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CONTRACT REPORT
SF/19A
Strawberry - Investigations on pre-planting runner treatments to eliminate *Colletotrichumacutatum* from planting stock

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AUTHENTICATION

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

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Strawberry: Investigations on pre-planting runner treatments to eliminate *Colletotrichum acutatum* from planting stock

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APPLICATION

Blackspot is a serious disease of strawberries, which, although prevalent in many other countries, was only confirmed for the first time in the UK in 1983 on imported strawberry runners. The disease is mainly introduced into new crops by planting infected runners. UK-produced runners are free of blackspot but imported runners may be infected and with the introduction of a common EC-market and free movement of plants, the risk of the introduction of blackspot infected runners is high. The objective of this project was therefore to investigate the possibility of eradicating blackspot from strawberry runners by using pre-planting fungicide or hot water treatments. Results indicate that the fungicides fenpropimorph (Corbel) or prochloraz (Sportak or Octave) both have potential as eradicant treatments. Further work is needed to identify the minimum fungicide concentration and dip time required for efficacy before the treatment can be used safely by growers.

Strawberry: Investigations on pre-planting runner treatments to eliminate *Colletotrichum acutatum* from planting stock

SUMMARY

In a two year study, fungicides were evaluated *in vitro* and *in vivo* for efficacy as treatments to eliminate *Colletotrichum acutatum* from planting stock. Initially *in vitro* tests were conducted to determine potential candidate fungicides for the *in vivo* tests. In the *in vitro* tests, test fungicides were incorporated at a range of concentrations into agar plates and tested for activity against both spore germination and mycelial growth of *C. acutatum*. Fenpropimorph (Corbel), penconazole (Topas), prochloraz (Octave) and propiconazole (Tilt) were most inhibitory against both spore germination and mycelial growth of *C. acutatum*.

Strawberry runners cv. Elsanta inoculated with *C. acutatum* were then treated with test fungicides at a range of concentrations and dip times, by submerging the runners in the fungicide solution. After treatment the runners were tested for the presence of blackspot to evaluate the success of the treatments. None of the fungicide treatments completely eliminated *C. acutatum* from plants, however strawberry runners dipped in fenpropimorph or prochloraz or in mixtures of these had the least incidence of blackspot after treatment. These two fungicides therefore show potential as eradicant treatments for the elimination of blackspot from runners, but further work is necessary to identify the optimum fungicide concentration and dip time.

ACTION POINTS FOR GROWERS

1. Current MAFF policy ensures that imported strawberry planting material is free of blackspot. If MAFF policy regarding this disease should change, and especially with regard to the common EC Market and free movement of plants between member-states using a plant passport, the risk of blackspot infected runners is likely to increase. Use of pre-planting runner dips to eradicate or reduce blackspot from suspect plant consignments is more desirable and possibly easier than treating the disease in the crop.

2. Screening for fungicides with activity against blackspot should continue, both for pre-planting treatments and for foliar sprays in the field to ensure that should blackspot become established in the UK, a treatment is available for control.

3. Fenpropimorph and prochloraz were most promising as pre-planting dips of the products tested. However further work is needed to establish the optimum dose and dip time required for effective control.

4. Currently neither prochloraz nor fenpropimorph have approval for use on fruiting strawberries. If further trials confirm these products as the most effective, then approval or off-label approval will need to be sought for their use in fruiting plantations.

5. Blackspot is a major disease of strawberry in other parts of the world. The incidence of the disease may increase in the near future in the UK. It is essential that treatments are available for use, to ensure that the disease does not become established in the UK.

EXPERIMENTAL

INTRODUCTION

An extensive review of blackspot has already been conducted by Swinburne (1991).

Strawberry blackspot caused by the fungus *Colletotrichum acutatum* was identified for the first time in the UK in 1983 in plants cv Brighton introduced to Southern England from California. Steps taken by MAFF to eradicate the outbreaks were apparently successful, apart from an outbreak in Cornwall, but in the summer of 1988 about a dozen outbreaks of blackspot were recorded in strawberry crops in South East England. All outbreaks were on or near crops originating from strawberry runners imported from Europe. Further outbreaks were recorded in 1989 and 1990. Blackspot of strawberry is still a notifiable disease, statutory action being required on confirmed outbreaks, requiring grubbing of affected plantations and banning of imported runner consignments where blackspot has been confirmed in tests carried out by Central Science Laboratory (CSL) at Harpenden.

Strawberry blackspot caused by the fungus *Colletotrichum acutatum* is primarily a fruit rot and is responsible for serious losses of ripe strawberry fruit particularly in Australia, New Zealand and the USA and more recently parts of Europe notably France. The disease becomes a serious problem when warm (>23°C) humid conditions prevail during fruit ripening and harvest. Since the disease attacks ripe fruit, an intensive programme of sprays would be needed near or during harvest which would be undesirable given the present public concern over the use of pesticides. In addition, experience from other countries where the disease is established indicates that it is difficult to control with the fungicides currently available. In the UK the disease has mainly been found on the cv Elsanta, where losses have been very small, but it is on the everbearer crops such as Rapella, with the long periods of fruiting under warm humid conditions in the late summer that the greater losses are likely to occur. The fungus also causes lesions on petioles, fruiting stalks and stolons, and can invade the crown remaining as symptomless, latent infection until conditions favour development and the fruit starts to ripen.

