

Control of clubroot in vegetable brassicac



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A report prepared for

**The Fresh Vegetable Industry
Development Committee of the
New Zealand Vegetable & Potato
Growers' Federation Inc., Bayer NZ
Ltd, Ciba-Geigy NZ Ltd, Crop Care
Holdings Ltd and Elliot Chemicals
Ltd**

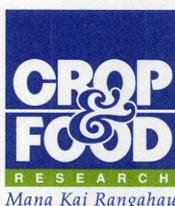
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November 1995

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Private Bag 4704, Christchurch, New Zealand*



Crop & Food Research Confidential Report No. 62

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brassicac**

H M Nott et al.

CONTENTS

	Page
1	EXECUTIVE SUMMARY 1
2	INTRODUCTION 2
3	CLUBROOT RESISTANCE IN VEGETABLE BRASSICAS 3
3.1	Objectives 3
3.2	Materials and methods 3
3.2.1	<i>Seed sources</i> 3
3.2.2	<i>Clubroot susceptibility testing</i> 6
3.3	Results and discussion 7
3.4	Conclusions and recommendations 15
4	PRELIMINARY GLASSHOUSE EXPERIMENTS ON CLUBROOT OF BRASSICAS 16
4.1	Preliminary experiment 1: Production of clubroot symptoms in glasshouse-grown plants 16
4.1.1	<i>Materials and methods</i> 16
4.1.2	<i>Results and discussion</i> 16
4.2	Preliminary Experiment 2: Effect of different ratios of infested field soil and potting mix on development of clubroot, and effect of benomyl on clubroot development 17
4.2.1	<i>Materials and methods</i> 17
4.2.2	<i>Results and discussion</i> 17
4.3	Preliminary Experiment 3: Identification of the race of <i>P. brassicae</i> present in infested field soil using the standard ECD host set 18
4.3.1	<i>Materials and methods</i> 18
4.3.2	<i>Results and discussion</i> 18
5	CHEMICAL CONTROL OF CLUBROOT 20
5.1	Objective 20
5.2	Materials and methods 20
5.3	Results 22
5.3.1	<i>Experiment 1</i> 22
5.3.2	<i>Experiment 2</i> 23
5.4	Discussion 28
5.5	Conclusions and recommendations 30
6	ACKNOWLEDGEMENTS 31
7	REFERENCES 32

1 EXECUTIVE SUMMARY

Control of clubroot (caused by *Plasmodiophora brassicae*) in vegetable brassicas has been identified by the New Zealand Vegetable & Potato Growers' Federation (VegFed) as an important priority for the fresh vegetable industry in New Zealand. In response, the NZ Institute for Crop & Food Research Ltd has undertaken a programme of research, funded by the VegFed Fresh Vegetable Industry Development Committee and appropriate agricultural chemical companies, to investigate the improved control of this disease. An initial report (Nott et al. 1994) and a NZ Commercial Grower article (Nott et al. 1995a) were prepared summarising current knowledge of clubroot of brassicas, and outlining possibilities for its control. Disease resistance and chemical and biological control were identified as areas of research with potential for improved control of clubroot.

Two research projects have now begun investigating clubroot control in vegetable brassicas. This report outlines their progress to date. The first project investigated the use of disease resistance to combat the disease in a field trial. A number of different brassica varieties and seedlines were evaluated for susceptibility to clubroot in a disease nursery maintained by Crop & Food Research at the Gore Research Station. Although no commercial brassica lines were shown to be totally resistant to race 17/15/12 of *P. brassicae*, several lines with field tolerance to clubroot have been identified.

In the second project, glasshouse trials were carried out to test the efficacy of a number of chemicals for clubroot control. Preliminary experiments established a suitable pot trial technique for testing chemicals. Soil drenches of cyprodinil, dichlofluanid, dichlorophen-Na, fluazinam, fludioxonil and mancozeb all reduced clubroot severity in treated plants, and gave levels of clubroot control equivalent to benomyl, a registered standard compound. While high rates of a formulation of flusulfamide (0.6 and 0.9 mg per plant) were phytotoxic to plants when applied as a soil drench, much lower rates (0.03 and 0.003 mg per plant) gave very good control of clubroot.

It is recommended that further field trials be carried out at a number of locations in New Zealand to test brassica lines identified as tolerant to clubroot for tolerance to different races of *P. brassicae*. Glasshouse and field experiments should also be undertaken to further assess chemicals that have been identified as having potential for clubroot control, and to determine the possibilities for incorporating both chemical and disease resistance approaches into an integrated strategy for clubroot control.

2 INTRODUCTION

Control of clubroot of vegetable brassicas has been identified by the New Zealand Vegetable & Potato Growers' Federation Inc. (VegFed) as an important priority for the fresh vegetable industry in New Zealand. Clubroot (caused by the soilborne obligate protostistan pathogen *Plasmodiophora brassicae*) can reduce marketable yields from vegetable brassica crops, and in severe cases completely destroy crops. The disease also has the potential to reduce the value of vegetable growing land by rendering it unsuitable for brassica production (Dixon 1988).

The first symptom of clubroot is wilting of infected plants, especially on warm days, although the plants usually recover overnight. As the disease progresses, leaves of affected plants turn yellow, the plants become stunted and may die in severe cases. The roots of infected plants become distorted and develop galls. In severe cases, root systems become very enlarged and swollen, and heavily infected roots eventually decay. Root damage reduces the capacity of plants to take up nutrients and water from the soil (Karling 1968; Biggs 1994).

While adequate control of clubroot is very important in vegetable brassica production, complete control of the disease has rarely been achieved, mainly because resting spores of *P. brassicae* remain viable in soils for many years (Nott et al. 1994). This research has focused on the two most promising approaches to clubroot control, i.e. the use of brassica lines resistant to the disease and the application of chemicals (Nott et al. 1994).

