VG97076

Integrated control of clubroot for the production of quality export and domestic crucifers.

Caroline Donald et al
Agriculture Victoria

Know-how for Horticulture™

VG97076

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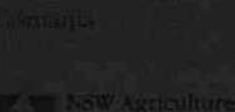

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Integrated control of clubroot for the production of quality export and domestic crucifers.

Final report for the project VG 97076
(June 2000)

Caroline Donald *et al*

Agriculture Victoria (Knoxfield)

Integrated control of clubroot for the production of quality export and domestic crucifers
Final report for project VG 97076

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Scope of the report
This report presents a summary of work conducted in Australia during the period July 1997 - June 2000 by the national clubroot research team. Whilst every attempt has been made to present as complete a summary as possible, some sections (ie section 4 'Field control of clubroot") have been restricted to key findings and research highlights to ensure the report is maintained at a manageable size. Details of individual 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 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:
1. Identifying treatments that control clubroot.
2. Developing more effective and sustainable methods of using these treatments.
3. Developing integrated management strategies that are effective in all states.
4. Determining the extent of variation in the pathogen population and the potential for use of tolerant crop varieties in Australia.

In the short term metham sodium (500 L/ha) has been used to control spot outbreaks, and limit further spread of the disease in recently affected regions.

Long-term management strategies have been developed based on

- good farm hygiene
- crop rotation
- liming responsive soils to pH 7.0-7.5
- maintaining high soil calcium in the first 3 weeks post-transplanting
- applying a preventative fungicide (fluazinam, Shirlan® 3 L/ha)

Recommendations arising from this work have been summarised and distributed to brassica growers nationally in the pamphlet "A guide to the prevention and management of clubroot in vegetable brassica crops".

A method of incorporation of treatments into the transplant row was developed and has:

- reduced the cost of treatments such as calcium cyanamide by approximately two thirds.
- improved the distribution and efficacy of fluazinam, doubling yields of conventionally treated plants in some soil types.
- minimised the impact of residues from treatments on the environment

This method of application is now the most effective method of application of fluazinam (Shirlan®) and the most cost effective method of applying calcium cyanamide (Perlka®) for clubroot control. It has also been used to improve fertiliser use efficiency and has increased yields of winter grown cauliflowers by 10 t/ha.

The pathogen population in Australia was found to consist of a number of distinct pathotypes of *P. brassicae*. Two varieties of broccoli (Yates '5737' and 'Dome') were tolerant of some, but not all of these pathotypes. 'Dome' is commercially available in Australia.

Clubroot can now be managed in the majority of Australian horticultural soils. The challenge remains to predict anticipated productivity losses from soil inoculum loads thereby enabling growers to choose the most cost-effective management strategy based on a simple soil test.

2 Technical summary

Clubroot is a soilborne disease affecting the vegetable brassicas. Infection of plant roots results in the development of misshapen, galled roots with impaired water and nutrient assimilation capacity. The cause of the disease is an obligate biotrophic plant parasite, *Plasmodiophora brassicae*, which can persist in soil for up to 10 years as resistant resting spores. Field, glasshouse and laboratory studies have led to the development of management strategies for clubroot based on maintaining the viable soil inoculum population below the threshold for disease. These strategies have been published in the pamphlet "A guide to the prevention and management of clubroot in vegetable brassica crops".

Clubroot can be managed by good farm hygiene together with crop rotation, manipulation of soil pH in responsive soils, increasing soil calcium and application of a preventative fungicide, such as fluazinam. Throughout Australia, field trials have highlighted the importance of liming to achieve a soil pH between 7.0-7.5. Lime does not kill the fungus, 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. Application of a calcium oxide based lime (hot lime, quicklime, Ground Burnt Agricultural lime) remains the most important 'first step' for effective clubroot control.

Both calcium and boron have been shown to reduce the severity of clubroot in laboratory and field trials. When applied with lime, these products have consistently increased marketable yields in responsive soils and resulted in economic returns of up to $2500/ha. Field studies and laboratory studies indicate that it is critical that infection is minimised in the early weeks post transplanting to enable healthy roots to become established. Calcium and boron have been most effective when applied in the first 3 weeks post-transplanting to soils at or near neutral pH.

Disease control and cost effectiveness of a number of the treatments has been optimised by strategic application into the transplant rows. A number of prototype machines were developed and evaluated in a series of field trials conducted at locations across Australia. These machines incorporate liquid or granular products in bands to an approximate depth of 15 cm along the transplant rows. At high soil inoculum loads, the most effective treatment combinations include incorporation of fluazinam (3 L/ha) into the transplant row. By incorporation, the amount of water required to apply fluazinam could be safely reduced from 2500 L/ha to 500 L/ha. This method of application also:

- reduced the cost of treatments such as calcium cyanamide by approximately two thirds
- improved the distribution and efficacy of fluazinam, doubling yields of conventionally treated plants in some soil types.
- minimised the impact of residues from treatments on the environment

The final prototype machine developed during the life of the project enables treatment and transplanting to be conducted simultaneously. This has resulted in a further saving to the grower of labour, time and fuel.

The pathogen population in Australia was studied using the European Clubroot Differential (ECD) series of brassica hosts. It was found to consist of a number of distinct pathotypes of *P. brassicae*. Sixteen triplet codes were identified from 23 pathogen collections from 5 states of Australia. Two of these pathotypes were identified repeatedly and were present in 3 of the 5 states surveyed. Two varieties of broccoli (Yates '5737' and 'Dome') were tolerant of some, but not all of these pathotypes in a series of field and glasshouse screening trials. This is the first report of commercially useful resistance in to *P. brassicae* in broccoli in Australia.

Clubroot can now be managed in the majority of Australian horticultural soils, however, selecting the most cost-effective strategy for a given paddock still involves considerable guess work. The future of clubroot research in Australia will be directed towards the development of predictive tools and disease models for clubroot that predict anticipated productivity losses from soil inoculum loads under a range of soil, climatic and cultural conditions. The aim of this research will be to provide decision support for growers, enabling them to choose the most cost-effective management strategy based on a simple soil test. This work will be conducted by the research team as part of HRDC funded project VG 00044 'Clubroot - Total Crop Management'.

3 Introduction

The Australian horticultural brassica industry produces $134 million worth of produce 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 (VG 306) 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.

In Victoria clubroot is endemic around Werribee South. Many properties South East of Melbourne are also affected. Clubroot became a problem in the Lindenow Valley in the late 1980s when a flood spread *P. brassicae* from one or several properties in the district to much of the valley. A single spot outbreak of clubroot in the Sunraysia district was identified in April 1998. This was the first, and to date, the only outbreak in the district. Further outbreaks reported from isolated properties in Trentham (100 km NW of Melbourne) and Cora Lynn (70 km SE of Melbourne) were confirmed in February 1999.

Nationally, recent clubroot outbreaks have occurred in Stanthorpe (Queensland, 1997) and Manjimup (Western Australia, 1993). Clubroot is now a significant problem in every state of Australia. Gatton (Queensland) is the only major production region that remains free of this disease. Growers in this region apply a voluntary summer brassica break, which avoids the period that favours infection by *P. brassicae*. During this time no brassicas are planted in the district. Together with high natural soil pH (approximately 8.0), this is likely to have prevented an outbreak in the region.

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 (VG 431) identified a number of new fertiliser, nutrient and fungicide treatments that reduced yield losses due to clubroot in Victoria and Western Australia. The primary aim of the current project was to develop these treatments into management strategies for clubroot that would be effective in all states of Australia. This was achieved by:

- Evaluation of treatments over a range of soil types and climates.
- Development and evaluation of the most effective methods of application for these soil treatments.
- Identification of the variation in the pathogen population in Australia.
- Identification of cultivars tolerant of one or several of the pathogen populations present in Australia.

4 Field Control of Clubroot

4.1 Summaries

4.1.1 General summary

More than 20 field trials have been conducted on commercial brassica growing properties to evaluate integrated management strategies to control and prevent the spread of clubroot. Trials were conducted at the following locations over a 3 year period:

- Werribee, Mornington, Lindenow and Trentham, Victoria.
- Manjimup, Western Australia.
- Forth, Tasmania.
- Stanthorpe, Applethorpe, Queensland.
- Castlereigh, New South Wales.

Recommendations arising from this work have been summarised and distributed to brassica growers nationally in the pamphlet "A guide to the prevention and management of clubroot in vegetable brassica crops".

A copy of this pamphlet accompanies this report.

4.1.2 Product summary

1. Limes

- 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, ease of handling and pH. Ground Burnt Agricultural lime (GBA, a calcium oxide lime) known as hot lime or quicklime 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, a calcium carbonate lime) 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.

- Trials have identified several soil types in Australia termed "lime non-responsive". It is very difficult to increase soil pH in these soils and other strategies such as strategic fungicide application need to be used. Fortunately, these soils are not common in mainland Australia. Such soils are generally high in organic matter and have a high cation exchange capacity.

- Control of clubroot was generally greater when lime was combined with other treatments (eg. calcium nitrate) than when used alone.

2. *Plant Nutrition*

Research worldwide indicates calcium, boron and magnesium inhibit gall formation due to clubroot. The benefits of several products containing these elements were demonstrated in field trials.

2.1 Nitrabor®

- This product is a calcium and boron fertiliser from Hydro. Campbells have a similar product. Both are now commercially available in Australia.
- It is more effective when used with lime. When applied with lime, these products have consistently increased marketable yields, especially in Victoria.
- Both calcium and boron have been shown to reduce the severity of clubroot in laboratory and field trials.
- Often shows poor clubroot control but increases yields by up to 40% due to improved nutrition.
- Further research is required to
  - Determine effects in high organic matter & mineral soils.
  - Refine the timing and method of application and determine differences in the effectiveness of various Ca & B products.
  - Investigate phytotoxicity to transplants reported in WA and NSW.
- Past analysis showed that when used alone, economic returns of up to $1,640/ha were realised from clubroot affected sites; 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 decomposes to urea.
- Must be applied at least 7-10 days before transplanting and irrigated immediately to initiate the break down process. Is toxic to plants if applied at transplanting.
- Provides moderate control of root galling.
- Consistently increased marketable yield of broccoli in Victoria in 1996 and 1997.
- Subsequent variable and poorer results are thought to be due to a change in product formulation, (ie no longer as fine and reactive as previously).
- Further evaluation in high organic matter & mineral soils required.
- Must be applied carefully - banding machines were purpose designed and built.
- Has previously given economic returns for broccoli of up to $3,700/ha from clubroot affected sites.

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, however, control of clubroot has been very poor.

3. Fungicides

Several fungicidal actives have been effective against clubroot in field trials. One of these has been registered.

3.1 Shirlan®

- Trials in this study 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 was obtained on heavy soils in Werribee in 1998 by incorporating Shirlan® in bands along the transplant rows immediately before transplanting. This success has been repeated in a number of trials in different states. This is now considered the most effective method of applying Shirlan®.
- (See section 5 'Application Technology')

4. Fumigants

4.1 Metham sodium

- Very effective in Queensland trials, efficacy dependant upon method of application. Extremely useful as a spot treatment for cleaning up localised infections particularly new infection sites. Long term, frequent use is not recommended because of potential enhanced biodegradation of the product.

4.2 Basamid®

- Extremely useful as a spot treatment for cleaning up localised infections, particularly new infection sites. Dispersal of active ingredient and therefore efficacy improved in lighter or sandy soils compared to heavy clays.

4. Composts

4.1 Greenwaste

- The greenwaste (Greenlife®) product provided good intial protection from clubroot (via moisture absorption? – like "kitty litter") in NSW in 1998, however, at the rates used it is thought to be cost prohibitive. Further assessment is needed. Other greenwaste products have resulted in poor control in VIC and WA.

4.2 Vermicompost

- Initial trials conducted in QLD produced some promising early results, however, there is a lot of variation in product formulation and some inconsistent results have subsequently been obtained.

4.2 Materials and Methods

Trial design

Unless otherwise stated, trials were designed as complete randomised blocks, with five replicates, and 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 (eg. lime, calcium nitrate, fluazinam, metham sodium).

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, calcium cyanamide and fumigant 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. Several treatments (Bion® and phosphorous acid) were applied as seedling drenches in the nursery 5-14 days before planting. All other treatments were applied in the field immediately before, during or immediately after transplanting. General methods of application include:

Broadcast:	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.
Band incorporated:	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.
Strip application:	Banding slightly below and offset from the transplants in two narrow strips either side of the transplant.
Soil injected:	Applied to raised planting beds through tines at 20 cm depth.
Plant dip:	Preplant transplant soak for 30 mins.
Seedling soak	Nursery drench application (watering can) applied at least 5 days before planting.
Spot drench (Shirlan control):	Liquid drench applied 100 ml/plant at planting.
Drench:	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 the following scale:
1-9 where 1 = no root galling and 9 = 100% roots galled.
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 (cabbages at Trentham), 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 bead 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.

4.3 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 the most recent results and key findings are presented for each state.

4.3.1 VICTORIA

Clubroot remains a serious problem over the summer months (Oct - March plantings) in Victoria. The disease is widespread in most of the major crucifer growing regions. A number of new, isolated outbreaks have been reported recently including a single report from Swan Hill which is of particular concern because of its close proximity to the Robinvale region. Many growers now routinely use calcium oxide based limes to manage soil pH at or near 7.0. The high cost and previously erratic control from fluazinam has limited uptake of this method of control. However, growers are slowly beginning to experiment with methods of application detailed in this report and have been pleased with the result. Crop rotation (or lack of) remains a concern, particularly in the most intensive production regions.

Trial sites:

Harry Velisha's property, Werribee Vic 1998	Clay sandy loam
Peter Schreur's property, Fiveways Vic 1998	
Russell Lamattina's property, Mornington Vic 1998	Sand
George Sabo's property, Trentham Vic 1999	Clay loam
Russell Lamattina's property, Mornington Vic 1999	Sand
Marco Mason's property, Werribee Vic 1999	Sandy clay loam
Institute for Horticultural Development, Knoxfield Vic 1999	Silty sand
George Sabo's property, Trentham Vic 2000	Clay loam
Con Ballan's property, Werribee Vic 2000	Light clay

Summary of results:

- Application of calcium oxide based limes continues to remain profitable (in lime responsive soils) and is an important 'first step' in the management of clubroot.
- Integrated strategies (lime, calcium/boron and fungicides) consistently effective.
- The most effective treatment combinations always included incorporation of Shirlan® into the transplant row as part of the treatment.
- By incorporating Shirlan® into the transplant row, the amount of water required to apply Shirlan® was reduced from 5000 L/ha to between 100 and 500 L/ha.
- New products Bion®, Bioflora®, compost and Nylate® were ineffective at the rates and/or methods of application used.

Effect of treatments on disease

In both trials incorporation of fluazinam (Shirlan®) into the transplant row significantly ($p=0.05$) reduced the severity of clubroot disease (Figs 1 &2). At Trentham, the average clubroot rating of cabbage assessed on a visual severity scale (1-9) was reduced from 7.1 to 6.6 (Fig.2) and at Werribee, the average clubroot rating of broccoli was reduced from 4.6 to 3.6 (Fig 1). Whilst nitrabor, lime and fluazinam dips all reduced the severity of clubroot disease at both sites, these effects were not significant ($p=0.05$).

Broadcast application of calcium cyanamide (1 t/ha) significantly reduced disease severity at both sites, as did calcium nitrate when applied with lime (Figs 1 &2). Application of fluazinam as a spot plant drench (Shirlan control, 100 ml/plant) was effective, significantly reducing root galling at both locations, however, the post planting seedling drench (applied as a continuous spray over the transplant rows) was ineffective at both locations (Figs 1&2).

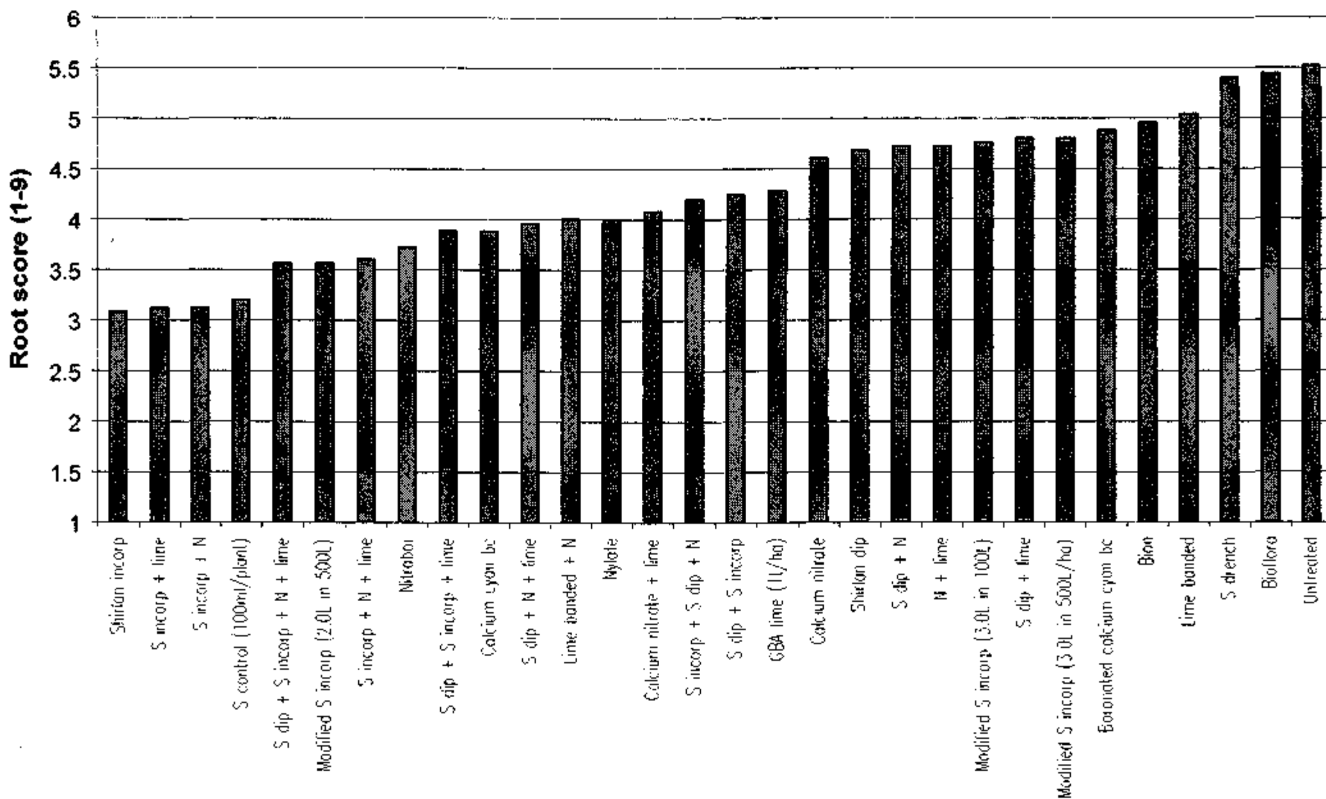

Figure 1: Effect of treatment on root galling of broccoli assessed at harvest, Werribee 2000.
LSD (p=0.05) = 1.3 (Sincorp = Shirlan incorporated into transplant row; Sdip = Shirlan transplant dip)

