THE EFFECT OF FUNGICIDE SPRAYS ON THE INCIDENCE OF APPLE CANKER (*NECTRIA GALLIGENA*) IN CV. BRAMLEY'S SEEDLING

L.R. COOKE

Applied Plant Science Division, Department of Agriculture for Northern Ireland, Newforge Lane, Belfast, BT9 5PX

B.S. WATTERS

Northern Ireland Horticultural and Plant Breeding Station, Department of Agriculture for Northern Ireland, Loughgall, Co. Armagh, BT61 8JB

ABSTRACT

In three trials between 1983 and 1994, copper oxychloride applied at 5% and 50% leaf-fall proved the most effective alternative to phenylmercury nitrate for the control of leaf-scar infection by *Nectria galligena* in cv. Bramley's Seedling. All spring-summer fungicide programmes reduced canker by at least 60% compared with an unsprayed control. DMI fungicides, including myclobutanil and penconazole (now widely used against apple scab), reduced canker to a similar extent as did dodine and/or dithianon, but were less effective than programmes including a spring-summer benzimidazole.

INTRODUCTION

The fungus *Nectria galligena*, the cause of European canker, infects apple trees during the spring-summer period through bud-scale scars and other damage points and in the autumn through leaf scars (Swinburne, 1971). Effective control depends on preventing infection during both periods. In Northern Ireland, where high rainfall favours canker, from 1978-1986, under an EC derogation growers of Bramley's Seedling apples were permitted to apply two autumn sprays of phenylmercury nitrate (PMN) following wet summers. Trials were initiated in 1983 to find more acceptable autumn fungicides. The *in vitro* activity of fungicides against *N. galligena* was used as a basis for selecting those to be assessed *in vivo*.

Swinburne *et al.* (1975) showed that spring-summer fungicide programmes cause a greater reduction in canker numbers than autumn fungicides alone. These authors found that whilst dodine and dithianon were equally effective in controlling apple scab (*Venturia inaequalis*), dithianon was significantly better in controlling canker. However, the most effective treatment was a summer programme of carbendazim, which not only prevented summer *N. galligena* infections, but also suppressed sporulation of the pathogen, preventing autumn leaf-scar infections. During the 1980's, new orchard fungicides belonging to the DMI group were developed for the control of apple scab. It was therefore decided to evaluate their impact on canker.

This paper compares the results of two trials between 1983 and 1990 (Cooke *et al.*, 1993) with those obtained from a trial during 1991-1994.

MATERIALS AND METHODS

Activity of fungicides against mycelial growth of *Nectria galligena in vitro*

The activity of fungicides against mycelial growth of *N. galligena in vitro* was determined by incorporating solutions or suspensions of fungicides into malt extract agar (Cooke, 1985). Diameters of growth zones were recorded after 7 days incubation at 20°C, and ED_{50} values determined using a log-logistic program. Hexaconazole was not available for *in vitro* testing.

Trees

Apple trees, cv. Bramley's Seedling on MM106 rootstock (Trials 1 and 2) or on M26 rootstock (Trial 3), were grown at the Northern Ireland Horticultural and Plant Breeding Station, Loughgall, Co. Armagh. They were planted in three (Trials 1 and 2) or four (Trial 3) fully randomised blocks in plots consisting of six (Trials 1 and 2) or five trees (Trial 3). Nylon mesh screens 2 m high were erected between plots to limit dispersal of sprays and inoculum. The leader shoot on each tree was inoculated with *N. galligena* (Swinburne *et al.*, 1975). Two months later, all trees were checked for the presence of active lesions around inoculation points.

Treatments

Details of fungicide formulations are given in Table 1. Treatments are specified in the captions to the Figures. During the spring-summer, treatments were applied as for the control of apple scab at *c.* 10-day intervals; in the autumn, where appropriate, two sprays were applied at 5 and 50% leaf-fall. Applications were made at high volume through a hand lance using a Nobilii sprayer (Trial 1) or a portable Fox Motori Wagon sprayer (Trials 2 and 3).

Trials

For Trial 1 (1983-1986), trees were inoculated in January 1983 and treatments applied from April 1983 until autumn 1985. Numbers of cankers were recorded twice a year from December 1983 until May 1985. In Trial 2 (1987-1990), trees were inoculated in January 1986. During 1986, all trees received a routine dodine programme (to allow time for canker to develop). Treatments were applied from spring 1987 until autumn 1989. Numbers of new cankers were recorded in March 1987, before the application of treatments, and twice a year thereafter. For Trial 3 (1991-1994), trees were inoculated in February 1990. During 1990, all trees received a routine dithianon programme. Treatments were applied from spring 1991 until autumn 1993. Numbers of new cankers were recorded as for Trial 2.

RESULTS

Activity of fungicides against mycelial growth of *Nectria galligena in vitro*

Carbendazim, prochloraz-manganese and PMN were most active (ED_{50} values <1 mg/l, Table 2). Dithianon, copper oxychloride and cupric ammonium carbonate were virtually inactive in inhibiting mycelial growth, but are known to inhibit spore germination.

TABLE 1. Details of fungicide formulations and application rates

Fungicide	Proprietary name	Manufacturer	Application rate (g/ha)		
			Trial 1	Trial 2	Trial 3
bitertanol	Baycor	Bayer	1000	-	-
captafol	Sanspor	Zeneca	3600	-	-
carbendazim	Bavistin	BASF	550	550	500
copper oxychloride	Cuprokylt	Universal	5000	5000	5000
cupric ammonium carbonate	Fungex	Rhône-Poulenc	-	4920	-
dithianon	Delan-Col	Zeneca		840[a]	840
dodine	Melprex	Cyanamid	600	600	-
fenpropimorph	Mistral	Rhône-Poulenc	1000	-	-
hexaconazole	Anvil	Zeneca	-	-	50
mancozeb	Karamate	Rohm & Haas	-	1680	1680
myclobutanil	Systhane	Rohm & Haas	-	66	66
penconazole	Topas	Ciba	-	50	50
penconazole+captan	Topas C	Ciba	-	50+950	-
penconazole+dithianon	Topas D	Ciba	-	-	50+500
phenylmercury nitrate	PMN	Farm Chemicals	250	-	-
prochloraz-manganese	Sporgon	AgrEvo	1000	-	-
thiophanate-methyl	Cercobin	Rhône-Poulenc	1000	-	1000

a 840 g/ha used for spring-summer treatments, 2520 g/ha used for autumn application

TABLE 2. Comparative ED_{50} values for fungicides against mycelial growth of *Nectria galligena*

Fungicide	ED_{50} (mg/l)
bitertanol	2.1
captafol	4.2
captan	10.3.
carbendazim	0.67
copper oxychloride	210[a]
cupric ammonium carbonate	130[a]
dithianon	>150
dodine	2.3
fenpropimorph	2.9
mancozeb	45.0
myclobutanil	5.7
penconazole	3.0
phenylmercury nitrate	0.11
prochloraz-manganese	0.04
thiophanate-methyl	5.0

a as mg Cu/l

Effect of treatments in field trials

Fewer new cankers developed in Trial 1 (Figure 1) than in the two later trials (Figures 2 and 3), probably because the site for Trial 1 was more exposed and less humid than that used for Trials 2 and 3. Of the autumn treatments evaluated in the first two trials, the most effective, apart from PMN, were copper oxychloride (Trials 1 and 2) and prochloraz-manganese (Trial 1). Unfortunately, AgrEvo decided not to develop prochloraz-manganese for use on apples. Other autumn treatments, including high-rate dithianon and cupric ammonium carbonate, did not reduce canker compared with the standard treatments (dodine and dodine/dithianon), so only copper oxychloride was selected for further evaluation.

Both trials confirmed the efficacy of spring-summer carbendazim in reducing numbers of new cankers. Carbendazim alone was tested in Trial 1, but in Trial 2 a tank-mix with dodine/dithianon was used, to reduce the risk of resistance. Although carbendazim + captafol proved outstandingly effective, it was not included in Trial 2 due to concern over the toxicity of captafol, which has subsequently been prohibited as a pesticide in the UK. The other spring-summer programmes evaluated in Trial 2 did not reduce canker compared with the standard and, surprisingly, plots treated with myclobutanil + mancozeb had significantly more cankers.

An unsprayed control was included in the 1991-94 trial, since in Trial 2 the similarity in canker levels between spring-summer programmes based on different types of fungicides suggested that they might be having little effect on canker. This possibility was disproved by the results of Trial 3, since all fungicide treatments reduced the number of new cankers by at least 60%, a finding in agreement with the conclusions of Swinburne *et al.* (1975).

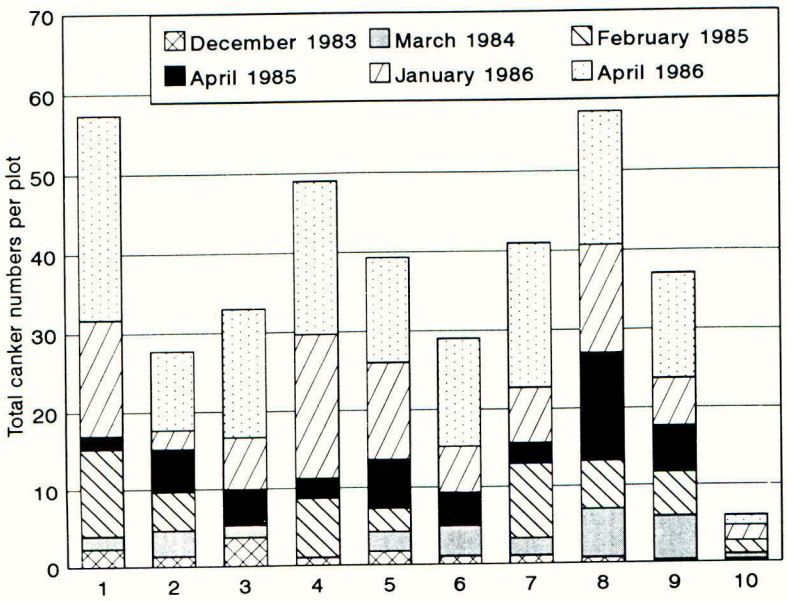


FIGURE 1. Trial 1 apple canker assessments. Treatments (spring-summer/autumn): 1 = dodine/none, 2 = dodine/PMN, 3 = dodine/copper oxychloride, 4 = dodine/carbendazim, 5 = dodine/thiophanate-methyl, 6 = dodine/prochloraz-manganese, 7 = dodine/bitertanol, 8 = dodine/fenpropimorph, 9 = carbendazim/none, 10 = carbendazim+captafol/none.

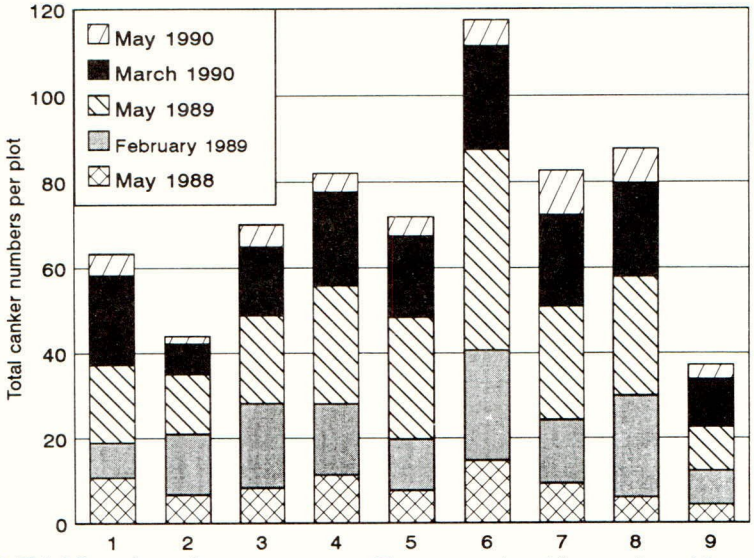


FIGURE 2. Trial 2 apple canker assessments. Treatments (pre-blossom/post-blossom/autumn): 1 = dodine/dithianon/none, 2 = dodine/dithianon/copper oxychloride, 3 = dodine/dithianon/cupric ammonium carbonate, 4 = dodine/dithianon/dithianon, 5 = myclobutanil/myclobutanil/none, 6 = myclobutanil/myclobutanil+mancozeb/none, 7 = penconazole/penconazole/none, 8 = penconazole+captan/penconazole+captan/none, 9 = dodine+carbendazim/dithianon+carbendazim/none.

