all other levels with the exception of the bottom of the cylinders where the concentration of spores was too dense to count. The rate at which spores settled was therefore approximately 25 cm/day.

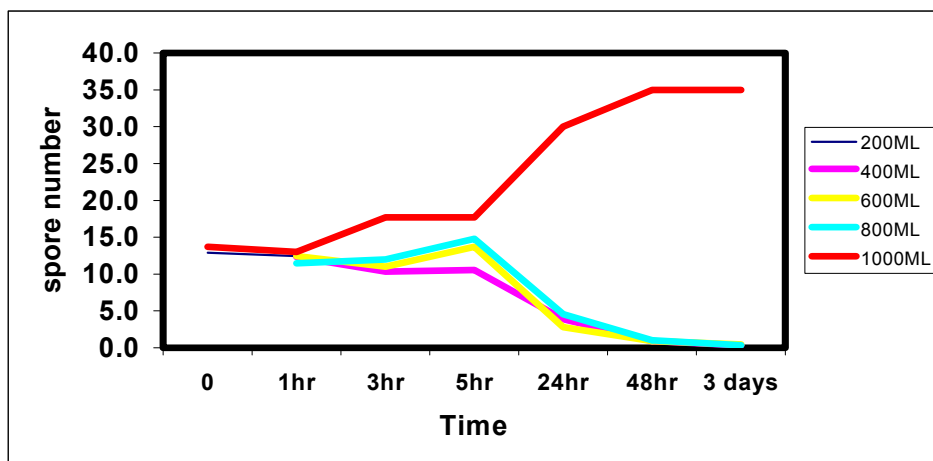


Figure 7: Number of spores ($\times 400 \times 10^4$) present over a period of 3 days, measured at 10 cm (200 mL) intervals in a 50 cm column containing 1 L of *P. brassicae* resting spores.

Implications: Resting spores of *P. brassicae* will settle to the bottom of an undisturbed body of water relatively quickly (25 cm/day). Growers using dam water to irrigate their own seedlings or farms should therefore ensure that the intake pipe is located in the stillest part of the dam and is mounted on a float to ensure that water is collected near the surface of the dam.

5.5.4 Can contaminated dams result in field symptoms of disease following prolonged irrigation?

Materials and Method

Bulk quantities of water were collected from dams and channels suspected to contain resting spores of *P. brassicae*. The presence of *P. brassicae* was confirmed using the PCR diagnostic test for clubroot (section 4). Broccoli transplants (cv. Marathon) were transplanted into 15cm diameter pots containing pasteurised soilless potting mixture. A plastic container was placed under each pot to retain run through water for reuse and to ensure that the pots remained moist at all times, maximising the likelihood of infection. Plants were watered daily with the appropriate water sample until it was depleted (approx. 2 weeks) after this time, town water was used.

Plants were removed from their pots, washed free of potting mixture and assessed for root galling due to clubroot (section 4) nine weeks after planting.

Results

No symptoms of clubroot were observed on any of the plants.

Implications: To date, even under 'ideal' conditions for infection, glasshouse studies have failed to result in the expression of disease symptoms on bait plants following repeated irrigation with

naturally infected dam water. Whilst dam water represents a low risk for field crops, it should not be used in commercial nurseries where tolerance for clubroot is zero.

5.6 Chemical amendment of the potting medium

Materials and method

Resting spores of *P. brassicae* were mixed through pasteurised soilless potting mixture to give a final concentration of 10^6 spores/g. Fungicides or lime were incorporated into the potting mixture at the recommended rate before it was used to fill plastic seedling trays (Table 3). Two different varieties of brassica were planted (Chinese cabbage and broccoli) and there were three replicates (each containing 20 seeds) of each treatment/variety combination. Emergence counts were made one and two weeks after sowing. Four weeks after sowing 10 plants from each replicate were removed from their cells. The roots of these plants were assessed visually for symptoms of root gall (section 4) and the dry weight of the seedlings was recorded.

Table 3: Chemical amendments screened for their ability to prevent clubroot in nursery grown transplants.

Treatment	Rate	Application method
Fluazinam (Shirlan)	Recommended: Seedling drench 25-50mL/1000plants/100L of water Applied 13.5mL/270 plants/1L of water/4kg of potting mix	Sprayed and incorporated into the potting mix prior to planting seeds
Flusulfamide (Nebijin)	Recommended: 0.6 mg/plant Applied 1.6mL/0.8L/4kg of potting mix	Sprayed and incorporated into the potting mix prior to planting seeds
Terraclor (PCNB)	Recommended: 500-750g per 100m ² Applied 2.25g/0.3m² potting mix (2.25g/1L water/4kg of potting mix)	Sprayed and incorporated into the potting mix prior to planting seeds
Lime (CaO)	Recommended: 1tonne per ha (1000kg/ha.3x10 ⁹ g) Applied 2.5g/kg of potting mix (10g/4kg of potting mix)	Incorporated into the potting mix prior to planting seeds
Unreated		nil

Results

Seedling germination was reduced by approximately 8% in the Shirlan treatment (Fig. 8). Other treatments did not affect germination.

Seedlings growth and the colour of leaves was also affected by Shirlan treatment. Seedlings grown in the Shirlan treated potting mixture were smaller than all other treatments.

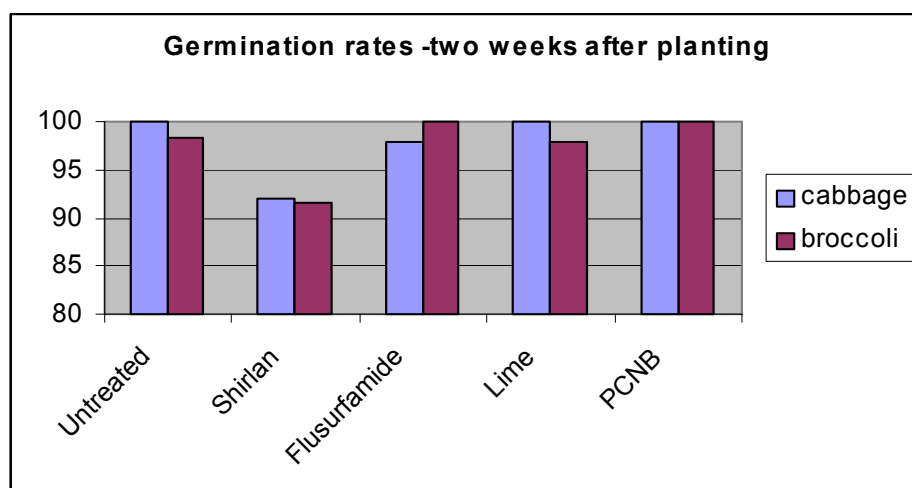


Figure 8: The effect of chemical amendments on the germination of Chinese cabbage and broccoli seeds.

Shirlan and lime (CaO) treatments completely eliminated visual symptoms of root galling, however, Shirlan was phytotoxic reducing both germination (Fig. 8) and plant weight (data not shown). Several plants in each of the flusulfamide and PCNB treatments exhibited symptoms of root galling (Fig. 9).

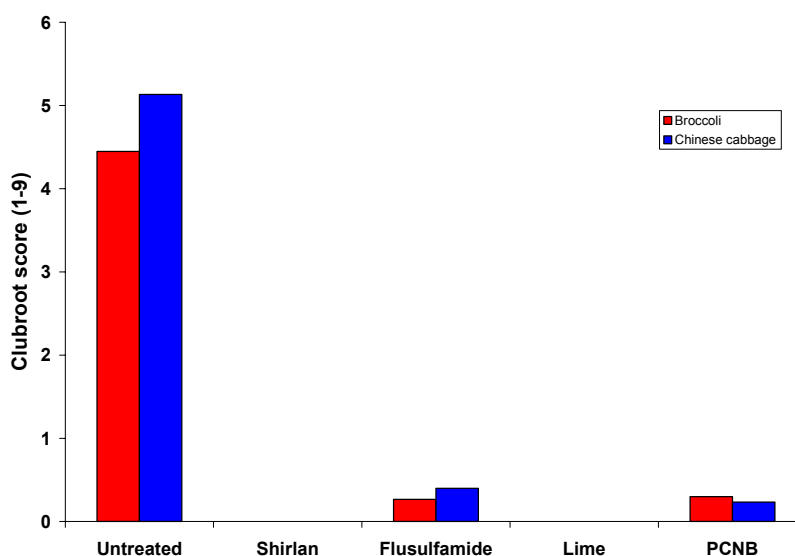


Figure 9: The effect of chemical amendments on the severity of root galling due to clubroot on 4 week old cell grown broccoli and Chinese cabbage.

Implications: *None of the fungicides completely eliminated clubroot without causing plant phytotoxicity. In contrast hot lime (CaO) completely eliminated clubroot without symptoms of phytotoxicity. This is a comparatively cheap and readily available treatment option.*

6 Monitoring, prediction and decision making

Introduction

The ability to quantify clubroot inoculum is crucial as disease severity (and yield loss) is related to inoculum concentration. Many clubroot researchers have studied this relationship, and although influenced by a number of factors (such as soil type, temperature, pH, etc.) it is generally possible to

develop a linear dose-response curve. Previous research by NRE (Cross and Porter, unpublished data) supported these views by demonstrating a linear dose response curve in a glasshouse trial. Such data has made it possible to begin correlating quantitative (real-time) PCR predicted soil inoculum levels and subsequent field disease levels.

Materials and Methods

Soil sampling

Fifty four soil samples were collected from farms growing brassicas crops across four states – Victoria, New South Wales, Tasmania and Western Australia. Soil samples were collected from the top 10 cm of soil at 1 m intervals in an X pattern over an area 20 x 20 m. A subsample of 500-750g of was taken from the bulk sample for PCR assay and soil pH analysis.

*PCR assay test for the detection of *Plasmodiophora brassicae* in soil samples.*

The FastDNA SPIN Kit for soil was used to extract PCR-ready genomic DNA from soil samples. For each soil sample duplicate extraction was performed and for each batch of soil samples a positive control (containing a known amount of spores added to the test soil) was included. Each sample of extracted soil DNA was tested in duplicate using a PCR (polymerase chain reaction) assay to detect DNA of *P. brassicae*, the cause of clubroot (PCR conditions as per Faggian *et al.*, 1999). In each PCR test negative and positive controls were included. Negative test outcomes were further scrutinised by one or both of the following approaches: (1) re-extraction of DNA from soil with and without addition of various known concentrations of clubroot spores followed by PCR assay, (2) dilution (1/10 and 1/100) of extracted DNA followed by PCR assay.

Real Time detection of amplification products using a dsDNA intercalating dye, Sybr-Green I (SG).

Real-time quantitative PCR is a relatively new technology that enables monitoring, in real time, of the progress of PCR amplification. The distinguishing feature of individual reactions is the point in time at which PCR products are first detected – the accumulation of PCR product is then plotted versus PCR cycle number. So, the more target sequence present at the start of the reaction, the sooner PCR products will be detected (i.e. the cycle number where product is first detected will be lower). Quantification of unknown samples can be achieved by plotting the amplification of a set of standards to create a standard curve (straight line) from which to read unknown values (cycle numbers) (Figs 10 & 11). The amount of PCR product (from field samples) can then be related to inoculum concentration, inoculum concentration to disease levels, disease levels to yield loss figures, and so on.

Real-Time Amplification was used to determine the amount of *P. brassicae* DNA in a soil sample based on the amount of amplification product produced during the exponential phase of a cycling program. An intercalating dye, Sybr-Green I, was used to assist in the quantification of the DNA. During the amplification phase of the reaction, the dye would bind to the amplified product and the fluorescent signal increase in proportion with increasing amounts of amplification product.

A protocol for DNA amplification and detection in real-time using Sybr-Green I intercalating dye was modified and optimised for clubroot DNA. For the first round nested PCR conditions were as per Faggian *et al.*, 1999. Second round PCR was performed in a real-time thermal cycler (Corbett Rotogene) using the Sybr-Green I dye. A standard curve was obtained by adding clubroot spores to soil samples (free of clubroot DNA) at various concentration (10^0 , 10^1 , 10^2 , 10^3 , 10^4 , 10^5 , 10^6 spores per gram of soil) and extracting DNA (Figs. 10 & 11).

In-field assessment of disease and yield loss

Roots from plants in the area from which soil was sampled were visually assessed for symptoms of disease 6 weeks after transplanting. Twenty plants were removed, their roots shaken free of soil and assessed for root galling using a 1-9 gall rating scale as described previously (section 4). The fresh weight of the above ground part of each plant was also recorded. If no disease was evident at 6 weeks, plants were reassessed at 10 weeks and again during harvest. At harvest, 2 x 10 m rows were harvested and a tonne/ha figure for each site was calculated.

Results

Standard curves for the real time detection assay were plotted by software (Figs 10 & 11).

The line of best fit obtained from undiluted DNA had a correlation coefficient (R-value) of $R=0.96$ (where 1 is a perfect fit) Fig. 10.

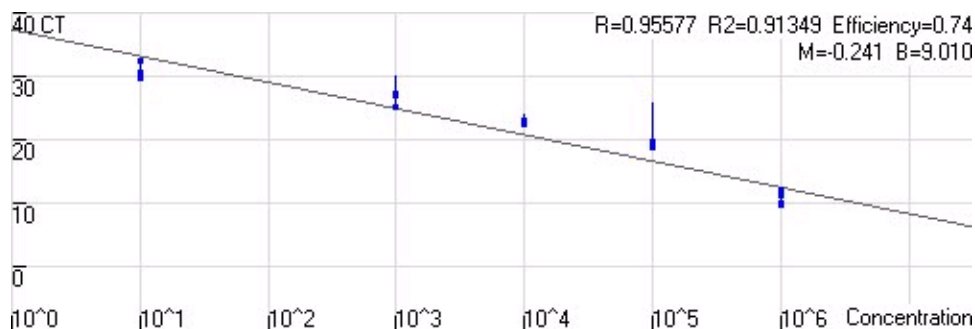


Figure 10: Standard curve obtained from undiluted DNA. The average Ct values of the triplicate reactions are graphed against clubroot spores concentrations (number of spores per gram of soil). Ct value – fluorescence values are recorded during every cycle and represents the amount of product amplified to that point. A point in which the fluorescence signal is first recorded as statistically significant is defined as the Ct.

The line of best fit obtained from DNA dilution (1/10) had a correlation coefficient (R-value) of $R=0.99$ (where 1 is a perfect fit) Fig 11.