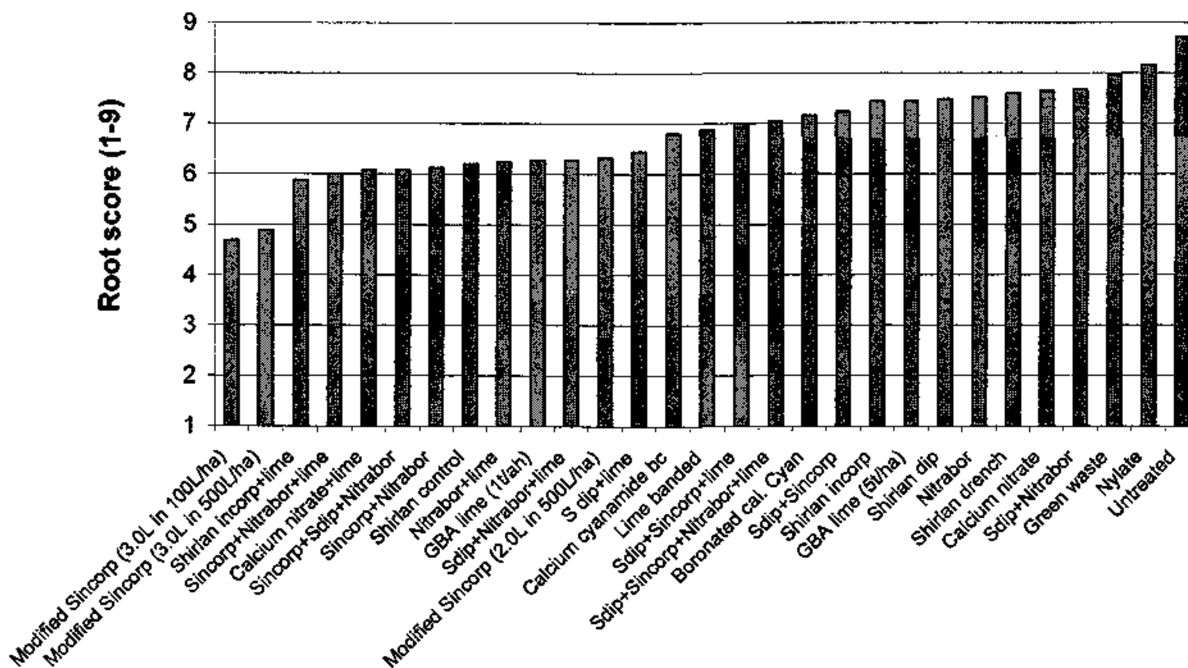

Figure 2: Effect of treatments on root galling of cabbages, 15 weeks after planting, Trentham 2000.
LSD (p=0.05) = 2.0. (Sincorp = Shirlan incorporated into transplant row; Sdip = Shirlan transplant dip)

Effects of treatments on yield and profitability

With the exception of banded application of lime with calcium nitrate at planting, the most effective treatments used in the Werribee (broccoli) trial included application of fluazinam alone or in combination with other treatments (Fig. 3). Incorporation into transplant rows (3 L/ha in 2500 L/ha) or application as a spot plant drench (Shirlan control, 3 L/ha in 5000 L/ha, 100 ml/plant) significantly increased yield by 3 and 3.3 t/ha respectively. A reduction in the volume of water used to incorporate fluazinam into the transplant rows from 2500 L/ha to 500 L/ha had no adverse effect on yield. However, reducing the rate of fluazinam from 3.0 L/ha to 2.0 L/ha caused a significant reduction in broccoli yield of 2.8 t/ha (Fig. 3).

Although expensive ($180/L), incorporation of fluazinam into the transplant rows returned a profit of $2500/ha above the untreated broccoli. (Fig. 5) (based on returns of $1/kg for broccoli). Whilst effective, broadcast application of calcium cyanamide returned only a modest profit (approximately $200/ha) due to the large cost of treatment. For this treatment to be profitable, banded application is essential. Application of Ground Burnt Agricultural lime (a very low cost input, approximately $190/ha) returned a profit of approximately $1200/ha (Fig. 5). This treatment remains a useful 'first step' in the treatment of clubroot.

At Trentham, disease development was rapid and severe. None of the treatments provided disease control sufficient to obtain consistent marketable cabbage heads, therefore, 15 weeks after planting, 5 heads were harvested per plot and weighed and the trial was concluded at this point. A number of treatments resulted in significantly increased plant fresh weight. The most effective treatment was incorporation of fluazinam into the transplant row with a reduced volume (500 L/ha) of water (Fig. 4). This treatment increased the average weight of cabbage heads by 1.9kg. Application of fluazinam in the same way at 3 L/ha in 100 L/ha or at 2 L/ha in 500 L/ha also significantly increased the average weight of cabbage heads compared with the untreated plants. Other treatments that significantly increased cabbage head weight included broadcast applications of lime (2.5t/ha, table 1), or calcium cyanamide (1 t/ha), calcium nitrate and lime and the spot plant drench (Shirlan control) application of fluazinam (Fig. 4).

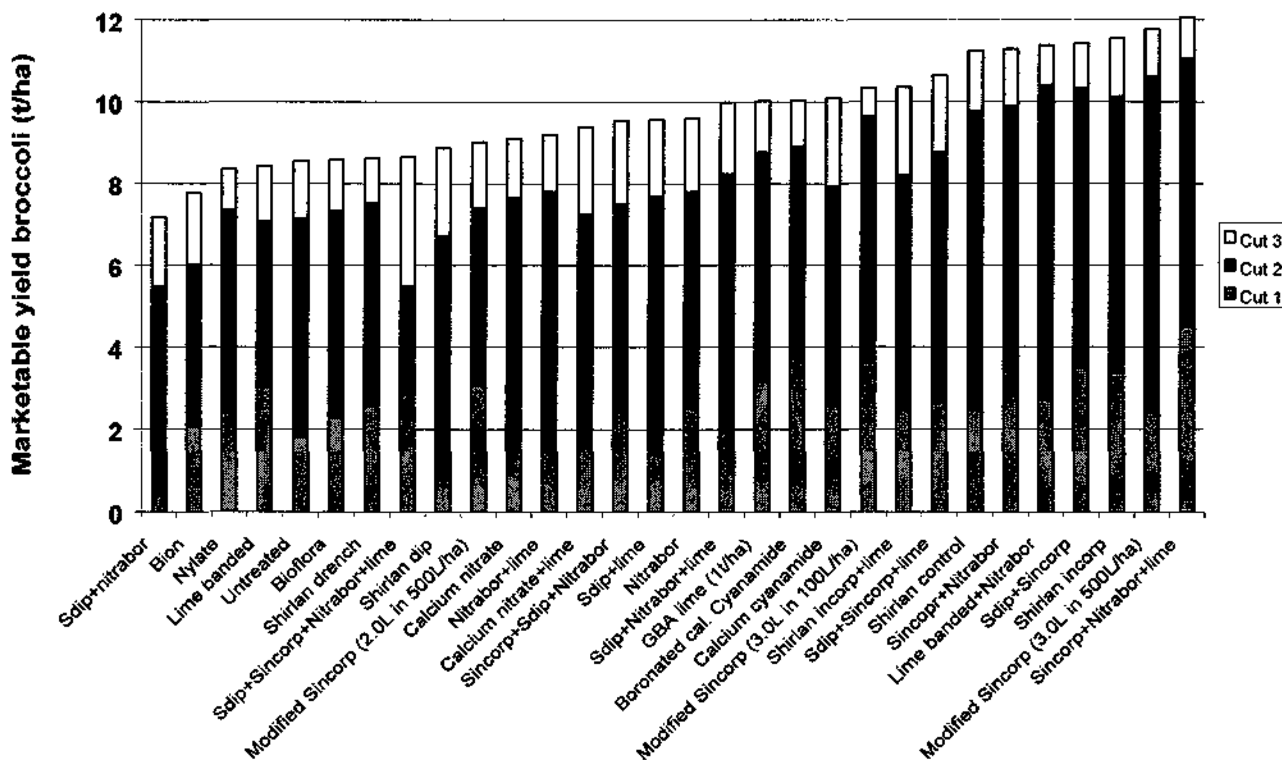

Figure 3: Effect of treatment on marketable yield of broccoli, Werribee 2000. LSD (p=0.05) = 2.7. (Sincorp = Shirlan incorporated into transplant row; Sdip = Shirlan transplant dip)

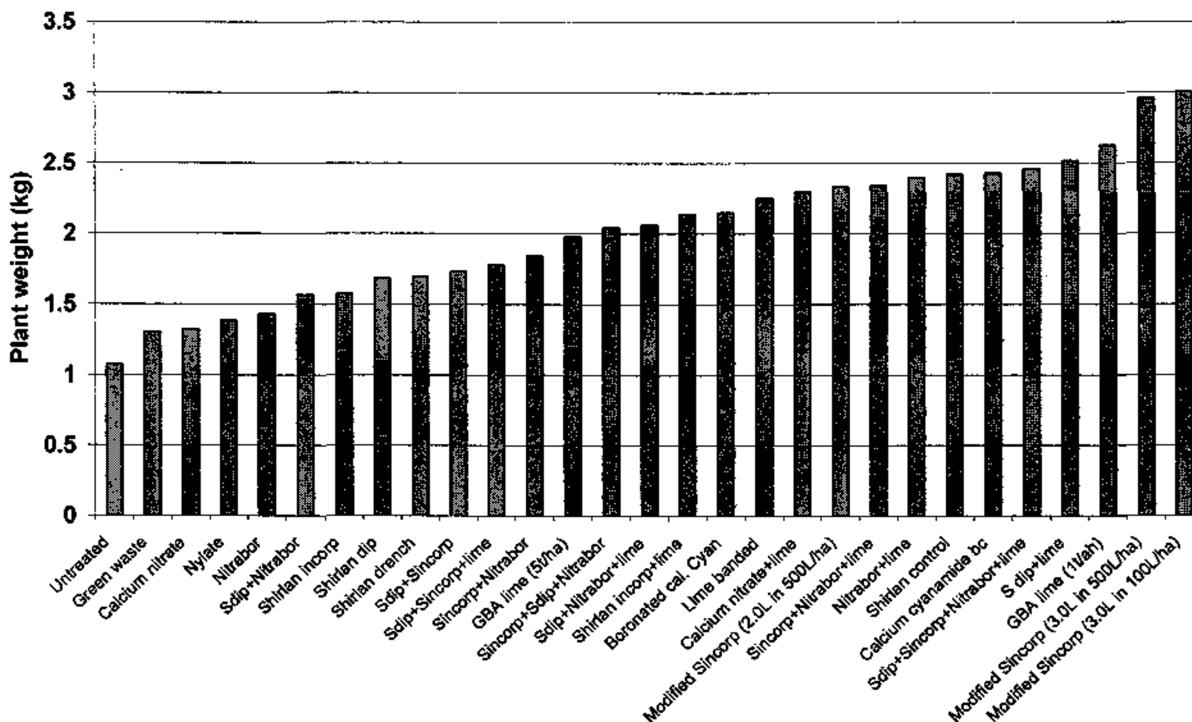

Figure 4: Effect of treatments on fresh weight of cabbages 15 weeks after planting.
LSD (p=0.05) = 1.10 (Sincorp = Shirlan incorporated into transplant row; Sdip = Shirlan transplant dip)

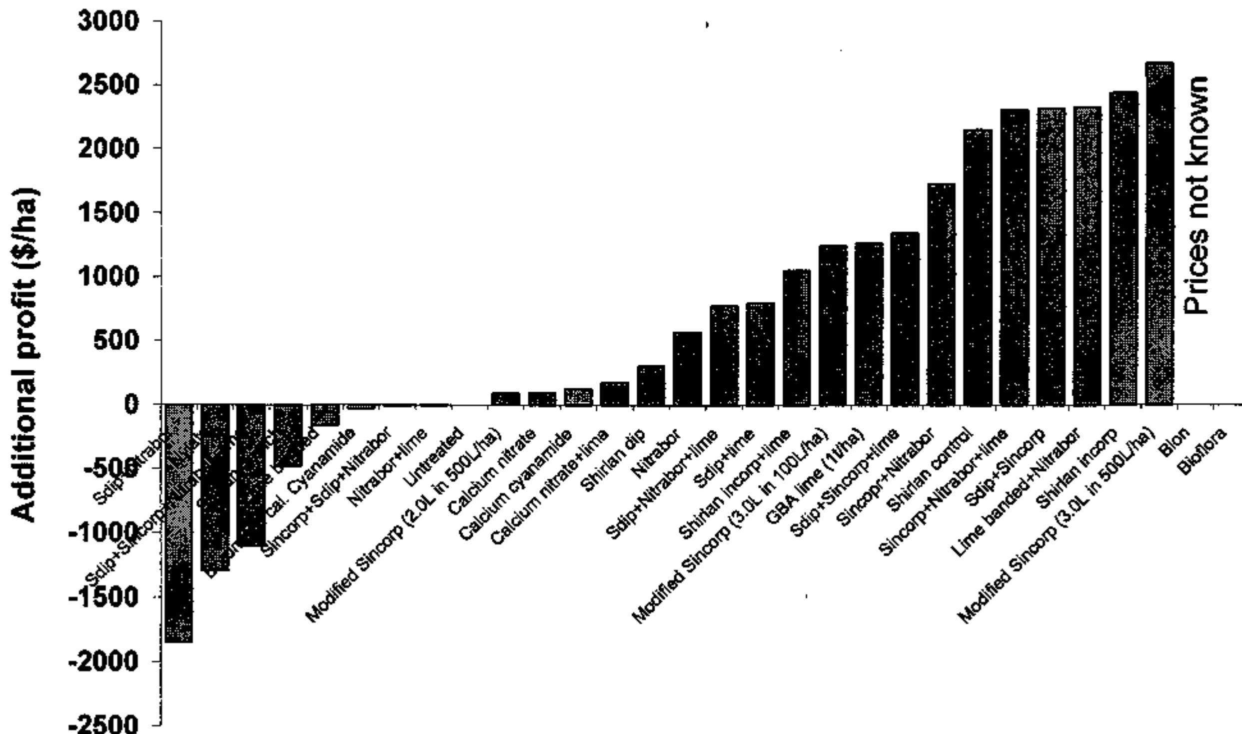

Figure 5: Additional profit, relative to untreated ($0) from treatment for clubroot, broccoli, Werribee 2000 (assuming returns $1/kg broccoli). (Sincorp = Shirlan incorporated into transplant row; Sdip = Shirlan transplant dip)

Table 1: Summary (factorial analysis) of main treatment effects from Victorian trials 2000.

Treatment	Clubroot score (Werribee 2000)		Yield of broccoli (t/ha) (Werribee 2000)		Clubroot score (Trentham 2000)		Fresh wt. cbbage (kg) (Trentham 2000)	
	-	+	-	+	-	+	-	+
Fluazinam (Shirlan®) band incorporated	4.6	3.6	9.1	10.6	7.1	6.6	1.9	2.0
Fluazinam (Shirlan®) dip	3.9	4.3	10.3	9.4	6.8	6.9	1.9	2.0
GBA lime broadcast	4.1	4.1	10.0	9.7	7.4	6.3	1.7	2.3
Nitrabor®	4.2	4.0	9.9	9.8	7.1	6.6	1.9	2.0
LSD (p=0.05)	0.5		1.0		0.7		0.4	

Table 2: Treatments used in Victorian trials, 2000.

TREATMENT	RATE	METHOD OF APPLICATION	COMBINATIONS
Limes GBA (Mitchells)	1t/ha, 2.5, 5.0 t/ha	broadcast (bc), incorp & irrigated 7-10 days before transplanting (DBT), also band incorporated AT.	+/- Shirlan®, Nitrabor®, Calcium nitrate
Nutrients Nitrabor®	125, 225 and 225 kg/ha	Split application (banded AT; 10 and 20 days after transplanting (DAT)).	+/- lime, Shirlan®
Campbells calcium nitrate	125, 225 and 225 kg/ha	Split application (banded liquid AT; solid 10 and 20 days after transplanting (DAT)).	+/- lime
Calcium cyanamide, boronated calcium cyanamide	1t/ha, equiv 1t/ha,	bc, incorp & irrigated 7-10 days BT Banded (machine 7-10 days BT)	
Bioflora	As recommended	Preplant incorporated into bands, foliar applications during crop.	
Green waste	25 t/ha	Broadcast and incorp. 7-10 days before planting	
Fungicides Shirlan spot drench (control)	3.0L/ha in 5000l/ha	100ml/plant AT	-
Shirlan continuous spray (drench)	3.0L/ha product in 2500L/ha water	Sprayed continuous band over plants AT	
Shirlan dip	0.5mL/L	Transplant dip 30 minutes	+/- Shir drench (incorp), Nitrabor®, lime
Shirlan incorporated	3.0L/ha in 2500L/ha water	Banded incorporation (machine) AT.	+/- Shir. dip, Nitrabor®, lime
Modified Shirlan incorp.1	3.0L/ha in 500L/ha water	Banded incorporation (machine) AT.	
Modified Shirlan incorp.2	2.0L/ha in 500L/ha	Banded incorporation (machine) AT.	
Modified Shirlan incorp. 3	3.0 L/ha in 100L/ha	Banded incorporation (machine) AT.	
Biocides Nylate	2000kg/ha as 10% dust	Banded incorporation AT.	
Acquired resistance Bion	5g a.i./100L & 50g a.i./ha	Preplant nursery drench & soil drench 1 WAT	

4.3.2 WESTERN AUSTRALIA

Clubroot has continued to spread in the Manjimup area and most farms are now considered to be infected. Most growers control the disease through crop rotation and raising soil pH. There has been little use of Shirlan® due to the application difficulties. Interest has increased in the product as the incorporation method of application (see section 5) has become available.

Overall awareness of clubroot and its management has increased substantially in the Manjimup area over the last three years. Awareness of the disease has also increased in other areas of WA (eg: Albany/Denmark).

Trial sites:

Vince Pedulla's, Manjimup 1998	Karri Loam
Tom Winfield's, Manjimup 1998	Karri Loam
Brad Wren's, Manjimup 1999	Gravel Loam
Tom Winfield's, Manjimup 1999	Karri Loam
Tom Winfield's, Manjimup 2000	Karri Loam

Summary of results:

- Crop rotation and liming are the most effective long term strategies for management of disease.
- Incorporation of fluazinam (Shirlan®) into the transplant rows was the most effective method of applying this product (Figs. 6 & 7).
- At least 500 L/ha of water should be applied with Shirlan® in the Manjimup area for effective incorporation of this product.
- E-2001, 'microbial enhancer' caused severe stunting in the plants in the first 6 weeks of the trial. The plants eventually grew out of the stunting but the harvest was delayed. Whilst control of clubroot appeared good from trial data in the year 2000 (Fig. 7), further work is needed before a recommendation could be made about this product as the roots never really grew enough for a large amount of clubroot infection to occur outside of the treated area.