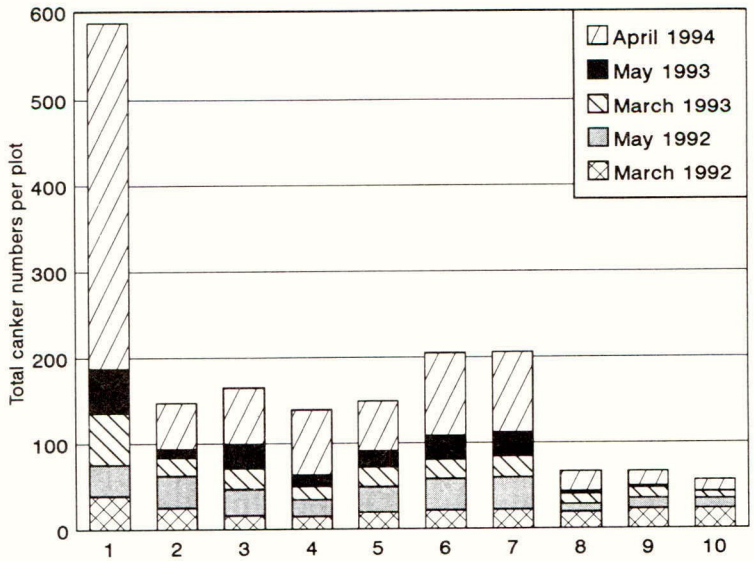


FIGURE 3. Trial 3 apple canker assessments. Treatments (pre-blossom/post-blossom/autumn): 1 = untreated, 2 = dithianon/dithianon/none, 3 = hexaconazole/hexaconazole/none, 4 = penconazole/penconazole/none, 5 = penconazole+dithianon/penconazole+dithianon/none, 6 = myclobutanil/myclobutanil/none, 7 = myclobutanil/myclobutanil+mancozeb/none, 8 = myclobutanil/myclobutanil+mancozeb/copper oxychloride, 9 = dithianon+thiophanate-methyl/dithianon+thiophanate-methyl/copper oxychloride, 10 = dithianon+carbendazim/dithianon+carbendazim/copper oxychloride

The three DMI fungicides, hexaconazole, myclobutanil and penconazole, reduced canker numbers to a level similar to that with dithianon. Myclobutanil tended to be slightly less effective than the other two DMI's, but the difference was not significant ($P>0.05$). Addition of dithianon to penconazole or mancozeb to myclobutanil did not affect canker incidence, in contrast to Trial 2 where myclobutanil + mancozeb gave more canker than myclobutanil alone.

The greatest reductions in canker numbers were achieved by the three treatments which included autumn copper oxychloride. The addition of autumn copper to the myclobutanil + mancozeb programme decreased canker incidence by 68% compared with the same programme without an autumn fungicide and resulted in a level of canker similar to that achieved by spring-summer benzimidazoles with autumn copper.

DISCUSSION

As an alternative to autumn PMN, copper oxychloride proved a more effective treatment than any of the newer fungicides evaluated, substantially reducing new canker infections, in contrast to earlier reports (Anon., 1969). The results show the value of autumn treatments in controlling canker, particularly in high rainfall areas. The DMI fungicides, myclobutanil and penconazole, now widely used for scab control in the UK, have a similar effect on canker to the older treatments, so the changed pattern of fungicide usage should not adversely effect canker. Where canker has become a major problem, the use of a programme including a spring-summer benzimidazole plus autumn copper remains the most effective option.

ACKNOWLEDGEMENTS

The authors thank staff of the Northern Ireland Horticultural and Plant Breeding Station, Loughgall for technical assistance, Miss S. Watson, Biometrics Division, for advice on trial design and analyses and the following companies for donating fungicides used in the trials; AgrEvo, Bayer (Ireland) Ltd., Ciba Agriculture, Farm Chemicals Ltd., Rhône-Poulenc Agriculture, Rohm & Haas (UK) Ltd., Universal Crop Protection and Zeneca.

REFERENCES

Anon. (1969) Field trials on canker control in orchards of Bramley's Seedling. *Annual Report on Research and Technical Work of the Ministry of Agriculture for Northern Ireland*, p. 99.

Cooke, L.R. (1985) Protection of apple trees from *Nectria galligena* canker by PP969. *1985 Fungicides for Crop Protection, BCPC Monograph No. 31* **2**, 469-472.

Cooke, L.R.; Watters, B.S.; Brown, A.E. (1993) The effect of fungicide sprays on the incidence of apple canker (*Nectria galligena*) in Bramley's Seedling. *Plant Pathology* **42**, 432-442.

Swinburne T.R. (1971) The seasonal release of spores of *Nectria galligena* from apple canker in Northern Ireland. *Annals of Applied Biology* **69**, 97-104.

Swinburne T.R.; Cartwright J.; Flack N.J.; Brown A.E. (1975) The control of apple canker (*Nectria galligena*) in a young orchard with established infections. *Annals of Applied Biology* **81**, 61-73.

EVALUATION OF FUNGICIDES FOR CONTROL OF RING SPOT (*MYCOSPHAERELLA DIANTHI*) ON HYBRID PINKS

T.M. O'NEILL

ADAS Horticulture, Brooklands Avenue, Cambridge, CB2 2BL

K.D. LOCKLEY

ADAS Bridgets, Staplake Mount, Starcross, Exeter, Devon, EX6 8PE

ABSTRACT

Seven fungicides (carbendazim, carbendazim + maneb, chlorothalonil, difenoconazole, penconazole, prochloraz and tebuconazole) applied at 14 day intervals from September 1993 to February 1994 to a protected crop of hybrid pinks, cv. Doris, significantly reduced ring spot on flower buds and leaves. Difenoconazole, penconazole and tebuconazole were more effective than carbendazin + maneb, a treatment commonly used by growers. After ten sprays of difenoconazole only 2% of flower buds were affected by ring spot compared to all buds on untreated plants. There was no reduction in flower stem length. Addition of a mineral oil to carbendazim + maneb improved control of ring spot on leaves but not on flower buds.

INTRODUCTION

Hybrid pinks are an important cut flower crop in England with a production area of around 50 ha. The area under protection (21.3 ha) in 1993 was greater than that of carnations (Anon, 1994). Plantings are usually left down for 18 - 36 months. The main production areas are in South West England and East Anglia. The principle diseases affecting commercial crops are ring spot (*Mycosphaerella dianthi*), rust (*Uromyces dianthi*), stub rot (*Fusarium avenaceum* and *Fusarium culmorum*) and wilt (*Fusarium oxysporum* f.sp. *dianthi*) (Harnett, 1986).

Infection by *Mycosphaerella dianthi* results in translucent ring spots, later turning tan coloured with a reddish border, on leaves, stems or flower buds; flower stems are unmarketable if buds become infected. Black sporulation is often visible on necrotic tissue. The fungus causes similar ring spot symptoms on other member of the *Caryophyllaceae*, especially carnation and sweet william (*Dianthus barbatus*) (Smith *et al.*, 1988). Infection by *M. dianthi* is favoured by high humidity (Fletcher 1984) and temperatures above 20°C (Novoa *et al.*, 1992). On hybrid pinks, ring spot is frequently found in the autumn in crops grown outdoors or in unheated polythene tunnels in South West England; it is less common in East Anglia (O'Neill, unpublished). The popular varieties Doris and Monica are particularly susceptible.

There are no fungicides in the UK with a label recommendation for control of ring spot on hybrid pinks and products currently used by growers appear to provide only partial control.

Fungicides previously reported to provide some control of ring spot include benomyl, benzimidazole-dithiocarbamate mixtures, chlorothalonil, dichlofluanid, iprodione, propineb, penconazole and triforine (Fletcher, 1984; Penaranda *et al.*, 1992). The waxy nature of the leaves of hybrid pinks makes them difficult to wet and non-ionic wetters are often added to try and improve spray cover and disease control.

Stem length influences the value of hybrid pinks and a difference of a few centimetres may be critical. Some growers have reported that propiconazole applied experimentally to the crop for control of rust reduced stem length. The same fungicide caused shortening of chrysanthemum flower stems (Dickens, 1990). It is therefore important that fungicides tested for control of ring spot are also examined for effect on crop growth.

The objective of the work described here was to evaluate seven fungicides and two spray additives for their effect on control of ring spot. The effect of treatment on flower stem length was also determined.

MATERIALS AND METHODS

Crop and site details

The experiment was located in a crop of cv. Doris in an unheated polythene tunnel in Cornwall. The soil was treated with metham sodium in November 1992 after removal of the previous crop (chrysanthemums). Hybrid pinks were planted on 4 March 1993 in beds at 30 x 25 cm spacing with 6 rows to a bed. The experiment was a randomised block design with four replicates. Plot size was 2 m x 1 m (2 m^2). A 1 m length of crop at the end of each bed, adjacent to the tunnel doors, was excluded from the experimental area.

Treatments

Fungicides were applied as high volume sprays (1500 l/ha) at 250 kPa pressure using an Oxford Precision sprayer with medium quality spray nozzles (Lurmark-04-F80). Sprays were applied every 14 days from 14 September 1993 to 19 January 1994. Treatments were: carbendazim, 0.5 g AI/l (Bavistin DF, BASF); carbendazim + maneb, 0.28 + 1.42 g AI/l (Kombat WDG, Hoechst); chlorothalonil, 1.1 g AI/l (Bravo 500, BASF); difenaconazole, 0.25 g AI/l (CGA 169374, Ciba Agriculture); penconazole, 0.1 g AI/l (Topas 100 EC, Ciba Agriculture); prochloraz, 0.46 g AI/l (Fisons Octave, Fisons plc); tebuconazole, 0.1 g AI/l (Folicur, Bayer). Also, carbendazim + maneb (0.28 + 1.42 g AI/l) was applied in mixture with a highly refined mineral oil, 4.85 ml AI/l (Actipron, Bayer) and a synthetic latex 2.25 g AI/l (Spraymate Bond, Newman). Finally, there was a programme treatment consisting of alternating sprays of carbendazim + maneb (0.28 + 1.42 g AI/l) in mixture with the highly refined mineral oil (4.85 ml AI/l), alternating with prochloraz (0.46 g AI/l). Control plots were sprayed with water.

Assessments

The number of calyces affected by ring spot was determined on 25 terminal buds selected at random, excluding buds within 20 cm of the plot edge. The leaf area affected by ring spot

was assessed on a whole plot basis. Plants were visually assessed for any signs of phytotoxicity at each assessment. Flower stem length was measured 15 weeks after the final treatment on 25 stems/plot in two replicate blocks, selecting buds with colour just showing.

Results were analysed by analysis of variance. Means were separated by Duncan's multiple range test.

RESULTS

Control of ring spot

A low level of ring spot (less than 1% leaf area affected and occasional buds affected) was evident when treatment commenced. No rust or other foliar disease was found. On untreated plants the incidence of buds affected by ring spot increased rapidly during September and October 1993 (Figure 1). The disease remained at a very low level on leaves until January 1994.