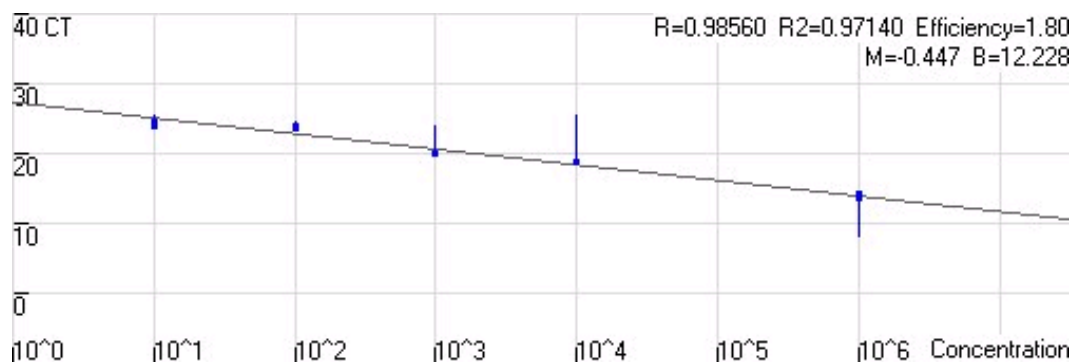


Figure 11. Standard curve obtained from diluted (1/10) DNA. The average Ct values of the triplicate reactions are graphed against clubroot spores concentrations (number of spores per gram of soil). Ct value – fluorescence values are recorded during every cycle and represents the amount of product amplified to that point. A point in which the fluorescence signal is first recorded as statistically significant is defined as the Ct.

A sample of results from PCR assay, Real-Time detection assay and visual assessment of disease symptoms is shown in Table 4. Plants from plots with a high predicted disease risk ($10^5 - 10^6$ spores/g soil) determined by (RT PCR) were severely galled and had a high root gall severity rating. There were a large number of predicted disease risks at or close to the threshold for symptom observation (approx 10^3 spores/g). Generally, plants grown in these plots exhibited no symptoms of root galling or only one or two plants were observed with one or several root galls. There were however, a large number of samples that consistently returned negative PCR and RT-PCR test results whilst the plants in the field were severely diseased (eg. Forth TAS and Manjimup WA samples – Table 4). A number of experimental approaches were taken to ensure that the extraction and/or PCR amplification procedures were functioning correctly. These included:

- Repeating the extraction in duplicate
- Spiking the sample with spores and repeating the extraction in duplicate
- Measuring total DNA (rather than just *P. brassicae* DNA)
- Dilution (1/10 and 1/100) of extracted DNA followed by PCR assay
- Repeating the extraction from negative soils together with those known to give a positive test result
- Checking the sampling and site history of each sample

These checks ruled out problems with the extraction kits, PCR assay and sampling technique and suggest interference from the particular soil samples. Work is ongoing to determine and eliminate the exact cause of the problem. This will be the focus of any future project.

Table 4. Selected results demonstrating predicted (using RT-PCR) and actual (in-field) development of disease.

State	Location	Results of PCR (duplicate DNA extractions)		RT results	Predicted disease risk	Visual assessment of root galling	Predicted vs actual disease
		+	+				
VIC	Trentham	+-	++	10^2	V. low	(Few plants with single galls only at 3 rd sampling)	Good
VIC	Werribee	-+	- +	10^3	Low	No symptoms of disease	Good
VIC	Werribee 2	-+	--	$<10^2$	V. low	No symptoms of disease	Good
WA	Baldivis	--	--	$<10^2$	V. low	No symptoms of disease	Good
WA	Bridgetown	- +	-+	10^2	V. low	Unknown but plants v. healthy	Good
WA	Manjimup	--	--	$<10^2$	Zero	Roots severely galled	V. poor
NSW	Badgery's Creek	++	++	10^5	High	Roots severely galled	Good
NSW	Kellyville	++	++	10^5	High	Roots severely galled	Good
NSW	Oakville	++	++	10^3	Low	No symptoms of disease	Good
TAS	Forth	--	--	10^3	Low	Roots severely galled	V. Poor

Discussion

Preliminary use of RT-PCR to predict yield loss due to clubroot disease indicated a strong relationship between the predicted and observed severity of disease symptoms (VG 99008). In the current national soil survey a number of soils were sampled from which DNA of *P. brassicae* could not be detected but field symptoms of disease were high. In spite of extensive (frustrating) attempts to identify the cause of this problem, it remains unknown and will be the focus of future work.

7 Treatment selection, application and crop growth

7.1 Introduction

A range of products were screened for their ability to control clubroot in the field in a previous project (VG 97076). This chapter details work undertaken to finalise rates, develop recommendations regarding timing and method of application and identify treatment combinations that will be effective in a range of soil types and climates throughout Australia.

7.2 Type, rate and timing of lime application in organic or buffered soils

Experiment 1: The ability of different types of lime (quicklime, lime kiln dust, cement kiln dust, G-lime and agricultural lime) to raise soil pH was compared in a long term field trial conducted over 2.5 years in the south west of Western Australia.

Materials and method

A formula developed for the region by the Chemistry Centre of Western Australia was used to estimate the rate required for each type of lime to increase soil pH to 7.2 (CaCl₂) (Table 5).

Table 5: Treatment details and application rates.

Lime	Neutralising value (%)	Fines (%) ^A	Relative Effectiveness	Rate applied (t/ha)
Aglime 1	79	89	3	17.0
Aglime 2	81	85	3	16.6
Aglime 3	77	88	3	17.5
Aglime 4	95	87	2	9.5
Aglime 5	72	80	3	18.7
G-lime 1	85	55	2	10.5
G-lime 2	94	34	2	9.6
Cement Kiln Dust 1	63	94	1	7.1
Cement Kiln Dust 2	85	100	2	10.6
Lime Kiln Dust 1	120	100	1	3.7
Lime Kiln Dust 2	115	100	1	3.9
Quicklime 1	155	99	1	2.9

^A Fines are regarded as any material that is less than 0.6 mm in diameter.

The required rate of each lime was evenly broadcast by hand across each plot and incorporated immediately by a single pass of a rotary hoe to a depth of approximately 25 cm. The plots were left undisturbed for the duration of the experiment. Eighteen cores of 2.5 cm² were randomly collected from each plot and combined into a bulk sample. Samples were collected more frequently during the first 3 months of the experiment to ensure the initial change in pH was detected. The Chemistry Centre of Western Australia measured soil pH (CaCl₂) according to the methods described by Rayment and Higginson (1992). The buffering capacity of the soil was measured using a method developed by the Chemistry Centre of Western Australia (D. Allen, pers comm). The data for both experiments was analysed using a mixed model in the REML procedure in GENSTAT 5 release 4.1, edition 3 statistical package.

Results

All of the limes reached maximum soil pH values within 0.2 pH units of one another, however, there were significant differences in the rate at which the lime products increased soil pH (Fig. 12). In general, the fine processed limes rapidly increased soil pH reaching a maximum 1 to 3 weeks after application. Up to 6 times as much agricultural lime was required to elicit a similar change in soil pH as the fine processed limes and maximum soil pH was not reached until 25 weeks after application (Fig. 10).

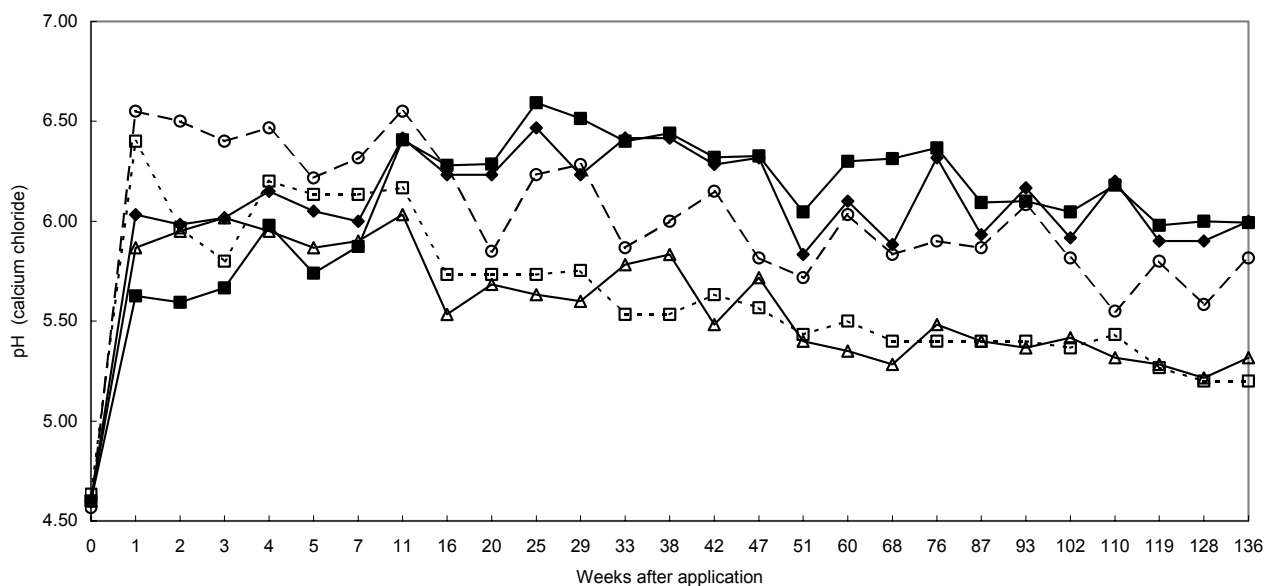


Figure 12: pH change over time following application of quicklime (□), lime kiln dust (△), cement kiln dust (○), G-lime (◆) and agricultural lime (■).

Implications: *With careful selection of an appropriate rate and time of application, each of the limes could be used to manipulate soil pH to manage clubroot. Fine processed limes can be applied up to 1 week before planting, however, the cheaper unprocessed limes must be applied 3-6 months in advance of planting to ensure that maximum soil pH is reached at the time of planting.*

Experiment 2: The efficacy of lime in the previously problematic organic Krasnozom soils of Northern Tasmania was demonstrated in a field trial.

Materials and method

A field trial was established in a Kindred paddock with history of clubroot in NW Tasmania. This field trial was established on 15 February 2001 using broccoli (cv marathon) transplants. The trial followed a randomised complete block design with 5 replicates. Ground Burnt Agricultural lime (3 t/ha) and/or the fungicide Shirlan (3 L/ha) were incorporated into the transplant row at planting using a purpose built machine. Gall severity was assessed 14 weeks after planting using a scale of 1-9 (section 4).

Results

A significant decrease in gall formation was recorded when ground burnt agricultural lime (GBA lime, hot lime, CaO) was incorporated into plots at a rate of 3 t/ha (Fig. 13). This was accompanied by a significant increase in mean head weight from 2.7g in untreated controls, to 67.2g in plots treated with GBA lime, and 73.0g in plots treated with GBA lime and 3L/Ha Shirlan (data not shown).

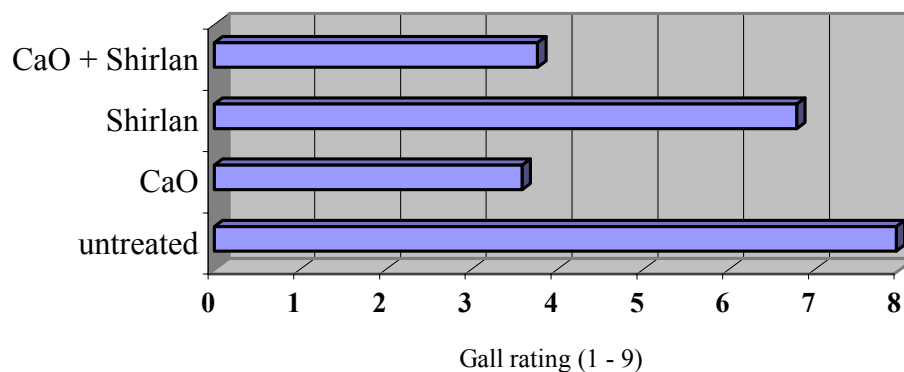


Figure 13: Severity of gall formation on plants treated with CaO and/or Shirlan at transplanting.

Implications: Incorporation of GBA lime for February plantings can be used to suppress clubroot sufficiently to obtain a marketable yield in a situation where severe stunting would have occurred. The efficacy of Shirlan and GBA lime has been clearly demonstrated in trials conducted in the mainland states, but previous seasons trials in Tasmania failed to demonstrate the efficacy of the treatments (VG 97076). It was proposed that this failure may be a result of either the transplanter slipping on the hillside and plants therefore not being transplanted into the treated band of soil, or the treatments being ineffective Kraznozam soils. The current results indicate that the treatments are effective in Kraznozem soils.

Experiment 3: The interaction of calcium and pH and their effect on the lifecycle of *P. brassicae* was determined in a series of laboratory experiments conducted in Victoria.

Materials and method

A sand solution culture system was developed to allow the sequential observation of broccoli root hairs infected with *P. brassicae*. Seedlings were grown in 5 ml tapered pippette tubes containing acid washed sand. Each tube was suspended in a buffered nutrient solution (Myers and Campbell, 1985) that moved up the tube toward the roots by capillary action. The solutions contained 2.5 (nutrient control), 5, 10 or 20 mM of calcium. A chloride control containing Cl^- from NaCl was included to test the effect of the highest concentration of Cl^- that occurred when additional calcium (<2.5 mM) was added as CaCl_2 .

The plants were inoculated with 200 μl of spore suspension (10^8 spores/mL) and maintained in a growth cabinet (temperature 25°C day, 20°C night, 90% relative humidity, 12 hr days). Ten days after inoculation, roots were rinsed free of sand, fixed in formyl acetic acid (FAA) and later stained with FAA Phloxine B to facilitate observation of the development of *P. brassicae* within the root hairs. Data analysis was by ordered logistic analysis (ordered data) or analysis of variance.

Results

The effect of calcium on the development of *P. brassicae* in root hairs was dependent upon pH. At low pH (5.5), root hairs rapidly became infected, although a significant delay in the developmental progress was caused by addition of calcium at the highest rates (10 and 20 mM). At pH 6.5, all calcium amendments significantly delayed the development of *P. brassicae*, compared to the nutrient control. As pH increased, the number of infected root hairs and the effect of calcium amendment decreased for all treatments. At pH 8, 75-95% of root hairs were uninfected 10 days after inoculation and there was no significant effect of calcium on the development of *P. brassicae* (Figure 14).