A field trial was undertaken with vegetable brassica seedlines, obtained from seed merchants in New Zealand and Japan, to test their susceptibility to *P. brassicae*. The trial, which was carried out in a clubroot disease nursery at Gore, Southland, aimed to identify resistant lines that could be further tested at a number of locations in New Zealand. The goal of this research is to compile a list of clubroot-resistant cultivars for New Zealand vegetable brassica growers.

In New Zealand only three fungicide products are registered for control of clubroot (O'Conner 1994). They are: benomyl (Benlate®), thiophanate-methyl (Topsin®) and chlorothalonil plus thiophanate-methyl (Taratek®). Metam sodium (Fumasol®) is also registered as a soil fumigant to control the disease. Recent investigations into the control of powdery scab of potatoes, caused by *Spongospora subterranea*, an organism closely related to *P. brassicae*, have shown that the chemicals mancozeb, fluazinam, dichlorophen-Na, flusulfamide and dichlofluanid can control this disease (Braithwaite et al. 1994; Falloon et al. 1994), and several of these compounds have been shown to control clubroot (Nott et al. 1994). These chemicals plus benomyl and two other candidate compounds were tested for their efficacy in controlling clubroot in glasshouse experiments.

3 CLUBROOT RESISTANCE IN VEGETABLE BRASSICAS

3.1 Objectives

The objectives of the research were to:

- obtain seed of all vegetable brassicas available in New Zealand and test them for clubroot resistance at a uniformly and heavily infested site at Gore,
- obtain seed of as many overseas lines as possible that have been bred for clubroot resistance, and test them at Gore, and
- select resistant lines that could be further tested against other races of clubroot at different sites in New Zealand, and from these trials compile a list of resistant cultivars for use by New Zealand vegetable brassica growers.

3.2 Materials and methods

3.2.1 Seed sources

New Zealand lines. Eight seed companies identified by VegFed were requested to supply seed of vegetable brassicas for clubroot testing. New World Seeds did not consider any of their lines to have resistance, but suggested two Japanese companies that could be approached because they were active in clubroot resistance breeding.

Yates Seeds supplied one cauliflower for testing, which was the only one of their lines considered to have any clubroot resistance. Watkins Seeds supplied five cabbage lines and one Brussels sprout line. Lefroy Valley Seed Co. supplied 16 lines and Webling & Stewart Seeds supplied 25 lines of various vegetable brassicas for testing. This response was less than expected, so Kings Seeds were then contacted and they supplied a further 25 lines of a wide variety of brassicas. Table 1 contains the list of varieties obtained and their suppliers.

Overseas Lines. Six Japanese seed companies were asked for seed of any vegetable brassicas that they had bred for clubroot resistance. Kyowa Seeds provided five lines of broccoli. Chinese cabbage seedlines were supplied by three companies: Nozaki Seed and Takii & Company (three lines each), and Sakata Seed Corp. (four lines) (see Table 1).

The two foremost researchers on clubroot in the USA and UK were asked for seed of resistant lines and information on other possible sources. Breeders in the USA have apparently not been active in this area for some years. Horticulture Research International (HRI) in the UK could only supply small quantities of breeding lines for testing. The only supposedly resistant line on the UK market is the broccoli, Trixie. Although this variety has yielded well in tests at HRI, it also produced very large clubs, has poor quality and is only sold for use in home gardens.

Requests for seed from breeders in France and the Netherlands were unsuccessful.

3.2.2 Clubroot susceptibility testing

The trial was carried out at the clubroot disease nursery at the Gore Research Centre, Gore, Southland. The disease nursery has been developed over 20 years and is heavily and uniformly infested with *P. brassicae*. The nursery area is used on a biennial cycle of sowing into brassicas and leaving fallow.

The area for the trial was ploughed and cultivated in early spring, and then sprayed with trifluralin herbicide and rotovated on 10 December 1994. The ground was left for a month to allow resistant weeds to germinate, and was then rotovated just before sowing on 17 January 1995. A cone seeder was used to sow the seeds in 6 m rows at 0.65 m spacing between rows, with two replicates sown in four blocks of 28 rows. Sowing rate was 10 seeds/m.

Ninety-seven lines of brassicas were tested, along with the 15 lines of the European Clubroot Differential (ECD) set. The ECD set comprises five lines each of the main brassica species including *Brassica campestris* (turnip), *B. napus* (swede and rape) and *B. oleracea* (cabbage and kale). The two standard swedes used in the clubroot paddock, Doon Major (susceptible) and Tina (resistant), were also included in the tests.

Ten weeks after sowing, the trial was scored for clubroot infection. Twenty plants from each replicate were dug out and the soil removed carefully so as not to break off any small clubs that may have developed on secondary roots. The plants were scored for clubroot severity using a four point scale: 0 = no disease; 1 = very small clubs on secondary roots; 2 = larger clubs on secondary or main root; and 3 = heavily infected roots. Plants that scored 0 or 1 were considered to be highly resistant to the disease.

A disease index was calculated using the formula:

$$\text{Disease Index} = \frac{\sum(\text{no. plants} \times \text{disease class})}{\text{total no. plants} \times 3} \times 100$$

This index gives a score of 100 if all plants were heavily infected (score 3), and zero if all plants were healthy.

Remaining plants of all lines having plants scored as resistant at 10 weeks were again checked for clubroot infection 20 weeks after sowing.

3.3 Results and discussion

Germination of the brassica lines was generally good. The later maturing *B. oleracea* lines were slower to emerge than the faster growing *B. campestris* lines, especially the Chinese cabbages. The cauliflowers were slow to germinate, with three lines in particular having very poor germination. Of the poor germinating lines, only two plants of Alverda, four of Snowball and seven of Deepheart developed. Broccoli line PC72, with only enough seed supplied for one replicate, also had poor germination resulting in only nine plants. The first signs of infection with clubroot were observed at six weeks, when wilting plants were seen during very dry spells (Fig. 1). At eight weeks, whole rows of susceptible plants were wilting badly when conditions were dry (Fig. 2). Clubroot severity scores for all the lines in the trial are summarised in Table 2, which gives the disease index for each line as the mean of the two replicates. The percentage of resistant plants (disease scores 0 and 1) is also given, which is usually close to the reciprocal of the disease index.