At the disease assessment on 12 October 1993, when two sprays of each treatment had been applied, the incidence of affected buds was significantly reduced by all treatments with difenoconazole most effective (Table 1). Difenoconazole was significantly more effective than the three standard commercial treatments (carbendazim, carbendazim + maneb and chlorothalonil). Addition of adjuvants to carbendazim + maneb did not significantly improve disease control on flower buds at this or subsequent assessments.

On 2 February 1994, two weeks after the tenth and final spray had been applied, all flower buds in untreated plots were affected by ring spot. There were very high levels of ring spot (more than 75% buds affected) in all other treatments apart from difenoconazole (2% affected), tebuconazole (35%) and penconazole (49%). Fifteen weeks after the final sprays had been applied, when a new spring flush of flowers had developed, there was a relatively low incidence of ring spot on flower buds and no significant differences between treatments. Lesions at this time were all very small. The occurrence of ring spot on leaves increased in early 1994 and by 2 February affected more than 10% of the leaf area of untreated plants. The severity of the disease on leaves was significantly reduced by all treatments with difenoconazole, tebuconazole, penconazole and carbendazim + maneb with mineral oil particularly effective. (Table 2). The addition of mineral oil to carbendazim + maneb improved control of ring spot on leaves.

Effect on plant quality

None of the fungicides had a visible effect on stem length at any time during the experiment and no significant effects were found at an assessment 15 weeks after the final treatment. Mean stem length ranged from 49.4 cm (penconazole) to 55.0 cm (chlorothalonil); the control was 49.7 cm. The use of mineral oil resulted in plants appearing a darker green, apparently due to loss of leaf bloom; this effect was visible after two sprays had been applied and became more evident with increasing number of sprays.

TABLE 1. Control of ring spot on flower buds of hybrid pinks.

Treatment		Dose (g AI/l)	% flower buds affected 12 Oct	9 Nov	6 Dec	2 Feb
1.	Control (water)	-	83 e	83 d	80 c	100 e
2.	Carbendazim	0.5	63 d	70 cd	60 bc	93 de
3.	Carbendazim + maneb	0.28 + 1.42	44 bc	59 c	47 b	91 de
4.	Chlorothalonil	1.1	57 cd	71 cd	63 bc	85 de
5.	Difenoconazole	0.25	20 a	22 a	8 a	2 a
6.	Penconazole	0.1	52 cd	38 ab	40 b	49 c
7.	Prochloraz	0.46	49 bcd	71 cd	63 bc	91 de
8.	Tebuconazole	0.1	35 b	53 bc	43 b	35 b
9.	C + M + mineral oil	0.28 + 1.42	55 cd	55 bc	60 bc	78 d
10.	C + M + synthetic latex	0.28 + 1.42	46 bc	58 bc	61 bc	79 d
11.	C + M + mineral oil alternating with prochloraz	0.28 + 1.42 0.46	47 bcd	53 bc	48 b	85 de
	SED (30 d.f.)		7.18	9.17	9.91	6.76

C + M - carbendazim + maneb
Treatment means followed by the same letter do not differ significantly (P=0.05).

TABLE 2. Control of ring spot on leaves of hybrid pinks.

Treatment		Dose (g AI/l)	Mean % leaf area affected 2 Feb	3 May
1.	Control (water)	-	14.3 f	15.8 e
2.	Carbendazim	0.5	10.3 e	8.3 bcd
3.	Carbendazim + maneb	0.28 + 1.42	8.3 de	12.3 de
4.	Chlorothalonil	1.1	6.3 cd	4.5 abc
5.	Difenoconazole	0.25	0.1 a	1.5 a
6.	Penconazole	0.1	1.3 a	0.9 a
7.	Prochloraz	0.46	9.3 de	9.8 cd
8.	Tebuconazole	0.1	2.3 ab	1.9 a
9.	C + M + mineral oil	0.28 + 1.42	4.5 bc	3.0 ab
10.	C + M + synthetic latex	0.28 + 1.42	7.8 cde	6.3 abc
11.	C + M + mineral oil alternating with prochloraz	0.28 + 1.42 0.46	7.0 cde	2.5 a
	SED (30 d.f.)		1.49	2.46

C + M - carbendazim + maneb
Treatment means followed by the same letter do not differ significantly (P = 0.05).

DISCUSSION

This work has identified three triazole fungicides which provide good control of ring spot on hybrid pinks with no detrimental effect on plant growth following ten high volume sprays. Difenoconazole was particularly effective. Penconazole and tebuconazole gave good control of bud infection. The level of control achieved was considerably better than that obtained with the standard fungicides currently used by growers (carbendazim; carbendazim + maneb; chlorothalonil). In a previous experiment (O'Neill, unpublished), carbendazim + maneb + mineral oil, chlorothalonil and prochloraz all gave moderate control of the disease on a protected crop of hybrid pinks cv. Haytor White, reducing leaf infection by 44 - 57%. In this experiment the treatments resulted in a similar reduction in leaf infection (33 - 56%) two weeks after the final spray; difenoconazole, penconazole and tebuconazole were all more effective.

Studies on the control of ring spot on carnation indicated that mineral oil alone inhibits spore germination of *M. dianthi* and its use at 0.5% improved the control from carbendazim + maneb (Nathaniels, pers. comm.). Non-ionic adjuvants have been reported to enhance the efficacy of carbendazim in controlling leaf spot of celery caused by *Septoria apiicola* (Amer *et al.*, 1993) In the experiment reported here, mineral oil added to carbendazim + maneb did not improve the control of ring spot on flower stems, but it did improve control of the disease on leaves. The addition of a synthetic latex spray additive to carbendazim + maneb did not significantly affect ring spot control on either stems or leaves.

ACKNOWLEDGEMENTS

We thank the grower who provided the site for this experiment and the Horticultural Development Council who funded the work.

REFERENCES

Amer, M.A., Hoorne, D., Poppe, J (1993) Septoria leaf spot disease of celery: action of adjuvants on plant surfaces. *Mededelingen van de Faculteit Landbouwwetenschapen Rijksuniversiteit, Gent* **58**, 1431-1440.

Anon. (1994). Agricultural and Horticultural Census: 1 June 1993 - United Kingdom and England. Government Statistical Service, Guildford.

Dickens, J.S.W. (1990). Studies on the chemical control of chrysanthemum white rust caused by *Puccinia horiana*. *Plant Pathology* **39**, 434-442.

Fletcher, J.T. (1984) Diseases of greenhouse plants, 351 pp, Longman.

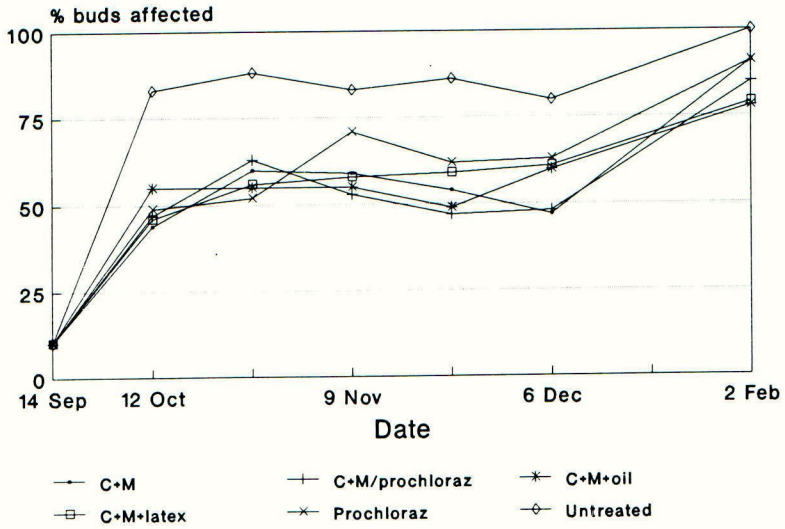
Harnett, R. (1986) Hybrid Pinks. ADAS leaflet P3028, 5 pp.

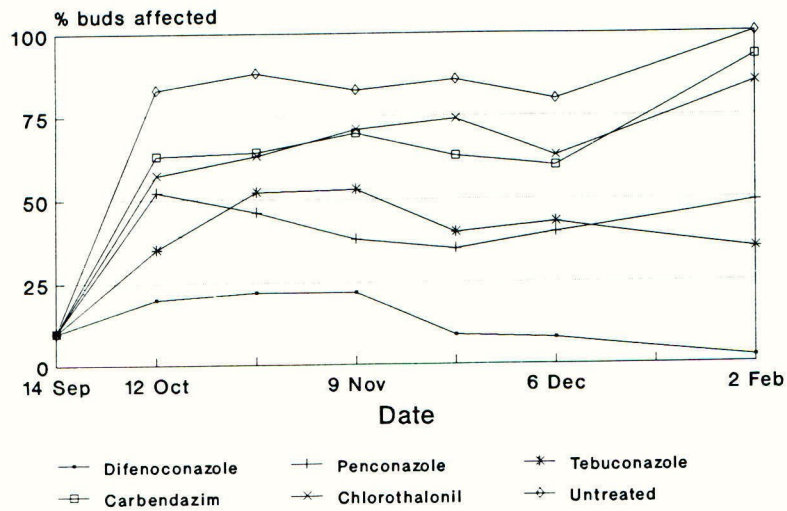
Penaranda, L.A.; Torres, H.J.; Arbeláez, G. (1992) Chemical control of the fairy ring spot of miniature carnation caused by *Heterosporium echinulatum*. *Acta Horticulturae* **307**, 247 - 250.

Novoa, C.; Casallas, J; Briceno, G; Orozco, M; de Granada, E.G. (1992) Some aspects on the biology of *Heterosporium echinulatum* (*Berk*) *Cooke*. *Acta Horticulturae* **307**, 233-240.

Smith, I.M.; Dunez, J.; Lelliott, R.A.; Phillips, D.H.; Archer, S.A. (1988) *European Handbook of Plant Diseases*, 583 pp, Blackwell Scientific Publications, London.

Figure 1. Effect of fungicides on number of flower buds affected by ring spot.





CHEMICAL CONTROL OF RUST ON MINT

R.A. HAGAN AND D.R. WALTERS

Plant Science Department, SAC, Auchincruive, Nr Ayr KA6 5HW

ABSTRACT

Infection of mint by the rust fungus *Puccinia menthae* is a serious problem for herb growers. Rust infection affects not only the appearance of the plants, which is important for the fresh and pot trades, but can also reduce yields markedly. An HDC-funded project was initiated to investigate the possibility for effective chemical control of rust on mint. Glasshouse trials evaluated 14 fungicides, 8 of which were chosen for field trials at 3 sites in the UK. Although most of the fungicides tested in the field reduced the levels of rust infection, the greatest reduction was obtained with a carbendazim & propiconazole mixture, which reduced rust infection by 63%. All the fungicides except triadimefon significantly reduced the loss of leaves associated with this disease and therefore increased yield. Even at the site with negligible amounts of rust, yield increases were noted with 3 of the fungicides. The formulation of triadimefon used in the field trials contains 5 times more active ingredient than the product which has an off-label approval for rust control on mint. This indicates the need for the identification and approval of more effective fungicides for rust control in this crop.

INTRODUCTION

Mints are grown commercially all over the world, mainly for their volatile oils, which are used to flavour many products including toothpastes, chewing gums and confectionery. In Britain, mint is principally grown for the fresh herb trade, for drying or processing and for the pot trade (Dumville, 1988).

Rust is the most important disease which affects the mint industry world-wide. The disease is caused by the fungus *Puccinia menthae* Pers., a pathogen restricted to certain members of the *Lamiaceae*. Under favourable conditions the fungus can cause severe damage to mint crops. In the early spring infected stems become abnormally thickened and distorted, while during the summer and autumn both leaves and stems may become covered in pustules ranging from yellow to almost black in colour. The rust not only affects the appearance of the plants, which is important for the fresh and pot trades, but it also affects yield by increasing defoliation and destroying oil glands (Harvey, 1979) .