Symptomless infected runners is the main way in which the disease is introduced into new crops and new areas. All disease outbreaks identified in the UK have been associated with imported runners. UK-produced runners are free of blackspot. To prevent the establishment of blackspot in the UK it is essential to ensure that planting material is blackspot free. This can be achieved either by using UK stock only or ensuring that imported stock is blackspot free. Current statutory arrangements ensure the latter as all imported runners are tested for blackspot at CSL Harpenden. However, with the introduction of a common EC-market and

free movement of plants between member states using a plant passport, the risk of introducing infected runners has increased considerably. The availability of pre-planting treatments to eliminate or reduce blackspot in suspect planting stock would reduce the risk of infection considerably. Research in other countries into these aspects have not, so far, resulted in a method of eradicating blackspot from runners. None of the fungicides tested were completely effective and hot water treatment gave variable results depending on the variety used and plant vigour. The aim of this project was, therefore, to evaluate the effectiveness of fungicides and hot water treatment for eradication of *Colletotrichum acutatum* from symptomless-infected strawberry runners.

Experimental objectives

To identify suitable pre-planting fungicide dips or hot water treatments alone or in combination to eliminate or significantly reduce *Colletotrichum acutatum* from runner stocks.

MATERIALS AND METHODS

Experiments were carried out in both the laboratory (*in vitro*) on artificial media and in the glasshouse on strawberry plants (*in vivo*).

(a) *In vitro* experiments

(i) Isolates of *C. acutatum*

Two isolates of *C. acutatum* were used for all experiments; viz. isolate 467 and isolate 474. Both were isolated in 1989 from infected strawberry plants cv. Elsanta from fields near Maidstone and Faversham in Kent, respectively. Cultures were maintained on potato dextrose agar (PDA; Oxoid) and regular checks were made to ensure the fungi retained their pathogenicity.

(ii) Fungicide amended agar

PDA was amended with fungicides to obtain concentrations of 0, 1, 5, 10, 50, 100 and 500 ppm active ingredient (a.i). Fungicides were added to the agar after autoclaving when the agar had cooled to 50°C. The fungicides tested are shown in Table 1.

(iii) Inhibition of spore germination

To test the effects of fungicides on germination of *C. acutatum* spores, a spore suspension of 10^3 spores/ml sterile distilled water with Tween 20 (0.1 ml/litre) was prepared for both isolate 467 and isolate 474. For each of the isolates, three replicate fungicide plates were inoculated with 0.1 ml of one of the spore suspensions. Plates were incubated at 25°C in the dark and developing colonies were counted after 2 and 6 days by holding the plate against a dark background.

(iv) Inhibition of mycelial growth

To test the effects of fungicides on mycelial growth, fungicide amended plates were inoculated centrally with a plug (0.5 cm diameter) of mycelium taken from the actively growing edge of a colony. Three replicate plates were used for each concentration and each isolate. Plates were incubated at 25°C in the dark. The growth of the fungus was measured in three directions at 120° intervals from each other, on alternate days commencing at day 3 and continuing until day 15 after plate inoculation.

Table 1 **Details off fungicide products tested for efficacy against *C. acutatum* in the laboratory**

Fungicide product	Active ingredient	% A.I. and formulation
Benlate	benomyl	50% WP
Bravo 500	chlorothalonil	500 g/l SC
Corbel	fenpropimorph	750 g/l EC
Elvaron	dichlofluanid	50% WP
Octave	prochloraz	50% WP
Rubigan	fenarimol	120 g/l SC
Systhane 6 Flo	myclobutanil	60 g/l EW
Thianosan	thiram	80% WG
Tilt	propiconazole	250 g/l EC
Topas	penconazole	100 g/l EC

(b) *In vivo* Experiments

(i) Source of plant material

Most experiments were carried out using strawberry runners cv Elsanta obtained from a UK commercial producer and which were consequently blackspot free and required inoculation prior to the experiments. Runners cv Elsanta were also obtained from a producer in Portugal and these were naturally infected with *C. acutatum*.

(ii) Inoculation of runners with *C. acutatum*

Plants were treated as far as possible to simulate natural field infection. Runners were potted up in 15 cm pots in compost (Fison, M2 grade) and allowed to establish for two weeks in a glasshouse (average temperature 25°C with misting at frequent intervals). After establishment the plants were inoculated with a spore suspension (10^6 spores/ml) of *C. acutatum* using a hand spray. The plants were then covered with plastic for 2 days to ensure optimum conditions for infection of temperature (25°C) and high humidity. The plants were then left for 4 weeks in the glasshouse before the experiments to ensure infections of *C. acutatum* were well established. Prior to experimental treatment the plants were removed from their pots, both tops and roots were trimmed to 6 cm and grouped into bundles of ten plants secured by a rubber band.