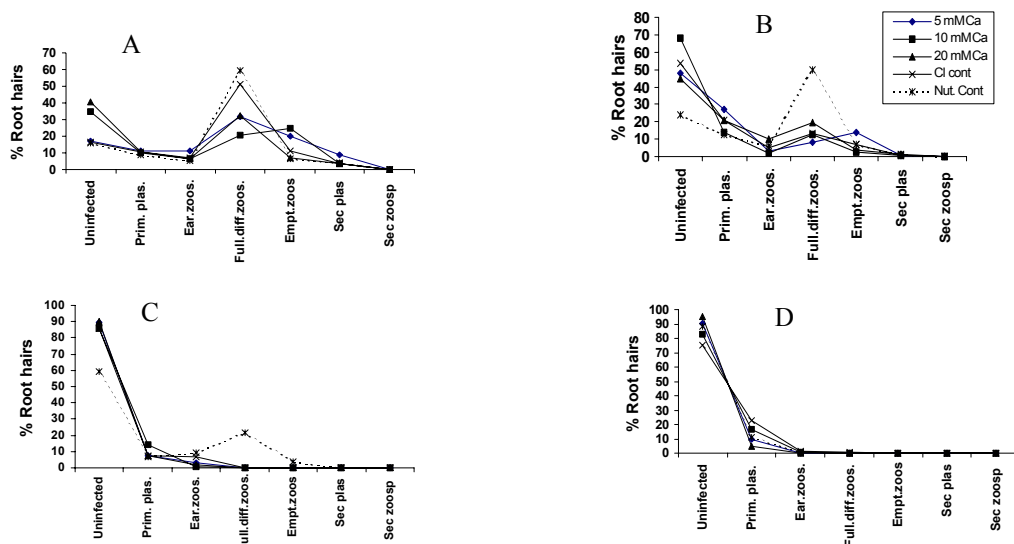


Figure 14. Effect of calcium and pH on the development of *P. brassicae* in broccoli root hairs, 10 days after inoculation (pH : A=5.5, B=6.5, C=7.5, D=8.0). The following abbreviations refer to lifecycle stages of *P. brassicae*: Prim plas = primary plasmodia, Ear zoos = early stages of differentiation of the plasmodium to form zoosporangia, Full diff zoos = fully differentiated zoosporangia, Empt zoos = zoosporangia from which the zoospores have been released, Sec plas = secondary plasmodia, Sec zoosp = secondary zoospores.

Implications: *Growers should aim to increase soil pH to between 7.0 and 7.5 by liming responsive soils. Calcium has an inhibitory effect on the development of clubroot disease, however, this effect is pH dependent (significant only at pH less than 7.0) so calcium amendments may be most effective in soils where it is difficult or undesirable to increase soil pH to 7.0. Optimum soil pH (7.0-7.5) should be reached at the time of planting and calcium applied at planting and in the first few weeks after planting to prevent early infection of the young transplant.*

7.3 The use of bait crops to reduce soil inoculum load

Introduction

Bait or 'catch' crops (Fig. 15) have been developed to work like a sponge for the clubroot pathogen which germinates and infects the crop but cannot multiply in its cells. If the pathogen cannot produce resting spores inside the bait host, destruction of the bait crop by incorporation would result in the death of the pathogen contained in the host. Bait crops were widely promoted in Australia as a non-chemical alternative for the control of clubroot. The seed of these crops are expensive and frequently successive crops were being recommended to achieve the required level of inoculum reduction. The effectiveness of these crops was evaluated in a series of field trials conducted on the same site in Victoria for two seasons.

Field evaluation in Australia

Single or two successive crops of radish bait were planted and raised (according to manufacturers instructions) before a crop of broccoli. These crops were compared with single or two successive cruciferous or non-cruciferous crops, or a single or two successive periods of fallow before planting broccoli.



Figure 15: Radish bait crop for clubroot

Results

In both trials (one trial was conducted under low disease pressure, the other under high disease pressure) there was no significant difference between the use of the radish bait crop and rotation for the same duration with a non-cruciferous crop or fallow.

Implications: *Growers can expect no significant benefit from the use of the crop evaluated as a bait for clubroot.*

7.4 Effect of nitrogen on disease development

Materials and method

Cell grown broccoli transplants (cv. Greenbelt) were planted into pots containing 5.5 kg sandy loam soil (pH = 6.4). The soil contained 16.3 mg/kg nitrate nitrogen (defined as low). All nutrients (except nitrogen) were added as recommended by soil analysis. Nitrogen was added as urea in 5 equal amounts 0, 3, 6, 9 and 12 weeks after transplanting. The total amount of urea added in each treatment is detailed in Figure 16. Resting spores of *P. brassicae* were added at 10 000 spores/g soil.

The total recommended nitrogen application for this soil (according to Incitec Analysis Systems Soil Analysis Report) was 136 kg Nitrogen/ha.

Results

Clubroot disease only became severe once the amount of nitrogen applied exceeded the rate recommended by soil analysis (Fig. 16).

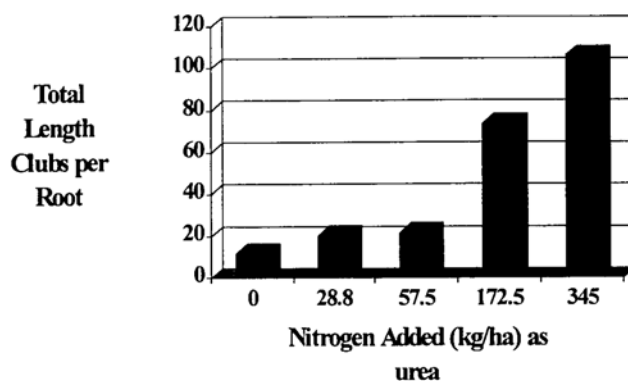


Figure 16: Influence of nitrogen (as urea) on the severity of clubroot on broccoli in soil inoculated with 10000 spores/g.

Implication: Growers should avoid excessive application of nitrogen as it can lead to increasing severity of symptoms of clubroot disease.

7.5 Influence of particle size on the efficacy of calcium cyanamide

Introduction

In 1997, a standard granular formulation of calcium cyanamide, Perlka®, was made commercially available to Australian brassica growers following successful trials (J. Cathcart, Horticultural Consulting Services, *pers. comm.*). In 1998 Perlka® was reformulated to a 'macrogran' formulation for the Australian market. Dust and fine granules were removed for ease of handling and improved spreading. In subsequent field trials to control clubroot the efficacy of calcium cyanamide was reduced. This led to a glasshouse investigation of the effect of particle size, rate and time of application on the efficacy of calcium cyanamide.

Materials and method

A resting spore suspension of *P. brassicae* was prepared as previously described (section 4) and mixed into pasteurised soilless potting mixture (Debco) in a cement mixer to give a final concentration of 10^6 spores/g of soil.

The trial was a completely randomised block design consisting of 10 pots of each of 18 treatment combinations and the appropriate inoculated controls. The following factors were analysed: formulation of calcium cyanamide, rate of application and time between application of calcium cyanamide and planting. Calcium cyanamide (as 'Powder' (P) (98 % w/w < 300µm), 'Standard formulation' (S) (68 % w/w > 850 µm and 31 % w/w 300-850 µm) or 'Macrogran' (M) (98% w/w

>850 μm) was incorporated into inoculated potting mixture at 0, 500 or 1000 kg/ha using a cement mixer. Pots were randomly arranged on a steel mesh bench in a glasshouse and were watered to field capacity before transplanting a single cell grown broccoli transplant (cv. Marathon) into each of 10 pots per treatment combination. A further 10 pots were planted in a similar manner at 7 and 14 days after treatment.

Plants were maintained under overhead irrigation (applied for 3 mins every 12 hrs) at $20 \pm 2^\circ\text{C}$ for the duration of the experiment. Six weeks after inoculation plants were removed from their pots, the roots shaken free of potting mixture and the severity of clubroot symptoms rated visually on a 1-9 scale as previously described (section 4).

Results

The 'powder' formulation was the most effective treatment used in the glasshouse (Fig. 17), significantly ($P = 0.05$) reducing root galling due to clubroot by between 61 and 81 % depending upon the duration between treatment application and planting (Fig. 17 and Table. 6). The standard formulation was less effective, causing 20 - 44% reductions in root galling, and the 'macrogran' was ineffective (1 - 16 % reductions). The three-way interaction was not significant, however, all two-way interactions were significant ($P = 0.05$) (Table 6). With the exception of the 'powder' formulation, increasing the plant back time (ie. time between treatment application and transplanting) generally caused a reduction in the severity of disease symptoms (Table 6). The high application rate (1000 kg/ha) was significantly more effective than the lower rate (500 kg/ha) of either the 'powder' or 'standard' formulations but this effect was not observed using the 'macrogran' formulation.

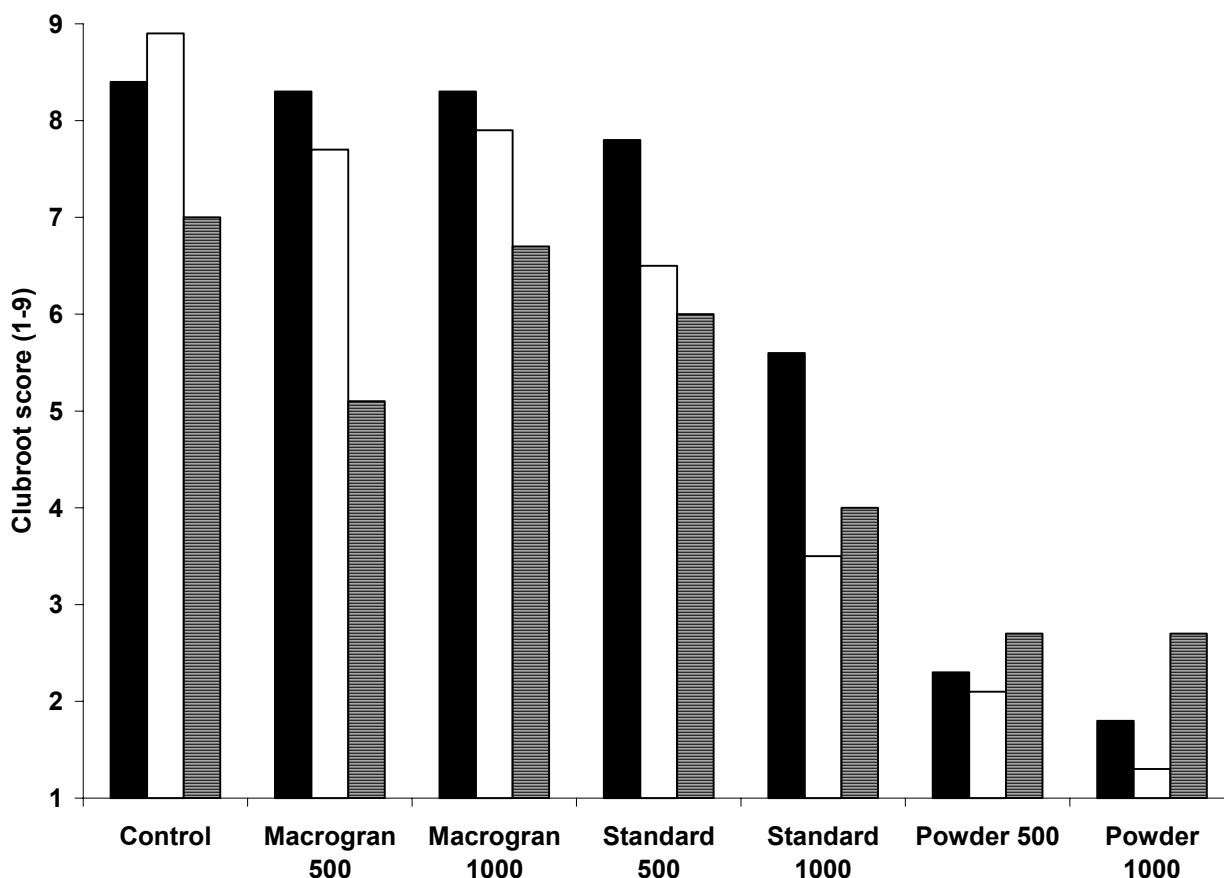


Figure 17: The effect of formulation (particle size), rate and timing of application (transplanting the same day (black bar), 7 (white bar) or 14 (shaded bar) days after treatment) of calcium cyanamide on the development of symptoms of clubroot disease on broccoli cv. Marathon.

Table 6: The effect of formulation of calcium cyanamide, rate of application and the time between treatment application and planting on the development of symptoms of clubroot disease (visually assessed on a scale 1-9) in the glasshouse, significant two-way interactions.

Formulation ^A	Planting (days after treatment)			Rate (kg/ha)	
	0	7	14	500	1000
Powder	2.1	1.7	2.7	2.4	1.9
Standard	6.7	5.0	5.0	6.8	4.4
Macrogran	8.3	7.8	5.9	7.0	7.6
l.s.d. ($P=0.05$)		0.7			0.5
Planting (days after treatment)					
0				6.1	5.2
7				5.4	4.2
14				4.6	4.5
l.s.d. ($P=0.05$)					0.5

^A All formulations of calcium cyanamide conform to the same chemical specifications (H. Gehrmann, Degussa AG *Pers. Comm.*). Formulations differ only in their particle size (powder, 98 % w/w < 300µm; standard, 68 % w/w > 850 µm and 31 % w/w 300-850 µm; macrogran, 98% w/w >850 µm).

Discussion

The effectiveness of calcium cyanamide was dependant upon the size of the particles in the product. The ‘powder’ containing small talc-like particles was the most effective formulation virtually eliminating root galling due to clubroot (Fig. 17). This formulation is currently used mainly for industrial purposes and is not commercially suited to agriculture, as handling and spreading such a fine powder is difficult (Rieder, 1981). The ‘standard’ formulation used in Australia until 1998 contains some small particles, however, larger granules are also present. This formulation was more effective than the ‘macrogran’ product, from which the small particle fraction was removed, indicating that the small particle fraction was likely to be responsible for the clubroot control observed in trials conducted before 1999. Smaller particles would have an increased surface area to volume ratio and are therefore likely to react more quickly in the soil upon contact with moisture. The decomposition of such particles would occur rapidly therefore the observed lack of response to increasing plant back time is as expected. Similarly, increasing the rate of application of calcium cyanamide is likely to be more effective for formulations containing these smaller particles. Increasing the rate of application of the ‘macrogran’ (large particles) in this study was ineffective. In contrast, this effect was significant for both the ‘powder’ and ‘standard’ formulations, however, the difference in observed symptoms of disease was greatest for the standard formulation. At the low rate application of calcium cyanamide as a ‘powder’ symptoms of clubroot were almost completely eliminated and any further reduction in symptoms of disease (by increasing the application rate) were relatively minimal (Fig. 17). By comparison, the effect of application of 500 kg/ha of the ‘standard’ formulation was marginal. Increasing the rate of this product to 1000 kg/ha would increase the amount of the reactive small particle fraction causing the observed increase in product efficacy (Fig. 17).

The ‘macrogran’ was developed to minimise dust hazards and improve the application of calcium cyanamide. In doing so, it appears that the active fraction of the product has largely been removed and activity against clubroot disease significantly reduced.

Implication: Reintroduction of the ‘standard’ formulation of Perlka® to the Australian market should immediately improve the observed level of disease control. There is potential for the efficacy of the product to be further improved by investigating an ‘optimum’ minimum particle size which spreads like a granule yet is small enough to be highly reactive in the soil.

7.6 Field control of clubroot

7.6.1 Product summary

Field trials and demonstration sites have been conducted in the following locations: Werribee, Mornington, Launching place, Boisdale, Meerlieu and Trentham Victoria, Manjimup and Perth Western Australia, Forth Tasmania, Castlereigh, Ebenezer, Kellyville, Oakville, Badgery's Creek and Schofields, New South Wales and Stanthorpe Queensland. The information that follows is a summary of results collected from these sites over the last three years.

