Project Title	Cut flowers: evaluation of drip-applied chloropicrin for control of soil-borne *Fusarium oxysporum*, *Rhizoctonia* species, *Sclerotinia sclerotiorum* and weed seeds (Phase II)
Project number:	PC 249a
Project leader:	Dr Tim O'Neill, ADAS Arthur Rickwood
Report:	Final report
Previous report	March 2006 (jointly with PC 213)
Key staff:	Dr Kim Green, Ms Amanda Shepherd, ADAS Arthur Rickwood Mr Geoff Thorpe, ADAS
Location of project:	Kent and ADAS Arthur Rickwood, Cambs
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Date project commenced:	1 April 2006
Date completion due:	30 August 2006
Key words:	Chloropicrin, soil disinfestation, *Fusarium oxysporum*, *Rhizoctonia* sp., *Sclerotinia sclerotiorum*, weeds

The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Grower Summary

Headline

- Results from two experiments on different soil types confirm that soil treatment with chloropicrin applied via drip-line irrigation can give broad-spectrum soil disinfestation, significantly reducing inoculum levels of the soil-borne pathogens *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum,* and provide effective control of broad leaf and grass weeds.
- Soil disinfestation of greenhouse soil with chloropicrin is now possible by use of drip-line irrigation; it is available only as a certificated contractor application service.

Background and expected deliverables

Where there is intensive and repeat cropping on a nursery, soil disinfestation can help to prevent damaging attacks of soil-borne pathogens such as fusarium, pythium, rhizoctonia and verticillium. In the cut flower sector soil disinfestation is commonly done before cropping chrysanthemum, lisianthus and stock. Following the recent loss of methyl bromide as a soil fumigant, there is keen interest in all potential alternatives for protected cut flower crops.

Chloropicrin is a broad-spectrum soil fumigant. Currently in the UK it is mainly used to disinfest soil prior to planting strawberry, the main target pathogen being *Verticillium dahliae*, cause of strawberry verticillium wilt. Treatment of open ground is usually by shank injection and this has been shown to give good control of *V. dahliae*. Under the Long Term Arrangements for Extension of Use (LTAEU) of pesticides, chloropicrin is now permitted for use as a pre-plant treatment for protected cut flowers. This follows two recent SOLAs (1579/2005 and 1948/2005) that permit pre-plant treatment for both protected and unprotected raspberry ground. The use of chloropicrin is strictly restricted to certificated contractor application only. The only safe and practicable method of application for this treatment in the confined environment of a glasshouse or ridged polytunnel is through the drip-tape irrigation system with the soil surface sealed with virtually impermeable barrier film prior to application. Throughout the world, drip application is a very common method of application for a wide range of soil treatments. The fumigant is suspended and delivered in the irrigation water by combining it with a carrier.

An initial experiment in December 2005, undertaken by ADAS in conjunction with K&S Fumigation Services Ltd, showed significant reductions in soil-borne *Fusarium oxysporum,*

Rhizoctonia solani and *Sclerotinia sclerotiorum* and provided effective weed control. Although some of the pathogens were eliminated at the soil surfaces, control at depth was less effective. Based on this work, K&S Fumigation have identified modifications that could further improve treatment efficacy.

A second experiment was therefore devised to try and increase the effectiveness of chloropicrin applied via drip-irrigation lines in controlling *F. oxysporum*, *S. sclerotiorum* and soil-borne *Rhizoctonia* and weed seeds. The expected deliverable is a novel, cost-effective treatment for disinfestation of soil before planting cut flowers.

Summary of the project and main conclusions

In an initial experiment (PC 249) carried out in December 2005 on a sandy loam soil, chloropicrin applied by drip-line irrigation at 200 L/ha gave significant reductions in soil-borne *F. oxysporum*, *R. solani* and *S. sclerotiorum*. The mean % kill of *F. oxysporum* in woody stem pieces (fresh and partially rotted), was 81% at the soil surface and 69% at 30 cm depth. Treatment was equally effective on fresh and part-rotted stem pieces. For *S. sclerotiorum*, the mean percentage kill was 95% at the soil surface and 52% at 30 cm depth (Table 1). At a position adjacent to the drip tape, control was better with 100% kill at the soil surface and 67% at 30 cm depth. The treatment also effectively controlled dicotyledon and grass weeds.