In the United Kingdom , triadimefon (5 % w/w formulation) is the only fungicide with an off-label approval for rust control on mint (Anon, 1994). Growers have, however, expressed doubts about its efficacy and so an HDC funded project was initiated to identify more effective alternatives.

MATERIALS AND METHODS

A fungicide screening programme was carried out in the glasshouse to select fungicides which would provide good control of the rust without causing phytotoxicity. Fourteen fungicides, belonging to groups with different modes of action, were selected initially and following glasshouse screening, 8 fungicides were selected for field trials (Table 1).

TABLE 1. The fungicides selected for field trials

MANUFACTURER	TRADE NAME	ACTIVE INGREDIENTS
Bayer	Bayleton	triadimefon
	Bayfidan	triadimenol
Ciba-Geigy	Hispor 45 WP	carbendazim & propiconazole
	Tilt 250 EC	propiconazole
Hoechst	Kombat WDG	carbendazim & mancozeb
ICI	Early Impact	carbendazim & flutriafol
	Impact Excel	chlorothalonil & flutriafol
Uniroyal Chemical	Plantvax 20 EC	oxycarboxin

Field trials were held at 3 sites in 1993. The 2 commercial plantings were near Norwich, Norfolk and near Ross-on-Wye, Herefordshire, with the third trial established at SAC, Auchincruive, near Ayr. Spearmint crops (*Mentha spicata*) were grown at all 3 sites and each trial was divided into 36 plots, each measuring 2 m x 3.5 m arranged in a randomised block design with 4 replicates.

Due to differing climatic and cultural practices, each site received a different spray regime. The Auchincruive crop was sprayed on 29/7/93 and again 3 weeks later (19/8/93), and was assessed 5 weeks following the second spray (23/9/93). The Norwich crop was sprayed on 19/7/93 and then 5 weeks later (23/8/93), and was assessed 3 weeks after that (13/9/93). The crop at Ross-on-Wye received only one spray on 16/7/93 and was assessed 3 weeks later (6/8/93). For the first application of fungicides, a compressed-air plot sprayer with a 2 m boom was used at a pressure of 2.0 bar. The second application of fungicides was administered using a propane gas plot sprayer operated at 2.5 bar. Both plot sprayers applied the fungicides in 200 l of water / ha.

An assessment was carried out at each site before spraying and harvesting, except at Auchincruive, where assessments were carried out only at the second spraying and at harvest. In each plot, 10 stems of mint were chosen at random , avoiding plants which were in the outside 20 cm of each plot. The height, number of leaves, number of missing leaves and number of infected leaves were all recorded. A pair of leaves in each third of the main stem were chosen and the percentage infection of rust assessed. A disease key for spearmint rust was created for use in these field trials (Figure 1). All the leaves were removed from the

stems and dried separately at 40 ^{o}C for 5 days and then weighed to give a measure of yield.

RESULTS

The results discussed in the following paragraphs are mainly from the Auchincruive site since this site had the highest levels of rust. The levels of rust at the Ross-on-Wye site were significantly lower, although fungicide performance was similar. At the Norwich site the levels of rust were insignificant and so the results from this site are only discussed in relation to the effect of the fungicides on plant growth.

TABLE 2 . Summary of the final assessment at the Auchincruive field trial

TREATMENT	MEAN % INFECTION	% LEAVES INFECTED	% MISSING LEAVES	TOTAL DRY WEIGHTS
Control	21.34	100.00	59.20	8.04
Triadimenol	11.61	98.34	35.79	9.43
Triadimefon	19.79	95.67	55.69	7.72
Propiconazole	11.68	97.66	34.73	9.17
Carbendazim & Propiconazole	7.91	96.28	26.72	11.81
Chlorothalonil & Flutriafol	14.01	99.39	34.29	8.97
Carbendazim & Flutriafol	14.91	100.00	38.43	11.38
Carbendazim & Mancozeb	13.52	100.00	46.07	8.04
Oxycarboxin	15.23	99.86	36.04	11.63
SED	1.112	1.149	3.359	1.262

At both assessments in the Auchincruive trial, all the fungicides except Triadimefon significantly reduced the mean infection. Best control, by the second assessment, was obtained with the carbendazim & propiconazole mixture, which produced a 63 % reduction in rust infection (Table 2).

At the first assessment, all the fungicides significantly reduced the percentage of leaves which were infected with rust. Indeed, 87 % of the leaves in the control were infected, compared with 55 % in the plots treated with carbendazim & propiconazole (Table 2). When the second assessment was carried out, infection levels in the plots were very high, with rust on almost all leaves in every treatment. Thus, in the controls, 100 % of the leaves were infected, while in the plots treated with fungicides, between 93 and 100 % of the leaves were infected.

Fungicide treatment had little effect on the percentage of missing leaves at the first assessment. However, at the second assessment, all fungicide treatments except triadimefon, reduced the percentage of missing leaves (Table 2). At the Norwich site, 3 of the fungicides significantly reduced the percentage of missing leaves, even though rust infection was negligible. Carbendazim & propiconazole, carbendazim & flutriafol and chlorothalonil & flutriafol reduced this loss by 12 , 9 and 7 % respectively (Table 2).

Fungicide treatment had little effect on total dry weights at the first assessment. Differences in plant dry weights emerged at the second assessment, when carbendazim & propiconazole, oxycarboxin and carbendazim & flutriafol all produced plants significantly heavier than the control. All other treatments, except triadimefon, produced plants slightly heavier than the control, although these differences were not significant (Table 2).

DISCUSSION AND CONCLUSIONS

Differences in rust control and plant growth appeared between the two assessments made at Auchincruive. The levels of rust infection in this trial were high, with a mean of nearly 22 % on the lower leaves of the control at the second assessment. The high levels of infection were due to the rust appearing soon after planting the crop. This early infection prevented the mint from becoming established, resulting in poor growth rates and reducing the expected yield. Unfavourable weather conditions delayed the first treatments and so the fungicides were applied to a crop which was already infected.

Despite this early infection, all the fungicides, except triadimefon, produced reductions in the mean percentage infection; however the percentage of leaves infected was not reduced. The high levels of rust increased the rate at which plants lost their leaves, although fungicide treatment (except triadimefon) significantly reduced this loss by the second assessment. A loss of leaves is a loss of yield, which was confirmed by the data on mean total dry weights. Fungicides may also increase yield even when disease pressure is very low, shown by the results from Norwich.

None of the fungicides produced any visible phytotoxic effects in the field trials and overall, carbendazim & propiconazole, used at 0.15 % w/v, performed best at Auchincruive.

Evidence from both the glasshouse experiments and field trials indicates that several fungicides may be suitable for the control of rust on mint. This is, however, only the first year of the field trials and it would be inappropriate to draw too many conclusions at this stage. Nevertheless, the data do indicate that triadimefon may not be a suitable fungicide for the control of rust on mint, since the product used in the field trials contained 5 times more active ingredient than would be present in the product sprayed onto commercial mint crops. If triadimefon performs as poorly in the 1994 field trials, then the information obtained from these field trials would back the need for new approvals of more effective fungicides.

ACKNOWLEDGEMENTS

We would like to thank the HDC for providing funding for this project. Thanks are due also to the growers Mr J. Bond and Mr J. Lambe for the use of their land and for their hospitality.The valuable assisstance of Dr N. McRoberts on statistical matters is much appreciated. We would like to thank the following companies for kindly donating samples of their products for inclusion in our trials: BASF, Bayer, Ciba-Geigy, Hoechst and Uniroyal Chemical Ltd.. SAC receives financial support from the Scottish Office Agriculture and Fisheries Department.

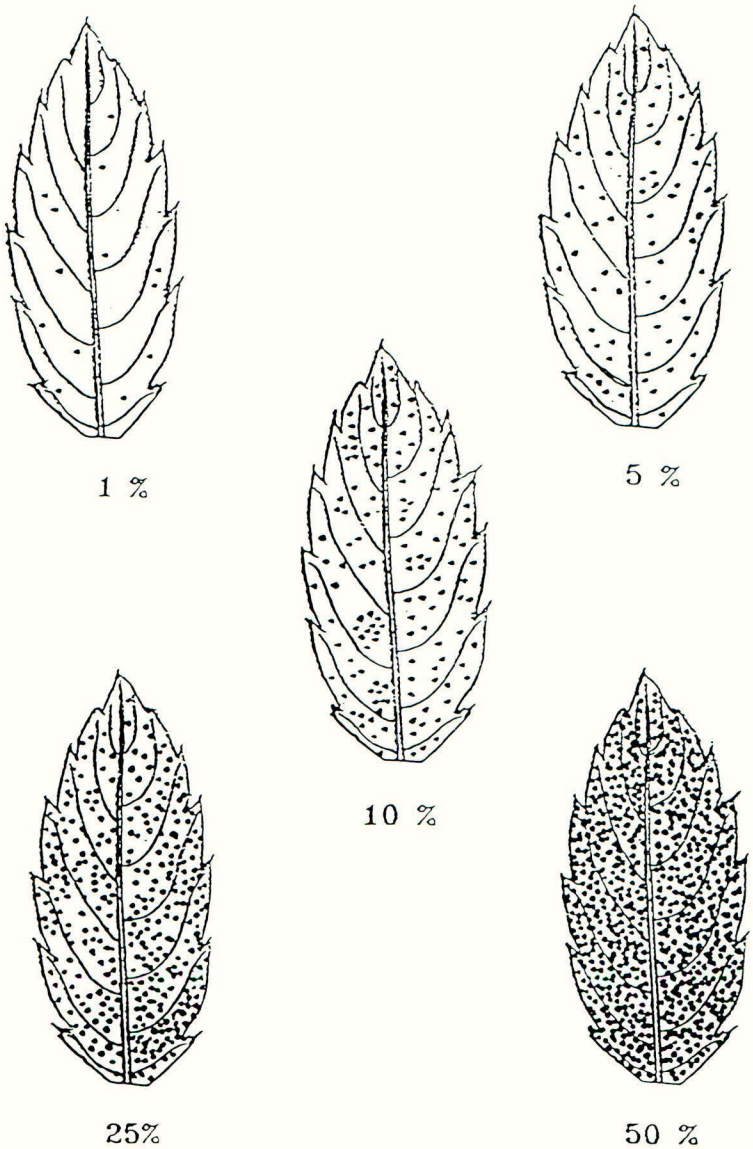
REFERENCES

Anon., (1994) The UK Pesticide Guide, G.W. Ivens (Ed.), CABI / BCPC.

Dumville, C. (1988) The herb industry. *Professional Horticulture* , **2** , 82-85.

Harvey, I.C. (1979) The impact of rust on peppermint crops in Canterbury 1978/79. *Australian Plant Pathology* , **8 (3)**, 44-45.

FIGURE 1. Standard area diagram used to assess the percentage rust infection on spearmint leaves.



THE POSSIBLE CONTROL OF *BOTRYTIS CINEREA* PERS. USING PARAFFINIC OILS

V.A. BOURBOS, M.T. SKOUDRIDAKIS

National Agricultural Research Foundation, Subtropical Plants and Olive Trees Institute of Chania, Laboratory of Plant Pathology, 73100 Chania Greece

V.C. HAITAS AND K.S. FOTIADIS

K.&N. Efthymiadis S.A., Dodekanissou 1, Thessaloniki 54626, Greece

ABSTRACT

In a two year trial carried out in a non-heated greenhouse at Chania, Crete, the activity of the paraffin-based oil Ultrafine was studied on cucumber against a strain of *Botrytis cinerea* resistant to benzimidazole and dicarboximide fungicides.