1. *Liming*

- All the various field trials have highlighted the importance of liming to achieve a soil pH between 7.0 to 7.5. Lime does not kill the disease, but creates soil conditions that are unfavourable for spore germination. It is almost always cost effective to use lime if soil pH is less than 7.0. Liming remains the most important “first step” for effective clubroot control. It is cheap and relatively easy to apply.
- There are a number of types of lime available. They vary in their cost, reaction rate, and ease of handling and pH. Ground Burnt Agricultural lime (GBA) known as hot lime or quicklime and has a pH of about 12 to 12.5. Unlike hydrated lime it produces exothermic heat when it reacts with soil moisture. It is highly reactive and will raise soil pH rapidly. It should be incorporated and irrigated in 7 days before transplanting. Agricultural lime (Aglime) has a pH of about 7. It reacts slowly and should be applied approximately 3 months before planting. Hydrated lime is intermediate between these two products.
- Control of clubroot was generally greater when lime was combined with other treatments.
- Most recent results have indicated that banded incorporation of lime can be extremely effective.

2. *Plant Nutrition*

Research world wide indicates calcium, boron and magnesium inhibit gall formation due to clubroot. The benefits of several products containing these elements have been demonstrated.

2.1 *Nitrabor®*

- * A calcium and boron fertiliser from Hydro. Campbells have a similar product. Both are commercially available in Australia.
- * More effective when used with lime.
- * Both calcium and boron have been shown to reduce the severity of clubroot in lab and field trials. When applied with lime, these products have consistently increased marketable yields, especially in Victoria.
- * Often shows poor clubroot control but increases yields by up to 40%.
- * Has shown phytotoxicity to transplants in WA and NSW.
- * Past analysis showed that when used alone, economic returns of up to \$1,640/ha were realised; but this was increased to \$2,500/ha when lime was added.

2.2 *Calcium cyanamide (Perlka®)*

- * Although calcium cyanamide is the active ingredient, it also contains 50% lime (CaO) and 20% nitrogen.
- * It is widely used in Europe for clubroot control. Recently available in Australia. Also used as a fertiliser eg lettuce as it converts to urea.
- * Generally must be applied at least seven days before transplanting and irrigated in immediately to initiate the break down process. Is toxic if applied at high rate at transplanting. Recent results have indicated that banded incorporation of very low rates (62 kg/ha) at transplanting is effective.

- * Moderate control of root gall, but yields usually consistently high.
- * Glasshouse trials have shown that recent mixed and poorer results were due to too coarse a product, ie not as fine and reactive as previously used. The supplier is returning to the original formulation for brassica production.
- * Needs careful application - banding machines were purpose designed and built.
- * Has previously given economic returns of up to \$3,700/ha.

2.3 *Dynamic Lifter®*

- * Poor clubroot control, especially at low (a half to 1t/ha) rates.
- * Increases soil organic matter.
- * Has previously increased marketable yields by up to 70% at high rates eg 4 t/ha.

3. *Fungicides*

Several fungicidal actives have been effective against clubroot in field trials. One of these has been registered, negotiations are currently underway to begin the registration process for a second in Australia.

3.1 *Shirlan®*

- * Trials have evaluated a range of application methods in an effort to address grower concerns that the recommended application for Shirlan was 'impractical'.
- * Excellent control of clubroot obtained by incorporation of the product into the transplant row (3 L/ha) immediately before transplanting. This is now considered the most effective method of applying Shirlan (7.4) and has been demonstrated Australia wide.

3.2 *Flusulfamide*

- * Recently proven effective in QLD and VIC trials at 0.6 mg/plant.
- * A New Zealand based company seeking approval from Japanese parent group to distribute and market this fungicide in Australia.
- * No residues detected even at the highest application rate (0.6 mg/plant).

4. *Composts*

4.1 *Greenwaste*

- * While the greenwaste (Greenlife®) product offered good initial protection from clubroot infection (via moisture absorption?) in NSW, it is thought to be cost prohibitive. Further assessment is needed. Other greenwaste products have resulted in poor control in VIC and WA.

Vermicompost

Early results from QLD very promising, however, there is a lot of variation in product and some inconsistent results have been obtained.

5. *Other*

- * A range of 'alternative' products including meat, soy and cottonseed meals and molasses have reduced the severity of root gall in pot trials conducted in Queensland. Field trials were problematic due to drought conditions.

7.6.2 Materials and Method

Trial design

Field work was conducted primarily for demonstration and extension of existing research results. However, most of these demonstrations were designed as complete randomised blocks, with at least 3 replicates. Collected data were subject to analysis of variance ($p=0.05$) using the GENSTAT statistical package, version 5.0 (Lawes Agricultural Trust, Rothamsted Experimental Station). Factorial analyses were conducted for all of the larger trials to determine significant main effects.

Site selection, preparation and maintenance

Field trials were performed on commercial brassica growing properties. All sites had a long history of brassica production and were known to be infested with *P. brassicae*, the cause of clubroot disease. Field trials were established between December and March of each year. Field sites were prepared and maintained according to the usual practice of the grower for commercial cropping.

Treatment application

Lime, fumigant and some of the calcium cyanamide treatments were applied to the soil 7-14 days before planting. The rate of lime required was estimated by determining (by soil test) the pH of the soil before treatment. All other treatments were applied in the field immediately before, during or immediately after transplanting. General methods of application include:

<i>Broadcast:</i>	Manually and evenly broadcast or sprayed onto preformed beds or a prepared soil surface. Immediately incorporated to a depth of approximately 30 cm with a single pass of a power harrow or rotary hoe.
<i>Band incorporated:</i>	Evenly incorporated to a depth of approximately 15 cm in bands 12.5 or 23 cm wide along the transplant row using a purpose built machine.
<i>Strip application:</i>	Banding slightly below and offset from the transplants in two narrow strips either side of the transplant.
<i>Soil injected:</i>	Applied to raised planting beds through tines at 20 cm depth.
<i>Plant dip:</i>	Preplant transplant soak for 30 mins.
<i>Seedling soak</i>	Nursery drench application (watering can) applied at least 5 days before planting.
<i>Spot drench (Shirlan control):</i>	Liquid drench applied 100 mL/plant at planting.
<i>Drench:</i>	Continuous fanjet spray over the transplant rows immediately after planting.

Measurement of soil pH

Soil samples were collected from the upper 10cm of all plots 3 weeks after transplanting to confirm expected effect of treatments on soil pH. A composite sample of 20 random cores was collected from each plot. Sub-samples of approximately 100g soil from each plot were oven dried and stored for later pH measurement. Soil pH was measured in a 1:5 (w/v) solution with distilled water and 0.01M calcium chloride.

Assessment of the severity of clubroot infection and gall development

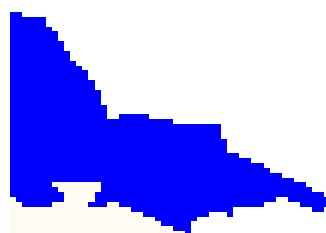
Plants were sampled from each plot at six weeks after transplanting. At least four plants were removed from each plot for assessment. The fresh weight of the above ground material was recorded. Plant roots were visually assessed for clubroot severity on a 1-9 scale as previously described (section 4). Where disease symptoms had failed to develop by the 6 week assessment or the produce was considered too valuable to destructively sample in this way, visual assessment of root galling was conducted as described above at harvest.

Assessment of marketable yield

At maturity, marketable yields were measured from the centre of each plot (buffers of at least 2m were left at either end of the harvestable area to allow for treatment run in/overlap effects). At each harvest the number of heads cut and the total plot weight of marketable heads was recorded. A marketable broccoli head was determined by the overall head size and stage of head development. Cauliflower curds were graded on a quality scale (Shellabear, 1994) to determine curds which were marketable. At least two, but up to six cuts were made to allow for variation in the rate of maturity.

7.6.3 Results

Most of the field research was conducted in Victoria and Western Australia, with the best treatments from these states, together with any locally important treatments being applied in Queensland, New South Wales and Tasmanian programs. In general, treatment lists and methods of application were refined throughout the life of the project, therefore, only a summary of key findings is presented for each state.



VICTORIA

Sites:	Meerlieu – broccolini	(severe clubroot)
	Werribee – cauliflower	(moderate-severe clubroot)
	Werribee – broccoli	(mild/patchy clubroot)
	Boisdale – cabbage	(very severe clubroot)
	Launching place – Chinese cabbage and broccoli	(mild/patchy clubroot)
	Werribee – broccoli	(moderate-severe clubroot)

Objectives

- Demonstration of effective treatments on a large scale.
- Use and evaluation of planter mounted banding machine.
- Evaluation of a new fungicide, flusulfamide (Nebijin). Development of appropriate rates and method of application. Collection of residue data.
- Evaluation of low rate application of calcium cyanamide at transplanting.

Outcomes

- Demonstrations were conducted on private properties at Werribee, Launching Place, Boisdale and Meerlieu. The most effective of these was an in-field demonstration of the most effective treatments conducted at the Werribee field days May 2003. The demonstration crop, machinery and static display attracted interest from local and interstate growers.
- Work cover expressed initial concerns with the safety of the planter mounted banding machine. These concerns related mainly to potential exposure of the operators to fungicide and fertiliser particles, the inability of the tractor driver to see the operators (planters) and the height of the fertiliser bin and fungicide tank. These concerns were addressed, delaying demonstration until the second year of the project, when these machines were demonstrated at field days in Victoria, Western Australia and Tasmania.
- The planter mounted banding machine was as effective as the previous preplant machine, however, this 'single pass' system halves the time taken to apply clubroot treatments and transplant as these processes can be conducted simultaneously. This represents considerable savings in terms of time, labour, fuel and wear and tear on the tractor.

- Flusulfamide (Nebijin) effective at 0.6 mg/plant (Figs. 18, 19 & 20). This is twice the rate recommended by the supplier. Residues were not detected in the produce at harvest at either of the application rates (0.3 or 0.6 mg/plant). A New Zealand based chemical company is currently in negotiation with the Japanese producers of this product to market it in Australia.
- Flusulfamide appears as good as Shirlan, however, the cost of treatment with this product is estimated to be substantially less than the cost of treatment using Shirlan as very low rates of product are used.
- Very low rate (62.5 kg/ha) of calcium cyanamide band incorporated at transplanting has proven effective for 3 successive years. At this rate, the cost of treatment is low, \$112/ha. No phytotoxicity has been observed (Figs. 19 & 20).

TREATMENT LIST – VICTORIA

- Untreated
- Shirlan 3L/ha band incorp.
- Nebijin 0.3mg/plant 50 mL/plant
- Nebijin 0.6 mg/plant 50 mL/plant
- Nebijin 0.9 mg/plant 50 mL/plant
- Nebijin 0.3mg/plant 100 mL/plant
- Nebijin 0.6 mg/plant 100 mL/plant
- Nebijin 0.9 mg/plant 100 mL/plant
- Nebijin 0.6 mg/plant band incorporated
- Perlka 62.5 kg/ha band incorporated (at transplanting)
- GBA lime (1 t/ha)
- GBA lime and Nitrabor (125, 250 and 250 kg/ha)

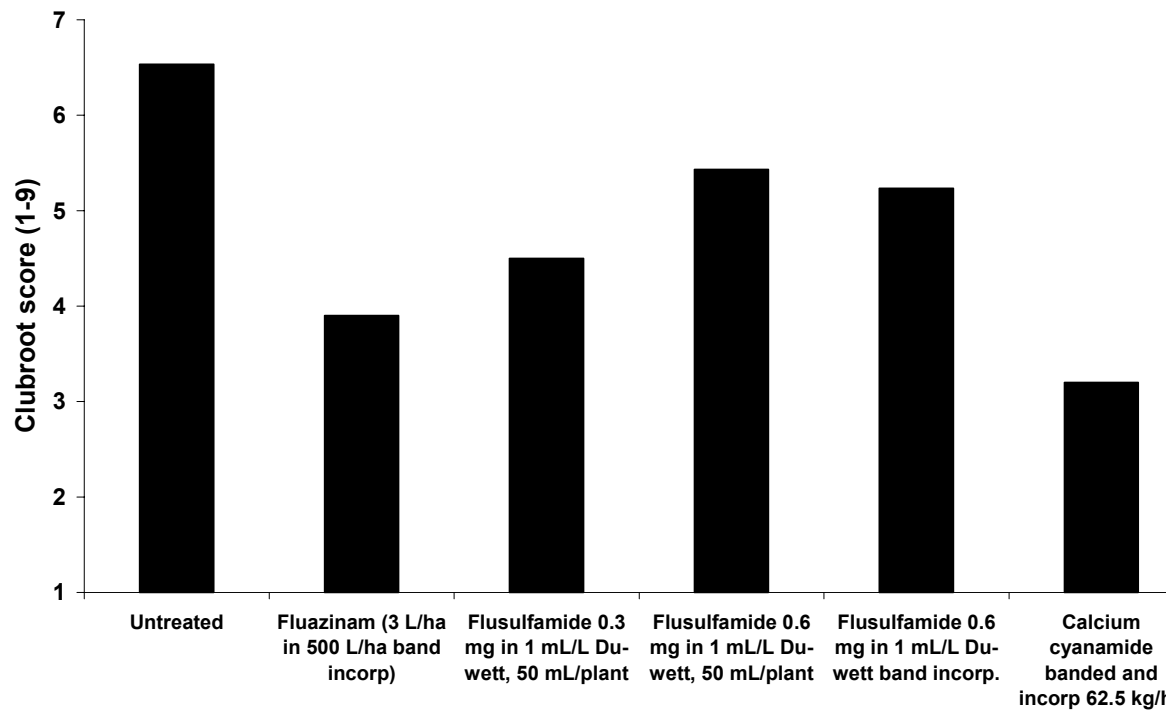


Figure 18: Effect of treatment on clubroot severity, Werribee (VIC), 6 weeks after transplanting. l.s.d. ($p=0.05$) = 1.0