In a second experiment carried out in May 2006 on a clay soil, despite use of the full rate of chloropicrin (400 L/ha), control of *F. oxysporum* was less effective than in experiment 1, with 51% kill at the soil surface and only 6% kill at 30 cm depth (Table 1). Persistence of chloropicrin in soil is greatest at low temperatures and this may account for the greater effectiveness against *F. oxysporum* in experiment 1. It is also possible that the drier stem material used in experiment 2 was less permeable to chloropicrin than the fresher material used in experiment 1. Control of S. *sclerotiorum* was improved at the higher rate with elimination of the pathogen at the soil surface, although efficacy again declined with depth. There was no positional effect, suggesting that drip tapes at 20 cm apart were sufficiently close. Inoculum levels of *R. solani* were reduced but not eliminated. Dicotyledon weeds were again controlled. Grass weeds were scarce even in the untreated control.

For future applications, the contractor plans to use a 300 L/ha rate, with tape positioning intermediate between that used for experiments 1 and 2. The turn-around time is around 3-4 weeks and the cost of treatment is similar to that which was charged for methyl bromide treatment of greenhouse soil.

Table 1. Comparison of chloropicrin efficacy applied by drip-line irrigation under different conditions for control of *Fusarium oxysporum* in woody stem pieces, sclerotia of *Sclerotinia sclerotiorum* and *Rhizoctonia solani*

	Experiment 1 (PC 249)	Experiment 2 (PC 249a)
Soil type	Sandy loam	Heavy clay
Month of treatment	December 2005	May 2006
Soil temperature at 15 cm	10°C	25°C
Chloropicrin rate	200 L/ha	400 L/ha
Drip tape spacing	35 cm	20 cm
Mean % kill of fusarium at:		
0 cm	81	51
15 cm	72	57
30 cm	69	6
Mean % kill of sclerotinia at:		
0 cm	95	100
15 cm	76	88
30 cm	52	78
Mean % kill of rhizoctonia at:		
0-15 cm	23	64

Financial benefits

In the cut flower sector, soil disinfestation can help to prevent damaging attacks of soil-borne pathogens such as fusarium, pythium, rhizoctonia and verticillium on for example, stock, lisianthus and chrysanthemum. For stock alone, annual production in the UK is estimated to be around 18 million stems, representing around 23 ha of crop. Assuming a return of 17p per stem, the annual UK crop production is worth around £1.3 million. The benefit to the industry from this project would be continued profitable production of stock despite the threat of fusarium from the soil.

Action points for growers

- Consider using chloropicrin applied via drip-line irrigation for disinfestation of greenhouse soil prior to cropping cut flowers where broad-spectrum disease and weed control is required.

Science Section

Introduction

With the loss of methyl bromide as a soil sterilant, alternative methods are urgently required for control of important soil-borne pathogens. Chloropicrin has recently received approval (SOLA 1579/2005 and 1948/2005) for use pre-planting of raspberry, both outdoors and under protection. The Pesticides Safety Directorate (PSD) have confirmed that this treatment can also be applied pre-planting of ornamentals. In several countries (e.g. USA, Israel, Italy) where methyl bromide was widely used, there is increased interest in application of soil fumigants, including chloropicrin, through drip irrigation lines on, or buried just beneath, the soil surface. This method is considered to be safer to the operator for application to greenhouse soils.

K&S Fumigation Services Ltd have recently purchased equipment for chloropicrin in-line injection. This equipment is used in Sicily to treat soil with chloropicrin and/or 1,3 dichloropropene (e.g. Telone II) prior to planting tomatoes for control of fusarium, nematodes and other soil-borne pests.