The results of the ECD set are presented in Table 3. Following the procedure of Buczacki et al. (1975), the race coding for *P. brassicae* race in this trial is ECD 17/15/12. The only lines fully resistant to the Gore clubroot race were ECD 03 and ECD 04, both of which are turnips. Of the kale/cabbage differentials, ECD 15 had almost completely resistant plants (one plant scored as Class 2 (98% resistant); see Fig. 3), and ECD 12 had two plants scored as Class 2 (95% resistant). The next most resistant line in the trial was the resistant swede Tina, with only three plants scored as Class 2 (93% resistant).

None of the commercial lines tested were fully resistant to *P. brassicae*. However, five lines had 75% or more of their plants scored as resistant. These were: broccoli Hanamori (80%), cabbages, Galaxy (78%) and Beverly Hills (75%), kohlrabi, Purple Vienna (83%) and Brussels sprout Dolmic (78%). The broccoli lines, Shigmori, Atsumori, Foed Hook and No. 133 had between 60% and 65% of the plants scored as resistant to clubroot. Cabbage, Rondy, kale, Palm Tree, kohlrabi, Gigante, cauliflower, All Year Round, and savoy cabbage, Taler, had between 63% and 73% of the plants scored as resistant. Brussels sprout, Titural, had 63% of the plants recorded as resistant with lines Pantera, Earlypick and Content having 58% resistant plants.

Resistance to clubroot is generally considered to be of the race-specific type in swedes and turnips (*B. napus* and *B. campestris*) and the non-specific, field resistance type in the cabbage/kale group (*B. oleracea*). The present results are in agreement with this generalization, with most of the *B. oleracea* lines having between 25% and 75% resistant plants, and the *B. napus* and *B. campestris* lines generally being totally susceptible (Chinese cabbages and Japanese greens) or highly resistant (Tina swede and ECD 03 and 04 turnips).



Figure 1. First symptoms of clubroot infection in a susceptible brassica line 6 weeks after sowing in the Gore disease nursery. Individual plants are wilting in dry conditions.



Figure 2. (above) A clubroot susceptible line (Wong Bok, centre) showing severe wilting under dry conditions 8 weeks after sowing in the Gore disease nursery. Other lines (Red Russian, right and NS25, left) are more tolerant to the disease and appear unaffected by the dry conditions.



Figure 3. Line ECD15 (middle) is highly resistant to the clubroot strain in the Gore disease nursery, compared with the two neighbouring lines (top and bottom) which are heavily infected with the disease.



Figure 4A. Two Chinese cabbage lines in the clubroot nursery at Gore. The line in the foreground (Wong Bok) appears highly susceptible to the disease and is severely stunted, while the line in the background (NS25) appears to be more tolerant.



Figure 4B. The same two lines (Wong Bok; foreground: NS25; background) are actually both heavily infected with clubroot when dug from the soil and assessed.

Table 2: Varieties of vegetable brassicas in clubroot trial, their type, disease index and proportion of plants scored as resistant.

Name	Type ^a	Disease Index	% Resistant	Name	Type ^a	Disease Index	% Resistant
Hanamori	B	23	80	NS25	H	99	3
Shigemori	B	35	65	Two Seasons	H	98	3
Atsumori	B	36	63	Chorus	H	96	3
Foed Hook	B	37	61	Mari	H	100	0
No131	B	42	60	T651	H	100	0
Nine Star	B	48	49	Yuki	H	100	0
Triplex	B	51	47	NU70	H	100	0
Italian	B	55	45	NY90	H	100	0
PC72	B	52	44	Shinki	H	100	0
Rio Grande	B	60	36	Fire Dragon	H	100	0
Purple Early	B	65	32	Satuko	H	100	0
Shogun	B	70	28	Tah Tsai	H	100	0
Takamori	B	74	25	Komachi	H	100	0
Duplex	B	79	18	Bouquet	H	100	0
Broccoli Raab	B	100	0	Wong Bok	H	99	0
Galaxy	C	22	78	Palm Tree	K	23	73
Beverly Hills	C	23	75	Red Russian	K	93	5
Rondy	C	33	66	Purple Vienna	L	17	83
Summer Green	C	43	53	Gigante	L	26	68
T21	C	48	51	White Danube	L	51	43
Summer Cross	C	53	45	Giant Red	M	87	13
Stonehead	C	52	45	Miike	M	92	8
Castello	C	52	45	Horned Mustard	M	95	5
Derby Day	C	57	38	Komatsuna	M	97	3
Tucana	C	59	38	Green Snow	M	100	0
Oxylus	C	55	33	Savanna	M	100	0
Winter Hero	C	70	33	Swatow	M	99	0
Charlie	C	70	30	Tsai Shim	O	57	40
Sabina	C	69	29	Hon Tsai Tai	O	99	3
Ormskirk	C	66	25	Joi Choi	O	100	0
Stilon	C	83	13	Mei Quing	O	98	0
All Year Round	F	37	64	Dolmic	P	28	78
Whiteacre	F	42	53	Titural	P	36	63
Violet Sicilian	F	47	53	Panatera	P	42	58
Dova	F	53	48	Earlypick	P	40	58
All Rounder	F	50	45	Content	P	39	58
Dok Elgon	F	55	40	Lunet	P	63	30
Arcade	F	55	40	Roxy	R	67	25
Armetta	F	58	38	Tina	S	14	93
Sernio	F	66	33	Doon Major	S	89	10
Ivory	F	87	10	Doon Major	S	91	8
Paradiso	F	90	8	Tokyo Cross	T	98	3
Orange Bouquet	F	92	5	White Stone	T	99	0
Misome	G	88	13	Taler	V	39	63
Santoh Frilled	G	100	0	Wivoy	V	63	33
Green spray	G	100	0	Salto	V	84	15
Tokyo Belle	G	100	0				
Mizuna	G	99	0				
Santoh Round	G	99	0				

***Brassica type**

B Broccoli
 C Cabbage
 F Cauliflower
 G Japanese greens
 H Chinese cabbage

K Kale
 L Kohl rabi
 M Mustard greens
 O Pac Choi
 P Brussels sprouts

R Red cabbage
 S Swede
 T Turnip
 V Savoy cabbage

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Table 3: Disease index and proportion of resistant plants in the ECD differential set.