(iii) Treatment with fungicide

Fungicide solutions were prepared in buckets at a concentration of either 100 or 500 ppm a.i. except for thiram (Thianosan) which was tested at a concentration of 1000 ppm a.i. The bundles of runners were soaked in the test treatment at outside ambient temperature (15-18°C) for either 10 or 30 minutes, except for thiram. For this treatment bundles of plants were soaked at 30°C for 1 or 4 hours using a heated water bath to maintain temperature. Treatments of dichlofluanid and fenpropimorph both at 100 ppm ai were also tested at 30°C for 10 and 30 minute dip times. Mixtures of fenpropimorph with prochloraz or dichlofluanid were also tested. After treatment half the runner sample was immediately tested for the presence of *C. acutatum*. The remaining half was re-potted and grown on for 6 weeks in the greenhouse, but assessed at frequent intervals for signs of phytotoxicity. At the end of the 6 week period these plants were also tested for *C. acutatum*.

(iv) Hot water treatment

Infected strawberry runners prepared as in (b)(ii) were dipped for 5 or 10 minutes in a heated water bath at a temperature of either 45°C or 50°C. After treatment plants were assessed as in (b) (iii).

(v) Assessment of runners for the presence of *C. acutatum*

The paraquat test (Cerkauskas, 1988) modified by Dr Roger Cook (CSL, Harpenden) was used in all experiments to test for the presence of *C. acutatum*. Petioles from the test plants were treated with 10% bleach for 10 minutes, rinsed three times with tap water, and then submerged in diluted paraquat (5g paraquat/litre) for 1 minute. The petioles were again rinsed to remove excess paraquat and then placed in high humidity chambers and incubated in the light at ambient room temperature. After 10 days petioles were assessed visually for the presence of blackspot, which was confirmed by microscopic examination. An additional, more sensitive, technique was used in later experiments. This technique was developed in France by Baudry and Mozieres (GRISP-DRAF-SRPV de Bordeaux). After treatment with paraquat and incubation (as described above) petioles were soaked in 100 ml sterile water and subjected to a stomacher for 1 minute. A dilution series was prepared from the suspension produced and 0.1 ml

of the appropriate dilution was plated out onto PDA amended with streptomycin (50 ppm), chloramphenicol (50 ppm), benomyl (5 ppm) and vinclozolin (2 ppm). Plates were assessed for presence of *C. acutatum* colonies after 5 days.

(vi) Phytotoxicity

Frequent observations for signs of phytotoxicity were made on the plants potted after treatment. The number of plants surviving after 6 weeks was recorded together with any obvious phytotoxicity symptoms such as yellowing, necrosis, etc. apparent on the plants.

RESULTS

(a) *In vitro* experiments

(i) Inhibition of spore germination and mycelial growth

C. acutatum was most sensitive to prochloraz, fenpropimorph, propiconazole and penconazole assessed by both spore germination and mycelial growth (Table 2). Other fungicides such as chlorothalonil and dichlofluanid, did inhibit spore germination but were not as effective in inhibiting mycelial growth. Myclobutanil was more effective in inhibiting mycelial growth than spore germination.

Table 2 The EC_{50}s (mg a.i./ litre) of spore germination and mycelial growth in the presence of fungicides for isolate 467 and isolate 474 of *C. acutatum*

Active ingredient	Fungicide product	Isolate 467		Isolate 474	
		Spore germination	Mycelial growth	Spore germination	Mycelial growth
benomyl	Benlate	<1[1]	<1	50	50-100[2]
chlorothalonil	Bravo 500	<1	>500	<1	>500
dichlofluanid	Elvaron	<5	100	<5	<50
fenarimol	Rubigan	<10	<5	<10	<5
fenpropimorph	Corbel	<1	<5	<1	1
myclobutanil	Systhane	<50	<5	<50	<5
penconazole	Topas	<5	<5	<5	<5
prochloraz	Octave	<1	<1	<1	<1
propiconazole	Tilt	<1	<1	<5	<1
thiram	Thianosan	<10	<50	<10	50

[1]<indicates that the EC_{50} is between this concentration and the previous tested.
[2] mycelial growth was identical at these two concentrations.

The overall response of the two isolates was the same apart from their response to benomyl. Isolate 467 was extremely sensitive to benomyl, while isolate 474 was not. In general most isolates of *C. acutatum* are less sensitive to benomyl (Smith, 1983) and it was unusual to find a sensitive isolate.

(b) In vivo experiments

(i) Inoculation of runners with *C. acutatum*

The technique used to inoculate plants with *C. acutatum* was in general successful, but did not consistently result in 100% infection. In the untreated

control *C. acutatum* was detected at levels varying from 20-100% with an average of 50% infection in experiments 1-3 and 70% infection in experiments 4-5. *C. acutatum* was detected in uninoculated control plants in two of the five experiments.

The incidence of *C. acutatum* in plants from naturally infected stock used in experiment 6 was very low and sporadic. Only 30% of runners were detected with *C. acutatum* in the untreated control (see Appendix 6).

(ii) Fungicide treatment

A total of six experiments were carried out. The results of experiments 1-3 are summarised in Table 3 and of experiments 4-6 in Table 4. The individual results for each experiment are given in Appendices 1-6. Each experiment was carried out with 20 plants per treatment apart from the first experiment when only ten were used. In experiments 1-3 (Table 3) where the paraquat test alone was used to test the efficacy of fungicide dip treatments, only fenpropimorph at 500 ppm ai appeared to eliminate *C. acutatum* from strawberry runners. Raising the temperature of the dip solution to 30°C did not appear to improve efficacy. There was also no consistent effect of increasing the dip time from 10 to 30 minutes.