In an initial experiment in 2005 (PC 249), undertaken jointly with K&S Fumigation Services Ltd, chloropicrin was applied through drip irrigation lines to glasshouse soil on a cut flower nursery in Hertfordshire. The experiment was undertaken to evaluate the effectiveness of the treatment for killing important plant pathogens. The trial demonstrated that soil treatment with chloropicrin applied via drip-line irrigation has potential for broad spectrum soil disinfection, significantly reducing inoculum levels of the soil-borne pathogens *F. oxysporum, R. solani* and *S. sclerotiorum,* and providing effective control of dicotyledon and grass weeds. Lack of phytotoxic effects on plants placed 5 m from the treated area, suggested that turn-around time with this treatment could be relatively quick (eg around 3-4 weeks).

A second experiment was designed to improve treatment efficacy. The modifications were: an increased rate of chloropicrin (from 200 L/ha to 400 L/ha, the full approved rate), an increased soil moisture content prior to application, and a reduced distance between T-tapes (from 35 to 20 cm). The duration for which the soil was covered was 15 days, 2 days longer than for experiment 1, and the soil temperature at 15 cm depth was higher (25^{o}C) during treatment.

In this second experiment using modified conditions, the efficacy of chloropicrin applied by drip irrigation was evaluated on *Fusarium oxysporum* in woody stem pieces (fresh and partly

rotted) of stocks, and on sclerotia of *Sclerotinia sclerotiorum*. Each was buried in replicate bags at three depths and at three distances from the irrigation lines. Efficacy against soil-borne *Rhizoctonia solani* and weeds was also determined.

The commercial objective is to optimise the efficacy of a practical alternative to methyl bromide for use in disinfestation of soil before planting cut flowers.

Materials and Methods

Site

The experiment (May 2006) was done on a clay-based soil, which had had farm yard manure incorporated in previous years, in a single span polythene tunnel at Appledore, Kent. The beds were sub-soiled and rotavated a few weeks prior to treatment, then watered 2 days prior to chloropicrin treatment and re-rotavated.

Chloropicrin application

Chloropicrin was applied by a contractor (K&S Fumigation Services Ltd) to 240 m^2 of soil in the tunnel at 400 L/ha (the maximum permitted rate) using a maximum concentration of 0.87 ml chloropicrin in 1 L water. The treated area was covered with virtually impermeable barrier film (VIF) which was dug in to a depth of c. 15 cm. The barrier film was suspended above the soil by c. 15-20 cm (as frequently done when methyl bromide was used) to help the vapour move across the whole area more easily. Drip tapes were set up along the treated bay at 20 cm apart, with 30 cm between drippers. The VIF covers were removed 15 days after treatment.

Fusarium oxysporum

Chloropicrin was tested against *F. oxysporum* using woody stem pieces of stocks naturally infected with *F. oxysporum* (collected in 2005). Nylon gauze bags each containing ten 2-cm long stem sections were prepared. The stem pieces were mixed with sufficient silver sand to separate the stem pieces in the bags. Both fresh (woody) and part-rotted stem pieces were used. For the fresh stem piece treatments, the sand in the bags was moistened prior to sample burial at the nursery and chloropicrin treatment. For the rotted stem piece treatments, the bags were buried in moist soil that was regularly watered for 4 weeks prior to sample burial at the nursery site and chloropicrin treatment. Treatments were as shown in Table 1 with three replicate bags of ten stem pieces per treatment:

Table 1. Treatments to evaluate the effect of chloropicrin on *Fusarium oxysporum*