Differential code	Type	Disease Index	% Resistant
ECD01	Turnip	38	60
ECD02	Turnip	13	88
ECD03	Turnip	2	100
ECD04	Turnip	0	100
ECD05	Chinese cabbage	100	0
ECD06	Forage rape	61	41
ECD07	Forage rape	93	8
ECD08	Forage rape	84	13
ECD09	Forage rape	68	33
ECD10	Swede	24	78
ECD11	Cabbage	19	83
ECD12	Cabbage	6	95
ECD13	Cabbage	50	50
ECD14	Cabbage	73	25
ECD15	Kale	2	98

In general, the broccoli lines were able to tolerate clubroot, even though some of the lines were assessed as highly susceptible to the disease. For example, Duplex continued to grow well and produced some good heads. Of the five reputedly resistant lines provided by Kyowa Seed, all plants produced good heads, including the line recorded as having only 25% resistant plants.

The Brussels sprouts lines were also able to tolerate the disease, although they were not given suitable growing conditions to produce large productive plants. It is unlikely, however, that the plants would have produced marketable product before degradation of the root system, because these varieties have a long growth phase. Broccoli varieties, on the other hand, are quick maturing, and are usually able to produce good heads before being seriously affected by clubroot.

The growing conditions in the trial were not ideal for cabbages. While Galaxy and Beverly Hills both had over 75% of plants classed as resistant, neither produced good, firm heads. The remaining lines all had 25% or more plants which appeared resistant to clubroot, except Stilon which had only 13% of plants classified as resistant.

For some of the vegetable brassica lines tested, there were marked differences between the two replicates in the number of plants recorded as resistant. Galaxy is an example of this, with all plants recorded as resistant in one replicate, while only 11 plants were recorded as resistant in the second replicate. Several other lines (Shigemori, Atsumori, All Year Round, Purple Vienna and Earlypick) also had similar differences. Lines scored as having resistant plants at 10 weeks were checked again at 20 weeks and, although there was still an occasional plant that appeared

uninfected, all lines were by then heavily infected and, at least, had clubs on the lateral roots.

None of the commercial lines appeared to be resistant to clubroot after 20 weeks. Several lines appeared to be able to tolerate the disease, apart from the ones scored as resistant at 10 weeks. However, this was under the climatic conditions at Gore, where there was a soil water deficit for only a short period in late summer followed by sufficient moisture to allow all but the worst infected lines to survive.

It must be noted that, although the clubroot paddock at Gore contains *P. brassicae* which is highly pathogenic to swedes and turnips, it may be less pathogenic to *B. oleracea* lines. A race of clubroot was isolated from a market garden near Christchurch, Canterbury, which attacked kale and cabbage, but not turnips and swedes (Lammerink 1986). It is therefore possible that lines shown to be resistant to race ECD 17/15/12 at Gore may be susceptible to other races.

Direct sowing the seed into clubroot infested soil, as in this trial, is a more severe test of susceptibility to the disease than transplanting seedlings into infested soil. *P. brassicae* is capable of infecting emerging seedlings, whereas transplants have a well developed root system before being exposed to the pathogen in field soil. It is therefore possible that partially resistant lines identified in this trial may show good resistance when grown from transplants.

3.4 Conclusions and recommendations

This trial has shown that no commercial vegetable brassica lines are totally resistant to *P. brassicae* race 17/15/12 present in the disease nursery in Gore. Several lines have been identified that show some resistance or field tolerance to this race. It is important that these lines are now tested at other sites in New Zealand to examine their resistance to other races of clubroot in other climatic conditions, soil types and cropping histories. Normal commercial transplanting should be used to establish future trials.

It is possible that vegetable brassica lines that do not show high levels of resistance to clubroot but have field tolerance to the disease may still be important components of strategies for clubroot control. Other parts of this research programme (see below) have examined chemical control of the disease, and it is proposed that the potential for biological control of clubroot is investigated in the future. All three components (disease resistance, chemical and biological controls) are likely to be important in an integrated approach to reducing the deleterious effects of this disease in commercial vegetable brassicas crops.

4 PRELIMINARY GLASSHOUSE EXPERIMENTS ON CLUBROOT OF BRASSICAS

4.1 Preliminary experiment 1: Production of clubroot symptoms in glasshouse-grown plants

4.1.1 *Materials and methods*

Clubroot infested soil was obtained from a commercial field where cauliflowers had exhibited clubroot symptoms. Three seedling trays were filled with a 1:1 mixture of field soil and potting mix and each tray was sown with about 20 seeds of either of the Chinese cabbages Wong Bok, Chi Hi Li (both commercially available in New Zealand) or Granaat (ECD 05). The trays were left on a glasshouse bench for 4 weeks. All the plants were then carefully removed from the trays by washing the root systems free of soil. The roots of each plant were scored for clubroot using a 0 - 3 scale of Buczacki et al. (1975): 0 = no clubroot; 1 = very light swelling on lateral roots; 2 = moderate swelling on lateral and/or tap roots; and 3 = severe swelling on the lateral and/or tap roots. A severity score was calculated using the following formula:

$$\text{Severity Score} = \frac{\sum(\text{no. of plants in category} \times \text{category score})}{\text{total no. of plants}}$$

4.1.2 *Results and discussion*

All three lines of Chinese cabbage used in this experiment exhibited severe clubroot symptoms (Table 4). This indicated that the field soil was heavily infested with *P. brassicae* and suitable for use as the inoculum source in further glasshouse experiments.