In experiments 4-6 (Table 4) both the paraquat test and a more sensitive culturing technique (Baudry and Mozieres, see section b(v)) was used to test the efficacy of the fungicide tests. As a result none of the treatments completely eliminated blackspot from the runners. Least blackspot was detected following treatment with fenpropimorph at 500 ppm ai or prochloraz at 500 ppm ai and mixtures of these two fungicides at various concentrations. Increasing the dip temperature to 30°C did not improve efficacy.

Table 3. Summary of results of Experiments 1-3 showing the percentage of plants with blackspot and percent plant survival following fungicide treatment of runners as a pre-planting dip

Active Ingredient	Fungicide Product	Concentration tested ppm a.i. (at 15°C unless stated)	Dip time	No. experiments tested	% runners in which blackspot detected post dipping	% Plant survival after 6 weeks	% Plants with blackspot after 6 weeks
Uninoculated control	-	-	-	3	6.7	83.3	6.7
Inoculated control	-	-	-	3	50.0	56.6	see note 1
chlorothalonil	Bravo	500	30 mins	2	65.0	60.0	16.7
chlorothalonil	Bravo	500	10 mins	1	70.0	10.0	100
dichlofluanid	Elvaron	100	10 mins	2	20.0	40.0	12.5
dichlofluanid	Elvaron	100	30 mins	1	0	10.0	0
dichlofluanid	Elvaron	100 (30°C)	10 mins	1	not tested	90.0	0
dichlofluanid	Elvaron	500	10 mins	1	10.0	60.0	0
dichlofluanid	Elvaron	500	30 mins	2	75.0	90.0	7.7
fenarimol	Rubigan	500	30 mins	1	100.0	40.0	100.0
fenpropimorph	Corbel	100	10 mins	2	0	90.0	0
fenpropimorph	Corbel	100 (30°C)	10 mins	1	50.0	70.0	0
fenpropimorph	Corbel	100	30 mins	2	25.0	55.0	0
fenpropimorph	Corbel	500	10 mins	1	0	100.0	0
fenpropimorph	Corbel	500	30 mins	1	0	20.0	0
myclobutanil	Systhane	100	10 mins	2	45.0	80.0	6.3
myclobutanil	Systhane	100	30 mins	2	55.0	50.0	0
myclobutanil	Systhane	500	10 mins	2	25.0	55.0	0
myclobutanil	Systhane	500	30 mins	1	20.0	0	-

Table 3 (continued)

Active Ingredient	Fungicide Product	Concentration tested ppm a.i. (at 15°C unless stated)	Dip time	No. experiments tested	% runners in which blackspot detected post dipping	% Plant survival after 6 weeks	% Plants with blackspot after 6 weeks
penconazole	Topas	100	30 mins	1	20.0	40.0	see note 1
prochloraz	Octave	100	30 mins	1	20.0	100.0	0
propiconazole	Tilt	100	10 mins	2	25.0	50.0	0
propiconazole	Tilt	100	30 mins	2	20.0	55.0	14.3
propiconazole	Tilt	500	10 mins	2	15.0	60.0	25.0
propiconazole	Tilt	500	30 mins	1	30.0	50.0	20.0
thiram	Thianosan	1000	30 mins	1	100.0	100.0	100.0
thiram	Thianosan	1000 (30°C)	60 mins	2	25.0	70.0	21.4
thiram	Thianosan	1000 (30°C)	4 hours	1	10.0	100.0	0

Notes

1. Blackspot tests were not completed.

Table 4. Summary of results of Experiments 4-6 showing the incidence of blackspot and percent plant survival following fungicide treatment of runners as pre-planting dip