	Soil treatment	Stem material	Position*	Depth (cm)
1	Untreated	Fresh	-	0
2	Untreated	Rotted	-	0
3	Untreated	Fresh	-	15
4	Untreated	Rotted	-	15
5	Untreated	Fresh	-	30
6	Untreated	Rotted	-	30
7	Chloropicrin	Fresh	1	0
8	Chloropicrin	Rotted	1	0
9	Chloropicrin	Fresh	1	15
10	Chloropicrin	Rotted	1	15
11	Chloropicrin	Fresh	1	30
12	Chloropicrin	Rotted	1	30
13	Chloropicrin	Fresh	2	0
14	Chloropicrin	Rotted	2	0
15	Chloropicrin	Fresh	2	15
16	Chloropicrin	Rotted	2	15
17	Chloropicrin	Fresh	2	30
18	Chloropicrin	Rotted	2	30
19	Chloropicrin	Fresh	3	0
20	Chloropicrin	Rotted	3	0
21	Chloropicrin	Fresh	3	15
22	Chloropicrin	Rotted	3	15
23	Chloropicrin	Fresh	3	30
24	Chloropicrin	Rotted	3	30

*with respect to dripper tape (See Figure 1)

Sclerotinia sclerotiorum

Effects of chloropicrin were tested against *Sclerotinia sclerotiorum* using sclerotia obtained from a flask culture of an isolate originally from lettuce. Nylon gauze bags were prepared (as for stocks stem pieces) using 20 sclerotia per bag. Bags were moistened for a period of 24 h prior to burial and chloropicrin treatment. Treatments were as shown in Table 2 with three replicate bags of 20 sclerotia.

Table 2. Treatments to evaluate the effect of chloropicrin on *Sclerotinia sclerotiorum*

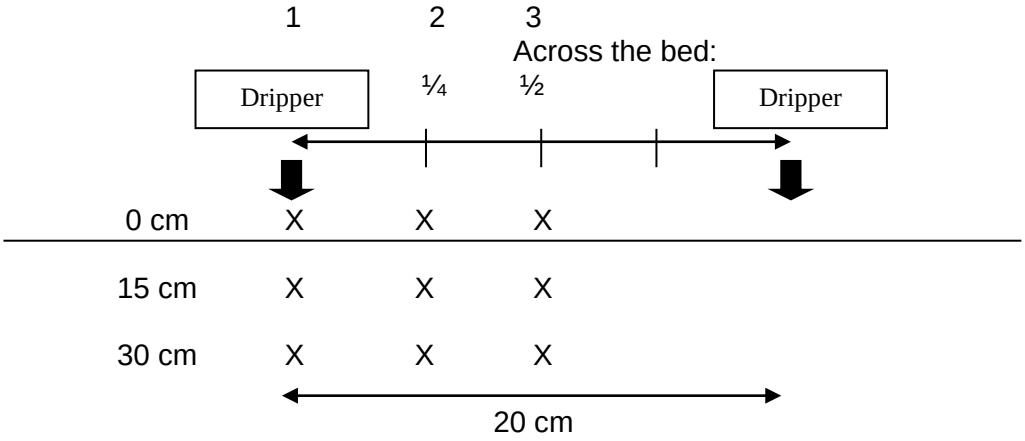
	Soil treatment	Position*	Depth (cm)
1	Untreated	-	0
2	Untreated	-	15
3	Untreated	-	30
4	Chloropicrin	1	0
5	Chloropicrin	1	15
6	Chloropicrin	1	30
7	Chloropicrin	2	0
8	Chloropicrin	2	15
9	Chloropicrin	2	30
10	Chloropicrin	3	0
11	Chloropicrin	3	15
12	Chloropicrin	3	30

*with respect to dripper tape (See Figure 1

Sample burial

For each position/depth treatment, one bag of fresh stem pieces, one bag of rotted stem pieces and one bag of sclerotia were labeled and placed into a nylon onion bag. Sample burial took place once the drip-line irrigation was in place but prior to sheeting and chloropicrin application. There were 30 continuous drip tapes across a bed at 20 cm apart. The central T-tape was selected for sample burial. The three replicates were positioned at approximately 1 m, 10 m and 20 m from the inlet end of the bed. For one replicate, bags were positioned adjacent to the drip tape by a dripper (position 1), and at a quarter (position 2) and a half (position 3) the distance across the bed to the next drip tape (Figure 1). This was repeated for the other two replicates.