Table 4: Mean severity scores for three Chinese cabbage varieties grown in a 1:1 mixture of clubroot infested field soil and potting mix.

Variety	Severity score
Wong Bok	2.1
Chi Hi Li	2.4
ECD 05	2.6

4.2 Preliminary Experiment 2: Effect of different ratios of infested field soil and potting mix on development of clubroot, and effect of benomyl on clubroot development

4.2.1 *Materials and methods*

Infested field soil and potting mix were combined in various ratios (Table 5). Ten cm diameter plastic pots were filled with 600 ml of one of the field soil and sand/peat potting mix combinations. The sand/peat potting mix contained a 4:1 mixture of composted bark and sand with added nutrients. Each pot was then planted with two 14-day-old Chinese cabbage Granaat (ECD 05) plants. Eight pots of each of the field soil/potting mix combinations shown in Table 5 were used. Four of these pots were left untreated while the other four were treated with the fungicide, benomyl. The fungicide was made up at the rate of 50 g benomyl (100 g Benlate®)/100 l water, according to label recommendations for soil drench application for clubroot control. Fungicide suspension (200 ml/pot) was poured over the soil in each pot, taking care to avoid contact between transplant leaves and fungicide. Pots were placed in metal trays on a glasshouse bench where temperature was maintained at approximately 20°C. The benomyl treated and untreated pots were kept in separate trays, as were the pots containing 100% field soil and 100% potting mix, to avoid cross-contamination with fungicide and/or *P. brassicae*. At 5 weeks after transplanting and fungicide treatment, the plants were removed from pots by washing root systems free of soil. The root system of each plant was scored for clubroot using a 0-4 scale: 0 = healthy, 1; = up to 5% roots galled; 2 = 5-20% roots galled; 3 = 20-50% roots galled; and 4 = >50% of roots galled. A severity score was calculated using an appropriately modified formula (see 4.1.1 above).

4.2.2 *Results and discussion*

Results from this experiment are summarised in Table 5. No clubroot was observed on Chinese cabbage plants grown in 100% potting mix. Very severe clubroot occurred in all pots containing clubroot-infested field soil, even at the lowest ratio of field soil to potting mix. Mean severity scores between 3.25 to 4.00 were recorded, similar to results for the 100% field soil treatment.

It was noted that plants growing in 100% field soil were small compared with those in 100% potting mix. Plant size increased as the ratio of potting mix in the combinations increased. Decreasing the ratio of field soil to potting mix had little effect on the severity level of clubroot in plants, as all plants that were not treated with fungicide were severely diseased. In pots treated with benomyl, no clubroot occurred except for one plant in the 1:7 field soil to potting mix treatment that had a disease score of 1 (up to 5% roots infected).

This experiment demonstrated that mixtures of infested field soil with sand/peat potting mix in the ratio of 1:7 resulted in severe clubroot in Chinese cabbage plants, and would be suitable for glasshouse experiments. Furthermore, the volume of fungicide suspension used in this experiment was excessive, so a smaller volume would be suitable for future tests.

Table 5: Mean severity scores for the Chinese cabbage ECD 05 grown in pots containing different ratios of infested field soil and potting mix, either untreated or drenched with benomyl.

Ratio field soil:potting mix	Mean severity score	
	Untreated	Benomyl-treated
1:0	4.00	0.00
1:1	3.75	0.00
1:3	3.25	0.00
1:7	4.00	0.12
0:1	0.00	0.00

4.3 Preliminary Experiment 3: Identification of the race of *P. brassicae* present in infested field soil using the standard ECD host set

4.3.1 Materials and methods

Three seeds of each of the 15 ECD host set were sown into each of ten (10 cm diameter) plastic pots containing a 1:7 mixture of *P. brassicae*-infested field soil with sand/peat potting mix (see 4.2 above). The pots were placed in metal trays on a glasshouse bench (temperature approximately 20°C). After six weeks, the plants were harvested by washing root systems free of soil, and the roots of each plant were scored for clubroot using a 0-4 severity scale (see 4.2.1 above).

4.3.2 Results and discussion

Results are summarised in Table 6. Following the system of Buczacki et al. (1975), the race of *P. brassicae* present in the field soil was identified as ECD 16/31/31.

A period of growth longer than the six weeks used in this experiment could give a better level of disease in the test plants, particularly in the Chinese cabbage differential 05. A period of 8-9 weeks, equivalent to about six weeks after full emergence, would be more suitable than the period used in this experiment.

Table 6: Number of plants recorded in each disease category and mean severity score of the ECD host set when grown in a medium containing infested field soil and potting mix (1:7 mixture).

Host code	Line group	Disease category ¹					Mean severity score
		0	1	2	3	4	
<i>Brassica rapa</i>							
ECD 01	fodder turnip	28	0	0	0	0	0.0
ECD 02	fodder turnip	29	0	0	0	0	0.0
ECD 03	fodder turnip	29	0	0	0	0	0.0
ECD 04	fodder turnip	29	0	0	0	0	0.0
ECD 05	Chinese cabbage Granaat	4	8	6	5	6	2.0
<i>Brassica napus</i>							
ECD 06	fodder rape Nevin	0	10	16	4	0	1.8
ECD 07	giant rape commercial	0	3	9	8	6	2.7
ECD 08	giant rape selection	0	7	11	3	5	2.2
ECD 09	New Zealand resistant rape	0	13	11	2	1	1.7
ECD 10	swede Wilhelmsburger	0	13	11	3	0	1.6
<i>Brassica oleracea</i>							
ECD 11	cabbage Badger Shipper	0	8	17	2	2	1.9
ECD 12	cabbage Bindsachsener	0	6	17	6	0	2.0
ECD 13	cabbage Jersey Queen	0	10	14	5	1	1.9
ECD 14	cabbage Septa	0	3	15	10	0	2.3
ECD 15	fimbriate kale Verheul	0	6	12	9	0	2.1

¹ Number of plants in each disease category

5 CHEMICAL CONTROL OF CLUBROOT

5.1 Objective

The objective of this research was to test the efficacy of a range of soil-applied chemicals for control of clubroot of Chinese cabbage.