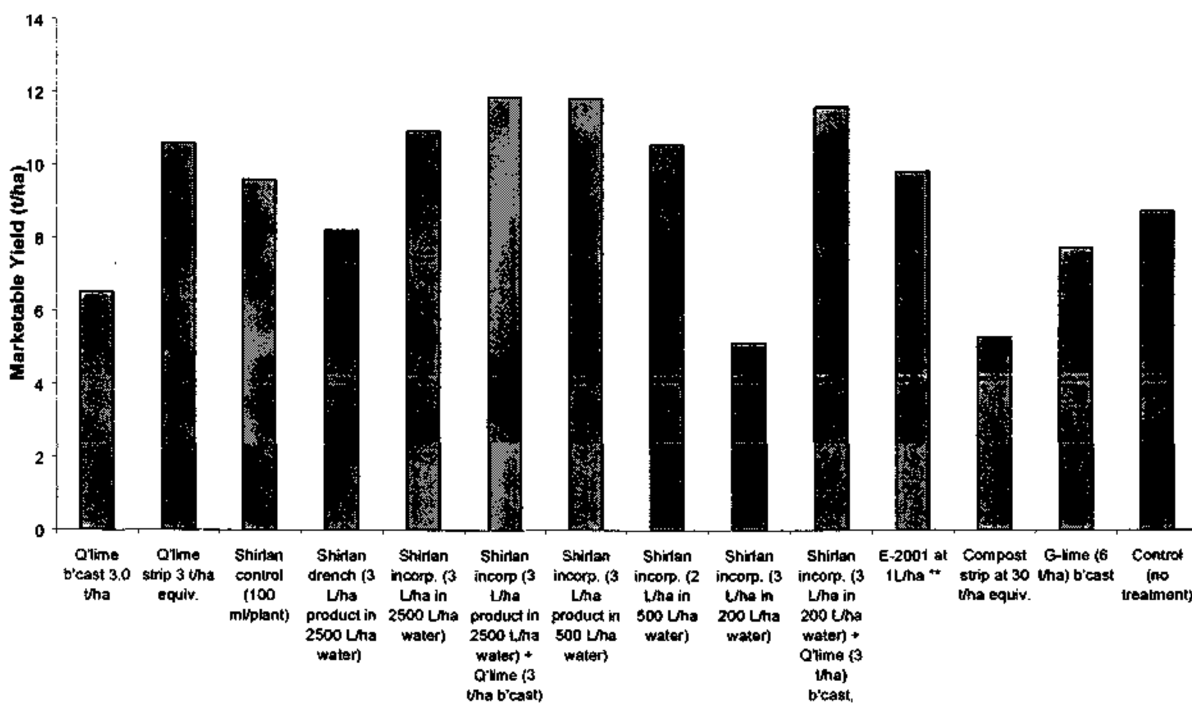

Figure 6: Effect of treatments on marketable yield of cauliflower, Manjimup, WA 1999/2000. LSD (p=0.05) = 2.57 **See note on E2001 in results summary.

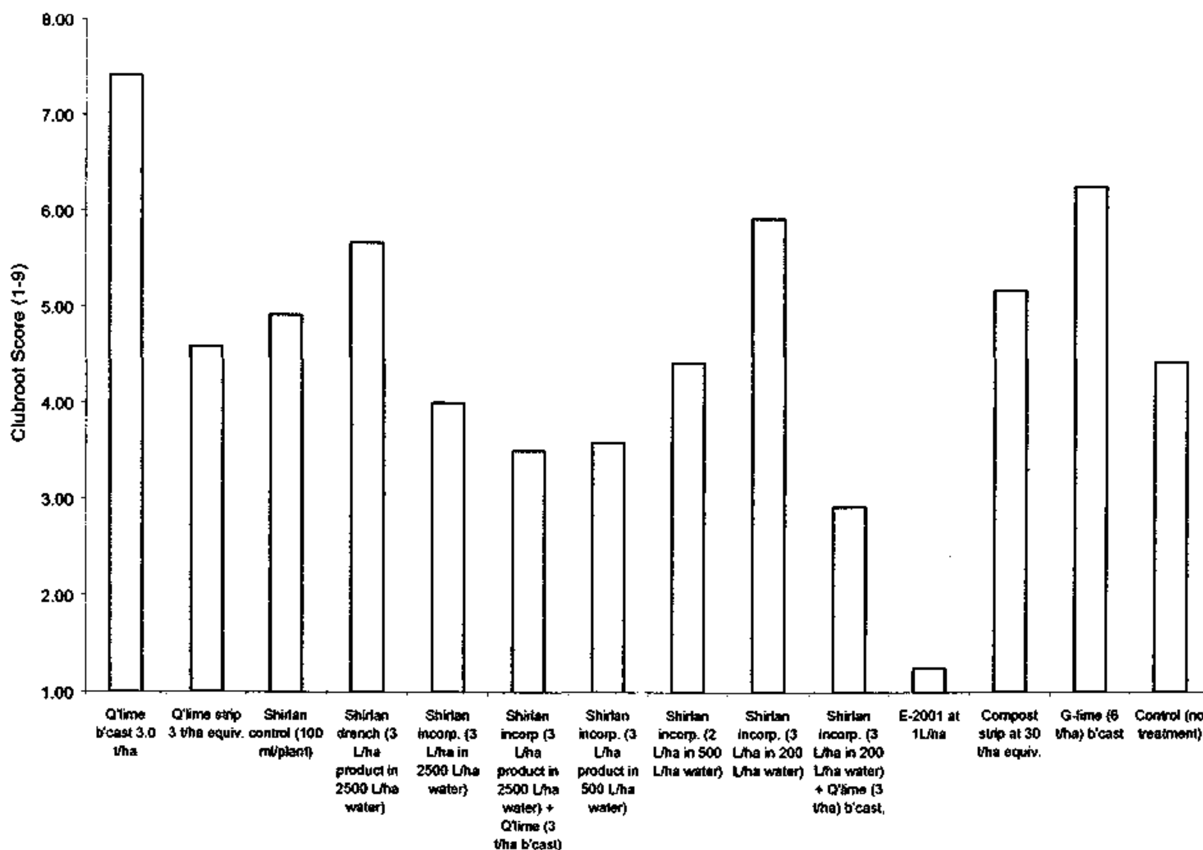

Figure 7: Effect of treatments on clubroot disease of cauliflower 6 weeks after transplanting, Manjimup, WA 1999/2000. LSD (p=0.05) = 2.8.

Table 3: Treatments used in Western Australian trials 1999/2000.

Two rows of cauliflower per plot. Four replicates.

T1 -	CaO (Quicklime) broadcast at 3.0t/ha, 2WBT
T2 -	CaO (Quicklime) strip at 3.0t/ha equivalent, 2WBT
T3 -	Shirlan control (3L/ha product in 5000L/ha water - 100ml/plant) at transplanting
T4 -	Shirlan drench (3 L/ha product in 2500L/ha water) over the top of plants along the length of row (immediately after transplanting).
T5 -	Shirlan incorporated (3L/ha product in 2500L/ha water applied prior to transplant and incorporated) (1DBT)
T6 -	Shirlan incorporated (3L/ha product in 2500L/ha water applied prior to transplant and incorporated) (1DBT) + quicklime (3.0t/ha incorporated 2WBT)
T7 -	Shirlan incorporated (3L/ha product in 500L/ha water) applied prior to transplant and incorporated (1DBT).
T8 -	Shirlan incorporated (3L/ha product in 500L/ha water applied prior to transplant and incorporated, 1DBT) + quicklime (3.0t/ha) broadcast, 2WBT.
T9 -	Shirlan incorporated (2L/ha product in 500L/ha water) applied prior to transplant and incorporated, (1DBT).
T10 -	Shirlan incorporated (2L/ha product in 500L/ha water applied prior to transplant and incorporated, 1DBT) + quicklime (3.0t/ha) broadcast, 2WBT.
T11 -	Shirlan incorporated (3L/ha product in 200L/ha water) applied prior to transplant and incorporated (1DBT).
T12 -	Shirlan incorporated (3L/ha product in 200L/ha water) applied prior to transplant and incorporated (1DBT) + quicklime (3.0t/ha) broadcast, 2WBT
T13 -	E-2001 microbial product to be applied at 160ml per plot.
T14 -	Compost applied at 30t/ha.
T15 -	G-lime broadcast at 6t/ha and incorporated 2WBT.
T16 -	G-lime applied in 25cm strip at an equivalent rate of 6 t/ha
T17 -	Control (no treatment)
T18 -	Control

Note:
2WBT = 2 weeks before transplanting.
1DBT = 1 day before transplanting.

4.3.3 QUEENSLAND

Since the initial outbreaks in 1997/98, clubroot has continued to spread in the Stanthorpe/Applethorpe area, with 22% of farms are now considered to be infected. The most severely infected farms and spot outbreaks are fumigated using metham sodium. Most growers continue to use this fumigant as it has been extremely effective not only controlling clubroot but also controlling weeds, which are a significant problem in the district. As field trials have only been conducted in Queensland for the last two years, metham sodium has given growers confidence that they can manage clubroot in the short term. The most recent trials confirmed that when applied correctly, Shirlan® can provide control of clubroot almost equal with low rate application of Metham sodium. The challenge for the future of the project in this state is to demonstrate to growers long term effectiveness of integrated control programs using limes, nutrients and fungicides. Researchers do not recommend continued frequent-long term use of metham sodium due to possible enhanced degradation after repeated application.

Trial sites:

Dario Semezin's, Applethorpe Queensland 1999	Sandy loam
Two trials Dario Semezin's, Applethorpe Queensland 2000	Sandy loam

Summary of results:

- All chemical soil treatments had a significant effect on increasing yield (Fig. 8) and profit margin (Fig. 10) of broccoli, when grown in soil containing *P. brassicae*, in comparison to untreated soil.
- Metham® sprayed onto soil then immediately incorporated by rotary hoeing at 500 litres or 350 litres per hectare increased yield by 92% and 74% (Fig. 8).
- Metham® applied through tines at the same rates increased yield by 59% and 39% respectively (Fig. 8).
- Shirlan® at 3 litres per hectare banded in planting rows increased yields by 54%, in comparison to untreated areas (Fig. 8).
- Plants in soil sprayed with Metham® applied at 500 litres or 350 litres per hectare then immediate incorporated by rotary hoeing, had significantly less clubroot than plants in soil treated with Shirlan® or untreated soils (Fig. 9).
- The number of plants harvested was significantly increased by all the Metham® and Shirlan® treated soils, except for the Metham® 350 litres per hectare applied through tines (Data not shown).

- Metham® 500 litres per hectare applied as a spray then immediately incorporated into soil by rotary hoeing significantly increased the average head weight per plant. This treatment also reduced the time from planting to harvest, shown by the significant increase in the number of plants harvested after the first 4 of 6 harvests (Data not shown).
- Phosphorous acid treatment of seedlings or vermicompost added to seedling mix had no effect on the number of plants harvested, head quality rating, total harvest or clubroot control of the cultivar Green Belt (Data not shown).

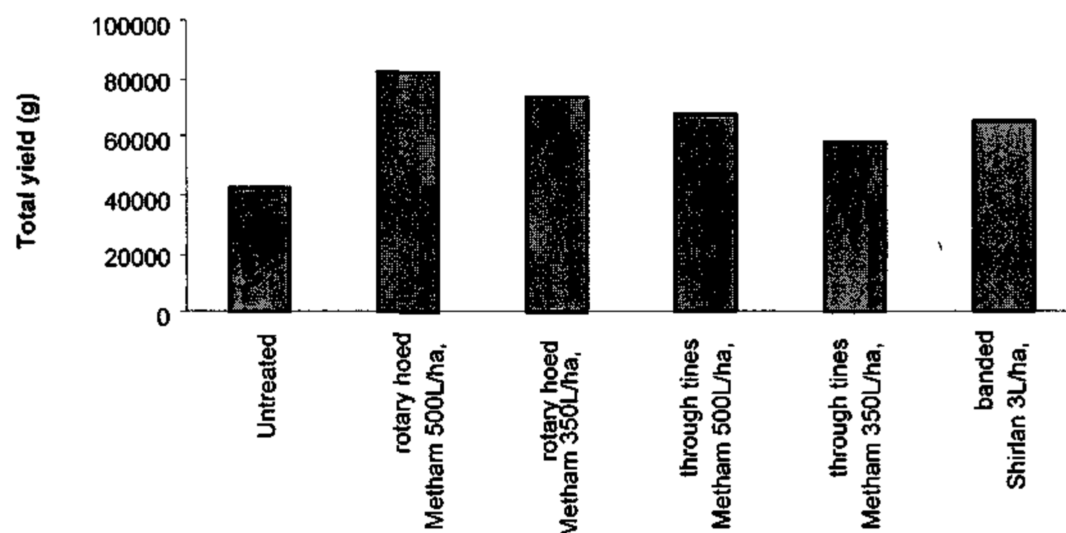

Figure 8: Effect of soil treatments on broccoli yield, Stanthorpe, QLD 1999/2000.

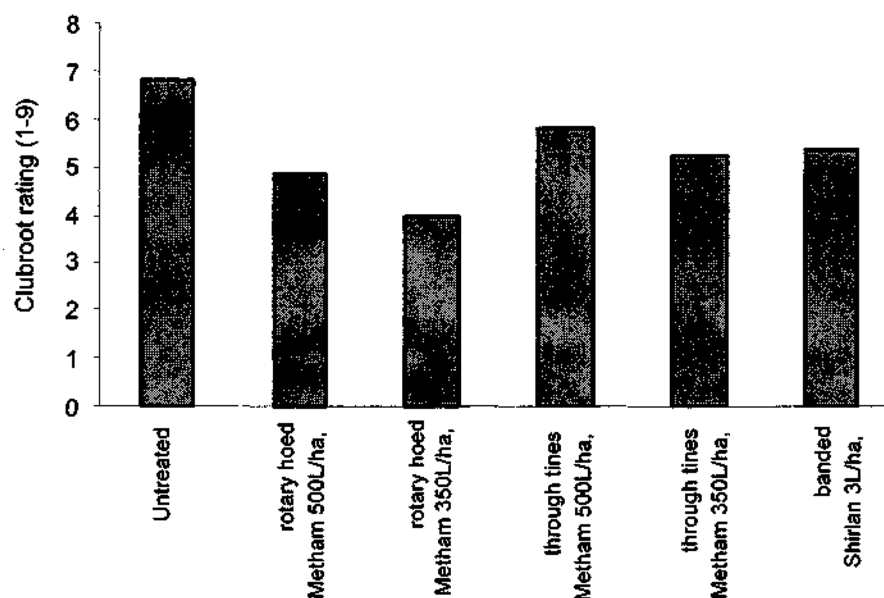

Figure 9: Effect of soil treatments on root galling of broccoli assessed at harvest, Stanthorpe, QLD 1999/2000.
(Clubroot rating refers to a scale of 1 to 9, where 1 = no clubs on roots and 9 = all roots with clubs.)

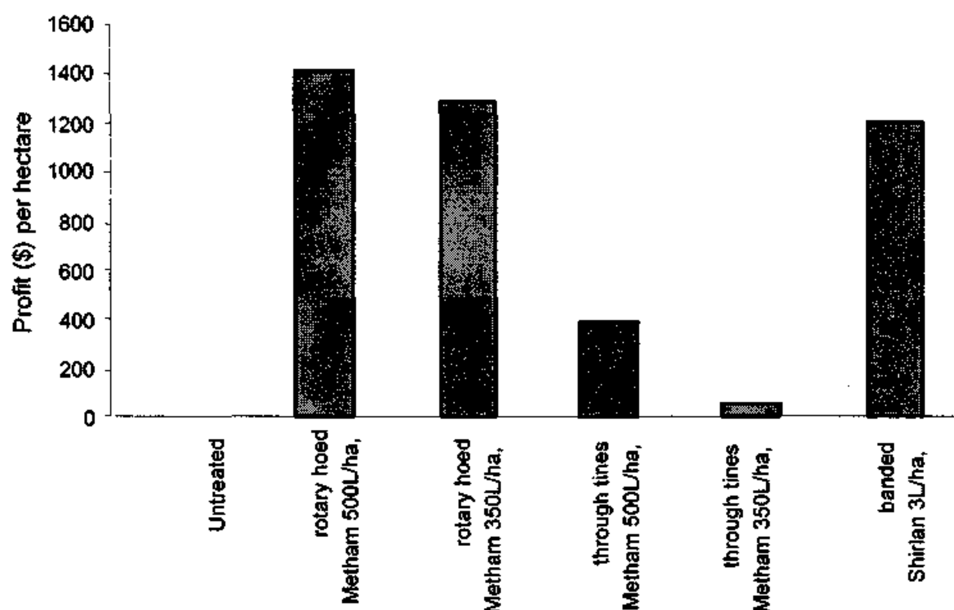

Figure 10: Effect of soil treatments on profit margin of broccoli grown in clubroot soil, Stanthorpe, QLD 1999/2000.

Table 4: Soil treatments used in Queensland 1999/2000.

Treatment	Rate	Method of application
1. Untreated		
2. Metham	500L/ha	Sprayed onto the soil surface of raised planting beds then immediately incorporated by rotary hoe.
3. Metham	350L/ha	Sprayed onto the soil surface of raised planting beds then immediately incorporated by rotary hoe.
4. Metham	500L/ha	Applied to raised planting beds through tines at 20cm depth.
5. Metham	350L/ha	Applied to raised planting beds through tines at 20cm depth.
6. Shirlan	3L/ha	Applied to raised planting beds as spray band (10cm) along (treated area) planting row with shallow incorporation by banding machine.

4.3.4 TASMANIA

Several environmental factors have created problems in the control of clubroot in the north west of Tasmania. Firstly, many of the local farms are on steep slopes. This posed difficulties for banding of treatments and lining up with treated areas. It was concluded that tractor slippage may have resulted in a number of treatments being planted outside of the treated bands. This together with the movement of treatments across beds (down the slope) may have caused the failure of all treatments to control the disease adequately on the steep Forthside site (Fig. 11). This was confirmed by the distribution of lime that could be seen in the red soils at this site.

In contrast, good control of clubroot using Shirlan® and lime (CaO) was achieved on the flat site at Harvest Moon (Fig. 12).