Figure 1. Position of samples buried prior to chloropicrin application.



X = sample position

At each selected position, samples were buried at 15 and 30 cm depth, with depth to the top of the bag. Smeared soil was loosened with a fork and back-filled. The bags for 0 cm depth were placed on the soil surface. The control bags were buried in untreated soil (0, 15 and 30 cm depth) at three separate positions away from the chloropicrin treated area.

Rhizoctonia solani

The effect of chloropicrin treatment on *Rhizoctonia solani* was determined using a semi-quantitative baiting technique based on the method of Paulitz & Schroeder (2005). Prior to soil treatment, 20 soil cores were collected from each of three areas (coinciding with the position of the three replicates for stocks fusarium/sclerotinia sample burial) of the bed to be treated, to a depth of 15 cm using a clean soil auger. The cores from each replicate were bulked to give three samples. The soil was air-dried and stored at ambient temperature until required. Each soil sample was mixed thoroughly and then used to fill 25 cells cut from a plug tray, placed in a plastic tray. The pots were maintained in a controlled environment cabinet in the dark at 16^{o}C for 2.5 days and watered lightly. Wooden toothpicks were then inserted vertically into the soil so that they were completely immersed (one toothpick per

cell). The trays were then replaced in the controlled environment cabinet. After 48 h, the toothpicks were removed and placed on plates of *Rhizoctonia*-selective medium (five toothpicks per plate, five plates per replicate). Rhizoctonia selective medium was made using water agar amended with carbendazim at 1 ug/ml and chloramphenicol at 100 ug/ml. After 24 h, the plates were examined under a dissecting microscope using a 5 mm grid underneath the plate. For each toothpick, the number of squares adjacent to the toothpick containing a colony of *R. solani* was counted (out of 22). Typical colony morphology for *R. solani* was described in Paulitz & Schroeder, 2005.

The soil sampling procedure for *R. solani* was repeated 15 days after chloropicrin application, when the polythene covers had been removed.

Soil samples

Soil temperature was recorded at 15-cm depth on the day of treatment in the bay that was to be treated with chloropicrin. Before the chloropicrin treatment was applied, 10 soil cores from 0-15 cm depth were taken and bulked to determine soil moisture content as a percentage of field capacity (FC).

Assessment of pathogen viability

Stocks stem pieces: After recovery the stem pieces were sifted from the sand and the remaining sand washed away. The stem sections were cut in half (transversely) then surface sterilised in 90% ethanol for 10 seconds. Surface sterilised pieces were then plated onto potato dextrose agar amended with streptomycin (PDA+S) (five stem pieces per plate) and incubated at 20^{o}C. The number of fusarium colonies (out of 10) present after 8 and 15 days was counted.

Sclerotia: After the samples have been recovered from soil, the sclerotia were sifted from the sand and rinsed in water to remove excess debris. Sclerotia were surface sterilised for 3 min in a 50:50 v/v mixture of 90% ethanol and 10% sodium hypochlorite, followed by three 1 minute rinses in sterile distilled water. The sclerotia were left to air dry on filter paper in a laminar flow cabinet. The sclerotia were cut in half using aseptic technique and then one half of each of the 20 sclerotia per bag was plated on to PDA+S (five halves per plate). The plates were incubated at 20^{o}C. The number of sclerotial halves (out of twenty) from which sclerotinia colonies developed after 7 and 14 days was recorded.

Weeds

Soil was collected from 0-5 cm, 10-15 cm and 20-25 cm before and after soil treatment from three replicate areas of the bed (20 cores bulked from each area). Each sample was

thoroughly mixed, but not sieved. For each soil sample, a seed tray was lined with paper towel and filled with soil. The seed trays were maintained in a polytunnel and watered as necessary to prevent the surface from drying out. The trays were checked weekly and numbers of emerged weeds recorded. The total number of emerged weeds was recorded after *c*. 20 days and the main species identified.