5.2 Materials and methods

Fourteen-day-old seedlings of Granaat Chinese cabbage (ECD 05) were transplanted (two seedlings per pot) into 10 cm diameter plastic pots, each containing 600 ml of a 1:7 mixture of *P. brassicae*-infested field soil and potting mix (see 4.2 above). The pH of the mixture was 5.6.

Sixteen different chemical treatments (Table 7) were applied to the pots, and two water control treatments were also included in the experiment. Each chemical was made up to 500 ml with water, and 100 ml of solution was poured evenly on to the soil surface in each pot, taking care to avoid contact between solutions and leaves of transplants. A randomised block design was used for this experiment, with five replicates and one pot (two plants) for each treatment in each replicate. Pots were placed on individual plastic saucers, to prevent cross contamination with chemicals, on a glasshouse bench at approximately 20°C and watered twice daily.

Plant height was measured at 1, 3, 5 and 6 weeks after transplanting and application of treatments. Six weeks after transplanting, the plants were removed from pots and their roots were washed free of soil. Fresh weight of roots and above ground plant parts were recorded for each plant. The roots of each plant were scored for clubroot using a 0-4 scale (see 4.2.1 above). Dry weight of plants was recorded after overnight drying at 80°C.

A second experiment (Table 8), using the chemical flusulfamide at three rates which were lower than tested in the first experiment, was carried out in a manner identical to that described above, except that only one water control was used. This experiment was undertaken because the rates of flusulfamide used in the first experiment were phytotoxic.

Data were analysed by analysis of variance, after appropriate transformation of data where necessary.

Table 7: Chemical treatments applied as drenches to Chinese cabbage transplants in pots containing *P. brassicae*-infested soil.

Treatment name	Product	Application rate		
		mg ai/plant	prod/plant	prod/l
Benomyl 100	Benlate 50WP	100.0	200.0	4.00 g
Benomyl 25	Benlate 50WP	25.0	50.0	1.00 g
Cyprodinil 100	Unix 75WG	100.0	133.3	2.67 g
Cyprodinil 25	Unix 75WG	25.0	33.3	0.67 g
Cyprodinil 12.5	Unix 75WG	12.5	16.7	0.33 g
Dichlofluanid 25	Euparen	25.0	50.0	1.00 g
Dichlofluanid 2.5	Euparen	2.5	5.0	0.10 g
Dichlorophen-Na 50	Mostox	50.0	116.0	2.31ml
Dichlorophen-Na 25	Mostox	25.0	58.0	1.15ml
Fluazinam 25	Shirlan	25.0	50.0	1.00ml
Fluazinam 12.5	Shirlan	12.5	25.0	0.50ml
Fludioxonil 25	Celest	25.0	1000.0	20.00ml
Flusulfamide 0.9	MTF 651 liquid	0.9	18.0	360.00ml
Flusulfamide 0.6	MTF 651 liquid	0.6	12.0	240.00ml
Mancozeb 100	Pencozeb	100.0	129.5	2.59 g
Mancozeb 25	Pencozeb	25.0	32.5	0.65 g

Table 8: Flusulfamide treatments applied as drenches to Chinese cabbage transplants in pots containing *P. brassicae*-infested soil.

Treatment name	Product	Application rate		
		mg ai/plant	prod/plant	prod/l
Flusulfamide 0.003	MTF 651 liquid	0.003	0.06	1.2 ml
Flusulfamide 0.030	MTF 651 liquid	0.030	0.60	12.0 ml
Flusulfamide 0.300	MTF 651 liquid	0.300	6.00	120.0 ml

5.3 Results

5.3.1 Experiment 1

All Chinese cabbage plants treated with both rates of flusulfamide died within one week of treatment. One plant treated with water and one treated with cyprodinil 12.5 were dead at time of harvest, probably as a result of the severe clubroot that developed in these treatments. All other plants survived to harvest.

Clubroot severity: Differences ($P < 0.01$) among treatments compared with the water controls were detected for mean severity score, and mean plant, shoot and root weights (Table 9). All treatments, except cyprodinil 12.5 and 25 reduced ($P < 0.01$) clubroot severity. The most effective treatments, benomyl (both rates), fluazinam (both rates), dichlorophen-Na 50, cyprodinil 100 and dichlofluanid 25, all gave mean severity scores less than 0.5. The mean severity scores for each of these treatments were not significantly different ($P > 0.05$).

Plant weight: Six of the treatments increased ($P < 0.05$) mean plant weight compared to the control. These treatments were; benomyl (both rates), dichlofluanid 2.5, dichlorophen-Na 25, fluazinam 25 and mancozeb 25. Cyprodinil 100, however, gave a mean plant weight which was less ($P < 0.01$) than the water controls.

Shoot weight: All treatments, except those containing cyprodinil and the dichlorophen-Na 50 treatment, increased ($P < 0.05$) mean shoot weights of plants. Benomyl 100 gave the greatest mean shoot weight. The cyprodinil 100 treatment reduced ($P < 0.01$) mean shoot weight compared with the controls.

Root weight: All treatments except cyprodinil 12.5 and 25 had mean root weights less than ($P < 0.01$) the controls. Cyprodinil 25 gave a mean root weight similar to the controls while cyprodinil 12.5 increased ($P < 0.01$) mean root weight. The cyprodinil 100 treatment gave very small roots which weighed less ($P < 0.01$) than those from all other treatments.

Shoot dry weight: Most of the treatments increased ($P<0.01$) shoot dry weight of plants compared with controls. The exceptions were the cyprodinil 12.5 and 25 treatments, dichlorophen-Na 50 and fludioxonil 25, which did not affect ($P>0.05$) shoot dry weight of plants, and the cyprodinil 100 treatment which reduced ($P<0.01$) mean shoot dry weight. There were no rate effects ($P>0.05$) on shoot dry weight for any of these chemicals.