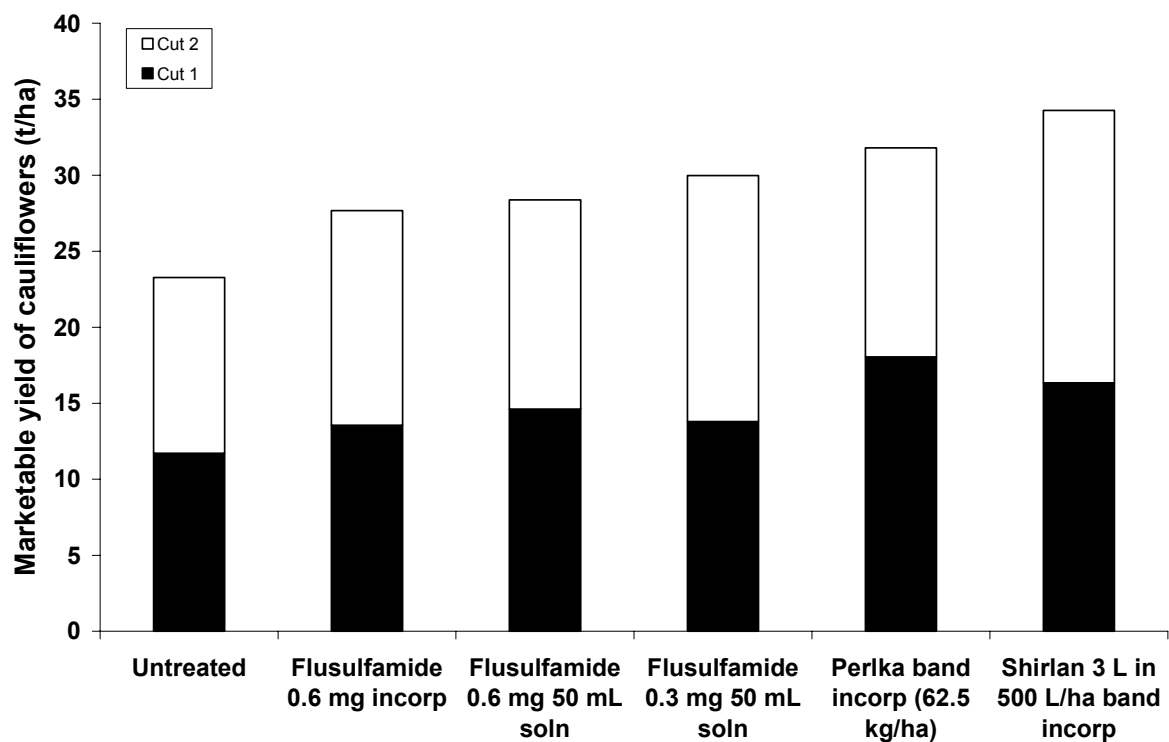


Figure 19: Effect of treatment on marketable yield of cauliflowers, Werribee (VIC). l.s.d. ($p=0.05$) = 7.9

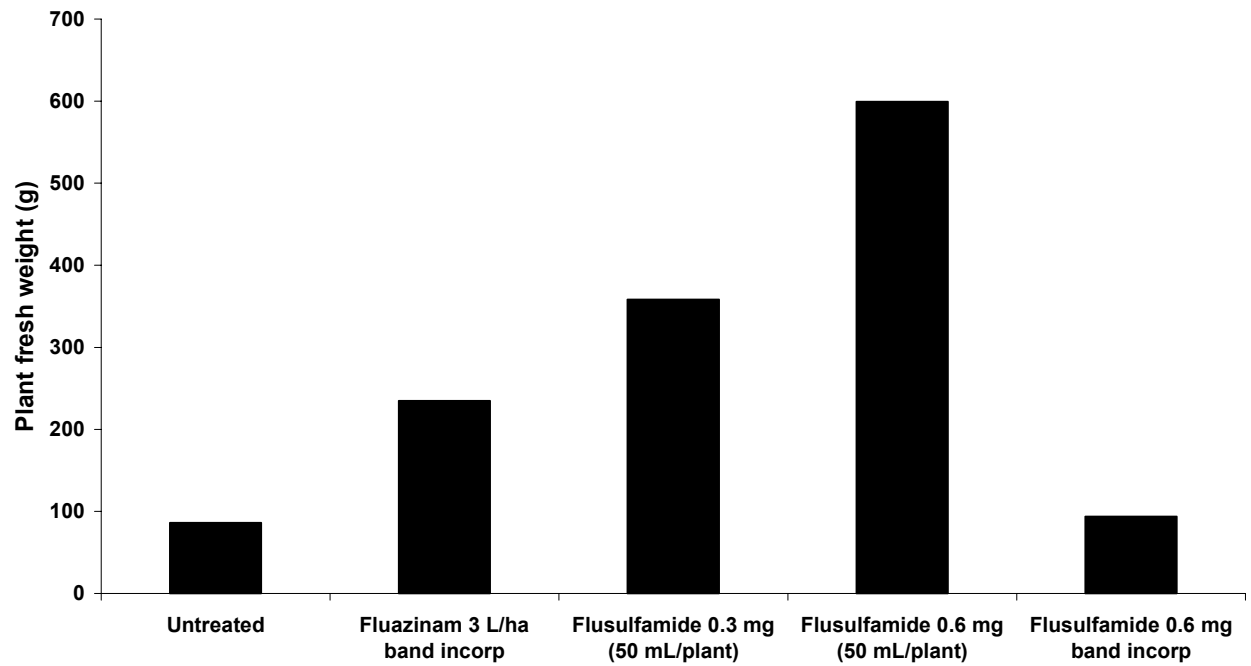


Figure 20: Effect of treatment on fresh weight of cabbage seedlings, Boisdale 8 weeks after transplanting. l.s.d ($p=0.05$) = 141



WESTERN AUSTRALIA

Sites: Manjimup – cauliflower (severe clubroot)
Perth – cauliflower (mild, patchy clubroot)

Objectives:

- Demonstration using larger plantings, of the most effective treatments.
- Use and evaluation of planter mounted banding machine.
- Evaluation of new fungicide flusulfamide in Western Australian soil types.

Outcomes:

- Many growers have now converted over existing transplant rigs to band incorporate products into the transplant row immediately before transplanting (Fig. 21). This has been a particularly effective and popular means of applying base fertilisers, improving yields and reducing the number of picks required (see IPM video).
- Banded incorporation of Shirlan (3 L/ha) band incorporated together with 2.5 t/ha Quicklime broadcast was the most effective treatment in 2001 trials (Figs. 22 & 23). Application with ag lime in subsequent trial less effective (Tables 7 & 8).
- A slightly lower rate of application (between 2 and 3 L/ha) than that used in eastern states appears effective in Manjimup soils.
- Flusulfamide ineffective at 0.6 mg/plant in Manjimup (Tables 7 & 8).
- Crop rotation and liming continue to be used effectively in Manjimup to manage clubroot.



Figure 21: Grower built banding machine (attaches to planter), Manjimup, WA.

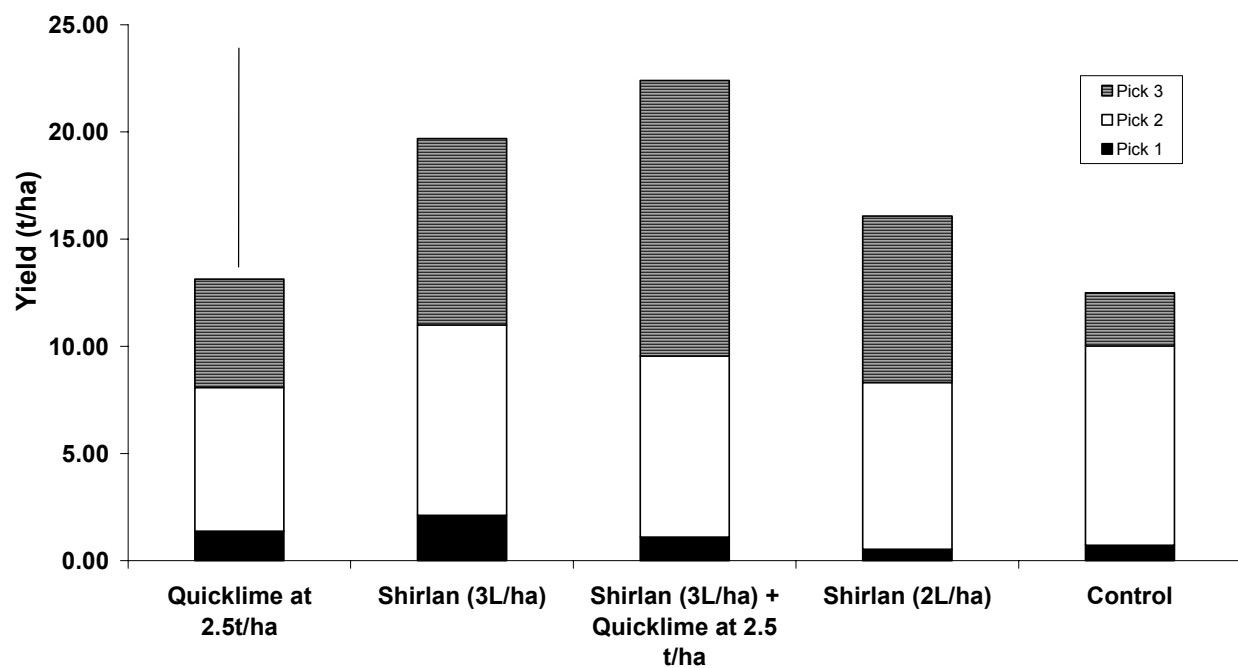


Figure 22: Effect of treatments on marketable yield of cauliflower, Manjimup, WA 2001/02.

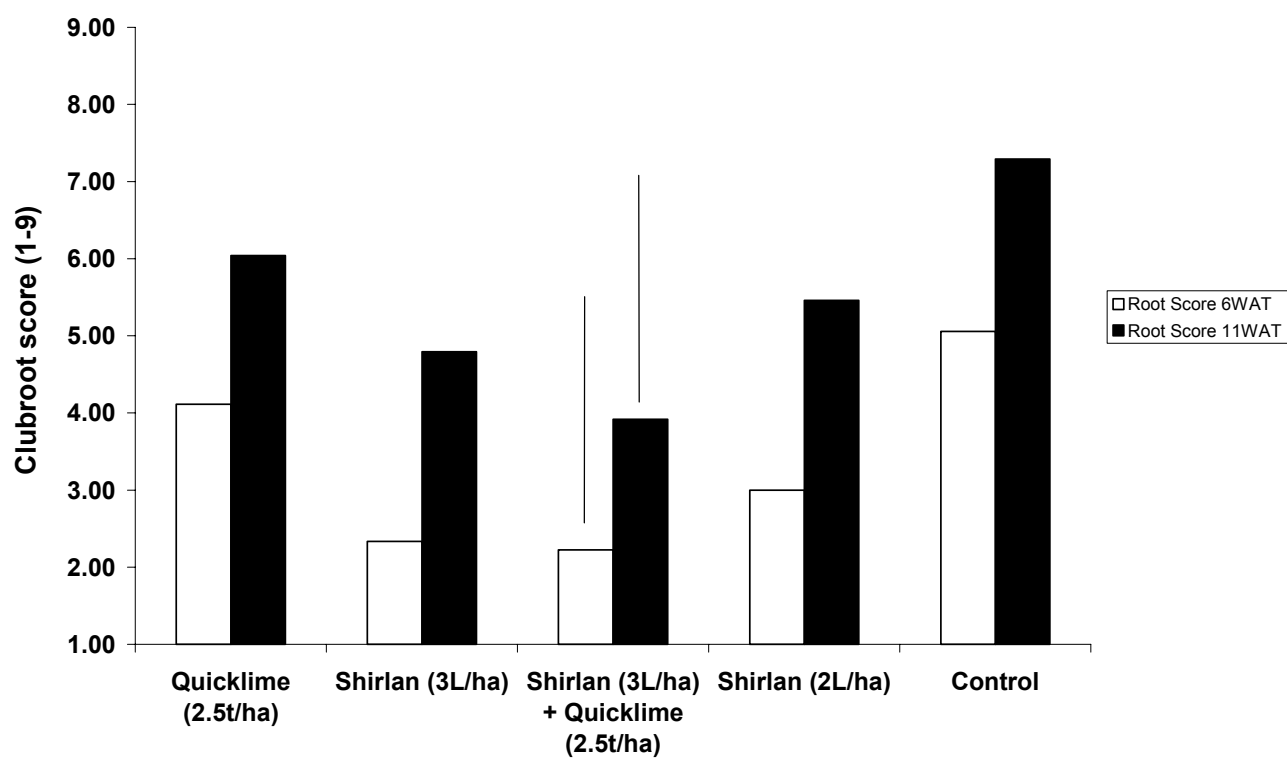


Figure 23: Effect of treatments on the severity of disease on cauliflower Manjimup 2001/02. l.s.d. = 3.0 (6WAT) and 3.1 (11WAT).

Table 7: Effect of treatments on the yield of cauliflower, Manjimup 2002/03.

Treat	Treatment	Marketable yield (t/ha)	Total yield (t/ha)
1	Fluazinam at 3 L /ha	15.83	23.08
2	Fluazinam at 2 L/ha	17.21	22.61
3	Fluazinam at 3 L/ha + ag. lime at 3 t/ha	13.58	20.66
4	Agricultural lime at 3 t/ha	7.50	11.94
5	Flusulfamide at 0.6 mg product /plant	6.63	10.46
6	Flusulfamide at 0.6 mg product /plant + ag. lime at 3 t/ha	3.68	8.48
7	Control (no treatment)	4.65	7.93
	lsd (5%) all treatments	9.37 (p = 0.023)	9.83 (p = 0.005)

Note: The harvested area was 8 m by 2 rows (1.6 m) = 12.8 m²

Table 8: Effect of treatments on disease development and plant growth, Manjimup 2002/03.

Treat	Treatment	Clubroot Score (6WAT)	Clubroot Score (14WAT)	Shoot weight (g) (6WAT)
1	Fluazinam at 3 L /ha	4.40	5.21	652.08
2	Fluazinam at 2 L/ha	4.33	5.04	565.48
3	Fluazinam at 3 L/ha + agricultural lime (calcium carbonate) at 3 t/ha	4.02	6.19	617.19
4	Agricultural lime at 3 t/ha	5.56	6.79	578.90
5	Flusulfamide at 0.6 mg product /plant	6.52	7.21	489.52
6	Flusulfamide at 0.6 mg product /plant + agricultural lime at 3 t/ha	6.04	7.88	553.96
7	Control (no treatment)	6.65	8.04	458.79
	lsd (5%) all treatments	2.222 (p = 0.089)	1.613 (p = 0.002)	181.2 (p = 0.354)

Number of plants checked at 6WAT = 8 per plot.

Number of plants checked at 14WAT = 8 per plot.

Treatments used in Western Australian trials.

Manjimup trial 1

3 replicates

Cauliflower variety Monarch

Soil type clay loam

- T1 - Quicklime broadcast at 2.5 t/ha (6.8 kg/plot), Neutralising Value = 131% with 100% fines, 2WBT.
- T2 - Shirlan incorporated (3 L/ha product in 1000 L/ha water) applied at transplanting and incorporated.
- T3 - Shirlan incorporated (3 L/ha product in 1000 L/ha water) applied at transplant and incorporated + quicklime (2.5 t/ha, 6.8 kg/plot) broadcast, 2WBT.
- T4 - Shirlan incorporated (2 L/ha product in 1000 L/ha water) applied at transplant and incorporated.
- T5 - Control (no treatment)

Manjimup trial 2

3 replicates

Cauliflower variety Monarch

Soil type clay loam

- 1 – Fluazinam at 3 L /ha
- 2 – Fluazinam at 2 L/ha
- 3 – Fluazinam at 3 L/ha + agricultural lime (calcium carbonate) at 3 t/ha
- 4 – Agricultural lime at 3 t/ha
- 5 – Flusulfamide at 0.6 mg product /plant
- 6 – Flusulfamide at 0.6 mg product /plant + agricultural lime at 3 t/ha
- 7 – Control (no treatment)



QUEENSLAND

Sites: Stanthorpe/Applethorpe (Sandy loam)

Objectives:

- ◆ Demonstrate effective alternatives to metham sodium application.
- ◆ Evaluate a new fungicide (flusulfamide) and determine appropriate rates of application.
- ◆ Evaluate a range of alternative products (meals, hay and processing byproducts).

