Evaluation of seven bacterial isolates for the biocontrol of *Botrytis cinerea* on strawberries in a commercial field.

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SUMMARY.

1. Seven antagonistic bacteria selected from screens, have been tested in field trials under commercial conditions for control of grey mould on strawberries.
2. Three isolates, F58, F168 and E44, showed significant control of the disease.
3. Isolate F58 consistently showed better control than a conventional fungicide treatment using 'Elvaron'.
4. Three different formulations were compared. In some cases, formulation influenced the results.
5. The three isolates which controlled the disease did not significantly affect fruit size or shape. Slight yield reductions were noted.
6. Isolates F168 and F58 are progressing into further field trials and investigations of the mechanism of action.

INTRODUCTION.

Grey mould, caused by the fungus *Botrytis cinerea* Pers. : Fr., is an important worldwide disease of green and ripe strawberry fruits. The main source of the disease on the fruits results from the infection of various parts of the inflorescence, including the sepals, petals, stamens, pistils and peduncles (Bristow *et al*, 1986). When senescing these flower parts provide an important source of mycelium capable of infecting strawberry receptacles (Jarvis, 1962). Later studies have shown that *B.cinerea* frequently grows down the filaments of infected stamens and into the receptacle. (Bristow *et al.*, 1986). In view of this the management strategy for controlling grey mould on strawberries should be aimed at reducing infection during flowering and also reducing subsequent infection which could result during fruiting.

The main source of inoculum responsible for grey mould is infection via conidia produced from mycelium in dead strawberry leaves. Healthy leaves are highly susceptible to infection at the bud or expanding stage, but are not so receptive when fully expanded, and slightly susceptible when senescent (Braun and Sutton, 1988). After the leaves have become infected,

B.cinerea remains quiescent in the epidermal cells until the leaves senesce and no symptoms are produced during this period. The pathogen may survive for several months in the dead leaves, and has the potential to sporulate. Therefore leaf development is an important factor which limits the duration of infection cycles and controls the rate of inoculum produced. The time scale from the initiation of infection in the leaves to the subsequent senescing and sporulation is 7-8 months in leaves infected during autumn and 6-8 weeks in leaves infected in April (Peng and Sutton, 1990b). Therefore another potential target for controlling grey mould is to reduce the amount of inoculum by protecting infection of the leaf buds and expanding leaves.

Currently grey mould is managed through an integrated programme of fungicidal treatment and cultural practices (Evans et al, 1988). Fungicides are currently the main form of control, although conventional fungicide programmes have become increasingly unacceptable to the grower and the public. Public concern with fungicide residues on the foliage (Peng and Sutton, 1990b), and problems of wash out from the soil and into the water systems has increased dramatically. The fungicides used are often ineffective in maintaining adequate levels of control, due to the difficulty in maintaining adequate coverage on rapidly developing flowers, maintaining spray-to-harvest intervals and the development of fungicide tolerance to the fungicide (Northover and Matteoni, 1986). Resistant strains of *B.cinerea* to the benzimidazoles, the dicarboximides and the diethofencarbs is widely reported (Elad, Yunis and Katan, 1992).

The problems associated with controlling grey mould has increased attempts to improve disease management programmes while reducing fungicide dependency (Sutton, 1990). An alternative to chemical application is the use of microorganisms as biocontrol agents. There are field examples where microorganisms have been as effective as fungicides. For example, Tronsmo and Dennis (1977) reported that *Trichoderma spp.* suppressed grey mould on strawberry fruits in the field, and that the best isolate was as effective as dichlofluanid.

Biocontrol agents offer several advantages compared to the use of fungicides. Notably the absence of chemical residues, but also the antagonists may offer wider protection during flowering, especially if they persist on the rapidly expanding flower. The biocontrol agent

needs to be well adapted to the phylloplane, which is a harsh environment with rapidly fluctuating temperatures, humidities and periodically high levels of ultra violet radiation (Dickinson, 1986). The survival and colonization of the antagonist maybe improved by amending the inoculum spray. The use of 'sticker' compounds to increase adhesion on the plant surface, u.v. protectors to reduce the harmful effects of the u.v. radiation, or nutrients to speed the colonization by the antagonists and compounds such as 'inositol' and di/trisaccharrides to increase the survival of the bacteria in the aerosol sprays (Cullen and Andrews, 1984).