Active Ingredient	Fungicide Product	Concentration tested ppm a.i. (at 15°C unless stated)	Dip time	No. experiments tested	% runners in which blackspot detected post dipping (1)	Result of B+M test for blackspot (2)	% Plant survival after 6 weeks (3)	% runners with blackspot after 6 weeks (1)	Result of B+M test for blackspot (2)
Uninoculated control	-	-	-	2	25	-ve	70	NT[4]	-ve
Inoculated control	-	-	-	3	70	+ve	80	30	+ve
dichlofluanid	Elvaron	100	30 mins	3	30	+ve	65	0	+ve
dichlofluanid	Elvaron	100 (30°C)	30 mins	1	0	+ve	(0)	-	-
dichlofluanid	Elvaron	500	30 mins	3	30	+ve	60	0	+ve
fenpropimorph	Corbel	100	30 mins	3	0	+ve	70	70	+ve
fenpropimorph	Corbel	100 (30°C)	30 mins	1	0	a few colonies	(0)	-	-
fenpropimorph	Corbel	500	30 mins	3	0	a few colonies	100	0	+ve
myclobutanil	Systhane	100	30 mins	1	0	+ve	(0)	-	-
myclobutanil	Systhane	500	30 mins	1	0	+ve	(0)	-	-
prochloraz	Octave	100	30 mins	2	20	+ve	75	0	+ve
prochloraz	Octave	500	30 mins	2	0	-ve	80	0	a few colonies
propiconazole	Tilt	100	30 mins	1	10	+ve	(0)	-	-
propiconazole	Tilt	500	30 mins	1	0	a few colonies	(0)	-	-
thiram	Thianosan	1000 (30°C)	60 mins	3	35	+ve	75	40	+ve
thiram	Thianosan	1000 (30°C)	4 hours	3	30	+ve	65	40	+ve
fenpropimorph + prochloraz	Corbel + Octave	100 + 100	30 mins	2	0	+ve	85	0	+ve
fenpropimorph + prochloraz	Corbel + Octave	100 + 500	30 mins	2	0	-ve	50	0	+ve
fenpropimorph + prochloraz	Corbel + Octave	500 + 100	30 mins	2	0	+ve	85	0	+ve
fenpropimorph + prochloraz	Corbel + Octave	500 + 500	30 mins	2	0	-ve	80	0	+ve
fenpropimorph + dichlofluanid	Corbel + Elvaron	100 + 100	30 mins	1	0	-ve	40	NT[4]	+ve

Notes to Table 4

1. Blackspot tested for by using the paraquat test.

2. Blackspot tested for by using the culture technique of Baudry and Mozieres (see Section b(v) p8). Test results expressed as positive or negative or as 'a few colonies' where the levels of *C. acutatum* detected were very low.

3. In experiment 4 none of the test plants survived to six weeks due to a crown rot. The exact diagnosis was not reached but thought to be due to *Phytophthora cactorum*. Survival percentages given as (0) indicate death due to crown rot.

4. NT = not tested

(iii) Hot water treatment

Hot water treatment of runners (Table 5) at 45°C for 5 minutes did not reduce blackspot and reduced plant survival compared to the untreated control. Increasing the dip time to 10 minutes did not improve disease control or reduce plant survival. Increasing the dip temperature to 50°C reduced blackspot incidence and plant survival. However these results are difficult to interpret as the untreated control also had reduced blackspot incidence and plant death. Efficacy of the hot water treatment was tested using the paraquat test only.

Table 5 Percentage of plants with blackspot and percent plant survival, following hot water treatment of runners

	Untreated		5 min		10 min	
	% with blackspot	% survival	% with blackspot	% survival	% with blackspot	% survival
45°C	100	80	100	40	100	60
50°C	20	0	9	10	9	10

(iv) Phytotoxicity

The effects of treatments on plant vigour as measured by the numbers or surviving plants after six weeks was variable. In the untreated controls the per cent plant survival varied from 0 to 100% with an average of 83.3% and 56.6% for uninoculated and inoculated control plants respectively in experiments 1-3 (Table 3) and 70% and 80% for the same controls in experiments 4-6 (Table 4). Of the fungicide dip treatments, fenpropimorph and prochloraz appeared to be the least phytotoxic. Treatment with penconazole appeared to be the most damaging with only 40% of plants surviving following treatment, and the remaining plants exhibiting phytotoxicity symptoms. There appeared to be no consistent effect of increasing fungicide concentration or dip time on plant survival.

In experiment 4 (Table 4) none of the plants (treated or untreated) survived to six weeks. This was due to crown rot, the exact cause of which was not diagnosed, but thought most likely to be due to *Phytophthora cactorum*.

(v) Detection of *C. acutatum*

The culturing method of Baudry and Mozieres proved more sensitive in detecting *C. acutatum* than the paraquat test alone. In general the detection of blackspot was greater when plants were treated immediately after fungicide treatment, than when plants from the same batch were tested six weeks later. (Tables 3 and 4)

DISCUSSION

The *in vitro* testing of fungicides was carried out to identify which fungicides might be active against *C. acutatum*. Chlorothalonil, dichlofluanid, fenpropimorph, penconazole, prochloraz and propiconazole are all effective in inhibiting germination of spores of *C. acutatum* and might have potential as fungicides for protection of plants and fruits against blackspot should the disease become established in the UK. Of these fungicide products only chlorothalonil and dichlofluanid are at present registered for use on strawberries. Treatment of the disease may only be necessary in warm, humid summers on everbearer strawberry varieties although the epidemiology of the disease, if it develops in the UK, is uncertain.

Fenarimol, fenpropimorph, myclobutanil, penconazole, prochloraz and propiconazole all reduced mycelial growth *in vitro* and are therefore good candidates for tackling the latent infection present in infected strawberry runners. *In vivo*, however, none of the products tested completely eliminated *C. acutatum* from runners. Fenpropimorph and prochloraz, and combinations thereof, appeared to be most effective in eradicating blackspot. Initial experiments, relying on the paraquat test to test the efficacy of treatments, appeared to show that fenpropimorph and prochloraz were successful in eradicating blackspot. However when the more sensitive culturing techniques were used to test efficacy, blackspot was detected following such treatments. Given the nature of the blackspot fungus, the strawberry runners and the fungicides available, it is probably unlikely that complete elimination of the fungus from planting material could be guaranteed. However, reducing levels of blackspot to trace levels, may be sufficient to minimise disease development in subsequent crops.