Statistical analyses

Data were analysed using ANOVA for stocks stem pieces and sclerotia (using a control + factorial design) and generalised linear models (GLMs) for rhizoctonia baiting. While ANOVA makes the assumption that data is normally distributed, GLMs allow analyses of data which do not follow a normal distribution, or where a transformation needs to be applied before normality can be assumed.

Results and Discussion

Treatment conditions

The soil moisture was 49% FC when sampled 1 day prior to chloropicrin treatment. The contractor confirmed that the clay-based soil was moist at the time of treatment and very sticky by the time the covers were removed post-treatment. Soil temperature was 25^{o}C 1 day prior to treatment and on the day of treatment. Temperatures remained high for 2 days after treatment.

Fusarium oxysporum

There was a significant effect of soil treatment on the survival of *F. oxysporum* in woody stem pieces, with a mean percentage kill of 38% for chloropicrin-treated samples, compared with 0.5% in the untreated control (Tables 3 and 4). For chloropicrin-treated soil, there was also an effect of depth, with significantly higher percentage kill at the surface and 15 cm depth (>50% kill) compared to 30 cm depth (Tables 3 and 4). There was no significant effect of stem material type (fresh or partially rotted), or position of stem pieces in relation to the drip tape on *F. oxysporum* survival in chloropicrin-treated soil. Control of *F. oxysporum* was less effective in this experiment compared with the previous experiment (PC 249) in which a mean percentage kill of 74% was obtained, irrespective of depth. The soil temperature for this experiment (25^{o}C) was considerably greater than in experiment 1 (10^{o}C), which may have contributed to the reduced efficacy as persistence of chloropicrin in soil is greatest at low temperatures and high soil moisture (Vanachter & van Assche, 1970). Also, the stem pieces used for this experiment were collected in 2005 and were perhaps drier and less easy to permeate than relatively fresh stem pieces used for the first experiment in winter 2005.

Table 3. Effect of chloropicrin treatment on mean survival of *Fusarium oxysporum* in stocks stem pieces at different depths in soil

Soil treatment	Depth (cm)	% stem pieces in which *F. oxysporum* remained viable	% kill
Untreated	0	98.5	1.5
Untreated	15	100.0	0.0
Untreated	30	100.0	0.0
Untreated mean		99.5	0.5
Chloropicrin	0	48.9	51.1
Chloropicrin	15	43.3	56.7
Chloropicrin	30	93.9	6.1
Chloropicrin mean		62.0	38.0

Full results are shown in Appendix 2.

Table 4. Analysis of variance for effect of chloropicrin treatment on survival of *Fusarium oxysporum* in stocks stem pieces

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.42144	0.21072	3.02	
Stem material	1		0.00011	0.00011	0.00	0.968
Treated vs untreated soil	3		1.89347	0.63116	9.05	<.001
Stem material*	3		0.00126	0.00042	0.01	0.999
Position*	2		0.36736	0.18368	2.63	0.083
Depth*	2		2.76475	1.38237	19.82	<.001
Stem material.Position interaction*	2		0.05444	0.02722	0.39	0.679
Stem material.Depth interaction*	2		0.05445	0.02722	0.39	0.679
Position.Depth interaction*	4		0.33999	0.08500	1.22	0.317
Stem material.Position.Depth interaction*	4		0.30445	0.07611	1.09	0.373
Residual	44	(2)	3.06917	0.06975		
Total	69	(2)	9.17535			

*Analysis for treated soil only

Sclerotinia sclerotiorum

There was a significant effect of chloropicrin treatment on sclerotial viability with means of 89% kill for chloropicrin-treated samples compared with 7% in the untreated controls (Tables 5 and 6). For chloropicrin-treated soil, there was also an effect of depth, with significantly higher percentage kill of sclerotia at the surface (100%) compared to 30 cm depth (78%) (Tables 5 and 6). There was no significant effect of position in relation to the drip tape on sclerotial survival in chloropicrin-treated soil. The treatment was slightly more effective than in experiment 1 (PC 249) where there was a mean percentage kill of 75%, again with decreasing efficacy with depth. The positional effect observed in experiment 1 (reduced percentage kill at 17.5 cm from the drip tape) was not observed in this experiment, due perhaps to closer positioning of drip tapes.