In 2000, a transplanter linked version of the prototype machine for incorporation of treatments into the transplant rows was used in Tasmania (Fig. 16A). This machine was developed to allow treatment and transplanting to be conducted in a single tractor pass, thereby ensuring that the plants are planted into the treated rows. Unfortunately, disease did not develop in the 2000 trial so this method has not yet been adequately demonstrated in Tasmania, although researchers expect that it will significantly improve the control of clubroot on the steep Tasmanian slopes.

A second difficulty has been the failure of the highly organic Krasnozem soils in the region to respond to amendment with lime. In general these soils have a high organic matter content and cation exchange capacity. Large amounts of lime are required to elicit a small change in soil pH. Lime cannot therefore be used routinely in these soil types because of the negative effects of high rate application of lime on soil micronutrients.

Trial sites:

Forthside Veg. Res. Station, Forth Tas, 1999	Red Kraznozem
Harvest Moon, Forth Tasmania, 1999	Sandy loam
Harvest Moon, Forth Tasmania, 2000	Sandy loam

Summary of results:

- Shirlan®, combined with lime (CaO), most effective treatment in both previously fumigated and unfumigated sites in the valley (Figs. 12 & 13).
- Poor control on steep slopes (Fig. 11), tractor slippage and treatment drift down slopes problematic. Product application problems need to be addressed with a single pass treatment and planting process to ensure plants are planted in treated areas.
- Large amounts of GBA lime (CaO), up to 10 t/ha required to elicit a small pH change from 5.8 to 6.5.

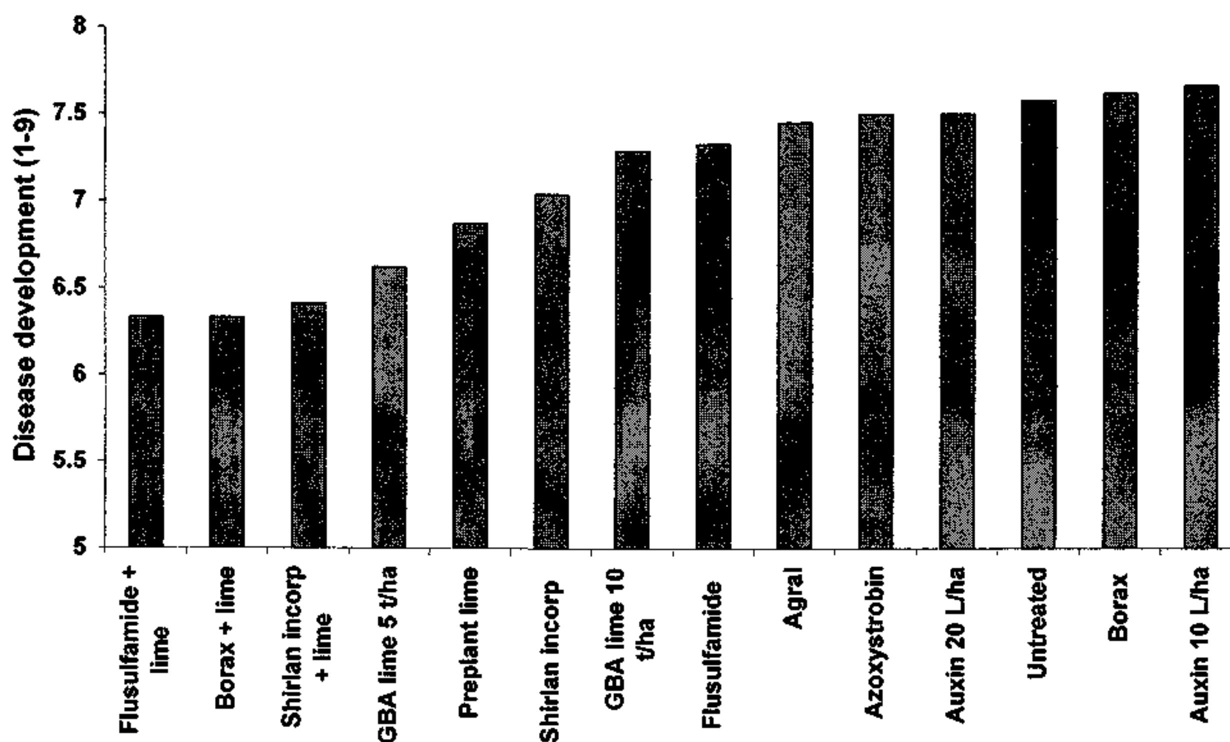

Figure 11: Effect of treatment on root galling of broccoli 6 weeks after planting, Forthside Vegetable Research Station, Tasmania 1999.

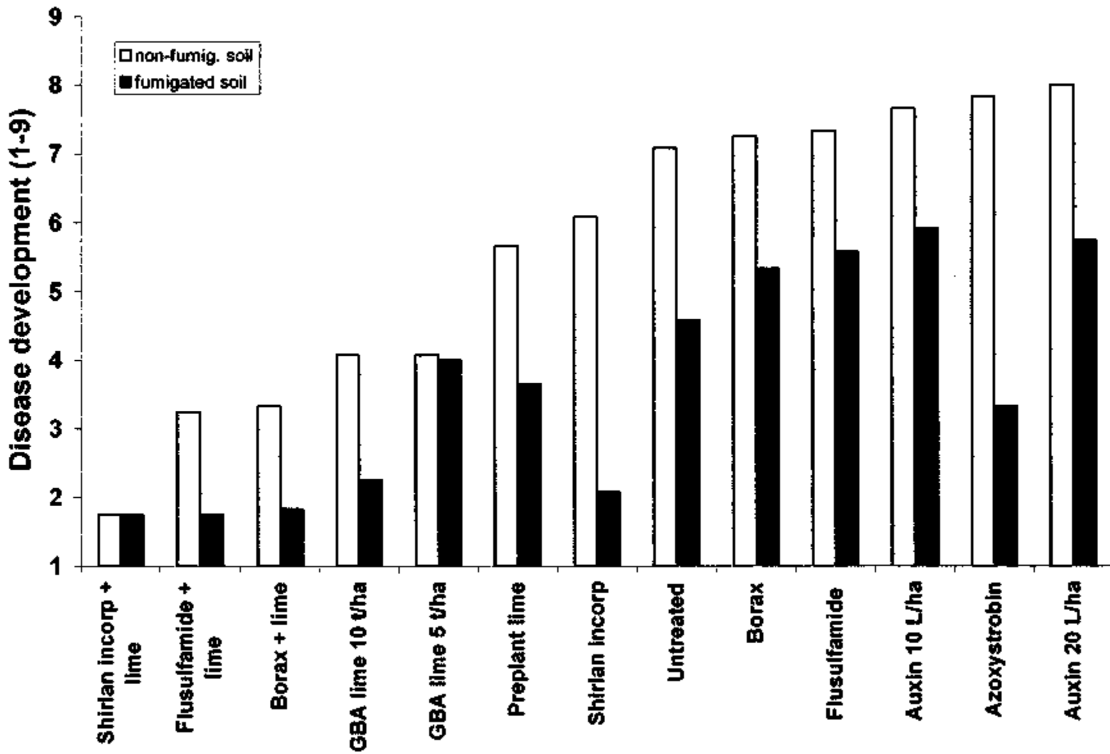

Figure 12: Effect of treatment on root galling of broccoli, 6 weeks after planting in previously fumigated (metham sodium) and non-fumigated soil. Harvest Moon trial, Tasmania 1999.

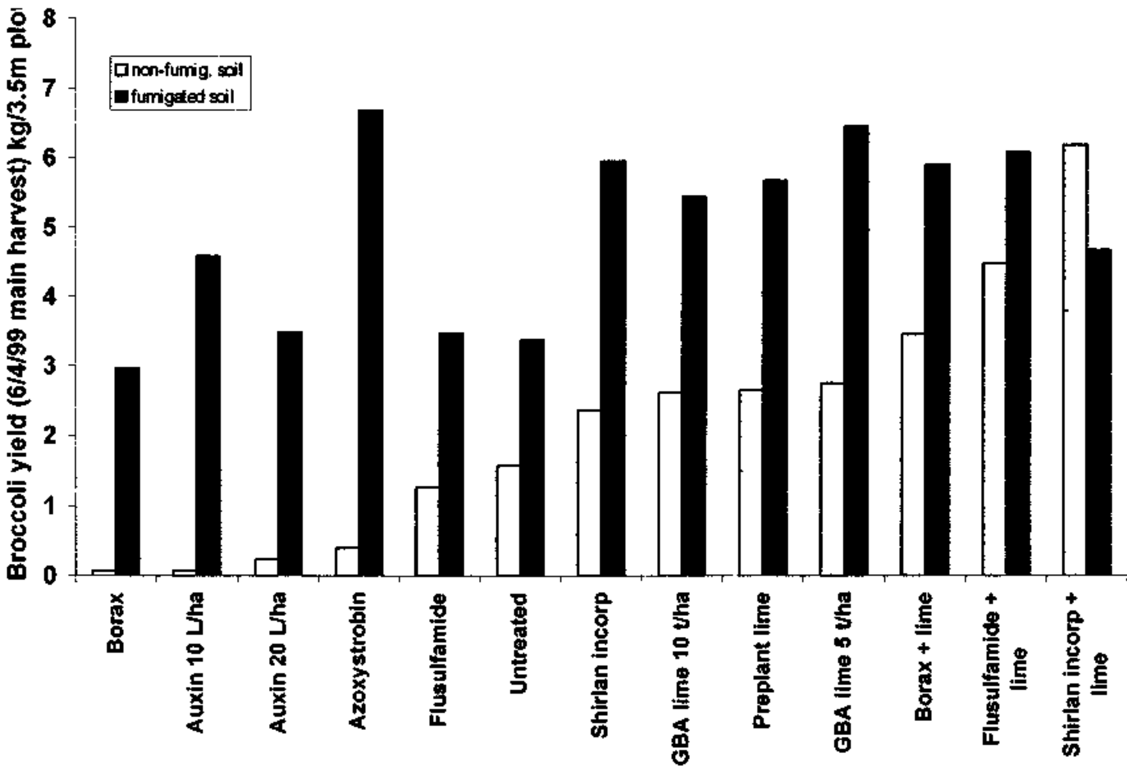

Figure 13: Effect of treatment on broccoli yield from previously fumigated (metham sodium) and non fumigated soil. Harvest Moon trial, Tasmania 1999. LSD (P=0.05) = 2.05

Table 5: Treatments used in Tasmanian clubroot trials 1999 (DBT= days before transplanting)

Treatment	Rate	Method of application
Control	Untreated	
GBA lime	5 t/ha	Broadcast and incorp 7-10 DBT
GBA lime	10 t/ha	Broadcast and incorp 7-10 DBT
Shirlan + lime	Shirlan 9 L/ha Lime 10 t/ha	Sprayed over entire bed and incorp preplant Broadcast and incorp 7-10 DBT
Flusulfamide + lime	Flusulfamide 9 L/ha Lime 10 t/ha	Sprayed over entire bed and incorp preplant Broadcast and incorp 7-10 DBT
Borax + lime	Borax 6 kg/ha in 9 L water/plot Lime 10 t/ha	Borax 0, 10 and 20 days after transplant Lime broadcast and incorp. 7-10 DBT
Preplant lime	10 t/ha	Broadcast and incorp preplanting
Shirlan	9 L/ha	Sprayed over entire bed and incorp preplant
Flusulfamide	9 L/ha	Sprayed over entire bed and incorp preplant
Borax	Borax 6 kg/ha in 9 L water/plot	0, 10 and 20 days after transplant
Agral	17 L/ha (applied in 9 L water/plot)	Watered in 4 and 6 weeks after transplant
Azoxystrobin	9 L/ha	Sprayed over entire bed and incorp preplant
Auxin	10 L/ha	Watered in 4 and 6 weeks after transplant
Auxin	20 L/ha	Watered in 4 and 6 weeks after transplant

4.3.5 NEW SOUTH WALES

Clubroot is a severe problem in both the Sydney basin and Bathurst regions of New South Wales. Rain has caused ongoing problems for NSW trials. One trial has been completed, however, treatment application was restricted due to wet weather (see note below). Unfortunately the clubroot trials planned for late summer and autumn 2000 were a wash-out due to the wettest autumn for 36 years. Although severe clubroot was present at the Castlereagh site in late autumn-early winter, trials were not undertaken to avoid potential criticsm if products did not perform to expectations in cold weather.

Current indications are that the integrated control program developed in Victoria will be directly applicable in NSW, however, this has not yet been fully demonstrated in trial work.

Trials: Rod Sherriff's property, Castlereagh, NSW 1998.
Fine sandy alluvial loam.

Summary of results:

- A number of treatments significantly reduced disease development and increased yield of cauliflowers (Figs. 14 & 15).
- Of these the most effective treatments included application of metham sodium (Figs. 14 & 15).
- In spite of substandard method of application (see note below), the Shirlan® and lime treatment reduced disease and significantly increased marketable yield of cauliflowers (Fig. 14 &15).
- Organic products Dynamic Lifter® and Neem® were ineffective (Figs. 14 & 15).

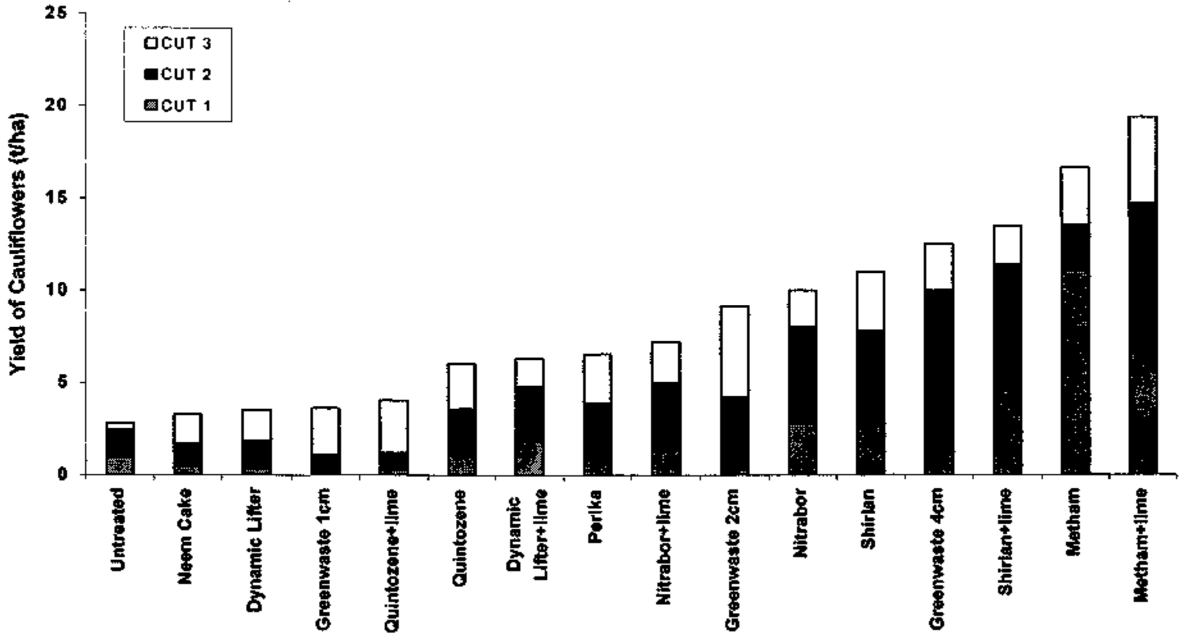

Figure 14: Effect of treatments on yield of cauliflower, Castlereagh NSW 1998.

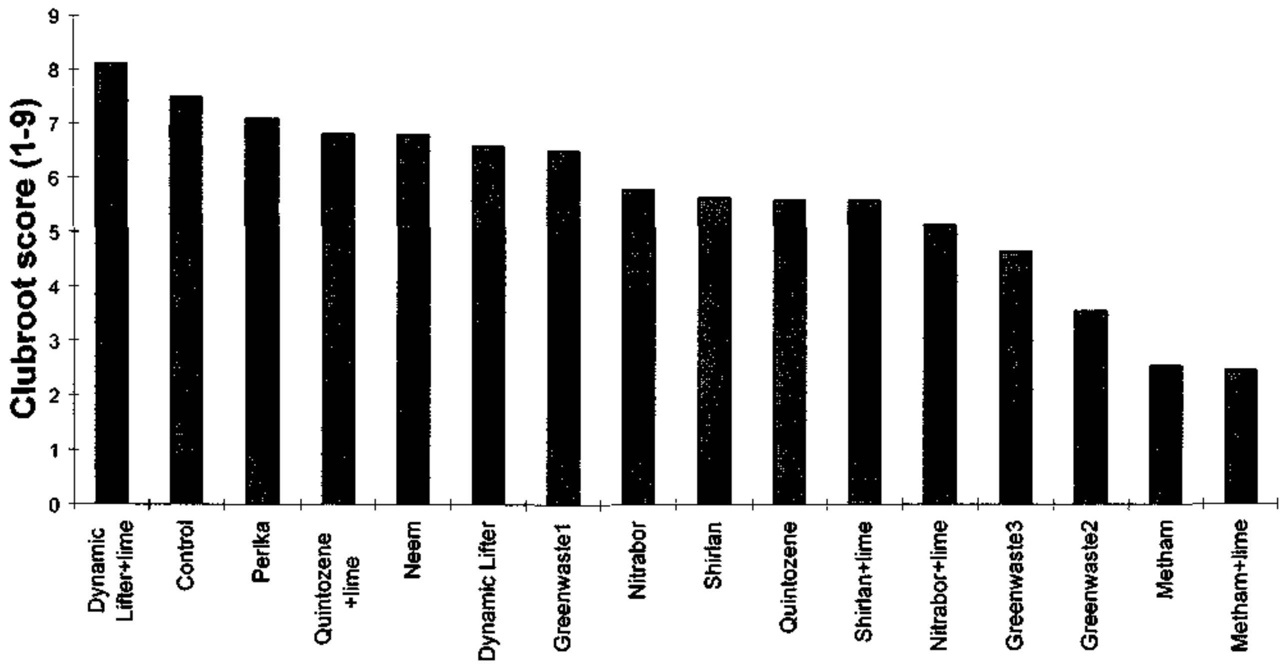

Figure 15: Effect of treatment on root galling of cauliflowers 7 weeks after planting, Castlereagh, NSW 1998.

Table 6: Treatments used in NSW clubroot trial 1998.