While these results have given strong indications on the efficacy of fungicide products it should be noted that all experiments, except experiment 6, were conducted using artificially infected runners. Naturally infected runners may behave differently. Experiment 6 was conducted on naturally infected runner stocks, but the disease incidence was too low for efficacy results to be reliable. All experiments were also conducted in the glasshouse on relatively small numbers of plants. It is therefore necessary to confirm the results obtained on larger numbers of plants under field conditions, to check disease development following treatment and subsequent survival of treated plants. Further studies are also needed to identify the optimum fungicide rate and dip time and the effects of treatments on other strawberry varieties. At present, neither fenpropimorph nor prochloraz are approved for use on fruiting crops, although they could be used during propagation, provided there is a year's interval between treatment and subsequent cropping. If larger scale trials confirm the initial results obtained with these products then approval (or off-label approval) would need to be sought.

Early trials with prochloraz conducted by Schering in the 1980s suggested that this product caused taint on fruit. This fact would need careful checking. The hot water treatment did not succeed in eliminating blackspot at temperatures where plant survival was guaranteed. Combining heat and fungicide by combining the two at lower temperatures did not appear to improve disease control. In addition such techniques may prove too difficult to do in practice compared to a simple cold fungicide dip.

The phytotoxicity effects of treatments are rather difficult to interpret. In several experiments poor survival was recorded in the untreated controls. Death of plants due to crown rot was implicated in experiment 4, but may well have been responsible for poor plant survival in other experiments.

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ACKNOWLEDGEMENTS

We wish to thank the Plant Health Division of the Ministry of Agriculture, Fisheries and Food for granting a Licence to enable the work to be undertaken. Thanks are also due to Dorothy Chambers, Sandra Reynolds and other staff at ADAS Wye for assistance with experiments.

APPENDIX 1

Experiment 1 30.01.1992 — 03.05.1992

Number of replicates : 5 plants

Dipping time : 30 min at ambient temperature (15-18°C)

Strawberry cv Elsanta

Active ingredient	conc. (ppm)	no. runners with blackspot (straight after dipping)	live plants (6 weeks after dipping)	no. runners with blackspot (6 weeks after dipping)
Control (-inoc)	-	2	5	1
Control (+inoc)	-	5	4	-*
Fenpropimorph	100	2	5	-
Myclobutanil	100	4	3	-
Penconazole	100	1	2	2
Prochloraz	100	1	5	0
Propiconazole	100	1	4	1
Chlorothalonil	500	5	3	-
Dichlofluanid	500	5	5	1
Fenarimol	500	5	2	2
Thiram	1000	5	5	5
45°C 5min	-	5	2	2
45°C 10min	-	5	3	3

* Failure of paraquat test due to drying out of humid chambers

APPENDIX 2

Experiment 2 10.04.1992 — 02.07.1992

Number of replicates : 10 plants

Dipping time : 10 or 30 min at ambient temperature (15-18°C)

Strawberry cv Elsanta

Active ingredients	**conc. (ppm)**	**dipping time (min)**	**no. of runners with blackspot (straight after dipping)**	**live plants (6 weeks after dipping)**	**No. of runners with blackspot (6 weeks after dipping)**
Control (- inoc.)	-	-	0	5	0
Control (+ inoc.)	-	-	2	0	-
Chlorothalonil	500	10	7	1	1
"	500	30	3	6	1
dichlofluanid	100	10	2	1	1
"	100	30	0	1	0
"	500	30	5	8	0
fenpropimorph	100	10	0	8	0
"	100	30	1	1	0
"	500	30	0	2	0
myclobutanil	100	10	8	8	1
"	100	30	3	4	0
"	500	10	0	3	0
"	500	30	2	0	-
propiconazole	100	10	0	0	-
"	100	30	2	3	0
"	500	10	3	3	1
"	500	30	3	5	1
thiram (30°C)	1000	60	4	4	3
50°C	-	5	0	1	1
50°C	-	10	0	1	1

APPENDIX 3

Experiment 3 21.07.1992 — 01.10.1992

Number of replicates : 10 plants

Dipping time : 10 min at ambient temperature (15-18°C)

Strawberry cv Elsanta

Active ingredients	conc. (ppm)	no. of runners with blackspot (straight after dipping)	live plants (6 weeks after dipping)	No. of runners with blackspot (6 weeks after dipping)
Control (-inoc)		0	10	0
Control (+ inoc)		3	9	0
dichlofluanid	100	2	7	0
"	500	1	6	0
dichlofluanid (30°C)	100	ND	9	0
fenpropimorph	100	0	10	0
"	500	0	10	0
fenpropimorph(30°C)	100	5	7	0
myclobutanil	100	1	8	0
"	500	5	8	0
propiconazole	100	5	10	0
"	500	0	9	2
thiram (1h) (30°C)	1000	1	10	0
(4h) (30°C)	1000	1	10	0

ND - not determined

APPENDIX 4

Experiment 4 11.12.1992 — 23.02.1992*

Number of replicates :10 plants

Dipping time : 30 min at ambient temperature (15-18°C)