Table 5. Effect of chloropicrin treatment on mean percentage of viable sclerotia of *Sclerotinia sclerotiorum* at different depths in soil

Soil treatment	Depth (cm)	% viable sclerotia	% kill
Untreated	0	95.0	5.0
Untreated	15	91.7	8.3
Untreated	30	91.7	8.3
Untreated mean		92.8	7.2
Chloropicrin	0	0.0	100.0
Chloropicrin	15	11.7	88.3
Chloropicrin	30	21.7	78.3
Chloropicrin mean		11.1	88.9

Full results are shown in Appendix 3.

Table 6. Analysis of variance of effect of chloropicrin treatment on percentage of viable sclerotia of *Sclerotinia sclerotiorum*

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.08094	0.04047	1.78	
Treated vs untreated soil	3		4.51431	1.50477	66.37	<.001
Position*	2		0.02421	0.01211	0.53	0.594
Depth*	2		0.21727	0.10863	4.79	0.019
Position.Depth interaction*	4		0.02259	0.00565	0.25	0.907
Residual	21	(1)	0.47615	0.02267		
Total	34	(1)	5.21686			

*Analysis for treated soil only

Rhizoctonia solani

In agreement with results from experiment 1 (PC 249), although the pathogen was not eliminated, the number of propagules of *R. solani* in soil was significantly reduced following treatment with chloropicrin (Table 7).

Table 7. Effect of chloropicrin treatment on the survival of *Rhizoctonia solani* in soil

	% toothpick sections from which colonies of *R. solani* developed
Untreated	64.2
Chloropicrin	23.0
F probability	<0.001
D.f.	145

Weeds

The majority of seedlings that grew in untreated soil were those of fat hen. There were few grass weeds. In agreement with results from the first experiment (PC 249), chloropicrin gave excellent weed control, irrespective of depth (Table 8).

Table 8. Effect of chloropicrin treatment on weed emergence

Treatment	Depth sampled (cm)	Mean no. of weeds emerged*	
		Dicotyledons	Monocotyledons
Untreated	0-5	7.3	0.3
Untreated	10-15	4.0	0.3
Untreated	20-25	4.3	0.0
Chloropicrin	0-5	0.0	0.0
Chloropicrin	10-15	0.0	0.0
Chloropicrin	20-25	0.0	0.0

*Assessed at 23 d for untreated soil and 21 d for chloropicrin-treated soil

Conclusions

- In agreement with results from a previous experiment (PC 249), soil treatment with chloropicrin applied via drip-line irrigation showed potential for broad spectrum soil disinfestation, significantly reducing inoculum levels of the soil-borne pathogens *F. oxysporum, R. solani* and *S. sclerotiorum,* and providing effective control of dicotyledon and grass weeds.
- Despite the higher rate of chloropicrin used (400 L/ha instead of 200 L/ha), the mean percentage kill of *F. oxysporum* was lower in this experiment (38%) compared with experiment 1 (74%), perhaps due to the use of stored woody stem pieces rather than fresh stem pieces.

- Control of *S. sclerotiorum* using chloropicrin was effective in both experiments, with mean percentage kill values of 95 and 100% at the soil surface, and 52 and 78% at 30 cm depth, for experiments 1 and 2 respectively. The increase in control in experiment 2 could have been due to the higher rate of chloropicrin applied.
- *R. solani* was not eliminated by the chloropicrin treatment in either experiment but inoculum levels were significantly reduced.
- Dicotyledonous weeds were eliminated in both experiments. Grass weeds were controlled by the chloropicrin treatment in experiment 1 but were scarce even in the untreated soil for treatment 2.
- In experiment 1 (December 2005 PC 249), a lack of phytotoxic effects on plants placed 5 m from the treated area suggested that it would be possible to treat beds when there was still a crop in the glasshouse under cool conditions. Further work is required to confirm this.