Outcomes:

- Two trials were conducted in the Stanthorpe/Applethorpe area (subsequent trials were abandoned due to drought).
- Metham (500L/ha) and [flusulfamide + DuWett (at double the recommended rate)] were equally effective at suppressing clubroot, while also increasing plant growth (Table 10). Flusulfamide + DuWett (at the recommended rate) appeared ineffective at controlling clubroot (Table 9).
- Green Hay + Urea at either of the rates applied, significantly reduced clubroot, but appeared deleterious to plant growth (Tables 9 & 10). The high rate of urea added to the soil (3.42 t/ha) may also cause excessive nitrogen leaching to the ground table.
- Cotton seed meal, at either 10 or 20 t/ha, proved ineffective at controlling clubroot in this trial (Tables 9 & 10). Cotton seed meal proved effective at controlling clubroot in glasshouse trials, although this was using a low inoculum level of clubroot spores (1000 per gram of soil).

Table 9: Results, Trial One - Queensland

Treatment	Clubroot Rating (Scale 1-9)	Av. Plant Weight (kg)	Av. Number of Plants in 8m Length at Harvest	Total Weight of Plants in 8m Length at Harvest
Control	5.14 a	3.37 bc	12.66 a	43.0 a
Cotton (20t/ha)	4.75 a	3.15 bc	12.50 a	42.2 a
Green Hay (15.8 t/ha) + Urea (3.42 t/ha)	2.87 c	2.88 c	12.50 a	36.2 a
Metham	3.03 bc	4.73 a	12.8 a	60.4 b
Flusulfamide (12mL/100L) + DuWett (100mL/100L)	4.31 ab	3.70 b	11.0 a	40.4 a
	P=0.0046	P=0.0009	P=0.393	P=0.014

Numbers followed by the same letter were not significantly different from each other at the $P < 0.05$ level.

Table 10: Results, Trail Two - Queensland

Treatment	Clubroot Rating (Scale 1-9)	Av. Plant Weight (kg)	Av. Number of Plants in 8m Length at Harvest	Total Weight of Plants in 8m Length at Harvest
Control	5.64 a	3.42 bc	13.2 a	45.1 ab
Cotton (10t/ha)	5.18 a	3.08 cd	12.16 a	37.5 b
Green Hay (3 t/ha) + Urea (3.42 t/ha)	2.97 b	2.39 d	9.83 b	24.3 c
Metham	3.84 b	4.37 a	13.2 a	56.7 a
Flusulfamide (24mL/100L) + DuWett (200mL/100L)	3.12 b	4.09 ab	12.8 a	52.4 a
	P=0.0046	P=0.0011	P=0.035	P=0.0004

Numbers followed by the same letter were not significantly different from each other at the $P < 0.05$ level.

Queensland treatment list – trial 1

	Treatment	Date Applied	Rate	Applic. Method
1.	Control	-	-	
2.	Cotton Seed Meal	16 th Jan	20 tons / ha	Broadcast
3.	Lettuce	7 th Feb		Planted commercial Rate
4.	Legume Hay Urea	16 th Jan	15.8 tons / ha 3.42 tons / ha	Broadcast
5.	Methane	16 th Jan	500L / ha	Applied to soil surface & rotary hoed in.
6.	Flusulfamide applied to DuWett	7 th Feb	12 mL / 100L 100 mL / 100L	Spot drench 50 mL each plant immediately after planting

Queensland treatment list – trial 2

	Treatment	Date Applied	Rate	Applic. Method
1.	Control	-	-	
2.	Cotton Seed Meal	16 th Jan	10 tons / ha	Broadcast
3.	Lettuce	7 th Feb		Planted commercial Rate
4.	Legume Hay Urea	16 th Jan	3 tons / ha 3.42 tons / ha	Broadcast
5.	Methane	16 th Jan	500L / ha	Applied to soil surface & rotary hoed in.
6.	Flusulfamide applied to DuWett	7 th Feb	24 mL / 100L 200 mL / 100L	Spot drench 50 mL each plant immediately after planting

The trials were planted on the 7th February with 3 different cauliflower varieties and harvested on the 16th and 17th April 2002. At planting, the soil was “generally satisfactory” with regard all nutrients, except organic carbon (1.6%), potassium (0.95 meq / 100g) and boron (1.4 mg/kg) which were classified as “below optimum”. The soil’s pH was 7.00 (1 : 5 Ca Cl₂). The grower conducted all fertiliser applications.



NEW SOUTH WALES

Sites: Bathurst (NSW) – cabbage (severe clubroot)
Castlereagh
Chinese market gardens at Kellyville, Oakville, Badgery's Creek (2) and Schofields
Korean market garden at Ebenezeer

Objectives:

- ◆ Provide 'low cost, low technology' treatment options for Asian market gardens.
- ◆ Demonstrate banded application of Shirlan and lime using precision incorporator.
- ◆ Demonstrate control of clubroot on heavily infested sites.

Outcomes:

- Excellent results using hot lime banded with precision incorporator (Figs. 24 & 25). Whilst only 32% of control cabbages were harvestable, 94% of hot lime treated cabbages were harvested. These cabbages were also an average of 3.6 kg heavier than the control plants.
- Although the Shirlan & Perlka had high severity of galling early (Fig. 24), the maturing plants were significantly healthier than the control (but hot lime plants were significantly healthier & larger than Shirlan & Perlka plants) & hence many were at least marketable (Fig. 26).



Figure 24: Effect of treatment on clubroot severity – Bathurst NSW 2001/02.

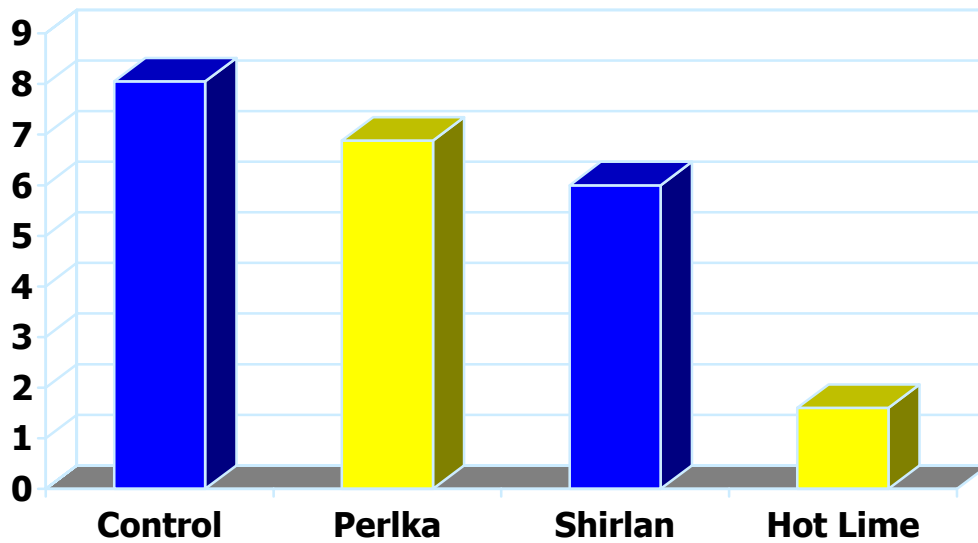


Figure 25: The effect of treatments on the severity of clubroot on cabbage, Bathurst, NSW 2001/02.

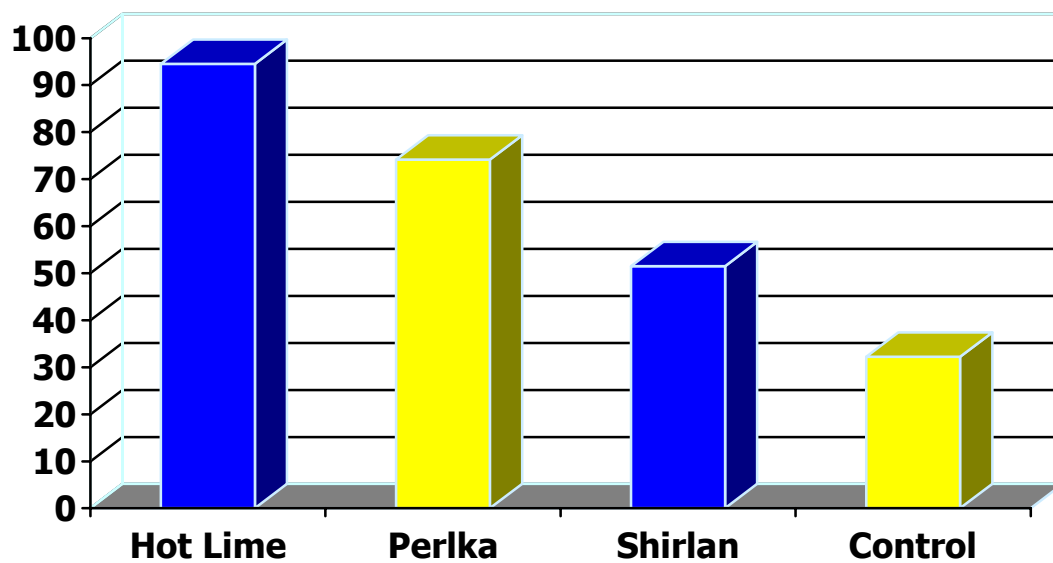
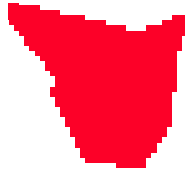


Figure 26: Effect of treatments on average harvestable yield of cabbage – Bathurst 2001/02.

Treatments used NSW

1. Hot lime banded at half a tonne/ha (ie ~ equivalent to 1.5 tonnes/ha broadcast).
2. Shirlan was banded but at a rate equivalent to 3 L ha.
3. Perlka 550 kgs/ha banded
4. Control, untreated.



TASMANIA

Sites: Several sites around Forth (NW Tasmania).

Objectives:

- ◆ Establishing the effectiveness of incorporated Ground Burnt Agricultural Lime (CaO, hot lime) and Shirlan (ai fluazinam) in the red Kraznozom soils of northwest Tasmania.
- ◆ Develop a curative treatment for clubroot that can be applied after the onset of symptoms.

Outcomes:

- ◆ Both GBA lime (Fig 27) and Shirlan (Fig. 28) provided effective control of clubroot in the red Kraznozom soils. It is proposed that the failure of these treatments in previous trials might be due to the transplanter slipping on the hillside resulting in plants not being transplanted into the treated band of soil. This potential problem is eliminated using the transplanter mounted banding machine.
- ◆ Incorporation of GBA lime for February plantings can be used to suppress clubroot sufficiently to obtain a marketable yield in a situation where severe stunting would have occurred.
- ◆ The significant increase in size of heads in the Shirlan and Shirlan & lime treatments (Fig. 27b) suggests that Shirlan contributed about 25% of the disease suppression in the Shirlan & lime treatment (Fig. 27a).
- ◆ Broadcast amendment of calcium cyanamide, borax, or GBA lime at 7 weeks after transplant (as potential curative treatments) did not significantly alter gall formation or the expression of clubroot in comparison to the untreated control (data not shown). We believe there is significant room for better infiltration of these amendments by using a greater volume of overhead irrigation.

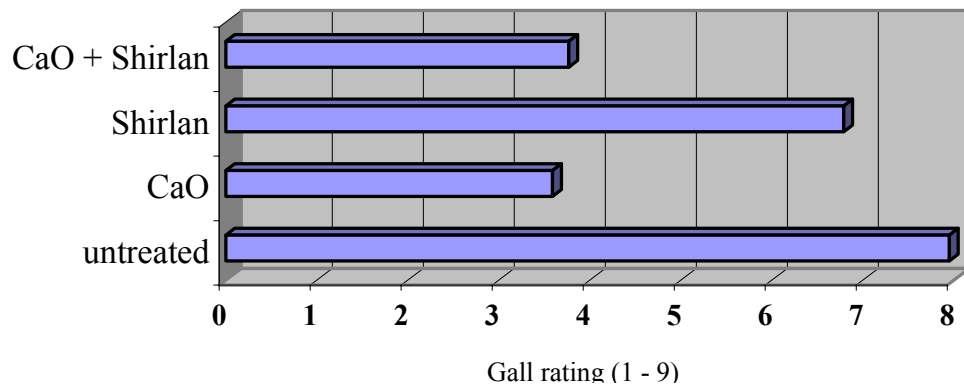


Figure 27a: Severity of gall formation in plants treated with CaO or Shirlan at transplanting (2001).