Strawberry cv Elsanta

Active ingredients	**conc. (ppm)**	**no. of runners with blackspot (straight after dipping) (1)**	**plating out test Baudry and (2) Mozieres**
Control (- inoc)	-	0	-
Control (+ inoc)	-	7	+
dichlofluanid	100	3	+
"	500	0	+
dichlofluanid (30°C)	100	0	+
fenpropimorph	100	0	+
"	500	0	very few colonies
fenpropimorph (30°C)	100	0	very few colonies
myclobutanil	100	0	+
"	500	0	+
propiconazole	100	1	+
"	500	0	very few colonies
thiram (1h)	1000	0	+
(4h)	1000	0	+

* No second assessment carried out because the plants died due to a crown rot. Isolations of the crown did not result in identification of the cause of the crown rot.

1. Tested using paraquat technique
2. Tested using plating technique of Baudry and Mozieres

APPENDIX 5

Experiment 5 13.03.1993 - 07.06.1993
Number of replicates : 10 plants
Dipping time : 30 min at ambient temperature (15-18°C)
Strawberry cv Elsanta

Active ingredient	conc. (ppm)	no of runners with blackspot (straight after dipping)(1)	plating out test (straight after dipping) (2)	live plants (6 weeks after dipping)	plating out test (6 weeks after dipping)(2)
Control (- inoc)	-	5	-	7	+
Control (+ inoc)	-	7	-	6	+
dichlofluanid	100	3	-	3	+
"	500	6	+	2	+
fenpropimorph	100	0	-	4	+
"	500	0	-	10	+
prochloraz	100	2	+	5	+
"	500	0	-	6	+
fenpropimorph +	100+100	0	+	7	+
prochloraz	100+500	0	-	0	-
"	500+100	0	+	7	+
"	500+500	0	-	6	+
thiram (1h) (30°C)	1000	7	-	5	+
(4h) (30°C)	1000	6	-	3	+
fenpropimorph + dichlofluanid	100+100	0	-	4	+

(1) tested using paraquat technique
(2) tested using plating technique of Baudry and Mozieres

APPENDIX 6

Experiment 6 — 16.04.1993 — 28.06.1993

Number of replicates : 10 plants

Dipping time : 30 min at ambient temperature (15-18°C)

Strawberry cv Elsanta with natural infection of *C. acutatum*

Origin : Portugal

Active ingredient	**conc. (ppm)**	**live plants (6 weeks after dipping)**	**no. of runners with blackspot (6 weeks after dipping) (1)**	**plating out test (2)**	**fruit with blackspot (6 weeks after dipping)**
Control	-	10	3	+	-
ichlofluanid	100	10	0	-	-
"	500	10	0	-	-
fenpropimorph	100	10	7	+	+
"	500	10	0	-	+
prochloraz	100	10	0	-	-
"	500	10	0	very few colonies	-
thiram (1h) (30°C)	1000	10	4	+	+
(4h) (30°C)	1000	10	4	+	+
fenpropimorph	100+100	10	0	+	+
+ prochloraz	100+500	10	0	+	+
	500+100	10	0	+	-
	500+500	10	0	+	+

(1) tested using paraquat technique

(2) tested using plating technique of Baudry and Mozieres

A D A S

G101291

Dr E R Moorhouse
Horticultural Development Council
18 Lavant Street
PETERSFIELD
Hants
GU32 3EW

Your reference

Our reference 5/RDS/561

Date 6 December 1991

Dear Ed

HDC LEVY FUNDING

Thank you for your letter of 2 December 1991 and for the two copies of the contract for project SF/19a on "Blackspot of strawberry: investigation on pre-planting runner treatments to eliminate Colletotrichum acutatum from planting stock". I have signed both copies and return one as requested.

We are grateful to HDC for agreeing to fund this work.

Yours sincerely

M J GRIFFIN

cc Dr Angela Berrie, Wye

MAFF Ministry of Agriculture, Fisheries and Food
Agricultural Development and Advisory Service
Government Buildings, Brooklands Avenue
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Chairman: Frank Thomlinson C.B.E. Chief Executive and Secretary: E.J. Kennedy

2 December.
~~19 November~~ 1991

Dr M J Griffin
Ministry of Agriculture
Fisheries and Food
Government Buildings
Brooklands Avenue
Cambridge CB2 2DR

Dear Mike

I enclose contract for the following project:

SF/19a: Blackspot of strawberry: investigation on preplanting runner treatments to eliminate Colletotrichum acutatum from planting stock.

Enclosed are two copies of SF19a, please sign both and return one to me in the usual way.

Yours sincerely

Dr ~~E R~~ Moorhouse

Contract between ADAS (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

PROPOSAL

1. TITLE OF PROJECT

Contract No: SF/19a

BLACKSPOT OF STRAWBERRY: INVESTIGATION ON PREPLANTING RUNNER TREATMENTS TO ELIMINATE COLLETOTRICHUM ACUTATUM FROM PLANTING STOCK

2. BACKGROUND

Strawberry blackspot caused by the fungus Colletotrichum acutatum was identified for the first time in Britain in 1983 in plants cv Brighton introduced to Southern England from California. Steps taken by MAFF to eradicate the outbreaks were apparently successful, apart from an outbreak in Cornwall. Until 1988 the disease was confined to the outbreak in Cornwall, but in the summer of 1988 about a dozen outbreaks of blackspot were recorded in strawberry crops in South East England. All outbreaks were on or near crops originating from strawberry runners imported from Europe. Further outbreaks were recorded in 1989 and 1990. Blackspot of strawberry is a notifiable disease, statutory action being required on confirmed outbreaks.