TREATMENT	RATE	METHOD OF APPLICATION
CONTROL		Untreated
METHAM	Equiv. 1.5L/10m^2	Banded in furrow and irrigated 10 days before transplanting
METHAM +LIME	Equiv. 1.5L/10m^2 + equiv 1.25 t/ha	Metham as above Lime incorporated into transplant rows immediately before transplanting.
NITRABOR	225, 25 and 225kg/ha	Split applications (side dressed 10,20 and 35 days after transplanting)
NITRABOR+LIME	As above + equiv of 1.25 t/ha	Nitrabor as above Lime incorporated into transplant rows immediately before transplanting.
DYNAMIC LIFTER	Equiv. 1t/ha	Incorporated into transplant rows immediately before transplanting.
DYNAMIC LIFTER+LIME	As above + equiv of 1.25 t/ha	Incorporated into transplant rows immediately before transplanting.
PERLKA	Equiv. 1t/ha	Incorporated into transplant rows and irrigated 10 days before transplanting
NEEM	Equiv. 1t/ah	Incorporated into transplant rows immediately before transplanting
SHIRLAN	25ml/100L	100ml per plant drench 5 days after transplanting
SHIRLAN+LIME	As above + equiv of 1.25t/ha	Shirlan as above Lime incorporated into transplant rows immediately before transplanting.
QUINTOZENE	Equiv. 20kg/ha	Incorporated into transplant rows immediately before transplanting
QUINTOZENE+LIME	As above + equiv of 1.25t/ha	Quintozene as above Lime incorporated into transplant rows immediately before transplanting.
GREEN WASTE1	1cm covering	Broadcast over plots 4 days before transplanting
GREEN WASTE2	2cm covering	Broadcast over plots 4 days before transplanting
GREEN WASTE3	4cm covering	Broadcast over plots 4 days before transplanting

NOTE:

- All treatments were rotary banded with prototype machine before immediately following with transplanter.
- "Lime" refers to Hydrated lime used at half the recommended Ag lime rate.
- At transplanting applications of Nitrabor® and Shirlan® were delayed due to wet conditions. The 10 day application of Nitrabor® was therefore doubled to compensate for this. However, this subsequently proved phytotoxic.

4.4 Discussion

In Victoria and Western Australia, consistent field results have been obtained over a number of seasons. Fluazinam incorporated into the transplant row is currently the most effective treatment in these states when crops are under high disease pressure. Application of lime (particularly calcium oxide based limes) remains good value for money and continues to return a profit at most field sites. Lime may be used effectively alone or together with crop rotation where disease pressure is low. In Queensland, recent new infections have directed project activity towards demonstrating effective eradication of spot infections. This has been achieved through use of metham sodium. Recent trials have demonstrated the effectiveness of incorporation of fluazinam into the transplant row, however, further work demonstrating sustained benefits of integrated control strategies will be required in both Queensland and New South Wales to increase grower confidence in sustainable integrated management strategies. Preliminary work suggests that on flat ground and valley basins NW of Tasmania, integrated strategies developed in Victoria and Western Australia can be directly applied. However, product placement is the major issue facing Tasmanian producers farming the steep kraznozem slopes of the Forth district. This has been addressed by the development of a tractor mounted tool bar to fit between the tractor and transplanter enabling treatment and transplanting to be conducted in a single tractor pass eliminating the potential for transplants to be planted outside the treated area due to tractor slippage.

Recommendations arising from this work have been summarised and distributed to brassica growers nationally in the pamphlet "A guide to the prevention and management of clubroot in vegetable brassica crops".

5 Application Technology

5.1 Summary

A method of incorporation of products into the transplant row was developed and evaluated during the life of the project. This method of application has:

- reduced the cost of treatment (Perlka®)
- improved product distribution and efficacy (Shirlan®)
- minimised the impact of residues from treatments on the environment

This method of application is now the most effective method of applying fluazinam (Shirlan®) and the most cost effective method of applying calcium cyanamide (Perlka®) for clubroot control.

In addition, when used to apply base fertiliser, this method improved the vigour of winter grown cauliflowers in Western Australia, increased final yield by 10 t/ha and reduced the time to harvest by 9 days.

The final prototype machine developed during the life of the project enabled treatment and transplanting to be conducted simultaneously. This represents a further saving to the grower of labour, time and fuel. Many growers have begun to modify their transplanting equipment to accommodate this method of treatment application.

5.2 Materials and Methods

Four commercial banding machines called "precision incorporators" (Fig. 16) were developed, produced and used in field trials conducted on commercial crucifer growing properties in Victoria, Western Australia, New South Wales, Tasmania and Queensland (see section 4.2) for site and assessment details).

The machines incorporated treatments to an approximate depth of 15 cm in two or three bands (the transplant rows). Early versions of the machines (Fig. 16B) were designed to incorporate products into 23 cm wide bands. This width was subsequently reduced to further reduce treatment cost to 12.5 cm (Fig. 16A).

Calcium cyanamide and lime were applied at a reduced rate (compared with the broadcast rate) 7-10 days before transplanting. The actual rate was approximately one third of the broadcast rate but was calculated for each trial based on the width of the treated area (band width). The fungicide fluazinam and other fertilisers were applied, immediately before transplanting.

Application of lime and fertiliser treatments was compared with the standard broadcast (calcium cyanamide and lime) or strip application (base fertiliser) (see section 4.2.3 for definitions). Banded incorporation of fluazinam was compared with the recommended spot drench 100 ml/plant (control) or the "grower preferred" continuous seedling spray, a continuous spray over the plants, applied immediately after planting.

A reduced rate of fluazinam (2 L/ha) together with reduced volumes of water (500 L/ha and 100 L/ha) were also compared with the standard 3 L/ha in 2500 L/ha incorporated into the transplant rows.

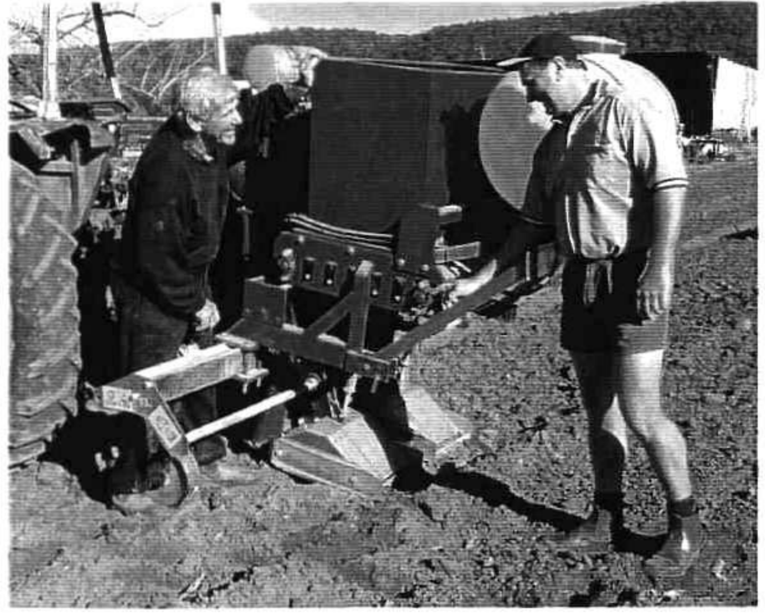

Figure 16: Precision incorporation machines designed to incorporate products into the transplant rows for improved clubroot control. A) Victorian machine designed to operate between tractor and transplanter for single pass operation B) NSW machine.

5.3 Results

5.3.1 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 reduced the cost of treatment by approximately two thirds, and has increased the profitability of the crop by up to 100% (Fig. 17).

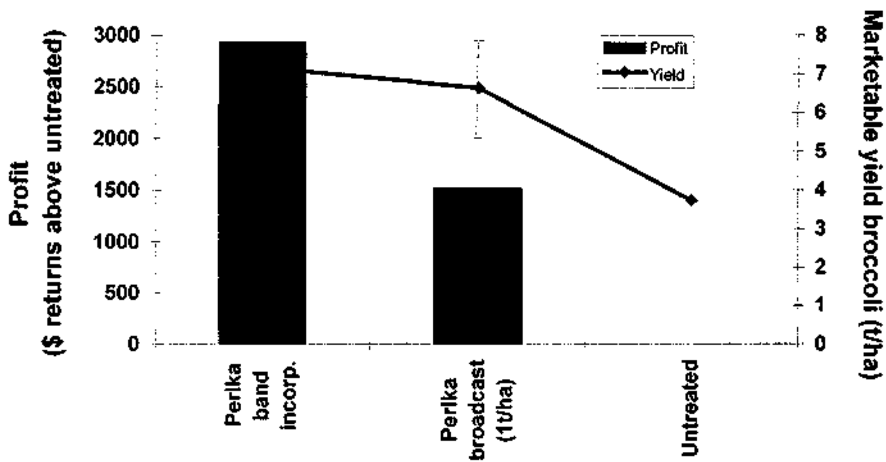

Figure 17: The effect of application method on the marketable yield (t/ha) and profitability ($/ha) of broccoli from Perlka® treated soil (based on $1/kg), Lindenow VIC 1997.

5.3.2 Improving product distribution and efficacy

5.3.2.1 Fluazinam (Shirlan) for control of clubroot

Fluazinam (Shirlan®) is a very effective treatment for controlling clubroot **only** when the product is distributed around the root zone at planting. The effectiveness of traditional continuous seedling sprays over the plants after transplanting, or spot drenches (100 ml/plant) applied at transplanting depends on the soil infiltration characteristics. In general, distribution is better in sandy soils than in heavy clay loams.

Incorporation of fluazinam into bands immediately before transplanting ensured that the product was evenly distributed around the root zone of the transplant where it could protect the roots from infection (Fig. 21). This method of application was extremely effective in trial work (Figs 18, 19 & 20) and significantly reduced the amount of water needed to apply the product from 2500 L/ha to 500 L/ha (Fig. 18).

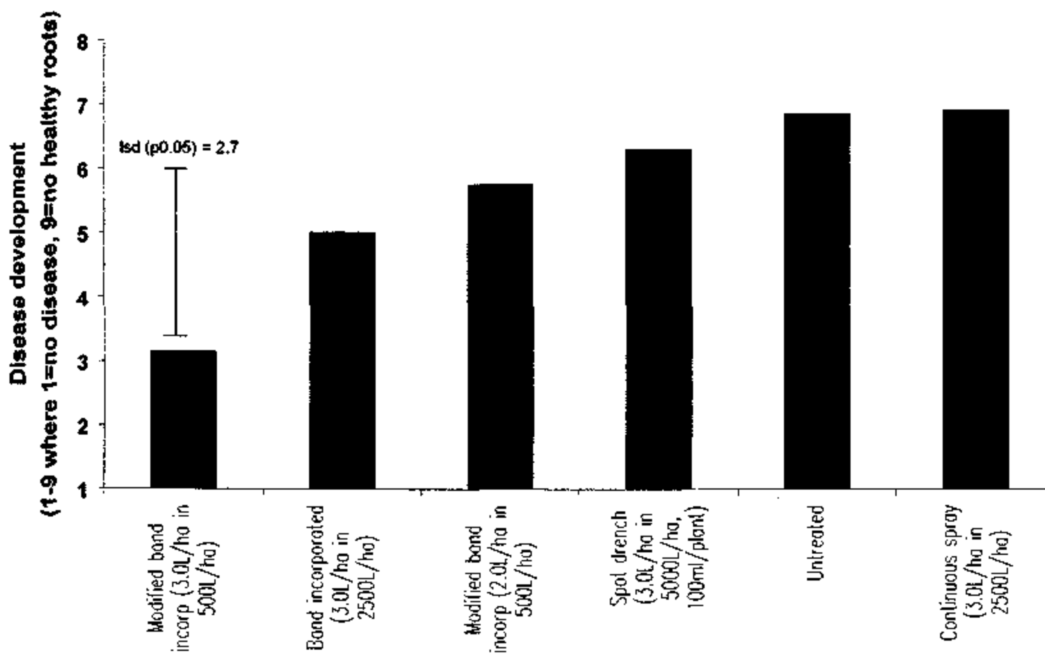

Figure 18: Effect of the method of application of fluazinam on the development of clubroot disease in cabbage (assessed immediately after harvest), Trentham VIC, 1999.

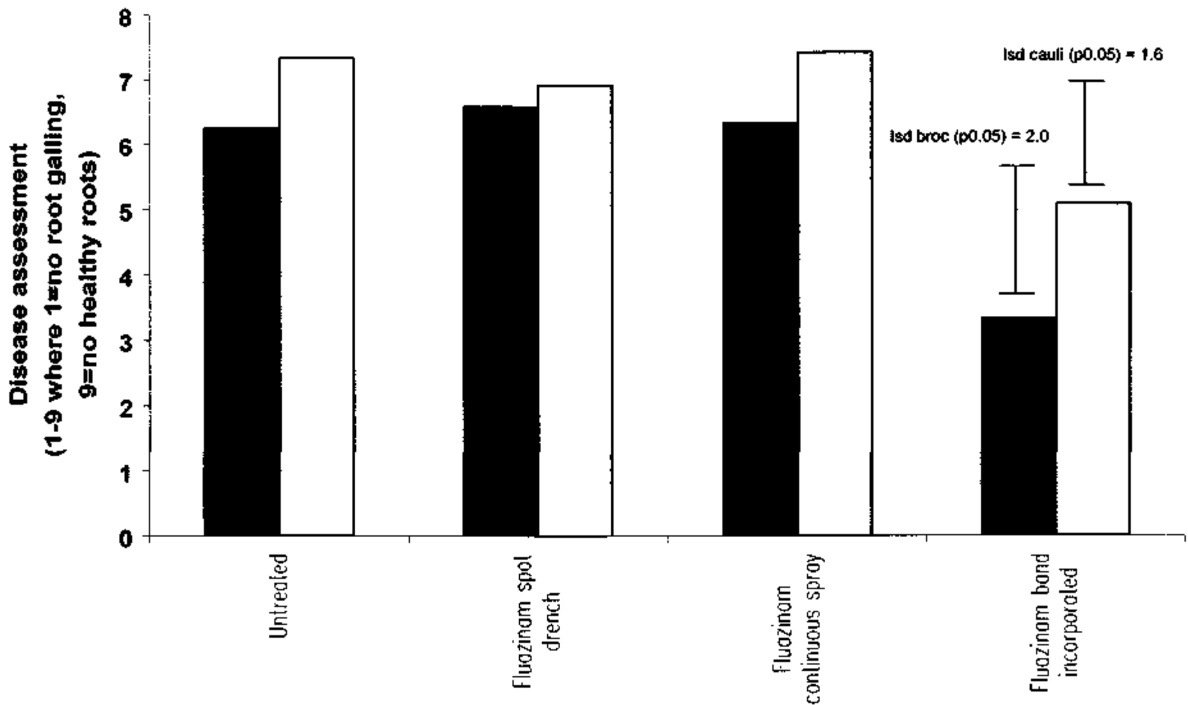

Figure 19: Effect of the method of application of fluazinam on the development of clubroot on broccoli ■ and cauliflower □ grown in field soil naturally infested with *P. brassicae*. Manjimup, Western Australia 1999.

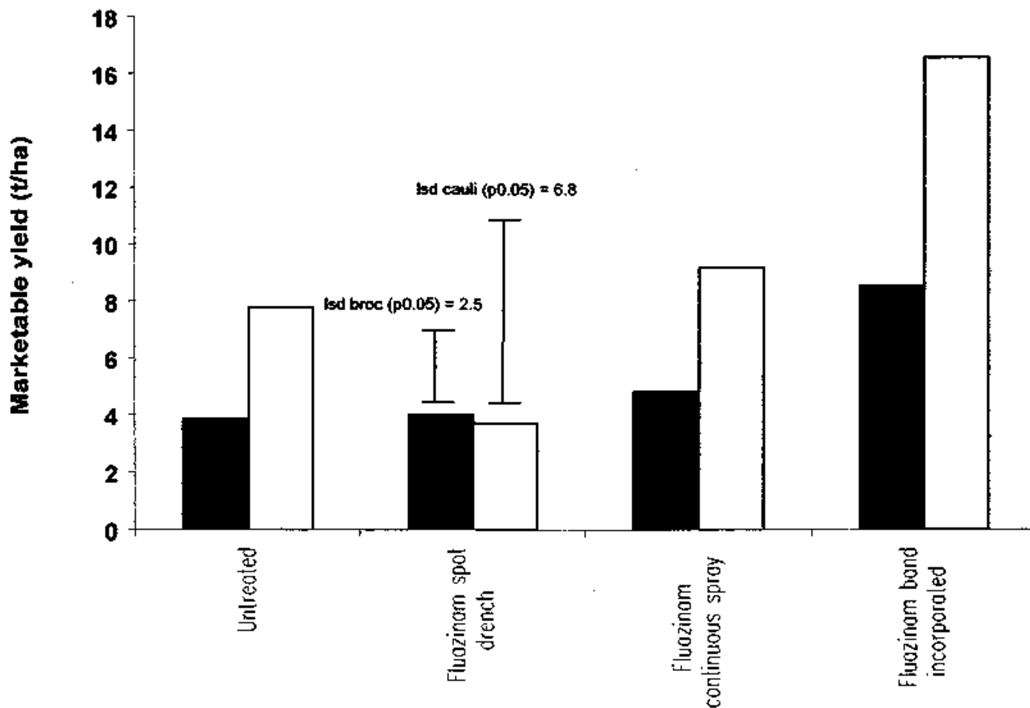

Figure 20: Effect of the method of application of fluazinam on the marketable yield of broccoli ■ and cauliflower □ grown in field soil naturally infested with *P. brassicae*. Manjimup, Western Australia 1999.