Root dry weight: All treatments, except cyprodinil 12.5 and 25, gave lower ($P<0.01$) mean root dry weights of plants than the controls. The lowest rate of cyprodinil increased ($P<0.01$) mean root dry weight, while the cyprodinil 25 treatment gave a mean root dry weight similar to that of the controls.

Percent dry matter: Only the cyprodinil 100 treatment increased ($P<0.01$) the percent dry matter in plant roots from 11.2% in the controls to 19.3%. None of the other treatments affected ($P>0.05$) proportions of dry matter in shoots or roots of plants.

Plant height: The effects of different treatments on height of the Chinese cabbage plants at different stages throughout the experiment are indicated in Table 10. Eight of the treatments (benomyl 100, cyprodinil 100, dichlofluanid 25, dichlorophen-Na (both rates), fluazinam 12.5, fludioxonil 25 and mancozeb 100) reduced ($P<0.01$) mean plant height one week after transplanting and treatment, compared with the control treatments. Rate effects were observed with mancozeb, where plants treated with mancozeb 100 were smaller ($P<0.05$) than plants in the mancozeb 25 treatment. Three weeks after treatment, only the cyprodinil 100 and dichlorophen-Na 50 treatments gave plants with mean heights less ($P<0.01$) than the controls. A rate effect was detected for dichlorophen-Na, with the plants treated with 50 mg being smaller ($P<0.01$) than those treated with 25 mg. No rate effects ($P>0.05$) were detected for the other chemicals. At 5 weeks after treatment, only the cyprodinil 100 treatment reduced ($P<0.01$) plant height compared with the controls, while treatments with benomyl (both rates), cyprodinil 25, dichlofluanid 2.5, fluazinam (both rates), fludioxonil 25 and mancozeb 25 all increased ($P<0.05$) mean plant height compared with the controls. No rate effects ($P>0.05$) were detected for any of the chemicals. At harvest (6 weeks after transplanting and treatment), the cyprodinil 100 treatment reduced ($P<0.01$) mean plant height while all of the other treatments increased ($P<0.05$) mean plant height relative to the controls. Again, no rate effects ($P>0.05$) were detected for any of the chemicals.

5.3.2 **Experiment 2**

All Chinese cabbage plants treated with flusulfamide 0.3 died within three weeks of treatment and three plants treated with flusulfamide 0.03 were dead at five weeks after treatment. All of the plants treated with flusulfamide 0.003 survived to be harvested at six weeks.

Table 11 summarises the results obtained from this experiment. Both of the flusulfamide treatments where plants survived to harvest reduced clubroot severity, and reduced root fresh and dry weights of plants compared with the controls (all effects statistically significant at $P < 0.01$). No rate effects ($P > 0.05$) were detected for this chemical. The treatments did not affect ($P > 0.05$) means of total plant weight, shoot weight, shoot dry weight or proportion of shoot or root dry matter. Furthermore, these treatments did not affect ($P > 0.05$) plant height at any time during the experiment (Table 12).

Table 10: Mean heights of Chinese cabbage plants grown for different periods after treating with different chemicals

Treatment	Time (weeks) after treatment							
	1		3		5		6	
	Log	mm	Log	mm	Log	mm	Log	mm
Benomyl 100	1.92**	83	2.20	157	2.36**	228	2.39**	248
Benomyl 25	1.99	98	2.23	168	2.33**	211	2.36**	231
Cyprodinil 100	1.86**	72	2.06	114	2.13	136	2.17	1487
Cyprodinil 25	1.99	98	2.22	168	2.32*	209	2.32*	208
Cyprodinil 12.5	2.00	100	2.24	174	2.29	195	2.31*	203
Dichlofuanid 25	1.93**	86	2.20	160	2.31	202	2.33*	211
Dichlofuanid 2.5	1.95	89	2.23	169	2.32*	207	2.34**	220
Dichlorophen-Na 50	1.80**	63	2.13	134	2.28	191	2.33**	214
Dichlorophen-Na 25	1.87**	74	2.20	159	2.31	207	2.36**	228
Fluazinam 25	1.96	92	2.21	163	2.34**	214	2.38**	239
Fluazinam 12.5	1.92**	84	2.21	160	2.34**	218	2.37**	236
Fludioxonil 25	1.93**	84	2.19	155	2.32*	210	2.35**	224
Mancozeb 100	1.89**	78	2.20	158	2.30	197	2.33**	215
Mancozeb 25	1.98	96	2.23	170	2.34**	218	2.38**	240
Mean of controls	2.01	101	2.22	165	2.27	184	2.27	188
LSD ($P<0.05$) ^a	0.07		0.05		0.04		0.04	
LSD ($P<0.01$) ^a	0.09		0.07		0.06		0.06	

* and ** indicate treatment means different ($P<0.05$ and 0.01 respectively) from mean of controls
^aLSDs for comparison of treatment means with mean of controls

Table 11: Mean parameters measured for Chinese cabbage plants grown in potting soil infested with *P. brassicae*.

Treatment	Severity Score	Plant weight		Shoot weight		Root weight		Shoot dry weight		Root dry weight	
		Log	g	Log	g	Log	g	Log	g	Log	g
Flusulfamide 0.003	0.30**	1.42	31.00	1.40	29.18	0.41**	1.76	0.48	2.13	0.06**	0.14
Flusulfamide 0.03	0.10**	1.31	23.81	1.30	22.20	0.39**	1.61	0.43	1.83	0.06**	0.15
Mean of Controls	3.70	1.40	29.50	1.30	23.16	0.76	5.26	0.41	1.67	0.18	0.50
LSD ($P < 0.05$)*	0.57	0.29		0.30		0.21		0.17		0.06	
LSD ($P < 0.01$)*	0.83	0.43		0.43		0.30		0.25		0.09	

** indicates treatment different ($P < 0.01$) from mean of controls.

* LSDs for comparison of treatment means with mean of controls.