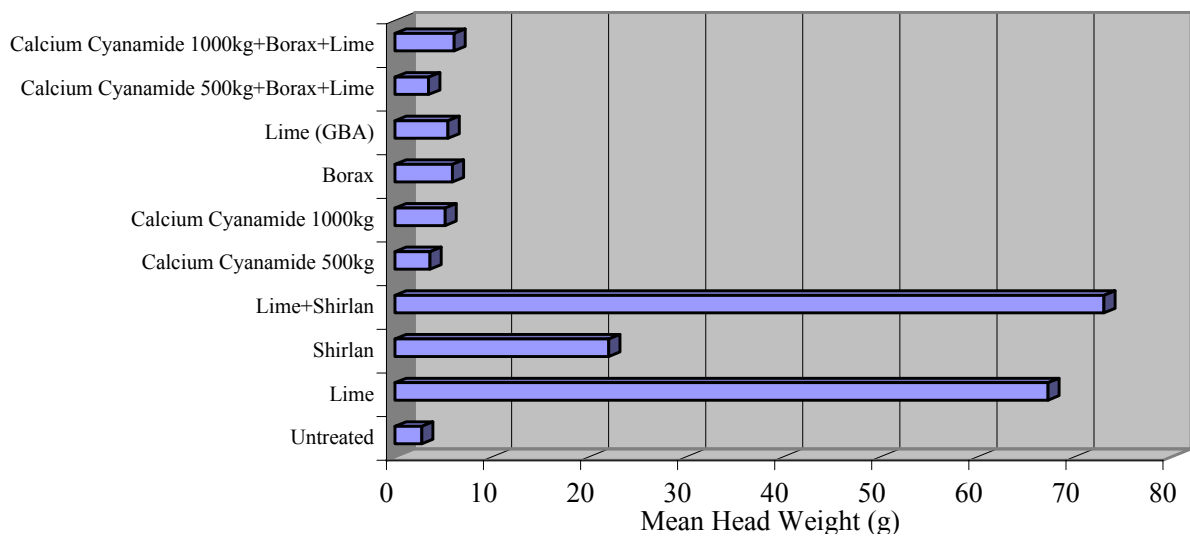


Figure 27b: Mean head weight of broccoli plants (2000) (l.s.d. = 18.5)

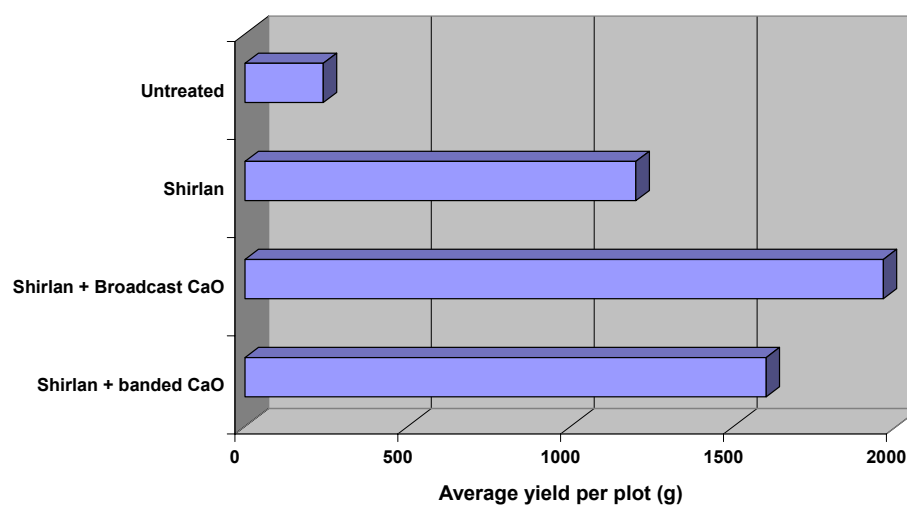


Figure 28: Average yield per plot (2001/2002 December planted trial) (l.s.d. 0.05 = 612)

Treatment list - Tasmania

	TREATMENT	RATE	Application Method	Time of application
A	Untreated			
B	Untreated			
C	Untreated			
D	Lime	3t/h	Band Incorporated	@ Transplanting
E	Shirlan	3l/h	Band Incorporated	@ Transplanting
F	Lime+Shirlan	3t & 3l/h	Shirlan Band Incorporated, lime broadcast or band incorp.	@ Transplanting
G	Calcium Cyanamide	500kg/ha	Broadcast	7 weeks post plant banded 3 inches on each side of row
H	Calcium Cyanamide	1t/h	Broadcast	7 weeks post plant banded 3 inches on each side of row
I	Borax	70 ppm	Broadcast	7 weeks
J	Lime (GBA)	3t/h	Broadcast	7 weeks
K	Calcium Cyanamide+Borax+Lime	500kg/h & 70 ppm & 3t/h	Broadcast	7 weeks
L	Calcium Cyanamide+Borax+Lime	1t/h & 70ppm & 3t/h	Broadcast	7 weeks

Trial conducted in a Kindred paddock with history of clubroot, planted with broccoli (cv marathon) transplants. There were 5 replicates of each treatment.

7.6.4 Discussion

The results presented in this report serve as a small snapshot of activities undertaken in the field for the duration of the project. During this time two new treatments have been developed, reports from individual states have been tested nationally and large numbers of growers have participated in field displays and demonstrations.

The following generalisations can be made:

- ◆ Application of lime as calcium oxide (to increase pH to 7.0-7.5 in responsive soils) is a 'good value for money' treatment consistently returning a profit from most field sites nationally.
- ◆ Shirlan (fluazinam) applied at 3 L/ha effectively controls clubroot but must be evenly distributed around the transplant root zone at planting. Lower rates (2-3 L/ha) may suffice in the Manjimup district of Western Australia.
- ◆ Banded incorporation of Shirlan (3 L/ha) at planting is the most effective method of application of this fungicide.
- ◆ Banded incorporation of a very low rate of calcium cyanamide has reduced the cost of treatment from \$1600/ha to \$112/ha.
- ◆ A new fungicide flusulfamide (Nebijin) applied with Du-wett (a wetting agent) has provided good control of clubroot at 0.6 mg/plant in Victoria and Queensland. In these states control has been on par with that provided by Shirlan. The effective rate is twice that recommended by the New Zealand based suppliers (the recommended rate being ineffective). No residues were found in treated produce and negotiations for registration and distribution in Australia are underway. This result was not repeated in Western Australia and the reason for the failure of the product to control clubroot in this state should be further investigated before registration.

A shed poster and accompanying series of field notes are currently being prepared to summarise key findings and recommendations.

7.7 Development of a transplant mounted banding machine

Introduction

A variety of machines have been used at sites around Australia to demonstrate the effectiveness of incorporating treatments into the transplant row.

Band incorporation of products along the transplant row:

- reduces the cost of treatment (Perlka)
- improves product distribution and efficacy (Shirlan)
- minimised the impact of residues from treatments on the environment
- improves availability of fertilisers to young transplants

Commercial banding machines called “precision incorporators” have been produced and used in trial work across Australia (Figure 29).

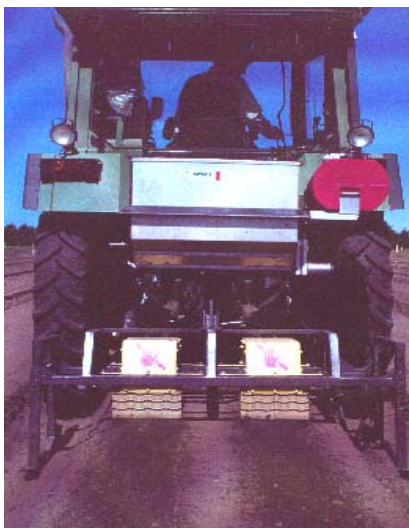


Figure 29: Precision incorporation machines designed to incorporate products into the transplant rows for improved clubroot control.

Reducing treatment cost

Where the cost of treatment is high (eg. Perlka broadcast at 1 t/ha, approx \$1600/ha), incorporation of the product in the transplant row has reduced the cost of treatment by approximately two thirds, and increased the profitability of the crop by up to 100% (Figure 30).

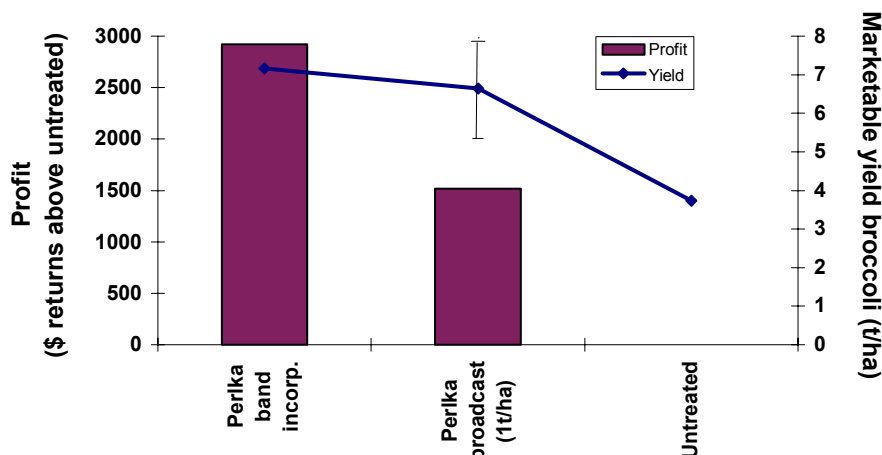


Figure 30: The effect of application method on the marketable yield (t/ha) and profitability (\$/ha) of broccoli from Perlka treated soil (based on \$1/kg), Lindenow.

Improving product distribution and efficacy

Shirlan is a very effective treatment for clubroot **only** when the product is distributed around the root zone. The effectiveness of traditional band sprays over the plants after transplanting or, drop application (100 ml/plant) applied at transplanting will depend on the infiltration characteristics of the soil. In general, distribution will be better in sandy soils than in heavy clay loams.

Incorporation of Shirlan into bands immediately before transplanting ensures that the product is evenly distributed around the root zone of the transplant where it can protect it from infection (Figure 34).

This method of application has been extremely effective in trial work and has significantly reduced the amount of water needed to apply the product (Figs 31-33).

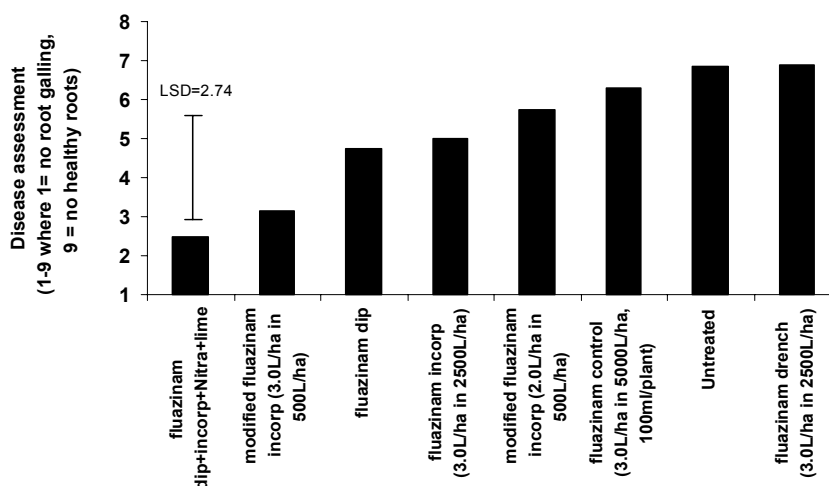


Figure 31: Effect of the method of application of fluazinam on the development of clubroot disease in cabbage (assessed immediately after harvest).

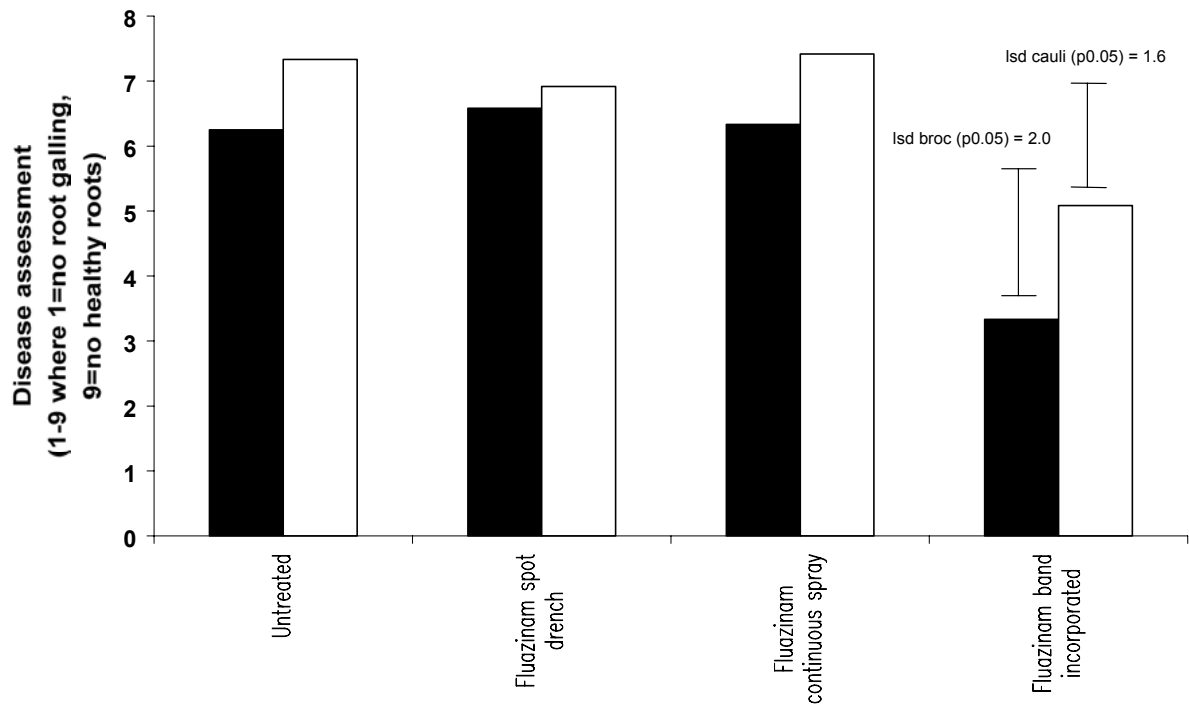


Figure 32: Effect of the method of application of fluazinam on the development of clubroot on broccoli ■ and cauliflower □ grown in field soil naturally infested with *P. brassicae*. Manjimup,

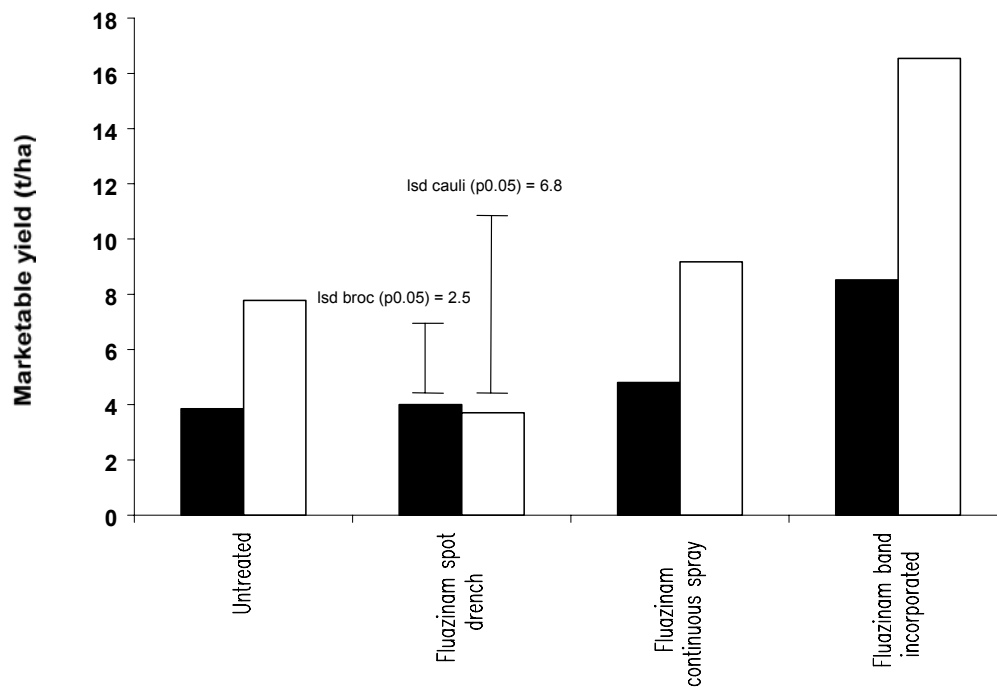


Figure 33: Effect of the method of application of fluazinam on the marketable yield of broccoli ■ and cauliflower □ grown in field soil naturally infested with *P. brassicae*. Manjimup, Western Australia.