Colletotrichum acutatum primarily causes a fruit rot, and is responsible for serious losses of ripe strawberry fruit in other parts of the world, particularly Australia, New Zealand and USA. In Britain the disease has mainly been found on cv Elsanta, but it is on the everbearer crops, with the long period of fruiting under warm humid conditions, that the greatest losses are likely to occur. The fungus also causes lesions on petioles, fruit stalks and stolons, but can also invade the crown remaining as symptomless latent infection until conditions favour development and fruit starts to ripen. Such latent infections then provide a reservoir of inoculum to initiate epidemics during fruiting provided suitable environmental conditions of high humidity and warm temperatures exist.

Blackspot is thought to survive between crops on infected debris in the soil, but for how long such debris remains as an inoculum source is not known. Strawberry plants remaining as weeds in subsequent crops are also a source of inoculum. Abroad, weeds are known to harbour blackspot, but whether this is also true for the UK is not known.

The disease is spread within crops on pickers' hands, and by rain splash, but the main means of spread into new crops, and by which the diseases was introduced into the UK, is as latent infections on symptomless infected runners. The availability of preplanting treatments to eliminate blackspot from suspect planting stock would therefore be of considerable value.

Strawberry blackspot has been the subject of detailed investigation in other parts of the world, but in the UK there are many questions that need to be answered,

particularly on the biology and epidemiology of the fungus. Some studies are already in progress at Harpenden Laboratory and at Wye. Further proposals for investigational work have been submitted for funding to MAFF Policy Division (see Appendix). In addition, Professor Terry Swinburne is proposing to study the biology of the latent infection process, as well as other mycological aspects. Close liaison will be maintained with both Professor Swinburne and Harpenden.

The results of all these studies will increase our knowledge of the blackspot fungus and its threats to the UK strawberry industry and also may influence MAFF policy on the fungus.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

The important of symptomless infected strawberry runners as sources of blackspot has already been stated. Present information suggests that UK produced strawberry runners are free of the disease. Imported strawberry runners, however, do carry the risk of blackspot infection, and are routinely tested at Harpenden Laboratory to ensure that only blackspot-free plants are imported. Should MAFF policy on testing imported runners change, then the availability of a preplanting runner treatment to eliminate the disease from planting stock would be of great value.

4. OBJECTIVE

To identify suitable pre-planting fungicide dips or hot water treatments or a combination of both to eliminate or significantly reduce Colletotrichum acutatum from runner stocks, as a precautionary measure to prevent losses in subsequent fruiting crops.

5. DESCRIPTION OF THE WORK

The investigations would be laboratory/glasshouse based. Two are proposed. One to test the efficacy of pre-planting fungicide dips (prochloraz, thiram, propiconazole, myclobutanil, fenpropimorph) and the second to test the efficacy of pre-planting hot water treatments (49.4°C and 30°C) alone and in combination with the above fungicides. Strawberry runners would be inoculated with C. acutatum, incubated to allow infection and then treated with the test fungicides or hot water treatments. The effectiveness of treatments will be tested by checking plants for blackspot using the paraquat test, and by growing on plants in the glasshouse under conditions favourable for development of blackspot to check for symptoms and also to check plants for treatment damage.

The paraquat test involves treating petioles/crowns with paraquat, which kills the tissue, stimulates the fungus to sporulate and enable detection by visual assessment. Inoculated and uninoculated controls would be included in each investigation, 50 strawberry runners would be used per treatment.

It is planned to do some preliminary in vitro fungicide screening work to test the efficacy of the proposed fungicides, the results of which may modify the choice of

products included in the proposal.

The temperature of 49.4°C has been selected based on a successful work in the USA to eliminate C.acutatum from American varieties. This temperature is higher than that tried by Dr Cook at Harpenden. The choice of the lower temperature of 30°C is based on seed treatments with warm fungicide soaks for up to 24 hours which was successful in eliminating deep seated infections from various vegetable seeds. Additional time is included to allow discussion with Professor Swinburne and Harpenden Laboratory.

6. COMMENCEMENT DATE AND DURATION

November 1991 for two years initially.

7. STAFF RESPONSIBILITIES

Project Leader: Dr A Berrie, Plant Pathologist, ADAS, Wye Advisory Centre, Olantigh Road, Wye, Ashford, Kent TN25 5EL
Telephone: 0223 812761

TERMS AND CONDITIONS

The Council's standard terms and conditions of contract shall apply.

Signed for the Contractor(s)

Signature M. J. Griffiths

Position R&D Manager

Date 6/12/91

Signed for the Contractor(s)

Signature.....

Position.....

Date.....

Signed for the Council

Signature.....

Position CHIEF EXECUTIVE

Date 2.12.91