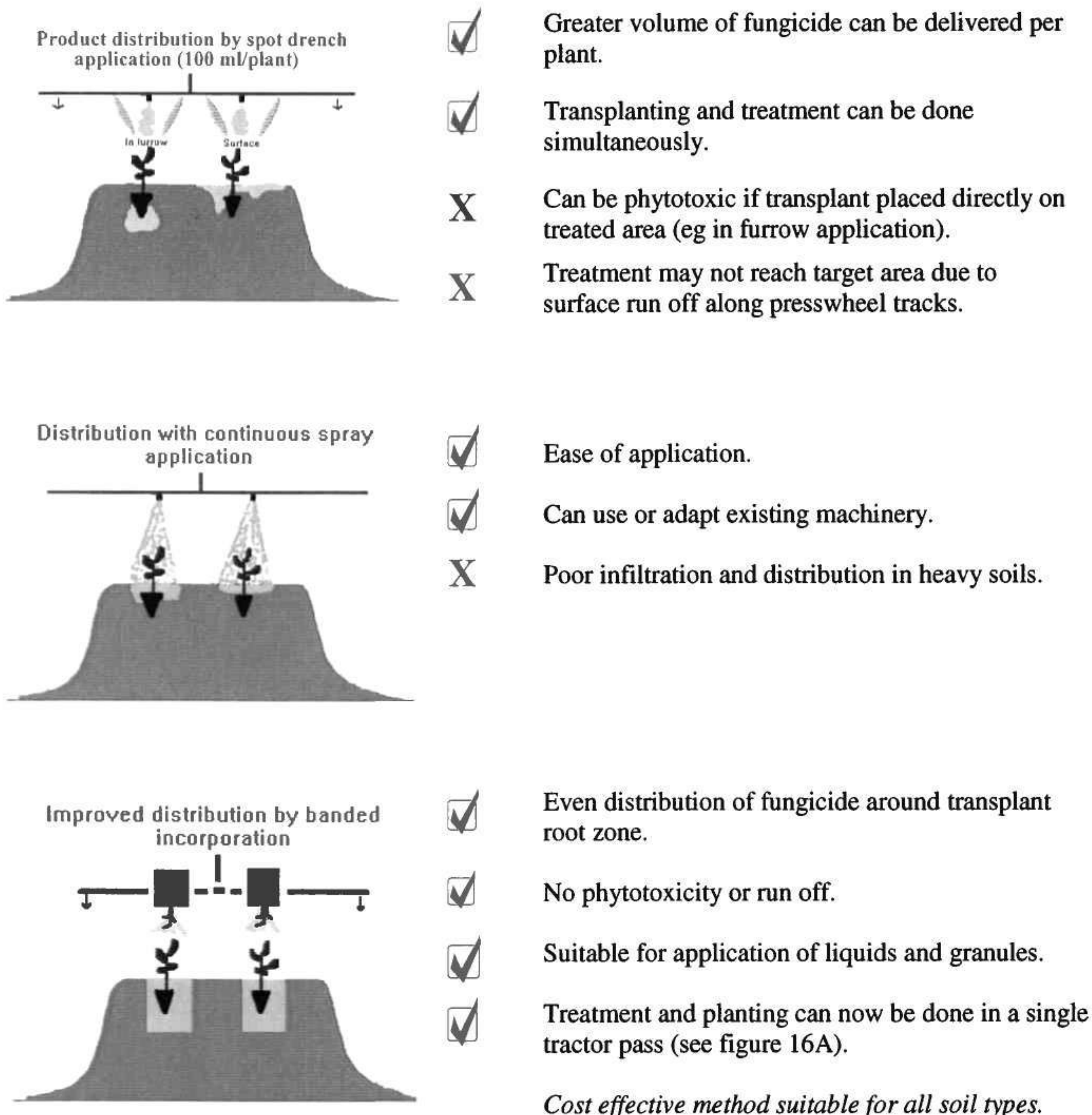

Figure 21: The effect of different methods of application on distribution (and efficacy) of fluazinam (Shirlan®).

5.3.2.2 Base fertiliser for improved vigour in winter grown cauliflowers

Having been used throughout the summer to demonstrate improved methods of application of fluazinam, the machine was evaluated in winter trials conducted in Western Australia to determine its ability to improve fertiliser use efficiency in export cauliflowers.

The machine was used to incorporate base fertiliser into the transplant row. This method was compared with the current method of banding fertiliser slightly below and offset from the transplants (strip application).

Incorporation of the fertiliser into the transplant row increased total yield of cauliflower (cv. Virgin) by approximately 10 t/ha and caused the crop to mature nine days earlier than the crop treated in the traditional way (Fig. 22). This effect was thought to be the result of slow growing winter transplants having immediate access to the fertiliser that was incorporated into the transplant row, compared with the fertiliser bands which may not be reached by the transplant roots until 4-6 weeks after planting.

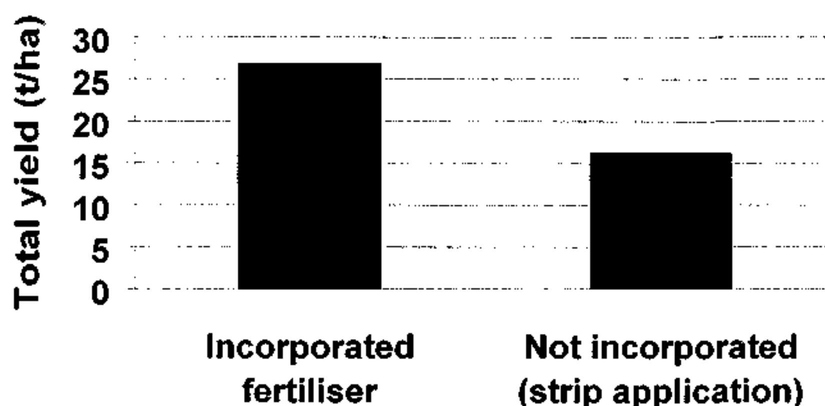

Figure 22: Effect of the method of application of base fertiliser on the yield of winter grown cauliflower (cv. Virgin), Manjimup, WA 1999.

5.3.3 Reducing soil residues

The efficacy of either lime (CaO) 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. Whilst increasing soil pH to 7-7.5 is desirable to prevent clubroot in brassica crops, high residual soil pH can be undesirable in some crops that are grown in rotation with brassicas. Potatoes for example, are more susceptible to scab diseases at high soil pH.

5.4 Discussion

Banded incorporation into the transplant row is now the most effective method of application of fluazinam (Shirlan®) and the most cost effective method of applying calcium cyanamide (Perlka®) for clubroot control.

In addition, when used to apply base fertiliser, this method improved the vigour of winter grown cauliflowers in Western Australia, increasing final yield by 10 t/ha and reducing the time to harvest by 9 days.

The final prototype machine developed during the life of the project enables treatment and transplanting to be conducted simultaneously. This represents a further saving to the grower in labour, time and fuel. Many growers have begun to modify their transplanting equipment to accommodate this method of treatment application. This method of application may also be suitable for treatment of other soilborne diseases in row crops.

6 Population Variation in *P. Brassicae*

6.1 Summary

A large number of 'races' of *P. brassicae* (the cause of clubroot disease) are known to exist worldwide. In Australia, it has been necessary to determine the extent of variation in our pathogen population to enable screening or breeding of tolerant crops to yield varieties that are commercially useful (ie. tolerant of the main "races" of clubroot in this country).

Screening was conducted using the European Clubroot Differential (ECD) Series of brassica hosts (an internationally accepted series of 15 standard hosts). Sixteen triplet codes were identified from 23 pathogen collections from 5 states of Australia. Data appear to support the existence of at least three distinct pathotypes within Australia.

A new molecular screening procedure for *P. brassicae* was developed in a complementary program (VG 99008). The method, known as 'microsatellite analysis' works by comparing the DNA from the pathogen collections. Similar collections will have similar patterns of DNA when visualised on a gel. This method was found to enable discrimination of *P. brassicae* isolates to levels consistent with that currently available using the ECD series. This molecular approach may therefore be a viable alternative to the time- and labour-intensive ECD method. As it is Polymerase Chain Reaction (PCR) based, it is fast, sensitive, and only requires small amounts of starting material.

6.2 European Clubroot Differential studies

6.2.1 Materials and Methods

Infected root material was collected from a range of commercial crucifer crops from different crucifer growing regions within Australia. Collections from new sites of infection were made as soon as disease symptoms became obvious. This material was catalogued and stored at -2°C for not longer than one year before use.

Screening studies were conducted with seeds of the ECD series (Buczacki *et al.*, 1975) obtained from Horticultural Research International, Wellesbourne, England. Three seeds were sown 2-3 cm deep into 125 mm diameter pots containing pasteurised soilless potting mix (Propine, BJH 9321), and maintained at 20 $\pm$ 2°C in a glasshouse (Fig. 33). Seedlings were thinned to one vigorous seedling per pot at the two leaf stage. Pots were arranged in a randomised block design. Trials 1-7 consisted of three replicates, each containing five seedlings of each host. Trials 8-23 consisted of four replicates, each containing three seedlings of each host.

Inoculum was prepared by homogenising clubbed roots with distilled water (1:3 w/v) at high speed in a mechanical blender. The suspension was filtered through 2 layers of muslin cloth. The spore concentration was quantified with a haemocytometer and adjusted to approximately 10^8 spores/ml by dilution with distilled water. Seedlings were inoculated 3-4 weeks after sowing (two leaf stage). Pots were thoroughly watered prior to inoculation and 200µl of the spore suspension was pipetted into a small depression in the potting mix, at the base of the seedling.

Inoculated plants were maintained under drip irrigation (applied three times daily for three minutes) at 20 $\pm$ 2°C in a polycarbonate greenhouse for 6-8 weeks, after which time roots were washed free of potting mix and rated for clubroot severity on a scale 0-3, (Buczacki *et al.*, 1975).

A disease index (DI) as used by Dobson et. al. (1983) was calculated for each host:

$$DI = \left(\frac{[n_0 \times 0] + [n_1 \times 1] + [n_2 \times 2] + [n_3 \times 3]}{n_0 + n_1 + n_2 + n_3}\right) x \left(\frac{100}{3}\right)$$

where n_0 is the number of plants in severity group 0; n_1 is the number of plants in severity group 1 etc.

This was used to assign a host reaction type (resistant DI=0; indeterminate 0<DI<33 susceptible DI ≥ 33). A numerical code based on susceptible ECD hosts was assigned to each isolate (Buczacki *et al.*, 1975).

Only those experiments which resulted in a DI of >80 on at least one of the ECD hosts have been reported. This arbitrary value, used by Crute *et al.* (1983), was adopted to ensure that the data reported were obtained using viable inoculum.

6.2.2 Results

Sixteen triplet codes were identified from 23 pathogen collections from five states of Australia. Triplet codes 16/3/12 and 16/2/31 were the most frequently assigned (four times each) and were present in three of the five states surveyed (Table 7).

Trials were repeated using inoculum prepared from the Manjimup and Donnybrook collections which had produced unique reactions of the ECD series (16/0/0 and 20/19/31 respectively). All attempts failed to reproduce the original host reactions (Table 7). It is possible that these collections contained more than one pathotype of *P. brassicae* resulting in different host reactions depending on the proportion of each pathotype in the inoculum used for each screening trial. Alternatively, the viability of the inoculum or environmental conditions at the time of the test may have restricted the expression of symptoms resulting in the 16/0/0 result. These inconsistent results highlight the problems associated with a screening method based on visual assessment of bait plants, and highlight the need for a faster and more accurate method of differentiating pathotypes of *P. brassicae*. Molecular techniques were investigated as a possible alternative (section 6.3).

Table 7: Reactions[1] of the European Clubroot Differential hosts to Australian collections of *Plasmodiophora brassicae*.

State of Australia	Location	Host	Host reaction type[1] *B. rapa*					*B. napus*					*B. oleracea*					ECD code[2]
		Host no.	*01*	*02*	*03*	*04*	*05*	*06*	*07*	*08*	*09*	*10*	*11*	*12*	*13*	*14*	*15*	
		Binary no.	*1*	*2*	*4*	*8*	*16*	*1*	*2*	*4*	*8*	*16*	*1*	*2*	*4*	*8*	*16*	
Western Australia	Donnybrook[B]	Cauliflower	?	R	S	?	S	S	S	R	R	S	S	S	S	S	S	20/19/31
Queensland	Stanthorpe	Cauliflower	R	R	R	R	S	R	S	S	?	S	R	R	S	S	R	16/22/12
Western Australia	Manjimup[AC]	Chinese cabb.	R	R	R	R	S	S	S	R	R	S	S	S	S	S	S	16/19/31
Western Australia	Manjimup[C]	Cauliflower	R	?	R	?	S	S	S	?	?	S	S	S	S	S	S	16/19/31
Western Australia	Donnybrook[B]	Cauliflower	R	R	R	R	S	S	S	?	R	S	?	?	S	S	?	16/19/12
Victoria	Lindenow	Chinese cabb.	R	R	R	R	S	S	S	R	R	?	S	S	S	S	S	16/3/31
Victoria	Werribee	Cauliflower	R	R	R	R	S	S	S	R	?	R	?	S	S	S	S	16/3/30
Tasmania	Weslyvale	Cauliflower	R	R	?	R	S	S	S	?	R	?	?	?	S	S	S	16/3/28
Victoria	Mornington	Cabbage	R	R	?	R	S	S	S	R	R	?	S	S	S	S	?	16/3/15
Western Australia	Manjimup	Cauliflower	?	R	R	R	S	S	S	?	R	?	R	S	S	S	R	16/3/14
Western Australia	Manjimup	Cauliflower	R	R	R	R	S	S	S	?	R	?	?	?	S	S	?	16/3/12
Victoria	Werribee	Broccoli	R	R	R	R	S	S	S	?	R	?	?	?	S	S	?	16/3/12
Western Australia	Manjimup	Cauliflower	R	R	R	R	S	S	S	?	R	?	R	?	S	S	?	16/3/12
Tasmania	Elphinstone	Broccoli	?	?	R	R	S	S	S	?	?	?	?	?	S	S	?	16/3/12
Western Australia	Manjimup	Cauliflower	?	R	R	R	S	S	S	?	R	?	R	?	?	?	S	16/3/8
Tasmania	FVRS	Wild Mustard	R	R	R	R	S	S	S	?	R	R	R	R	?	?	R	16/3/0
Western Australia	Waneroo		R	R	R	R	S	?	S	R	R	?	S	S	S	S	S	16/2/31
New South Wales			R	R	R	R	S	?	S	R	R	R	S	S	S	S	S	16/2/31
Victoria	Werribee	Broccoli	R	R	R	R	S	?	S	R	R	R	S	S	S	S	S	16/2/31
Victoria	Lindenow	Broccoli	R	R	R	R	S	R	S	R	R	R	S	S	S	S	S	16/2/31
New South Wales	Bathurst	Cabbage	R	R	R	R	S	?	?	R	R	?	S	S	S	S	S	16/0/31
Victoria	Lindenow	Broccoli	R	R	R	R	S	?	?	R	R	?	S	?	S	S	S	16/0/29
Western Australia	Manjimup[AC]	Chinese cabb.	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	16/0/0

[1] R = Resistant (DI = 0); S = susceptible (DI ≥33); ? = indeterminate (0<DI<33)

[2] Preliminary race numbers assigned on the basis of definite susceptible reactions only.

[A] and [B] Inoculum prepared using the same pathogen collections., [C] Pathogen collections from the same property.

6.3 Molecular Screening

6.3.1 Materials and Methods

Two commonly employed molecular methods, microsatellite analysis and rDNA (ribosomal DNA) sequencing, were used in an attempt to distinguish Australian *P. brassicae* isolates. Where available, the data were compared with known ECD results.

DNA extracted from purified *P.brassicae* resting spore suspensions was either sequenced or PCR amplified to examine microsatellite DNA.

rDNA Sequence Analysis. The intergenic transcribed spacer 1 (ITS1), 5.8S and part of the 18S and ITS2 regions were amplified by PCR from genomic DNA isolated from *P. brassicae* using the primers of Faggian *et. al.* (1999). PCR products were cleaned using a Qiagen PCR purification kit and sequenced directly using an ABI PRISM™ Ready Reaction Dye Terminator Cycle Sequencing kit (Perkin-Elmer, Scoresby, Vic., Aust.) on an Applied Biosystems Model 373 Automated DNA Sequencer Stretch.

Microsatellite Analysis. *P. brassicae* DNA was amplified using three different microsatellite primers (MS1 – CTGCTGCTGCTGCTG; MS2 – CACCACCACCACCAC; MS3 - GACAGACAGACAGACA). The temperature cycling regime was 35 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min, using 1 U of *Tth* Plus DNA polymerase (Fisher Biotech) and 2 mM $MgCl_2$. The PCR products were visualised on a 2% agarose gel stained with ethidium bromide.

6.3.2 Results

Ribosomal DNA sequence data were compared using the GCG package (Program manual for the Wisconsin Package, Version 8. 1994. Genetics Computer Group, 575 Science Drive, Madison, Wisconsin USA 53711), revealing very little sequence variation. Isolates were approximately 99% homologous within the region examined, and therefore could not be correlated with ECD data.

Microsatellite analysis, while only producing between 3 and 7 visualisable bands, enabled discrimination of *P. brassicae* isolates to levels consistent with that obtained with the ECD series.

Seven isolates were assigned ECD race numbers identifying six distinct pathotypes (Table 8). Microsatellite analysis of the same seven isolates produced six different banding patterns, therefore also identifying six distinct pathotypes (Figure 23). However, two Victorian isolates (Vic_1 & Vic_2) had identical microsatellite profiles but different ECD race numbers, while a Victorian isolate (Vic_2) and the NSW isolate had different microsatellite profiles but identical ECD race numbers. This can be attributed to the technical problems associated with achieving consistent ECD results, or a lack of sensitivity of the microsatellite technique.

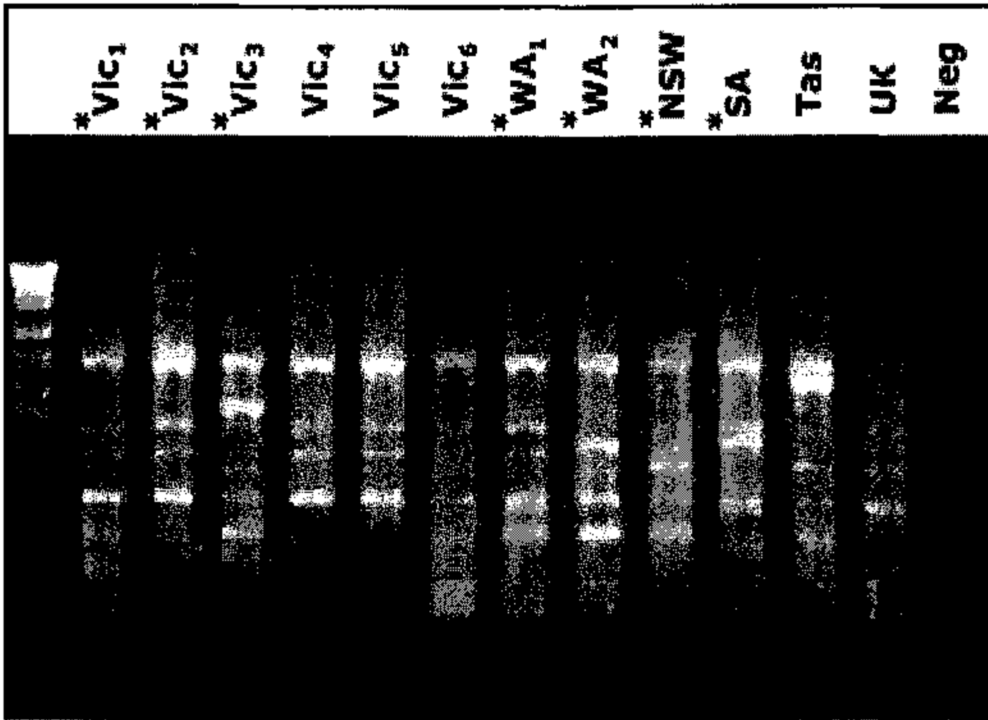

Figure 23: Microsatellite PCR products of *P. brassicae* isolates using primer MS1.
* ECD data available.

Table 8: ECD results *P. brassicae* isolates

Vic_1	16 / 03 / 12	WA_1	16 / 19 / 31
Vic_2	16 / 02 / 31	WA_2	20 / 19 / 31
Vic_3	16 / 00 / 12	NSW	16 / 02 / 31
		SA	16 / 31 / 31

6.4 Discussion

The ECD series is currently the most widely accepted method of classifying populations of *P. brassicae*. However, environmental conditions, resting spore viability and the potential existence of mixed pathotypes of *P. brassicae* within infected roots can complicate the interpretation of tests and the comparison of international data. In spite of this, the data reported appear to support the existence of at least three distinct pathotypes within Australia (16/19/31, 16/3/12 and 16/2/31).