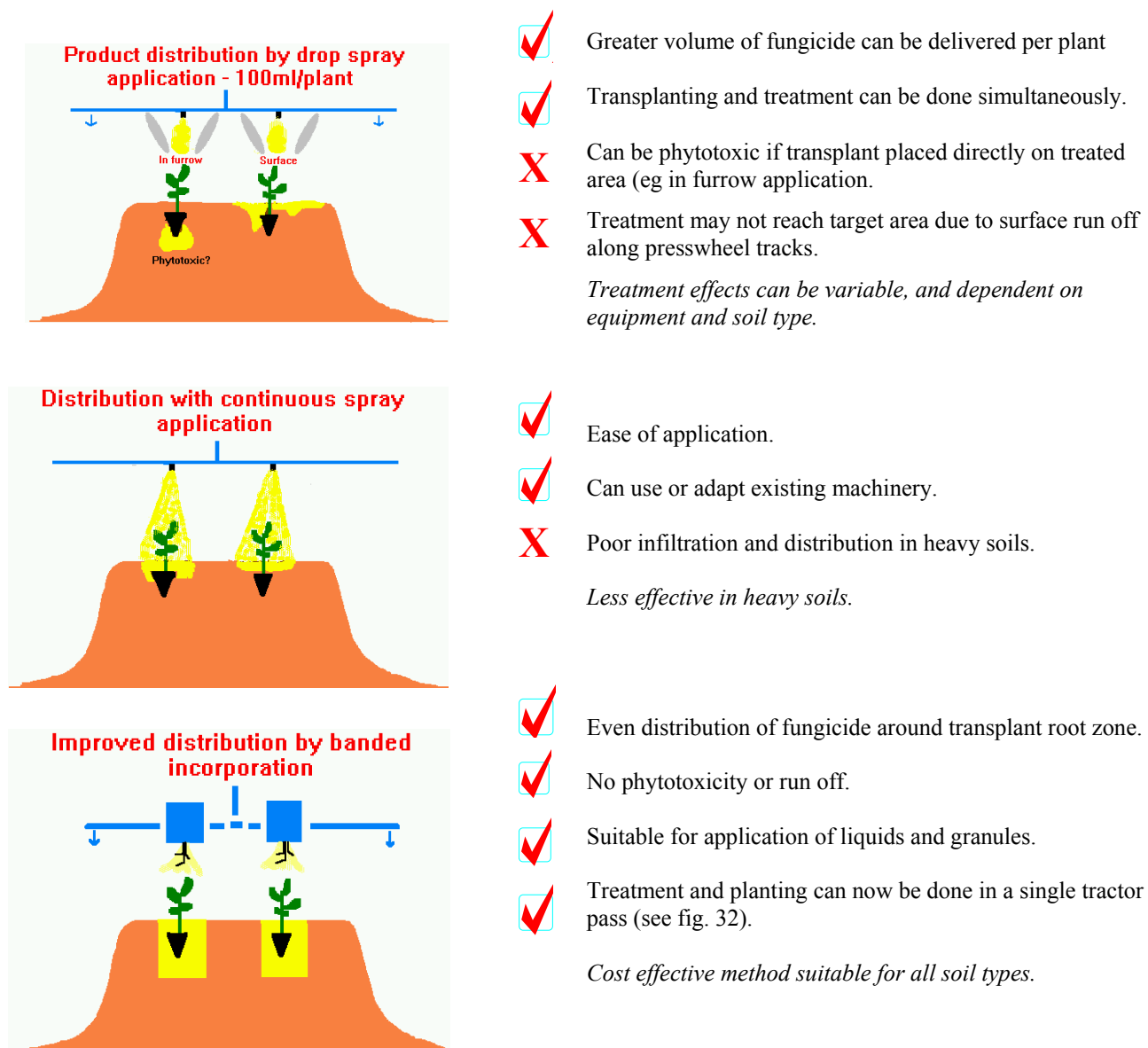


Figure 34: The effect of different methods of application on distribution (and efficacy) of Shirilan.

Reducing soil residues

The efficacy of either lime or Perlka was not significantly changed by band incorporation, compared to broadcast incorporation in Victorian trial work. However, in each case the amount of product used was reduced by approximately two thirds. This has not only reduced the cost of treatment but has reduced the amount of potential carry over into subsequent crops. This is an important consideration for some rotation crops. Potatoes for example, are more susceptible to scab diseases at high soil pH.

Improved availability of fertilisers to young transplants

Incorporation of base fertiliser into the transplant row at transplanting increased the total yield of winter cauliflower by approximately 10 t/ha (Fig. 35) and caused the crop to mature 9 days earlier than the crop treated in the traditional way.

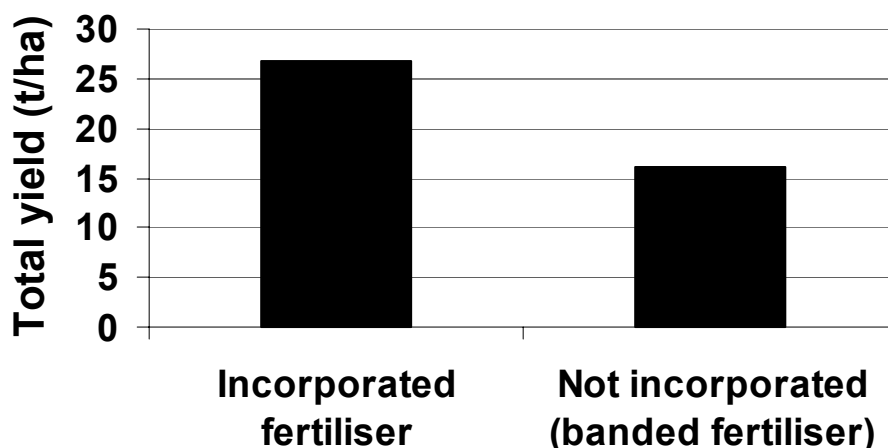


Figure 35: The effect of incorporation of base fertiliser into the transplant row at transplanting compared to the traditional banded method of fertiliser application. Winter cauliflower trials, Manjimup.

A modified transplanter designed to incorporate liquid and/or granular products into the planting row at transplanting was built for the project in 2000 (Fig. 36), however, problems with the Victorian Workcover Authority prevented the use of this machine in field trials and demonstrations in that year. These problems were rectified and the Authority approved the machine for use in subsequent trials and demonstrations.



Figure 36: Transplant mounted machines for simultaneous treatment and planting. Victoria (left) and Western Australia (right).

These machines were used the 2001/02 and 2002/03 seasons trials and demonstrations (see field reports) and were as effective as previous two part units. The advantages of the transplant mounted models for commercial operators are:

- ◆ single pass treatment and planting saving fuel, tractor wear and labour
- ◆ single pass treatment and planting ensures that plants are always placed in the centre of the treated row. This is particularly important on sloping ground or where formed beds are not used making it difficult for the operator to plant accurately into the treated bands.

Since the release of these results many growers, particularly those in the Manjimup district, have converted existing transplant machines to apply fertiliser in this way (Fig. 37). These growers have been unanimously pleased with their investment in this type of machine and report increased profits of approximately \$4000/ha using this method of application. (see brassica IPM video).



Figure 37: Grower designed and built modified planter in use – Manjimup WA.

8 Technology Transfer

A range of technology transfer activities has been undertaken as part of this project. These have included field demonstrations, field walks, grower seminars, oral presentations at conferences and grower group meetings, publication of posters, fact sheets, scientific papers, newsletters and articles in the press. These are detailed below:

Industry publications

Donald, C, Czernaikowski, B and Porter, I. Keeping clubroot out of nurseries. Poster. Department of Primary Industries. June 2003.

Donald, C, Czernaikowski, B and Porter, I. Identifying likely sources of clubroot contamination in the nursery. Fact sheet for nurseries number 1. Department of Primary Industries. June 2003.

Donald, C, Czernaikowski, B and Porter, I. Design the nursery to minimise your clubroot risk. Fact sheet for nurseries number 2. Department of Primary Industries. June 2003.

Donald, C, Czernaikowski, B and Porter, I. Monitor and restrict access to the nursery. Fact sheet for nurseries number 3. Department of Primary Industries. June 2003.

Donald, C, Czernaikowski, B and Porter, I. Wash then sterilize or disinfect. Fact sheet for nurseries number 4. Department of Primary Industries. June 2003.

Donald, C, Czernaikowski, B and Porter, I. Keep seeds, water and soil free of contamination. Fact sheet for nurseries number 5. Department of Primary Industries. June 2003.

Donald, C, Czernaikowski, B and Porter, I. Monitor for clubroot in the nursery. Fact sheet for nurseries number 6. Department of Primary Industries. June 2003.

Donald, C, Czernaikowski, B and Porter, I. Know what to do if clubroot is detected. Fact sheet for nurseries number 7. Department of Primary Industries. June 2003.

James L. Club root "Fact Sheet" NSW EPA & NSW Agriculture (English and Cantonese).

Scientific papers

Donald, E.C., Porter, I.J. and Lancaster, R.A. (2001) Band incorporation of fluazinam (Shirlan) into soil to control clubroot of vegetable brassica crops. *Australian Journal of Experimental Agriculture* **41**(8), 1223-1226.

Donald, E.C., Lawrence, J.M. and Porter, I.J. (2002) Evaluation of a fluorescent staining technique as an indicator of pathogenicity of resting spores of *Plasmodiophora brassicae*. *Australasian Plant Pathology* **31**, 373-379.

Donald, E.C., Porter, I.J. and Lancaster, R.A. Strategic application of lime, fertilisers and fungicides for improved control of clubroot. *Cruciferae Newsletter* **24**, 81-82.

Conference presentations and papers

Donald, E. C., Lancaster, R.A. and Porter, I.J. (2003) A novel method of application improves the control of clubroot in vegetable brassica crops. (Abstract). International Congress of Plant Pathology. Christchurch NZ Feb 2003 Vol. 2 p271.

Donald, E.C. Lawrence, J.M. and Porter, I.J. (2003) Commercial disinfectants fail to kill resting spores of *P. brassicae*. (Abstract) International Congress of Plant Pathology. Christchurch NZ Feb 2003. Vol. 2 P 65.

Faggian, R., parsons, S, Donald, E.C., Lawrie, A.C. and Porter, I.J. (2003) Molecular detection and quantification of *P. brassicae*. (2003) (Abstract). International Congress of Plant Pathology. Christchurch NZ Feb 2003 Vol. 2 p82.

Metcalf, D.A., Doyle, R.E., Archer, S.C., Donald, C and Porter, I. (2003) Management of clubroot in red ferrosols in northwestern Tasmania. (Abstract). International Congress of Plant Pathology. Christchurch NZ Feb 2003 Vol. 2 p134

Porter, I.J., Donald, C.E., Faggian, R. (2003) Integrated management of clubroot (*Plasmodiophora brassicae*) in vegetable brassica crops. (Abstract). International Congress of Plant Pathology. Christchurch NZ Feb 2003 Vol. 1 p111.

Donald, E.C., Lancaster, R.A., Lawrence, J.M. and Porter, I.J. (2001) Application advances improve the control of clubroot in Australia. Proceedings of the Second Australasian Soilborne Diseases Symposium, Lorne Victoria, 5-8 March, 2001. Porter I.J. *et. al. (eds.)*. p129.

Faggian, R., Parsons, S., Porter, I.J. and Lawrie, A.C. (2001) Detection of clubroot in Australia: Progress towards a reliable molecular diagnostic assay. Proceedings of the Second Australasian Soilborne Diseases Symposium, Lorne Victoria, 5-8 March, 2001. Porter I.J. *et. al. (eds.)* p5.

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Donald, E.C., Porter, I.J. and Lancaster, R.A. Strategic application: a means of improving control of clubroot. Brassica 2000, 3rd ISHS International Symposium on Brassicas. (Abstract). Warwick, UK. Sept. 2000.

Project Newsletters

Donald, E.C. and Porter, I.J. Clubroot - Galls and All Special Edn 'A guide to the prevention and management of clubroot in vegetable brassica crops.' September 2000.

Donald, C and Porter, I. Clubroot – Galls and All. Issue 10, July 2001.

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Lancaster, R. (July, 2000) Clubroot Newsletter (No. 10) – WA edition

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Lancaster, R. (Aug., 2002) Clubroot Newsletter (No. 12) – WA edition

General (media) publications

James, L. New success in clubroot prevention and management. Good Fruit and Vegetables, March 2001. P25-27.

Stephens, P. Molasses implicated in brassica clubroot control. Good Fruit and Vegetables, September 2000.

Elsley, K. Pest war progress, vegetable growers given heart. The Land 17th August 2000 p61

Bray, S. Brassica research priorities discussed. Good Fruit and Vegetables, October, 2000.

Lancaster, R (editor) (March 2003) 'Clubroot – Ongoing management is required'. Better Brassica Newsletter no. 2

Lancaster, R (editor) (March 2003) 'Diagnostic test for clubroot'. Better Brassica Newsletter no. 2

Countryman newspaper – Horticulture Insert (2003) 'Clubroot test world first breakthrough'. 5 June 2003, page 8.

Field Demonstrations and Displays

VIC: Werribee Vegetable Expo 1st and 2nd May 2003 (field demonstration and display)
Werriree (Gilbert Chandler seminar) 15th Oct 2002
IHD (McMillan Horticulture students, tour and display) 16th Oct 2002
Werribee (Seminar to Diploma of Horticulture students) 17th Oct 2001
Werribee (Diamond back moth conference farm tour - talk) 29th Nov 2001

WA: Denmark (presentation on clubroot management). 19th Feb 2003.

NSW: Sydney basin field grown vegetables conference,(seminar) Richmond, 14th July 2000
Annangrove 8th July (Presentation on clubroot and the use of hot lime at Hills District Chinese Growers workshop)
SMARTtrain farm chemical user training courses (7 courses, clubroot control strategies presented as IDM and control option examples)
Kemps Creek 12th July 2002 (Presentation on clubroot and the use of hot lime to Australian Chinese Growers Assoc.)
Castlereigh – hot lime demonstration (May – Sept)
Hot lime demos on 4 Chinese market gardens at Kellyville (2 applications), Oakville, Badgery's Creek (2) and Schofields from November to March.
Ebenezer – Hot lime demonstration on Korean market garden (Dec 2002)
Presentation on Club root demos sites at Australian Chinese Growers Assoc biennial picnic day at Catherine Fields 8th June 2002, 400 present.

QLD: Gatton (seminar, farm visits and grower meeting) 9th August 2001

TAS: Forthside (seminar and field display) 13th Dec 2001

Clubroot research meetings (with HAL commodity group representatives)

23rd – 24th August, 2000

23rd – 24th July, 2002

9 Acknowledgments

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11 Appendix I - Nursery Fact sheets 1-7.

12 Appendix II – Nursery audit sample submission sheet