Technology transfer

1. Soil treatment for cut flowers. *HDC News* **126**, page 4.
2. Presentation at South Holland Growers Club entitled Fusarium wilt of stocks-research update 2006, planned for 20 November 2006 (Tim O'Neill).

References

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Acknowledgements

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Appendix 1

Experiment diary

Date	Task
12.04.06	Bags of stocks stem pieces prepared. Half of them buried in moist soil, remaining half stored in the laboratory
28.04.06	Bags of sclerotia prepared
08.05.06	Bags of sclerotia put in tray of water to soak
09.05.06	Bags of rotted stem pieces recovered from soil and washed. Bags of fresh stem pieces moistened. All samples sent to G. Thorpe, Kent
10.05.06	Pre-treatment soil samples collected Sample bags buried
11.05.06	Chloropicrin treatment applied
15.05.06	Pre-treatment soil samples used to set up weed emergence test, to test % field capacity, and dried for rhizoctonia bait test
22.05.06	7 d weed emergence assessment (pre-treatment soil samples)
26.05.06	Covers removed 15 d after treatment
30.05.06	15 d weed emergence assessment (pre-treatment soil samples)
30.05.06	Sample bags recovered and post-treatment soil samples collected
31.05.06	Stem pieces and sclerotia plated out (chloropicrin treatments)
01.06.06	Stem pieces and sclerotia plated out (untreated controls)
01.06.06	Weed emergence tests with post-treatment soil samples set up
05.06.06	Sclerotia assessment 1 (4-5 days)
06.06.06	23 d weed emergence assessment (pre-treatment soil samples)
09.06.06	8 d weed emergence assessment (post-treatment soil samples)
09.06.06	Stock stem piece assessment 1 (8-9 days)
09.06.06	Rhizoctonia bait test set up
11.06.06	Sclerotia assessment 2 (8-9 days)
12.06.06	Toothpicks inserted in soil for rhizoctonia baiting
14.06.06	Toothpicks plated on agar
15.06.06	Rhizoctonia incidence assessed
16.06.06	Stock stem piece assessment (15-16 days)
23.06.06	40 d weed emergence assessment (pre-treatment soil samples) and 21 d weed emergence assessment (post-treatment soil samples)
30.06.06	28 d weed emergence assessment (post-treatment soil samples)
14.07.06	42 d weed emergence assessment (post-treatment soil samples)

Appendix 2. Full results of the effect of chloropicrin treatment on survival of *Fusarium oxysporum* in stocks stem pieces

Table 1. Effect of depth in soil, distance from drip-tape and condition of stem tissue on mean % survival of *F. oxysporum*

Soil treatment	Depth in soil (cm)	Distance from drip tape (cm)	Mean % survival in stem pieces	
			Fresh	Part rotted
Untreated	0	-	97.0	100.0
	15	-	100.0	100.0
	30	-	100.0	100.0
Treated	0	0	45.1	35.1
		5	50.0	70.0
		10	63.3	30.0
	15	0	16.7	33.3
		5	50.0	26.7
		10	63.3	70.0
	30	0	83.3	93.3
		5	86.7	100.0
		10	100.0	100.0

Appendix 3. Full results of the effect of chloropicrin treatment on survival of *Sclerotinia sclerotiorum* sclerotia

Table 1. Effect of depth in soil and distance from drip-tape on mean % survival of *S. sclerotiorum*

Soil treatment	Depth in soil (cm)	Distance from drip tape (cm)	Mean % survival
Untreated	0	-	95.0
	15	-	91.7
	30	-	91.7
Treated	0	0	0.0
		5	0.0
		10	0.0
	15	0	11.7
		5	3.3
		10	20.0
	30	0	20.0
		5	20.0
		10	25.0