The fungicidal activity of Ultrafine applied alone at the rate of 2 lit/100 l and combined with full and half rate of benomyl (30 and 15 g/100 l) and vinclozolin (50 and 25 g/100 l) was evaluated against benomyl, vinclozolin and (carbendazim + diethofencarb) applied alone at full rate (30, 50 and 25+25 g/100 l, respectively).

Disease control was evaluated by assessing fruits, leaves and stems (internodes) infested by the pathogen. Ultrafine found to control disease 38.8 - 47.5 % while benomyl and vinclozolin, applied alone, controlled *Botrytis cinerea* 2.5-6.3 and less than 7.5 %, respectively. Ultrafine in combination to benomyl and vinclozolin increased disease control to 88.8-100 and 86.3-100 %, respectively. The application of (carbendazim + diethofencarb) provided excellent (100 %) control to all crop parts examined.

INTRODUCTION

Spray oils have been used in agriculture since the end of 19th century for their insecticidal, herbicidal and fungicidal activity (Gauvrit, 1994). Insect control provided by spray oils was found to exclude several insect-transmitted viruses from planting material (Basky *et al*, 1987; Bell, 1989; Harrington *et al*, 1989).

Spray oils mainly used in pest management are plant-derived oils (natural oils) and mineral (petroleum) oils coming from crude oil.

The development of highly refined paraffin-based oils (superior oils) and new application methods increased the number of crops and plant stages when oils can safely be sprayed. In addition, these oils could control several pest species besides the traditional targets of oil sprays (scale insects and mites) by safely applying them in a wider range of temperatures (5-42^{o}C) (Haitas, 1991).

Paraffin-based oils contain a higher proportion of paraffinic hydrocarbons, saturated (single bond), straight or branched-chain molecules. Furthermore, they possess a lower proportion of naphthenes, aromatics and unsaturates being less phytotoxic than conventional mineral oils.

Recently, the use of spray oils applied alone or combined with fungicides against fungal diseases has been widely reported (Steurbaut, 1992). Mineral oils applied to banana provided effective control of the fungus *Mycosphaerella musicola* especially when used in combination with benzimidazole fungicides (Stover and Simmonds, 1987). In Cuba, the use of mineral oils alone or combined with benomyl, thiophanate or copper fungicides was found to control effectively *Mycosphaerella citri* (Saenz and Lewis, 1985). It has also been reported (Hall, 1983) that several fungal diseases of apple trees were successfully controlled when mineral oils were applied in combination with half rates of the fungicides captan, dinocap, mancozeb and benomyl.

Laboratory tests carried out against *Botrytis cinerea* proved that iminoctadine triacetate, (carbendazim + diethofencarb), benzimidazole and dicarboximide fungicides increase their activity when applied in combination with paraffin-based oil, even against resistant strains of the fungus (Bourbos, Skoudridakis, 1993).

MATERIAL AND METHODS

Two field experiments were carried out in 1993 and 1994 on the F1 hybrid cucumber Condessa RZ grown in a non-heated greenhouse. The experimental design used was the randomised complete block design (RCBD). Each plot contained 10 cucumber plants and was replicated twice.

Cucumber plants were artificially inoculated with a native strain of *Botrytis cinerea* resistant to benzimidazole and dicarboximide fungicides. Inoculation was carried out in full-production cucumber plants under favourable conditions for disease development (temperature: 21-23° C; humidity: 80-90%). The trial plots were artificially inoculated by lightly injuring all cucumber-fruits, 10 leaves and 5 stems (internodes) per plant, followed by a spray of spore suspension containing 1.2-1.4 x 10^6 spores of the fungus/ml. The suspension was uniformly applied from a handsprayer of 1 litre volume with low pressure.

The first fungicide application was carried out 2-3 hours after inoculation, when the surfaces sprayed with spore suspension had already dried. All products were applied three times at 8 day intervals using a knapsack sprayer with low pressure.

The fungicides applied were: Benlate 50WP (benomyl), Ronilan 50WP (vinclozolin) and Sumico 25/25WP (carbendazim+diethofencarb) at the rate of 60, 100 and 100 g/100 l, respectively, while the oil tested was the paraffin-based Ultrafine oil at the rate of 2l/100 l. The fungicides benomyl and vinclozolin were also used in full and half rate combined with 2 lit/100 l of Ultrafine.

Ultrafine is a paraffin-based narrow-range oil (superior oil) with a high content of paraffin (98.8% w/w), having a 50 percent distillation point of 414° F, very low viscosity (60-63 sec SUS in 37.8° C) and unsulfonated residue higher than 92% (highly refined).

Disease control was calculated using Abbott's method (Puntener, 1981) by counting 8 days after last application the number of cucumber-fruits, leaves and stems (internodes) infested with *Botrytis cinerea*.

Data was analysed statistically over years while means separation followed the Duncan's multiple range test (P : 0.05)

RESULTS

The fungicides benomyl and vinclozolin failed to control *Botrytis cinerea* when applied alone at full rate providing 5-6.3 and 0-7.5% disease control respectively (Table 1) In contrast, the fungicide carbendazim+diethofencarb gave complete disease control.

The paraffin-based oil Ultrafine applied alone was found to reduce *Botrytis cinerea* below the untreated control providing 38.8-42.5% disease control. Furthermore, Ultrafine applied in combination with the fungicides benomyl and vinclozolin provided a significantly higher reduction in disease severity. Depending on the crop part examined the combination of Ultrafine with half and full rate of benomyl provided 91.3 and 88.8 (fruits), 95.0 and 97.5 (leaves) and 100% (internodes) disease control respectively. The combination of Ultrafine with half and full rate of vinclozolin provided 95.0 and 98.8 (leaves), 86.3 (fruits) and 100% (internodes) disease control respectively. No sign of phytotoxicity was observed in any treated plot.

Table 1. Disease control provided by the superior oil Ultrafine against *Botrytis cinerea* on greenhouse cucumber. Crete, Greece.

Treatment	Rate g ml/100 l	Efficacy (%) Fruits	Stems (internodes)	Leaves
Control	-	0 a	0 a	0 a
Ultrafine	2.000	38.8 c	42.5 b	38.8 c
Benomyl	60	6.3 b	5 a	6.3 b
Benomyl+Ultrafine	30 +2.000	91.3 d	100 c	95. d
Benomyl+Ultrafine	60 +2.000	88.8 d	100 c	97.5 d
Vinclozolin	100	7.5 b	0 a	7.5 b
Vinclozolin+ Ultrafine	50 +2.000	86.3 d	100 c	95. d
Vinclozolin+ Ultrafine	100 +2.000	86.3 d	100 c	98.8 d
(Carbendazim+diethofencarb)	100	100	100 c	100 d

1. mean of two years
2. efficacy was calculated by Abbott's method.
3. means followed by the same letter do not differ significantly at P : 0,05.

DISCUSSION

The paraffin-based oil Ultrafine when applied to cucumber plants grown in the greenhouse provided a significant reduction in *Botrytis cinerea* compared to the untreated control. Similar fungicidal activity of spray oils has been reported against the fungus *Mycosphaerella musicola* (Calpouzos, 1968).

The addition of Ultrafine to the fungicides benomyl and vinclozolin was found to significantly increase their efficacy against the resistant strain of the pathogen even when applied in combination with a half rate of the fungicides.

As indicated above, a high reduction in disease severity following the use of Ultrafine in combination with fungicides is due not only to the direct activity of Ultrafine to spore germination but also to the indirect activity of the oil, improving tenacity and spreading. Furthermore, the possibility of synergism between Ultrafine and fungicides, and the possible influence of Ultrafine to the mechanism of resistance development has also to be further investigated.

REFERENCES

Basky, Z., Kajati, I., Kiss, E., Kolber, M. and Nasser, M.A.K., 1987. Inhibition of aphid transmission of plant viruses by light summer oils. Mededelingen-van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent, **52:3a,** 1027-1031.

Bell, A.C., 1989. Use of oil and pyrethroid sprays to inhibit the spread of potato virus Y in the field. Crop Protection **8:1,** 37-39.

Bourbos, V.A. and M.T. Skoudridakis, 1993 (in Greek). Possibility to control *Botrytis cinerea* Pers. with the use of summer oils. Proceedings of the 16th Scientific Meeting of the Greek Society of Horticultural Science, Volos-Greece, 90.

Calpouzos, L., 1968. Oils in Fungicides, Vol. **2**, pp 367-393. Ed. D.C. Torgason, Academic Press, New York.

Gauvrit, C., 1994. Les huiles en phytosanitaire: les cas des herbicides. Phytoma-La Defense de vegetaux, No **458**, 37-41.

Haitas, V.C, 1991 (in Greek). The paraffinic oils in agriculture. Ed. K.&N. Efthymiadis, 28p.

Hall, F.R., 1983. Pesticide usage patterns for Ohio apple orchards. Journal of Economic Entomology, **76:3**, 584-589.

Harrington, R., Bartley, E., Riley, D.K., Efrench-Constant, R.H. and Clark, S.J., 1989. Resurgence of insecticide-resistant *Myzus persicae* on potatoes treated repeatedly with cypermethrin and mineral oil. Crop Protection, **8:5**, 340-348.

Puntener, W., 1981. Manual pour essais de plein champ. Protection des vegetaux, 2e edition. ed. CIBA-GEIGY S.A., Bale, Suisse, 205p.

Saenz, M. and Lewis, F., 1985. Preliminary results on the possibility of using mineral oil and its mixture with various fungicides to control greasy spot (*Mycosphaerella citri*). Ciencia y Tecnica en la Agricultura, Protection de Plantas, **8:1**, 7-15.

Steurbaut, W., 1992. Adjuvants for use with fungicides. Adjuvants for agrochemicals, 3-7 Aug. 1002, Cambridge, U.K.

Stover, R.H. and Simmonds, N.W., 1987. Bananas, third edition. Ed. Longman Scientific and Technical, U.K., 468p.

PROTECTION OF FLOWER BULBS USING ANTIFUNGAL PLANT METABOLITES

E.J. SMID & L.G.M. GORRIS

Agrotechnological Research Institute (ATO-DLO), Bornsesteeg 59,
P.O. Box 17, NL-6700 AA Wageningen, The Netherlands

ABSTRACT

Fifteen essential oil components were screened for antifungal activity towards *Penicillium hirsutum*. The most potent inhibitors of *in vitro* growth were found to be carvone and several aldehydes tested. Growth suppression by carvone was reversible, whereas exposure to the aldehydes resulted in an irreversible inhibition. Storage of tulip bulbs in an atmosphere containing the volatile essential oil components significantly reduced the natural *Penicillium* infection. Treatment of tulip bulbs with the six antifungal compounds had no effect on the total stalk length and the flowering capacity.

INTRODUCTION

Fungal diseases of flowerbulbs cause economically important post-harvest losses. The chemical fungicides currently applied for fungal disease control are effective, but isolates resistant to some of them have already been identified. Also, because of governmental opinion and the reduced acceptance by the public of chemically derived fungicides, there is an urgent need for environmentally friendly alternatives. In view of previous achievements with other crops, the use of antifungal plant metabolites seems to offer a sound alternative.

Many different plant species produce essential oils as secondary metabolites. These oils are composed of complex mixtures of volatile compounds which have several biological activities. The active compounds (*i.e.* aldehydes, ketones and terpenes) are thought to play a role in plant defence against phytopathogenic microorganisms. Essential oils, administered as oils from which the active compounds evaporate, are increasingly utilised outside their natural source in the control of pests and diseases.

In addition to a previous communication (Smid *et al.*, 1994) we report on the potency of several essential oil components to counteract fungal storage rot of tulip bulbs, a fungal disease caused by the wound-dependent pathogen *Penicillium hirsutum*.