Screening using the ECD series is also time consuming requiring at least 8 weeks per pathogen collection. Microsatellite analysis was found to enable discrimination of *P. brassicae* isolates to levels consistent with that currently available using the ECD series. This molecular approach may therefore be a viable alternative to the time- and labour-intensive ECD method. As it is PCR-based, it is fast, sensitive, and only requires small amounts of starting material.

7 Evaluation of clubroot tolerant varieties

7.1 Summary

Glasshouse and field trials were conducted in Victoria, Queensland and Western Australia to screen a range of varieties of broccoli, cauliflower, cabbage and Chinese cabbage for tolerance of the pathotypes of *P. brassicae* (the cause of clubroot) known to exist in Australia (Section 6.3.2, Table 7). Whilst no resistance has yet been discovered in either cauliflower or cabbage, a large number of resistant Chinese cabbage cultivars have been identified and most recently several cultivars of broccoli have exhibited tolerance to some (but not all) of the collections of clubroot in Australia. Among these Yates varieties Y5737 and Dome (which is genetically very similar to Cathederal) are the most tolerant. Dome is commercially available in Australia.

It is important to note however, that a variety that is tolerant to one collection of *P. brassicae* will not necessarily be tolerant to all pathotypes of *P.brassicae* (Fig. 24).

7.2 Materials and Methods

Inoculum preparation

Glasshouse trials were artificially inoculated with resting spore suspensions prepared from clubroot galls collected from commercial vegetable brassica farms. The galls were washed and stored in a freezer (-2°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. In Victorian trials, the concentration of spores in the suspension was adjusted to 10^8 spores/ml. A small volume of this was used to inoculate each plant (200µl for direct seeded trials, 2 x 200 for transplanted trials). In Queensland trials, the spore suspension was mixed into the soil to give a final concentration of 500 000 spores per gram of soil.

Field sites were selected with a long history of intensive brassica production and a recent history of severe clubroot disease. The native soil inoculum at these sites was used.

Root galls from each trial were retained for pathotype identification using the European Clubroot Differential series of hosts (see section 6.2.1).

Glasshouse trials - Victoria

Three pot trials were conducted in Victoria using different sources of inoculum (Table 9). Varieties of four crops *B. oleracea* (broccoli, cabbage and cauliflower) and *B. campestris* (Chinese cabbage) were screened. Where possible the same varieties of each crop were used in each trial, however, broccoli varieties Y5737 and Dome only became available during the second year of work and were consequently used in only one of the three glasshouse trials (Table 9). For each crop, a commercially available, clubroot susceptible control was chosen.

Table 9: Host crop varieties and inoculum sources used in Victorian glasshouse (GH) and field screening trials.

VARIETY	CROP	SUPPLIER	GH 1 16/3/12 Werribee	GH 2 16/2/30 Cora Lynn	GH 3 16/3/12 Trentham	FIELD 16/3/31 Knoxfield
NW-702 (Cathedral)	Broccoli	New World	✓	✓	✓	✓
N-248	Broccoli	Nozaki seed	✓	✓	✓	✓
N-320	Broccoli	Nozaki seed	✓	✓	✓	✓
Y5737	Broccoli	Yates	x	x	✓	✓
Dome	Broccoli	Yates	x	x	✓	✓
Trixie	Calabrese (Broccoli)	Thompson & Morgan	✓	✓	✓	✓
Marathon	Broccoli control	Lefroy Valley Seeds	✓	✓	✓	✓
N-337	Cabbage	Nozaki seed	✓	✓	✓	✓
N-276	Cabbage	Nozaki seed	✓	✓	✓	✓
Green coronet	Cabbage control	Hendersons	✓	✓	✓	✓
55	Cauliflower	Juji-ribon	✓	✓	✓	✓
65	Cauliflower	Juji-ribon	✓	✓	✓	✓
N-713	Cauliflower	Nozaki seed	✓	✓	✓	✓
Marba	Cauliflower control	Hendersons	✓	✓	✓	✓
Bejo 1746 Rokko F1	Ch. cabbage	Bejo	✓	✓	✓	✓
Banko F1	Ch. cabbage	Bejo	✓	✓	✓	✓
Bejo 1771 Bilko F1	Ch. cabbage	Bejo	✓	✓	✓	✓
Bejo 1785 F1	Ch. cabbage	Bejo	✓	✓	✓	✓
Utage No. 70	Ch. cabbage	Nozaki seed	✓	✓	✓	✓
N-181	Ch. cabbage	Nozaki seed	✓	✓	✓	✓
Yamami No. 90	Ch. cabbage	Nozaki seed	✓	✓	✓	✓
Mari	Ch. cabbage	Sakata	✓	✓	✓	✓
Yuki	Ch. cabbage	Sakata	✓	✓	✓	✓
Komachi	Ch. cabbage	Sakata	✓	✓	✓	✓
CR 75 Hybrid Strong	Ch. cabbage	Wantabe	✓	✓	✓	✓
W R 60	Ch. cabbage control	Lefroy Valley Seeds	✓	✓	✓	✓

Glasshouse trials 1 and 2. Two seeds were planted into soilless potting mixture in 15 cm diameter plastic pots. At the two leaf stage, the seedlings were thinned to one per pot and were inoculated by pipetting 200 µl of spore suspension into a small depression at the base of the plant. There were 12 replicate plants of each host crop variety.
Glasshouse trial 3. Each cell grown transplant was planted into soilless potting mixture in 15 cm diameter plastic pots and inoculated by pippetting 2 x 200 µl aliquots into small depressions at either side of the transplant. There were 7 replicate plants of each host crop.
Maintenance and assessment. Plants were maintained for the duration of the experiment under drip irrigation (applied for 3 mins every 12 hrs) at 20 $\pm$ 2°C in a glasshouse. Six weeks after inoculation the plants were removed from their pots. The roots were shaken free of potting mixture and were visually assessed for symptoms of clubroot on a 1-9 scale (where 1 = no disease, 9 = no healthy roots).

Field trial - Victoria

Six beds 1.6 m wide (wheel centre to wheel centre) x 25 cm high were prepared on a light brown silty sand (pH $CaCl_2$ = 4.9) at the Institute for Horticultural Development, Knoxfield. This soil had a history of cabbage production and was known to be severely infected with clubroot in the previous growing season. Each bed contained one randomly placed replicate plot (13 plants) of each variety planted over 2 m. The trial was planted in late summer (16th March). Irrigation was applied as required through moving pipe irrigation. The premergant herbicide Dual was applied (3 L/ha) the day after transplanting and the fertiliser Rustica blue (@600 kg/ha) was applied one week after transplanting. Between 7 and 8 weeks after transplanting (9th May), three plants were dug from each plot, their roots shaken free of soil and visually assessed for disease symptoms on a scale 1-9 (where 1 = no disease, 9 = no healthy roots).

Glasshouse trial - Queensland

A clubroot spore suspension (Table 10) was used to inoculate soil to give a final inoculum concentration of 500 000 spores/g soil. Each (20 cm diameter) pot was filled with 5.5 kg of the inoculated soil. Pots were planted with 8 varieties of broccoli (including Greenbelt, a susceptible variety used widely through out the district) and two varieties of cauliflower (as cell grown transplants) (Table 10). There were 6 replicate pots of each variety. Pots were placed in an unheated plastic tunnel (23 $\pm$ 5°C) and were watered every two days to saturation.
10 weeks after planting the plants were removed from their pots and their roots washed free of soil. Roots galls were removed from the plants and dried (60°C, 4 days). The average dry weight of root galls was recorded for each variety.

Table 10: Host crop varieties and inoculum sources used in Queensland glasshouse (GH) and field screening trials.

VARIETY	CROP	SUPPLIER	GH 1 16/22/12	FIELD 1	FIELD 2
NW-702 (Cathedral)	Broccoli	Yates	✓	✓	X
N-248	Broccoli	Nozaki seed	✓	✓	X
N-320	Broccoli	Nozaki seed	✓	✓	X
Y5737	Broccoli	Yates	✓	✓	✓
Trixie	Calabrese (broccoli)	Thompson & Morgan	✓	✓	X
Y5736	Broccoli	Yates	✓	✓	X
Y5738	Broccoli	Yates	✓	✓	X
Greenbelt	Broccoli control		✓	✓	✓
N-713	Cauliflower	Nozaki seed	✓	X	X
Mosaic	Cauliflower		✓	X	X

Field trials - Queensland

The 8 different types of broccoli evaluated in the glasshouse, were also transplanted into a sandy loam in the Stanthorpe district. Cultivars were planted in double rows on raised beds and were arranged in a randomised block with 6 replicates. Plants were grown according to normal commercial practice. Fertiliser application was conducted according to recommendations from soil analysis. Plants were grown to maturity, harvested, weighed and visually rated for clubroot severity (scale 1-9 where 1=no clubbing, 9=no healthy roots) and head quality (scale 0-5 where 0=not marketable, 5=dome shape, even colour, tight even florets).

The following season, 6 replicates of the most effective variety and Greenbelt were planted on the same site, raised to maturity and assessed as described previously.

Field trial - Western Australia

Varieties of broccoli and cauliflower used locally, together with several varieties of broccoli known from previous trials (Qld and Vic) to have some tolerance to clubroot, were raised to transplant stage in a commercial nursery. Cell grown transplants of each variety were planted in a randomised block design with 6 replicates on a commercial brassica growing property in Manjimup, WA. Plants were grown according to normal commercial practice. Six weeks after planting, 4 plants were removed from each plot and their roots assessed for galling symptoms due to clubroot. The remaining plants were grown to maturity, harvested and weighed to determine yield.

Data analyses

Data from Victorian trials were analysed by analysis of variance using Genstat 5 release 3.2 (Laws Agricultural Trust, Rothamsted Experimental Station).

7.3 Results

Glasshouse trials - Victoria

Two of the three sources of inoculum (Werribee - Trial 1 and Trentham - Trial 3) were allocated the same race number (16/3/12) following screening against the European Clubroot Differential series of hosts (Table 9). There was very limited tolerance to this race of *P. brassicae* in the *B. oleracea* varieties used. In the first trial, cv. Trixie was the only variety with significantly reduced root galling compared to Marathon, the clubroot susceptible broccoli control (Fig. 24a). In the same trial, N276 was more tolerant of clubroot than Green coronet, the susceptible cauliflower control. No tolerance was identified in any of the lines of cauliflower (Fig. 24a).

In trial 3, cv. Y5737 was used for the first time. This was the only variety of *B. oleracea* (broccoli, cabbage or cauliflower) to exhibit significantly reduced root galling compared to the

appropriate control (Fig. 24c). With the exception of Bejo 1785 F1, all of the Chinese cabbage varieties were significantly more resistant than WR 60 the susceptible Chinese cabbage control (Fig. 24c).
Trial 2 was inoculated with *P. brassicae* sourced from Cora Lynn Victoria. This pathogen collection was assigned race number (16/2/30) using the ECD series of differential hosts (Table 9). A number of broccoli lines (NW 702, N-248 and N320) exhibited significantly reduced root galling (Fig. 24b). One line of cabbage (N-276) and one line of cauliflower (55) were also significantly less galled than their respective susceptible controls (Fig. 24b). All lines of Chinese cabbage, including Bejo 1785 F1 exhibited significantly less root galling than WR 60 the susceptible control (Fig. 24b).

Field trial - Victoria

Whilst all of the lines of Chinese cabbage exhibited significantly less root galling than WR 60, tolerance of this race of *P. brassicae* (16/3/31) was not observed in any of the lines of *B. oleracea* (broccoli, cauliflower or cabbage) (Fig. 25).

Glasshouse trial - Queensland

Broccoli varieties N-320, Y5736, Y5737 and NW 702 ('Cathederal', now replaced by 'Dome') exhibited significantly less root galling compared to Greenbelt the susceptible control. Root galling was not significantly reduced in the cauliflower varieties (Fig. 26).

Field trials - Queensland

In contrast to the results obtained in the glasshouse, only Y5737 resulted in significantly reduced root galling compared to Greenbelt, the susceptible control in the field (Fig. 27). Two attempts were made to determine the pathotype of *P. brassicae* at this site using the ECD series and galls collected at the end of the trial. In both instances, infection of the ECD hosts was insufficient to assign a race number. It is thought that the galls used were immature.
Tolerance of Y5737 to *P. brassicae* at this site was confirmed in a second field trial that was conducted the following year. Root galling was again significantly reduced compared to Greenbelt in this trial (Fig. 28).

Field trial - Western Australia

The onset of disease at this sight was rapid and very severe. There was some variation in the relative susceptibility of commercial cauliflower varieties to clubroot measured at 6 weeks after planting (Fig. 29), however, all varieties were severely infected and most produced no measurable yield. Broccoli varieties 'Dome' and 'Y5737' were more tolerant of clubroot than any of the varieties commonly used in the district (Fig. 29 and 30). These were the only two varieties to produce any yield from the field trial (Table 11). Due to the severity of disease which restricted the size of the data set, statistical analyses of these results could not be completed.

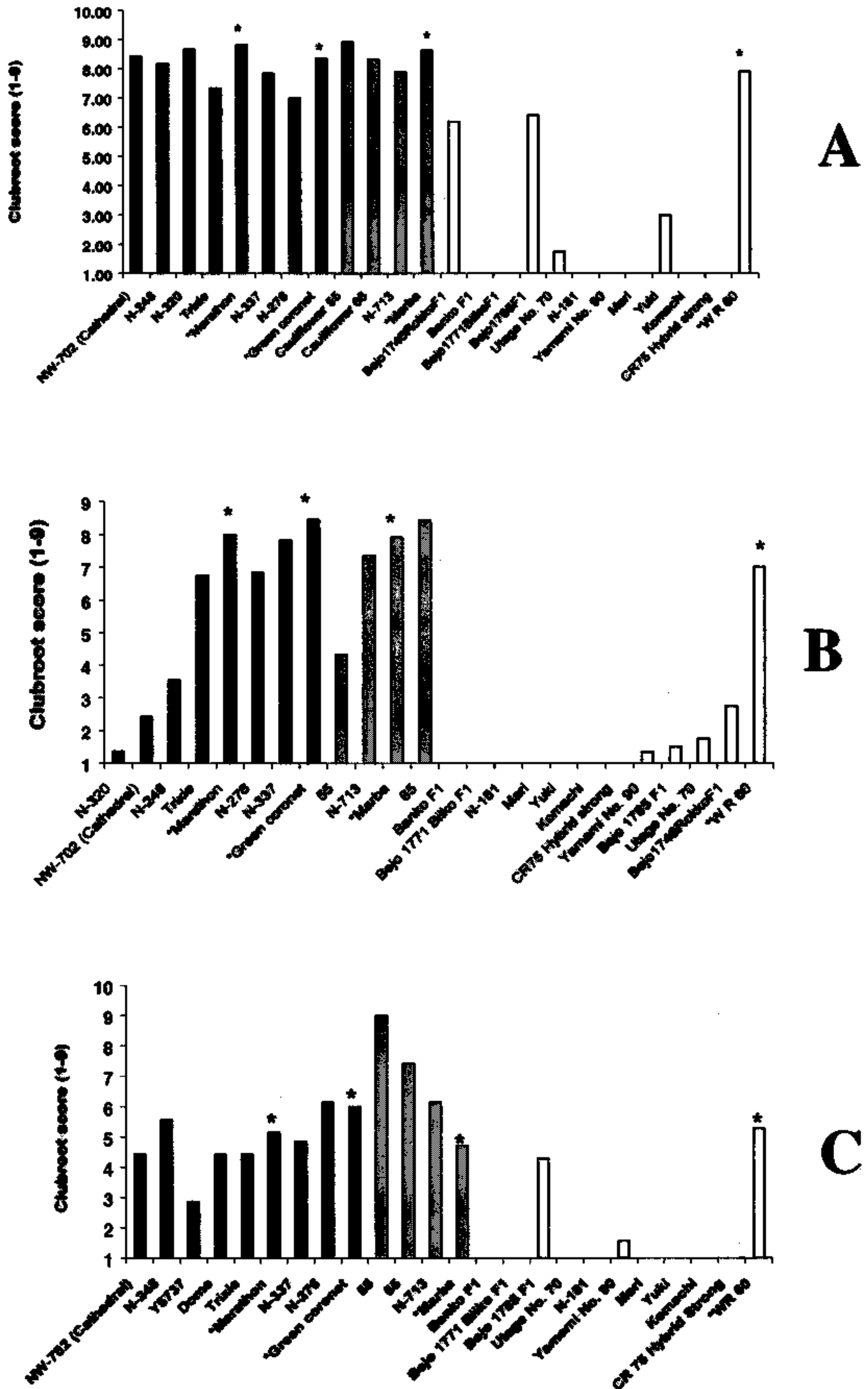

Figure 24: Glasshouse assessment of the relative susceptibility of *B. oleracea* broccoli (black area), cabbage (striped area), cauliflower (shaded area) and *B. campestris* Chinese cabbage (white area) varieties to three Victorian sources of inoculum of *P. brassicae*; A Werribee collection 16/3/12, B Cora Lynn collection 16/2/30 and C Trentham 16/3/12. (* = varietal control)

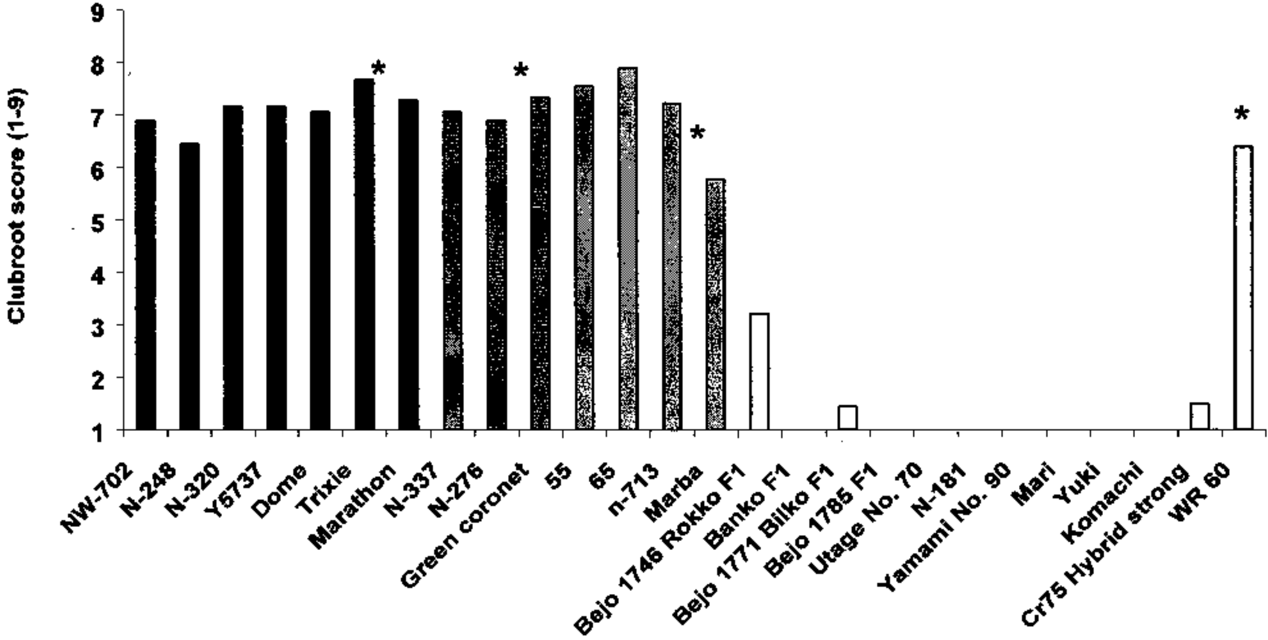

Figure 25: Field assessment of the relative susceptibility of *B. oleracea* broccoli (black area), cabbage (striped area), cauliflower (shaded area) and *B. campestris* Chinese cabbage (white area) varieties to *P. brassicae* (16/3/31). Institute for Horticultural Development , Knoxfield, Victoria.