Table 12: Mean heights of Chinese cabbage plants measured at different times after treating with different rates of flusulfamide.

Treatment	Time (weeks) after treatments applied							
	1		3		5		6	
	Log	mm	Log	mm	Log	mm	Log	mm
Flusulfamide 0.003	2.01	102	2.25	179	2.34	220	2.34	221
Flusulfamide 0.03	1.94	87	2.20	158	2.29	193	2.29	196
Mean of controls	2.04	109	2.26	183	2.32	208	2.32	211
LSD ($P < 0.05$) ^a	0.08		0.07		0.07		0.08	
LSD ($P < 0.01$) ^a	0.12		0.10		0.11		0.11	

^aLSDs for comparison of treatment means with mean of controls.

5.4 Discussion

These experiments have established a pot trial method for testing the efficacy of chemicals as soil drenches to control clubroot of Chinese cabbage, and have used the method to test a range of products for controlling the disease. We have compared the test compounds with a product (benomyl) that is registered as a transplant soil drench for control of clubroot of cabbage (O'Connor 1994).

All of the chemicals tested (cyprodinil, dichlofluanid, dichlorophen-Na, fluazinam, fludioxonil and mancozeb) reduced the severity of clubroot in Chinese cabbage plants when the chemicals were applied as soil drenches in pots containing soil infested with *P. brassicae*. Control of clubroot was reflected in the size and weights of plants with little disease at the end of experiments that lasted for six weeks. The efficacious treatments increased plant shoot weight, and reduced root weight compared with heavily infected plants that resulted from control (water) treatments. Increased shoot weight resulted from improved growth of plants treated with efficacious chemicals, while reduced root weight was due to the much smaller root mass in healthy plants than in those heavily infected with clubroot.

Flusulfamide was the most active of the chemicals against clubroot. While the product containing this chemical (MTF 651) was phytotoxic when applied as a soil drench at a rate containing as little as 0.3 mg per plant, in a repeat experiment flusulfamide gave control of clubroot at rates (0.03 and 0.003) that were much lower (up to 1000-fold less) than were required for most of the other chemicals tested. Although the results of Experiments 1 and 2 are not directly comparable, the severity of clubroot in control plants was similar in both experiments. In Experiment 2, 0.003 mg of flusulfamide per plant gave control of clubroot that was similar to that

achieved with 25 mg of benomyl per plant, and even greater rates of some of the other chemicals tested.

MTF 651 is a product containing only 5% of flusulfamide. It is possible that the formulation constituents, rather than the active ingredient, may have caused or enhanced the phytotoxic effects observed in this study. Very high amounts of the product were required, compared with the other products tested, in order to apply the required amount of active ingredient. MTF 651 may be an unsuitable product for soil drench application and be better suited for soil incorporation. This method of application was not tested in the present study, as comparison of different application techniques was not undertaken in a test designed to determine relative efficacy of chemicals. Furthermore, as excellent efficacy of this chemical has been recently demonstrated elsewhere against both clubroot of cabbage and cauliflower (Dixon et al. 1994) and powdery scab of potato (Dixon et al. 1994; Nott et al. 1995b), further evaluation of flusulfamide is warranted. Future trials with this chemical should include investigation of alternative application techniques and the use of different chemical formulations, preferably with higher proportions of active ingredient.

Of the other chemicals tested, dichlofluanid, fluazinam, fludioxonil and mancozeb all gave levels of clubroot control that were equivalent to that achieved with benomyl. All of these chemicals gave low disease severity and increased shoot weight in treated plants compared with controls. Dichlorophen-Na was less efficacious than these other chemicals, and while reducing clubroot severity, did not increase plant shoot weight compared with controls. Cyprodonil was the least efficacious of the chemicals tested; although this chemical reduced clubroot severity, the rate required to achieve control caused severe stunting and elevated proportion of dry matter in the test plants.

These experiments have demonstrated only very few rate effects for the chemicals tested. However, as most of the compounds showed efficacy for clubroot control, further research to determine suitable rates of application is warranted. The glasshouse method developed in the present study could be used to determine comparative rate effects of different chemicals, and provide results that could better indicate more precisely appropriate rates for subsequent field testing.

Evaluation of the most effective chemicals under field conditions should proceed so their potential for clubroot control in commercial vegetable brassica crops can be established. These trials should be carried out with a number of different vegetable brassica types, including cultivars and seedlines that have shown partial resistance to the disease (Section 3). The possibility of incorporating chemical and disease resistance strategies into an integrated approach to clubroot control could thus be investigated.

5.5 Conclusions and recommendations

Glasshouse experiments have shown that several chemicals (dichlofluanid, dichlorophen-Na, fluazinam, fludioxonil, flusulfamide, and possibly mancozeb) show potential for control of clubroot in Chinese cabbage. Further testing of chemicals in glasshouse experiments should attempt to establish comparative rate effects. Efficacious chemicals should also be tested in field trials with several vegetable brassica types, and at different locations, to determine their potential for control of the disease in commercial brassica crops in different soil types and climatic conditions. These experiments should also consider different methods of chemical application (soil drench, soil incorporation, seedling dip), paying attention to appropriate rates of application. Furthermore, the trials should investigate the potential for incorporating disease resistance and chemical controls into an integrated strategy for clubroot control.

6 ACKNOWLEDGEMENTS

This research was financially supported by the Fresh Vegetable Industry Committee of the New Zealand Vegetable and Potato Growers' Federation Inc., Bayer NZ Ltd, Ciba-Geigy NZ Ltd, Crop Care Holdings Ltd and Elliott Chemicals Ltd. The assistance of Mr S D Armstrong, with establishment and scoring of the cultivar field trial, and Mr A R Wallace (biometrician, Crop & Food Research), with statistical analyses of data from the fungicide experiments, is gratefully acknowledged.

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