MATERIALS AND METHODS

Fungal strains and plant material

Penicillium hirsutum CBS 127.90 and CBS 734.74 (ex *Lilium*), *P. hirsutum* CBS 210.57 (ex *Hyacinthus*) and *P. hirsutum* CBS 2349.75 (ex *Tulipa*) were obtained from the "Centraalbureau voor Schimmelcultures" (CBS), Baarn, The Netherlands. Tulip bulbs c.v. 'Apeldoorn' were obtained from commercial growers.

Growth medium

P. hirsutum cultures were maintained as spore suspensions on standard phosphate buffered saline supplemented with 15 % (v/v) glycerol at -80°C. Fungi were routinely grown on potato dextrose agar.

In vitro assay

Plates were inoculated by applying 1 µl of a suspension containing 10^3 spores at the centre of the plate. A watch-glass, holding 10 µl of the volatile oil, was placed in each Petri-dish. Subsequently, the petri-dishes were sealed with a plastic ring and incubated at 19°C.

In situ assay

For each treatment with essential oil compounds, 20 tulip bulbs were placed on a grid in 20-L plastic containers. Two petri-dishes, each containing 125 µl of the volatile component, were placed under the grid. The containers were sealed and stored in the dark at 17°C. After 1 or 3 weeks in the presence of the volatiles, the containers were opened and the bulbs were stored for 12 weeks under dry conditions at 5°C in the absence of the volatiles. Subsequently, the bulbs were planted in soil and stored for another 6 weeks at 2°C in the dark, whereupon they were transferred to the greenhouse to set flower.

RESULTS

In vitro selection of effective volatiles

Using the *in vitro* screening assay, the effect of fifteen purified components of different essential oils was tested on four strains of *P. hirsutum* (Table 1). With fenchone and eucalyptol, no growth inhibition of the fungi tested could be detected. Menthone, pulegone, linalool, menthol, terpineol, menthylacetate and limonene oxide were found to exhibit full inhibition over a period of 4 and 8 days, which is considered short term. Long-term growth inhibition (between 30 to over 120 days) was observed in the presence of carvone, cuminaldehyde, perillaldehyde, cinnamaldehyde, salicylaldehyde and benzaldehyde. The most effective compounds (*i.e.* carvone, cuminaldehyde, perillaldehyde, cinnamaldehyde and salicylaldehyde) were selected for further investigation.

Mode of growth inhibition

Antifungal compounds can exert either a fungistatic or a fungicidal effect. To discriminate between these two possibilities, spores of *P. hirsutum* were seeded on agar plates and subsequently exposed to the volatiles for different periods of time (1, 2, 3 and 4 weeks). After these treatments, the spores were allowed to germinate in an atmosphere without the volatiles and the residual growth of the fungi was monitored.

Full suppression of *P. hirsutum* CBS 201.57 ex *Hyacinthus* spores was found with carvone and cuminaldehyde for at least 4 weeks. However, exposure to carvone for 1 to 4 weeks did not affect the viability of the spores since they formed a mycelial mat at the same rate as non-exposed spores after transfer to fresh medium in the absence of carvone.

However, spores exposed to cuminaldehyde for 1 to 3 weeks resumed growth with a few days delay, whereas spores exposed to the compound for 4 weeks did not germinate at all. Full inactivation of the spores was observed with perillaldehyde after 2 to 4 weeks of exposure. With cinnamaldehyde, after one week of exposure no germination of spores was found. From these results it follows that carvone acts as a fungistatic agent, whereas the aldehydes can exert a fungicidal effect depending on the length of exposure.

TABLE 1. Growth inhibition of different *Penicillium hirsutum* strains by essential oil components administered via the gas phase. Inhibition is expressed as the number of days at which full suppression of fungal growth is observed in the *in vitro* assay.

Compound	*Penicillium hirsutum*			
	CBS 127.90 ex *Lilium*	CBS 201.57 ex *Hyacinthus*	CBS 349.75 ex *Tulipa*	CBS 734.74 ex *Lilium*
Benzaldehyde	> 63	33	> 63	> 63
Carvone	8	39	39	60
Cinnamaldehyde	> 63	26	0	> 63
Cuminaldehyde	32	60	> 120	> 120
Eucalyptol	0	0	0	0
Fenchone	0	0	0	0
Limoneneoxide	5	0	> 63	0
Linalool	5	12	0	0
Menthol	8	8	12	8
Menthone	5	5	5	5
Menthylacetate	8	0	0	0
Perillaldehyde	32	67	26	60
Pulegone	0	5	5	5
Salicylaldehyde	> 63	> 63	> 63	> 63
Terpineol	5	5	5	0

In situ suppression of *Penicillium*

The effect of essential oils on the development of natural fungal infection on tulip bulbs was studied in containers each with 20 bulbs. After one week incubation in control containers without essential oil components, 68% of the bulbs clearly showed *Penicillium* at the surface. In the presence of cinnamaldehyde and cuminaldehyde growth of the fungus was observed in 42% and 3% of the cases, respectively. Treatment with carvone, perillaldehyde and salicylaldehyde completely suppressed fungal growth on the bulbs. After

incubating the bulbs for 3 weeks in control containers, 90% of the bulbs were found to be infected with *Penicillium*. Again, no significant reduction of fungal surface growth was observed with cinnamaldehyde. However, storage in the presence of cuminaldehyde, carvone, perillaldehyde and salicylaldehyde reduced *Penicillium* infection to 45, 66 and 66% of the bulbs, respectively.

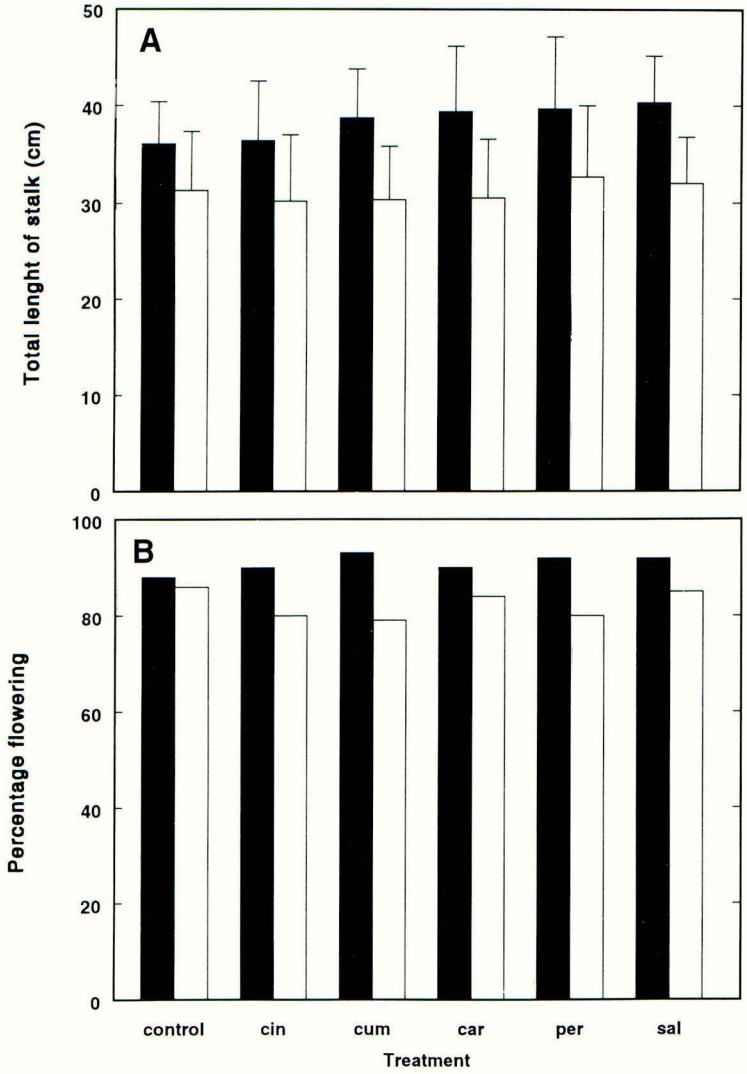


Figure 1. Effect of treatment with different volatiles on A) total stalk length of flowering tulip bulbs and B) on flowering capacity of tulip bulbs. For each treatment, bulbs were stored in the dark at 17°C in the presence of the test compound for 1 week (black bars) or 3 weeks (white bars), before they received a standard flowering treatment.

Phytotoxicity of essential oil components

The essential oil components could be biologically active, thus their effect on flower development of the tulip bulbs was tested. Bulbs were treated with carvone, perillaldehyde, cuminaldehyde, salicylaldehyde, cinnamaldehyde and water for 1 week or 3 weeks. After a standard treatment, the bulbs were allowed to set flower in the greenhouse. Exposing the bulbs to essential oil components for 1 or 3 weeks did not significantly affect the total stalk length nor the flowering capacity (Fig. 1). All bulbs which were treated for three weeks, including the water-treated control, had reduced stalk lengths compared with the bulbs which had been exposed for one week to the compounds. Conceivably, this difference was due to the different age of the two groups of bulbs. It can thus be concluded that none of the antifungal plant metabolites tested showed an effect on flower quality.

DISCUSSION

Using an *in vitro* screening system, six different essential oil components with strong antifungal activity against *P. hirsutum* and therefore with a good potential for development into practice were identified from a panel of fifteen compounds. With one exception (carvone), all of these were aldehydes (cuminaldehyde, perillaldehyde, cinnamaldehyde, salicylaldehyde and benzaldehyde). In studies at our Institute on the use of essential oils to control fungal storage diseases of potato, the same compounds were found to be amongst the most potent antifungals (Gorris *et al.*, 1993; 1994).

As a result of their hydrophobic nature, essential oil compounds probably accumulate in the membrane of target organisms, causing loss of membrane integrity and dissipation of the proton motive force (Sikkema *et al.*, 1992; Sikkema, 1993). Accumulation of the cyclic hydrocarbons in the phospholipid bilayer could also interfere with proper protein-lipid (in 't Veld *et al.*, 1992) and protein-protein interactions which could eventually cause the observed growth inhibition.

Whereas carvone exerted a fungistatic effect on *P. hirsutum*, all aldehydes tested irreversibly inhibit fungal growth after short- or long-term exposure. The variation in effectiveness between the aldehydes may be explained by the different concentrations of the compounds in the gas phase due to differences in volatility (Vaughn & Spencer, 1991). This could explain the observation that cinnamaldehyde, which is the most potent inhibitor in the *in vitro* assay, does not effectively suppress *Penicillium* infection on tulip bulbs in the *in situ* assay.

Since non of the tested antifungal compounds exhibited phytotoxic effects which could adversely affect their market quality, these compounds are promising tools for the control of postharvest *Penicillium* infection on flower bulbs.

ACKNOWLEDGEMENT

The authors are grateful to J.A.M. van Beek, A.H.J. Jansen and O. Vrees for excellent technical assistance. This work was partly supported by "Produktschap voor de Siergewassen", The Hague, The Netherlands.

REFERENCES

Gorris, L.G.M.; de Witte Y.; Smid, E.J. (1993) Plant essential oil components for the control of fungal storage diseases of potato. Abstracts *12th Triennial Conference of the European Association for Potato Research* EAPR '93, Paris, France. p105-106.

Gorris, L.G.M.; Oosterhaven, J; Hartmans, K.J.; de Witte, Y.; Smid, E.J. (1994) Control of fungal storage diseases of potato by use of plant-essential oil components. *Brighton Crop Protection Conference - Pests and Diseases 1994.*

in 't Veld, G.I.; Driessen, A.J.M.; Konings, W.N. (1992) Effect of the unsaturation of phospholipid acyl chains on leucine transport of *Lactococcus lactis* and membrane permeability. *Biochimica Biophysica Acta*, **1108**, 31-39.

Sikkema, J.; Poolman, B.; Konings, W.N.; de Bont, J.A.M. (1992) Effects of the membrane action of tetralin on the functional and structural properties of artificial and bacterial membranes. *Journal of Bacteriology*, **174**, 2986-2992.