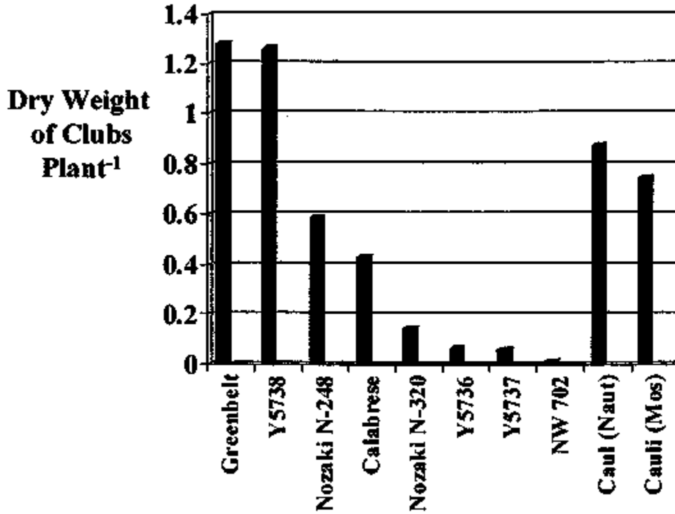

Figure 26: Glasshouse assessment of the relative susceptibility of *B. oleracea* broccoli and cauliflower varieties to a Stanthorpe (QLD) collection of *P. brassicae* (16/22/12) 1998/1999.

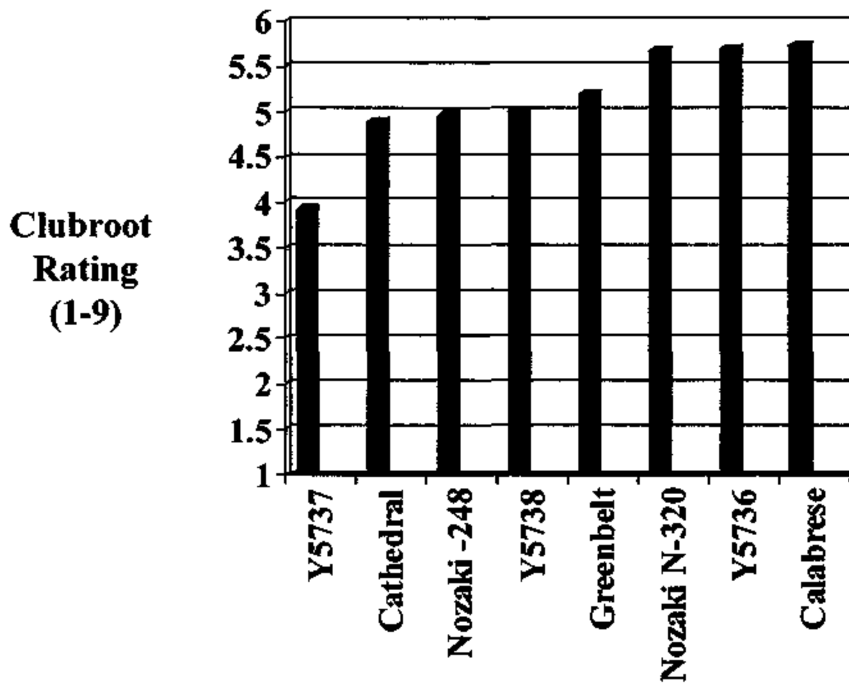

Figure 27: Field assessment of the relative susceptibility of *B. oleracea* broccoli (black area) and cauliflower varieties to *P. brassicae*. Stanthorpe, QLD, 1998/99.

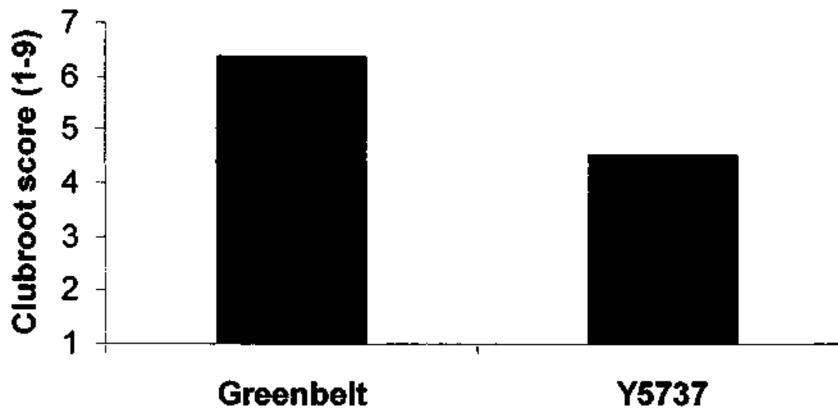

Figure 28: Field assessment of the relative susceptibility of *B. oleracea* broccoli cv. Y5737 and the widely used commercial variety 'Greenbelt', Stanthorpe, QLD 1999/2000.

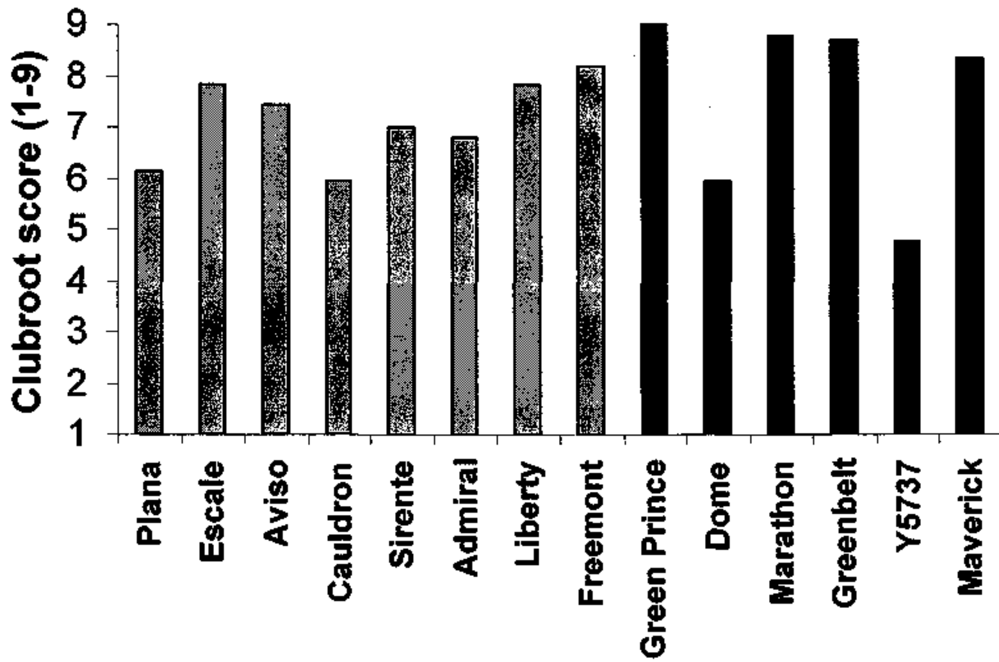

Figure 29: Field assessment of the relative susceptibility of *B. oleracea* broccoli (black area) and cauliflower (shaded area), Manjimup, WA 2000 (assessed 6 weeks after transplanting).

Table 11: Yield of broccoli - Resistance screening trial, Manjimup, WA 2000.

Broccoli cultivar	Yield (t/ha)
Marathon	0
Maverick	0
Greenbelt	0
Green Prince	0
Dome	3.91
Y5737	3.96

Note: There was no marketable yield from any of the cauliflower cultivars used. (Statistical analyses not completed as data set restricted).

Figure 30: Varieties 'Dome' and 'Y5737' in Western Australian field trials, Manjimup, WA 2000.

7.4 Discussion

Many of the cultivars of Chinese cabbage (*B. campestris*) were tolerant of a number of Australian collections of *P. brassicae*. In contrast, no tolerance was found in any of the varieties of cauliflower and cabbage. Some tolerance was observed in several cultivars of broccoli, however, it is important to note that these cultivars were not universally tolerant to all Australian collections of clubroot. Among the most promising cultivars are Yates 'Y5737' and 'Dome'. The later of these is the male sterile (commercial form) of 'Cathedral', also known as NW 702.

Crute *et al.* (1980) present a summary of the various known sources of resistance to clubroot in *B. oleracea* (broccoli, cauliflower and cabbage) and the attempts to incorporate these into commercial crop species. By analysis of international data collected using the European Clubroot Differential (ECD) series of host plants, Crute *et al.* (1983) found that, in contrast to clubroot resistance expressed in *B. campestris* and *B. napus*, resistance in *B. oleracea* genotypes, with the exception of cv. Badger Shipper (ECD line 11), appeared to be non-differential. These authors conclude that "the extent of inherited non-differential variation will determine whether resistance to clubroot in *B. oleracea* is of value for use in cultivar development".

This study highlights the importance of knowing the variation within the Australian *P. brassicae* population (Section 6) and understanding the limit of this tolerance in any new 'clubroot tolerant' variety before it is promoted. It is clear from this study that current tolerance in broccoli is incomplete and must be assessed regionally. It is also apparent however, that where tolerance is expressed, it will form a useful component of any integrated management system for clubroot.

8 Technology Transfer

A large number of technology transfer activities have been associated with the project. Amongst the most effective have been:

- Regular field days
- Grower meetings
- Project newsletter "Clubroot - Galls and All"
- Seminar evenings

Technology transfer activities and publications produced during project are detailed below.

8.1 Extension activities

Clubroot Seminars

-Manjimup, Western Australia (16th October, 1997)
-Stanthorpe, Queensland (25/2/98)
-Gatton, Queensland (26/2/98)
-Lenswood, South Australia (9/9/98)
-Albany, Western Australia (18/2/99)
-Werribee Vegetable Expo, Vic (3/5/99)
-Bathurst New South Wales (22/11/99)
-Stanthorpe Queensland (21/12/99)

Clubroot Presentations At Grower Meetings

-Werribee, VIC (27/8/97)
-At IHD (11/11/97)
-Bathurst, NSW (30/10/98)
- Myrtleford, VIC (new growers group) (10/12/98)
-Clubroot Committee Meetings (every two months), Manjimup (1995-1999)
-Clubroot Committee Meetings (quarterly), Stanthorpe (1997-1999)
- Sydney basin field grown vegetables conference, Richmond NSW (16/7/99)
-New Zealand grower seminars (5) 9/99
- Glenormiston vegetable apprentices, VIC (14/5/99)
- West Gippsland growers meeting, VIC (12/8/99)
-Nurserymen, water treatment workshop, VIC (16/8/99)
-Chinese vegetable growers, NSW (3/10/99)
-HRDC commodity groups (12/1099)
-Hawksbury, NSW (16/11/98)
-Werribee VIC (26/11/99)
-Richmond, NSW (14/7/00)

Clubroot Field Days

(including trial inspection, presentation of results, discussion, BBQ and drinks)

-Werribee, Vic, Harry Velisha's property (6/4/98)
-Manjimup, WA, Tour of several sites (31/3/98)
-Manjimup, WA, field tour, machinery demonstration and seminar (18/3/99)
-Forth, TAS, field walk, site inspection (29/3 /99)
-Grower visits and machinery display – Werribee Vegetable Expo, Melb. (6 and 7/5/99)
-Stanthorpe, QLD (2/00)
-Manjimup, WA, Tour of several sites (17/4/00)

Presentation To National And International Industry Groups

-Muirs horticultural distributors (27/8/97)
-Indian delegation (2/9/97)
-Chinese Department of Agriculture (12/9/97)
-Japanese Horticulture Students (5/12/97)
-Japanese horticulture group (28/9/98) and (1/12/99)
-Crop Care resellers and Muirs representatives (14/7/98)
-Werribee Vegetable Expo seminar series (3/5/99)
-Glenormiston Vegetable apprentices (14/5/99)
-Florida Vegetable growers (12/1/00)
-HRDC (22/5/00)
-Glenormiston Vegetable apprentices (25/8/00)

Other

-Radio interview (ABC radio rural report - Victoria 5/11/97)
-Television interview (Totally Wild Children's Television 1998)
-Manned display Werribee Veg. Expo (May 97 and May 99)
-Clubroot Industry day at IHD (chemical/fertiliser reps, seed companies in attendance 13/10/98)
- Radio Interview (ABC Rural Report – Bunbury, WA 14/4/99)

8.2 Publications

Papers

Porter, I.J., Donald, E.C. and Cross, S.J. (1998) Field evaluation of Fluazinam against clubroot (*Plasmodiophora brassicae*) of cruciferous vegetable crops. '*Tests of Agrochemicals and Cultivars*' No. 19, (*Annals of Applied Biology* **132**, Supplement), pp. 12-13.

Faggian, R., Bulman, S.R., Lawrie, A.C. and Porter, I.J. (1999) Specific Polymerase Chain Reaction Primers for the Detection of *Plasmodiophora brassicae* in Soil and Water. *Phytopathology* **89**: 392-397.

Donald, E.C, Porter, I.J. and Lancaster, R.A. Application of fluazinam (Shirlan) to control clubroot of vegetable brassica crops. Submitted to *Aust. J. Agric. Res.*

Oral Presentations And/Or Papers – Conferences

Donald, E.C., Porter, I.J., Faggian, R. and Lancaster, R. (1997) Towards integrated control of clubroot – Field evaluation of treatments for clubroot control. In 'APPS 11th Biennial Conference Handbook', p. 38. (Abstract). (Perth, Australia).

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Donald, E.C. and Porter, I.J. (1999) Improving integrated control of clubroot by strategic application of lime, fertilisers and fungicides. In 'Proceedings of the first Australasian soilborne disease symposium', pp. 185-186. (Brisbane, Australia).

Donald, E.C., Porter, I.J. and Cross, S.J. (1999). Variation in Australian Populations of Plasmodiophora brassicae. In 'APPS 12th Biennial Conference Handbook', pp. 330. (Abstract). (Canberra, Australia).

Faggian, R., Donald, C., Porter, I.J. and Lawrie, A.C. (1999). The use of molecular techniques to investigate the genetic variation of Australian clubroot isolates. In 'APPS 12th Biennial Conference Handbook', pp. 335. (Abstract). (Canberra, Australia).

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Donald, E.C. and Porter, I.J. (1999) Calcium ion and fluazinam as factors which suppress development of clubroot. In 'APPS 12th Biennial Conference Handbook', pp. 365. (Abstract). (Canberra, Australia).

Donald, C. and James, L. (2000) Review of the national clubroot project. In 'Proceedings of second annual Sydney basin field-grown vegetables conference'. (Richmond, New South Wales).

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

Newsletters/Industry Publications

Donald, C. and Porter, I. (1997) *Clubroot - Galls and All.* (No. 5)

Donald, C. and Porter, I. (1998) *Clubroot - Galls and All.* (No. 6)

Donald, C. and Porter, I. (1998) *Clubroot - Galls and All.* (No. 7)

Donald, C. and Porter, I. (1999) *Clubroot - Galls and All.* (No. 8)

Donald, C. and Porter, I. (2000) Clubroot - Galls and All. (No. 9)

Lancaster R (May., 1997) Clubroot Update in AgMemo - Agriculture WA, Manjimup

Lancaster R. (July, 1998), *Cauliflower Clubroot Newsletter* (No.7)

Lancaster R. (Nov., 1998), *Cauliflower Clubroot Newsletter* (No.8)

Lancaster R. (August, 1999), *Clubroot Newsletter* (No.9)

Lancaster R. (July, 2000), *Clubroot Newsletter* (No.10)

General Publications

Cheah, L-H., Porter, I., Donald, C. and Falloon, R. (2000) Clubroot hits the road. *Grower* **55(4)** 15-18.

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Faggian, R., Porter, I. and Donald, C. (1997) Molecular Detection of *Plasmodiophora brassicae.* Poster.

Lancaster, R., Donald, C. and Porter, I. (1997) Clubroot seminar booklet, Manjimup, WA October, 1997.

Porter, I., Donald C. and Lancaster, R. (1997) National Clubroot Project. Poster

Reports

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Stephens, P. (1999) Clubroot control. Report to growers, DPI Queensland.

9 Recommendations

Recommendations to brassica growers arising from this work have been summarised and distributed to brassica growers nationally in the pamphlet "A guide to the prevention and management of clubroot in vegetable brassica crops." A copy is included with this report.

The pamphlet outlines a range of integrated strategies that enable clubroot to be managed in the majority of Australian horticultural soils. Selecting the most cost-effective strategy for a given paddock, however, still involves considerable guess work to estimate the amount *P. brassicae* in the soil based on previous cropping history, cultural practice, soil type and a range of other variable factors. The development of a quantitative assay for *P. brassicae* in soil is an immediate and urgent priority. Such an assay would enable growers to select the most cost-effective treatment option based on a simple soil test. A quantitative assay is currently being developed as part of the HRDC funded project VG 99008 "A rapid diagnostic test for clubroot".

Once available, this assay will enable the effect of integrated management strategies on the amount of viable *P. brassicae* in soils to be studied for a variety of different soil types. This final phase of clubroot research in Australia will provide growers with a predictive tool to manage *P. brassicae* below the threshold for disease in their soil type.

Other research priorities include:

- Demonstration programs in each state to facilitate further uptake of effective clubroot control strategies.
- Development of effective on-farm hygiene procedures, including best practice protocols for the production of clubroot free brassica transplants.

A new project "Clubroot - Total Crop Management" has been developed to address these priorities and will encompass whole production systems - seed to transplant to mature crop, providing best practice protocols on farm that develop effective seedling treatments, hygiene procedures, and predictive farm management strategies.

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