Sikkema, J. (1993) Microbial transformation of tetralin. PhD. Thesis, Agricultural University Wageningen, Wageningen, The Netherlands.

Smid, E.J.; de Witte, Y.; Vrees, O.; Gorris, L.G.M. (1994) Use of secondary plant metabolites for the control of postharvest fungal diseases on flower bulbs. *Acta Horticulturae* (in press).

Vaughn, S.F.; Spencer, G.F. (1991). Volatile monoterpenes inhibit potato tuber sprouting. *American Potato Journal*, **68**, 821-831.

INTEGRATED CONTROL OF ROOT DISEASES ON ORNAMENTAL ERICACEOUS PLANTS

A.M. LITTERICK, S.J. HOLMES*
Scottish Agricultural College, Department of Plant Science, Auchincruive, Ayr, KA6 5HW, UK. *Present address ADGEN Diagnostic Systems, Watson Peat Building, Auchincruive, Ayr, KA6 5HW

ABSTRACT

The main root and stem-base pathogens of ericaceous crops are *Rhizoctonia, Cylindrocarpon, Cylindrocladium, Pestalotiopsis, Fusarium, Pythium* and *Phytophthora* spp. The importance and sources of these pathogens on nurseries are described and the influence of cultural and environmental factors and fungicides on the incidence and severity of disease are discussed. A blueprint for integrated control of root and stem-base diseases of ericaceous plants is proposed, based on an understanding of the host/pathogen relationships and modern nursery practice.

INTRODUCTION

Intensive production methods and large scale monoculture have led to increased incidence of root disease on container-grown ornamental ericaceous plants (Holmes & Litterick, 1990). Effective and economic disease control measures are essential if the UK nursery industry is to remain competitive within Europe. The lack of effective fungicides and the restrictions on their use mean that disease control must depend increasingly on integrated systems involving management and correct fungicide selection and use.

Work carried out at SAC Auchincruive has shown that the main root and stem-base pathogens of ericaceous crops are *Rhizoctonia, Cylindrocarpon, Cylindrocladium, Pestalotiopsis, Fusarium, Pythium* and *Phytophthora* spp.. Detailed epidemiological investigations have identified the sources of some of these diseases on nurseries. Pathogens can be controlled or eliminated from ericaceous crops through the use of strict nursery hygiene, management of cultural and environmental conditions and judicious fungicide use (Litterick & Holmes, 1993). The results obtained from this work have been used to develop a blueprint for control of the diseases studied on ericaceous crops.

SOURCES OF DISEASE

Isolations from diseased ericaceous plant material taken from UK nurseries have shown that *Rhizoctonia, Cylindrocarpon, Cylindrocladium, Pestalotiopsis,* and *Fusarium* spp. are most frequently located in the lower foliage (1 to 4 cm above compost level), stem base and upper roots (up to 1 cm below the compost surface). *Rhizoctonia* and *Pestalotiopsis* spp. have been isolated from shoot tips of *Calluna* and *Erica* spp.. *Pythium* and *Phytophthora* spp. are most frequently isolated from the fine roots at all levels below the compost surface. Some

Phytophthora spp. can be isolated from stems, branches and foliage at all levels above the compost surface (Hoitink & Powell, 1990).

Rhizoctonia, Cylindrocarpon, Cylindrocladium, Pestalotiopsis, and *Fusarium* spp. were often isolated from samples of used compost, capillary matting, pots, trays, polythene and other nursery materials and from plant fragments on the nursery. There are few records of these pathogens having been isolated from new or sterilised nursery materials, or sterile compost components.

THE EFFECT OF ENVIRONMENT AND MANAGEMENT PRACTICES ON DISEASE

Detailed studies on the effects of environmental and cultural factors on disease caused by *Rhizoctonia* spp. have been carried out on Calluna and *Erica* spp.. It has been shown that disease caused by *Rhizoctonia* spp. is minimised if the pH of propagation compost is maintained below 4.5 (Holmes & Litterick, 1990). Rhizoctonia infection and disease is greatest where humidities are high. For example the growth of *Rhizoctonia* spp. mycelium on heather foliage kept in an atmosphere of 70 - 85% relative humidity (r.h.) was approximately 15% of that of mycelium on heather foliage kept in an atmosphere of 90 - 100% r.h. (Litterick & Holmes, 1990). It has been shown that poor compost drainage, low compost air-filled-porosity (i.e. below 12%) and poor nutrition and delayed potting can all increase the likelihood of disease caused by *Rhizoctonia* spp. (Litterick, 1991).

FUNGICIDE PHYTOTOXICITY

It has been shown that a number of fungicides routinely applied to heathers and other nursery stock species may cause foliar browning and reduced root development. (Holmes & Litterick, 1990). The likelihood of damage is greater where combinations of two or more fungicides are used and damage is most severe on cuttings and young plants. The specificity of fungicides which are effective against the main causal pathogens means that comprehensive protection often requires the use of up to three fungicides (see Table 1).

Fungicide use should be minimised on cuttings and growing crops. Cultural and hygiene measures can be taken to prevent disease during these stages of production unless chemicals are needed to control disease outbreaks. A full fungicide programme can be used to prevent and control disease on stock plants from which cuttings are taken, since phytotoxicity occurs much less frequently on older plants.

THE EFFECT OF FUNGICIDES ON DISEASE

There is a shortage of approved products to control root and stem-base diseases on ericaceous crops and many of the available products have to be used at the growers' own risk. Research has shown that the following fungicides prevent or control the main root diseases of ericaceous crops. (Litterick & Holmes, 1993; Hoitink & Powell 1990, Table 1.)

Table 1. Fungicides available to control disease on ericaceous crops

Disease	Fungicide	Active Ingredient	Manufacturer
Rhizoctonia	Basilex*	tolclofos-methyl	Fisons
	Rovral*	iprodione	Rhone-Poulenc
	Terraclor 20D*	quintozene	Uniroyal
Pythium and Phytophthora	Fongarid	furalaxyl	Ciba Agric.
	Aaterra WP*	etridiazole	Zeneca/ICI
	Filex*	propamocarb-hydrochloride	Fisons
Phytophthora only	Aliette*	fosetyl-aluminium	Rhone-Poulenc
Cylindrocladium	Octave	prochloraz-manganese	Fisons
Cylindrocarpon Pestalotiopsis and Fusarium	Bavistin*	carbendazim	BASF

*NB Products marked with an asterisk do not have a manufacturers recommendation for use on ericaceous plants and therefore must be used at the growers own risk.

BLUEPRINT FOR DISEASE CONTROL

An integrated control programme must be practical, economic and durable. Continued disease control depends on careful attention by the grower to all aspects of the programme. The following blueprint is based on results gained from 6 years work on diseases of ericaceous crops.

Blueprint for disease control

1. Use only new or sterilised nursery materials and equipment. Use a good proprietary sterilant regularly on pots, trays, benches, pathways, trolleys etc.

2. Use only fresh or sterilised compost components and avoid contact of pots with nursery soil.

3. Burn or remove discarded, old or diseased plants from the nursery.

4. Stock plants should be potted or trimmed annually to maintain vigorous, healthy growth. Cuttings should be taken from tips rather than bases of shoots.

5. Space stock plants to minimise plant to plant contact and reduce humidity levels.

6. Reduce humidity around cuttings as swiftly as is practical.

7. Check that the propagation compost has a pH of around 4.0

8. Minimise plant stress by providing environmental and cultural conditions which promote healthy plant growth. In particular ensure consistent irrigation, adequate compost air-filled-porosity (12 - 15%) and optimum levels of fertiliser.

9. Root-rots and stem-base diseases should be correctly diagnosed to prevent the costly and ineffective application of a fungicide.

10. Avoid incorporating fungicides into the propagation compost and treat growing crops with fungicide only where disease is present or during a period of high disease risk.

11. Treat stock plants with a fungicide programme to prevent and control disease caused by *Rhizoctonia, Cylindrocarpon, Cylindrocladium, Pestalotiopsis, Fusarium, Pythium* and *Phytophthora* spp. Use high volume sprays or drenches to ensure that the chemical penetrates the foliage canopy.

12. Use fungicides according to the manufacturers recommendations and be aware that many of the above chemicals must be used at the growers own risk (see Table 1).

FUTURE RESEARCH

Further work is necessary to determine the effect of environmental and cultural factors on the incidence and spread of diseases caused by *Cylindrocarpon, Cylindrocladium, Pestalotiopsis, Fusarium, Pythium* and *Phytophthora* spp.. As more work is completed, the above blueprint can be modified and extended to cover all the major root pathogens of ericaceous crops

ACKNOWLEDGEMENTS

This work was supported by The Horticultural Development Council. SAC receives financial support from the Scottish Office Agriculture and Fisheries Department.

REFERENCES

Hoitink & Powell (1990) Fighting Phytophthora. *American Nurseryman,* **May 15**, 67-73

Holmes, S.J. & Litterick, A.M. (1990) Integrated approaches to controlling *Rhizoctonia* spp. on protected hardy ornamental nursery stock. *Brighton Crop Protection Conference - Pests and Diseases 1990*, **1**, 379-384.

Litterick, A.M. (1991) An investigation into the epidemiology and control of *Rhizoctonia* spp. on *Calluna vulgaris* and *Erica* spp. PhD Thesis, Glasgow University.

Litterick, A.M. & Holmes, S.J. (1990) Control of soil-borne diseases of heathers and heaths. *Proceedings Crop Protection in Northern Britain Conference 1990*, 193-198.

Litterick, A.M. & Holmes, S.J. (1993) Development of fungicide programmes for control of Rhizoctonia diseases on heathers. *Proceedings Crop Protection in Northern Britain Conference 1993*, 283-288.

BIOLOGICAL CONTROL OF FUSARIUM WILT OF BASIL (OCIMUM BASILICUM L.)

G. MINUTO, A. GARIBALDI, M.L. GULLINO

DI.VA.P.R.A. - Patologia vegetale, Via Giuria 15, 10126 Torino, Italy

ABSTRACT

Fusarium wilt of basil, caused by Fusarium oxysporum f. sp. basilici, is a major problem in the cultivation of basil (Ocimum basilicum) on the Riviera Ligure in Northern Italy. Four trials carried out in 1993 and 1994 using antagonistic Fusarium spp. as a soil treatment and/or seed dressing showed the possibility of using such strains for effective wilt control. With high disease incidence, soil treatment with the antagonistic Fusarium strains provided satisfactory and long lasting disease control.

INTRODUCTION

Fusarium wilt, incited by Fusarium oxysporum f. sp. basilici[1], is a major problem in the cultivation of basil (Ocimum basilicum), a cash crop grown under glasshouse (approximately 65 ha) in the Riviera Ligure, in Northern Italy, as well as in the French Riviera. Such a crop, although of minor importance on a national basis, is of high economic importance for local growers. Moreover, it represents a typical Mediterranean crop, used for fresh consumption as well as for the preparation of the world famous "pesto" sauce. F. basilici was first reported in Russia (Kvartskhava, 1957) and, more recently, in France (Mercier and Pionnat, 1982), Italy (Tamietti and Matta, 1989) and the USA (Wick and Haviland, 1992; Davis and Marshall, 1993).

Control of this pathogen is complicated by the very limited availability of registered fungicides: in Italy, the only permitted compounds effective against Fusarium wilt are benzimidazoles, which can be applied only as seed dressing (Minuto et al., 1994). In practice, Fusarium wilt management relies on the integration of different control measures, such as soil disinfestation, raised bench cultivation and seed dressing. However, since the pathogen can be transmitted by infected seeds (Martini and Gullino, 1991), soil disinfestation is only partially effective. Moreover, seed dressing alone does not completely reduce Fusarium wilt severity (Minuto et al., 1994). For all these reasons, the level of control achieved is often unsatisfactory while the

[1] Fusarium oxysporum f. sp. basilicum is renamed into Fusarium oxysporum f. sp. basilici (Hawksworth, 1974).

cost is high. Effective alternatives are needed in order to ensure growers more choice.

Saprophytic Fusarium spp., isolated from Fusarium-suppressive soils, known for their antagonistic activity against several formae speciales of Fusarium oxysporum (Tramier et al., 1983; Alabouvette et al., 1993; Garibaldi et al., 1992), have largely been tested on several crops such as carnation and cyclamen (Garibaldi et al., 1992; Minuto et al., 1994 a). During the past few years, the possibility of using such antagonistic Fusarium spp. against Fusarium wilt of basil was investigated. The first positive results obtained (Minuto et al., 1994 b) stimulated further research, in order to determine the best timing and methods of application.

MATERIALS AND METHODS

Four experimental trials have been carried out in 1993 and 1994 in an experimental glasshouse at the Centro Orticolo Sperimentale of the Chamber of Commerce of Savona, located at Albenga (Northern Italy). Plots of 2 m^2 were prepared in raised benches. A randomised block layout with three replicates was used.

Cultural practices. Two basil varieties, "Genovese a foglie giganti", commonly used in the region, and "Fine verde", very susceptible to Fusarium wilt, were grown on a substrate commonly used for basil, following the practices normally adopted by local growers. The temperature in the glasshouse was maintained throughout cultivation in eccess of 15 ^{o}C; the maximum temperature reached was 32 ^{o}C. Two g/m^2 of basil seeds were used.

Inoculation with the pathogen. The same isolate of Fusarium oxysporum f. sp. basilici, obtained from wilted plants, and prepared as chlamydospores suspended in talc (Locke and Colhoun, 1974) was used in 1993 and 1994 for soil infestation. After steaming, the substrate was infested with F. basilici by using 10^3 cfu (Colony Forming Units)/ml of soil in 1993, $5x10^3$ cfu/ml of soil in 1994. Artificial soil infestation with the pathogen was carried out in 1993 only before the first trial, and in 1994 before each trial.

Treatment with the antagonist. Antagonistic strains of F. oxysporum, known for their good biocontrol activity against several Fusarium wilts and widely tested on different crops (Garibaldi et al., 1992) have been used. F. oxysporum strain 251/2 RB, made resistant to benzimidazoles by UV treatment (Garibaldi et al., 1988) was used as chlamydospores suspended in talc (Locke and Colhoun, 1974) or as a formulated granular product, consisting of chlamydospore suspensions in sodium alginate, coded as FG, prepared by S.I.A.P.A. (Galliera, Bologna). Strain F I/11 was obtained by protoplast fusion (Migheli et al., 1992). Fusarium moniliforme strain TF4, isolated from a Fusarium suppressive

soil in France (Tramier et al., 1983) was kindly provided by Dr. R. Tramier. Micromax, a preparation based on F. oxysporum FO 47 (Alabouvette et al., 1993), commercialised in Italy by SCAM, was also tested.

Antagonistic Fusarium were used as a soil treatment (10^5 cfu/ml) for the formulation and/or seed dressing ($3x10^7$ - 10^8 CFU/g). Micromax was used as a soil treatment at 10^5 and $3.4x10^5$ cfu/ml of soil. Soil infestation with the antagonists was carried out at the same time as soil infestation with the pathogen. An interval of 10-15 days was left before seeding basil, in order to permit both pathogen and antagonist to colonise the soil.

The efficacy of the different treatments was evaluated by counting, at regular intervals, the number of healthy and infected plants. Differences between treatments were determined using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Disease incidence was very high in all trials (Tables 1-3); the high susceptibility of the variety "Fine verde" was confirmed (Table 1). Soil treatment with the tested

Table 1 - Efficacy of different treatments against Fusarium wilt on two basil varieties in two trials (Albenga, 1993).

Variety	Antagonist applied as soil treatment*	Antagonist applied as seed dressing**	% healthy plants Trial 1	% healthy plants Trial 2
Genovese a foglie giganti	--	--	87.6 a	68.7 a
Genovese a foglie giganti	251/2 RB 10^5 cfu/ml	251/2 RB $3x10^7$ cfu/g	94.6 a	88.4 a
Genovese a foglie giganti	FI/11 10^5 cfu/ml	FI/11 $3x10^7$ cfu/g	93.8 a	88.7 a
Fine verde	--	--	35.8 c	24.0 b
Fine verde	251/2 RB 10^5 cfu/ml	251/2 RB $3x10^7$ cfu/g	65.2 b	66.5 a
Fine verde	FI/11 10^5 cfu/ml	FI/11 $3x10^7$ cfu/g	82.4 ab	68.8 a

* carried out only once, 14 days before sowing trial no 1
** carried out at each trial
^ values of the same column with the same letter do not differ significantly (Duncan's Multiple Range Test, P =0.05)

Table 2 - Efficacy of different treatments against Fusarium wilt on the cv Fine verde (Albenga, Trial 1/1994).

Antagonist	applied as soil treatment	applied as seed dressing*	% healthy plants at 28/3	% healthy plants at 20/4
251/2 RB	10^5 cfu/ml	--	89.2 a^	62.5 ab
FI/11 RB	10^5 cfu/ml	--	92.7 a	69.8 a
T F4	10^5 cfu/ml	10^8 cfu/g	82.9 ab	40.2 c
251/2 RB	10^5 cfu/ml	10^8 cfu/g	86.7 a	48.3 bc
FI/11 RB	10^5 cfu/ml	10^8 cfu/g	90.0 a	63.6 ab
251/2 RB	--	10^8 cfu/g	73.0 bc	12.4 d
251/2 RB FG°	10^5 cfu/ml	--	63.9 cd	11.3 d
251/2 RB FG°	$1,5x10^5$ cfu/ml	--	64.8 cd	8.6 d
Control	--	--	56.2 d	2.4 d

* ^ see Table 1
° formulated by S.I.A.P.A., Galliera (Bologna)

Table 3 - Efficacy of different treatments against Fusarium wilt on the cv Fine verde (Albenga, Trial 2/1994).

Antagonist	applied as soil treatment	applied as seed dressing*	% healthy plants at end of trial
251/2 RB	10^5 cfu/ml	--	56.3 ab^
T F4	10^5 cfu/ml	10^8 cfu/g	70.8 a
T F4 RB	10^5 cfu/ml	10^8 cfu/g	57.2 ab
251/2 RB	10^5 cfu/ml	10^8 cfu/g	64.7 a
251/2 RB	--	10^8 cfu/g	19.4 c
251/2 RB FG°	10^5 cfu/ml	$5x10^7$ cfu/g	16.2 c
Micromax	10^5 cfu/ml	--	27.3 c
Micromax	$3.4x10^5$ cfu/ml	--	48.3 b
Control	--	--	20.1 c

*,^ see Table 1; ° see Table 2

antagonistic Fusarium sp. strains provided comparable disease control (Tables 2 and 3), significantly increasing the percentage of healthy plants. The results obtained in 1993 (Table 1) show that one soil treatment can effectively protect subsequent basil crops for several months. The exclusive use of 251/2 RB for seed dressing (trials 1/1994 and 2/1994, Tables 2 and 3) did not control Fusarium wilt. The combination of soil treatment with seed dressing generally did not improve wilt control in comparison with soil treatment alone. The formulation of strain 251/2 RB prepared by S.I.A.P.A., coded FG, was not effective: when used for soil treatment (trials 1/1994 and 2/1994; Tables 2 and 3) it did not reduce disease incidence. Micromax was partially effective at the highest dosage tested (Table 3).

The results obtained, in the presence of a high disease pressure, due to a very severe artificial soil infestation with F. basilici, confirm the possibility of using antagonistic Fusarium spp. for effective control of Fusarium wilt of basil (Minuto et al., 1994 b). In order to obtain consistent and long-lasting wilt control, the ratio between antagonist and pathogen propagules must be at least 20. The long lasting effect shown by one soil treatment with antagonistic Fusarium sp. protects the crop for several months.
The good results obtained on the variety "Fine verde", highly susceptible to Fusarium wilt, are more easily achieved with the cv "Genovese a foglie giganti", widely grown in Italy and less susceptible to Fusarium wilt.

The poor performance shown by the commercial preparation of strain 251/2 RB (FG) stresses once more the need to find good and reliable methods for producing large quantities of microbial preparations (Rhodes, 1993).

ACKNOWLEDGEMENTS

Work supported by Regione Liguria.

REFERENCES

Alabouvette, C., Lemanceau, P., Steinberg, C. (1993) La lutte biologique contre les maladies d'origine tellurique. Phytoma, n. 452, 36-40.

Davis, R.M., Marshall, K.D. (1993) First report of Fusarium wilt of basil in California. Plant Disease, 77, 537.

Garibaldi, A., Brunatti, F., Cugudda, L. (1988) Attivita' antagonistica nei confronti della tracheofusariosi del garofano di Fusaria non patogeni resistenti a benomyl. Atti Giornate Fitopatologiche, 2, 481-490.

Garibaldi, A., Aloi, C., Parodi, C., Gullino, M.L. (1992) Biological control of Fusarium wilt of carnation. In: Biological control of plant diseases. Progresses and

challenges for the future (Tjamos E.C., Papavizas G.C., Cook R.J. eds.), Plenum Press, New York, 105-108.

Hawksworth, D.L. (1974) Mycologist's Handbook, C.M.I., Kew, U.K., 231 pages.

Kvartskhava, P.A. (1957) On a study of the infectious wilting (fusariosis) in evgenol basil. Trud. Sukkumsk. opyt. Sta. efiromasl. Kul't, 2, 101-113 (in R.A.M.,38, 613).

Locke, T., Colhoun, J. (1974) Contribution to a method of testing oil palm seedlings for resistance to Fusarium oxysporum f. sp. elaidis Toovey. Phytopath. J.,79,77-92

Martini, P., Gullino M.L. (1991) Trasmissibilita' per seme di Fusarium oxysporum f. sp. basilicum agente della tracheofusariosi del basilico. Informatore Fitopatologico, 41 (9), 59-61.

Mercier, S., Pionnat, J.C. (1982) Présence en France de la fu sariose vasculaire du basilic. Comptes Rendus des Seances de l'Academie d'Agriculture de France, 68, 416-419.

Migheli, Q., Piano, S., Enrietti, S., Gullino, M. L. (1992) Protoplast fusion in antagonistic Fusarium spp. IOBC/WPRS Bull., XV/1, 196-198.

Minuto, A., Aloi, C., Mocioni, M., Garibaldi, A. (1994) Effetto della concia del seme di basilico sulla trasmissione di Fusarium oxisporum f. sp. basilicum. Atti Giornate fitopatologiche, 3, 293-296.

Minuto, A., Migheli, Q., Garibaldi, A. (1994 a) Evaluation of antagonistic strains of Fusarium in the biological and integrated control of Fusarium wilt of cyclamen. Crop protection, in press.

Minuto, A., Mocioni, M., Garibaldi, A. (1994 b) Preliminary trials on biological control of Fusarium wilt of basil. Acta Horticulturae, in press.

Rhodes, D.J. (1993) Formulation of biological control agents. In: Exploitation of Microorganisms (Jones D.J. coord.), Chapman & Hall, London, 411-439.

Tamietti, G., Matta, A. (1989) La tracheomicosi del basilico causata da Fusarium oxysporum f. sp. basilicum in Liguria. La difesa delle piante, 12, 213-220.

Tramier, R., Pionnat, J.C., Tebibel, N. (1983) Role of the fungi in the induction of suppressiveness into substrates to Fusarium wilt of carnation. Acta Horticulturae, 141, 55-59.

Wick, R.L., Haviland, P. (1992) Occurrence of Fusarium wilt of basil in the United States. Plant Disease, 76, 323.