Effective antagonists can be isolated providing an efficient screening protocol is used. Once an effective antagonist has been found, its potential to control the disease can be enhanced by manipulating conditions within the spray to maximise its antagonistic effect. This can transform a mediocre biocontrol agent into a very effective one.

The purpose of this study was to examine the effect of seven bacteria, which had been successfully isolated using *in vitro* and *in vivo* screens, against *B.cinerea* and their subsequent ability to control grey mould of strawberries in a commercial environment. The intention was to select one, or preferably two antagonists from this field trial which showed potential for controlling grey mould. These isolates would then be used for studies to improve their field performance.

MATERIALS AND METHODS.

1. Field Trial Design.

The field trial was located in a commercial field of 'Elsanta' strawberries in their third season at Ratling Court Farm, near Aylesham. A randomised block design was used with 28 treatments (Appendix 1), containing 6 strawberry plants (Figure 1). The plants in each treatment were arranged in a 2 by 3 design with the 2 plants straddling the bed. Neighbouring treatments were separated by a minimum of 2 guard plants (Figure 2).

The plot was treated the same as the remaining part of the commercial field . The plants

FIGURE 1. Experimental trial in a commercial field of 'Elsanta' at Ratling Court Farm, near Aylesham.

FIGURE 2. Treatment plot containing 6 strawberry plants arranged in a 2 by 3 design with 2 guard plants on either side.

were grown on raised black plastic, with plants being spaced 12cm apart.They were mown off twice (early July and the beginning of March). Similar chemical treatments were applied up until the antagonists were added (at the white bud stage). No further chemical treatment was applied after this point. The plants were watered by an underground trickle irrigation system.

2. Inoculum Production and Application.

Seven potential antagonists were used in the field trial which had been selected from earlier screens. The antagonists were applied in three different forms; the nutrient broth in which they had been grown i.e. including any metabolites produced by the isolates over the 3 day incubation period. This would be significant if the mode of inhibition was through antibiotic production.

The second application included the addition of a 5% Molasses solution with the nutrient broth. Molasses was shown to be a reasonable u.v. protectant in the laboratory when compared with two other compounds, 'Speswhite clay' and 'Neosyl silica'. My laboratory research however has shown that molasses at concentrations above 5% would counteract the inhibitory effects shown by some of the isolates. This meant that a much lower concentration had to be used than in previous work, despite giving a lower protection against the u.v. light. Other *in vitro* work has shown that despite all the antagonists inhibiting spore germination when resuspended in fresh nutrient broth, the addition of molasses resulted in uncontrolled germination and excessive mycelial extension.

The third application involved washing the bacteria free of metabolites and resuspending in fresh nutrient broth. Thus any inhibitory effect observed would be due to antagonism occurring directly in the field.

The three formulations were prepared as follows;

(i) Nutrient Broth + Metabolites.

A two litre flask containing 750ml of sterile nutrient broth was inoculated with 10 plugs of nutrient agar plus the antagonist and incubated at 20^0C for 5 days on an orbital shaker at 180rpm.

(ii) Nutrient Broth + 5% Molasses + Metabolites.

The inoculum was prepared as in (i), except the nutrient broth also contained 5% molasses.

(iii) Resuspended in Nutrient Broth.

A 5 day old culture grown as in (i) was resuspended in 750ml of fresh nutrient broth, to produce a final suspension of $1x10^{8}$-$1x10^{11}$ cfu per ml.

The controls used in this trial were;

- Nutrient Broth.
- Nutrient Broth + 5% Molasses.
- No Treatment.
- Elvaron (Fungicide).

The antagonists were applied to the flowers using a hand held spray gun on 10th, 14th, 19th, 24th and 29th May. The 'Elvaron' control however was applied only on alternate dates to coincide with the recommended spray application. The treatments were applied to groups of flowers until run-off, during a period approximately 2 hours before sunset. This ensured there was a minimal exposure of the treatments to ultra violet radiation. During the treatments, no other chemicals were applied to the field trial site. The applications were stopped approximately 14 days before the first harvest.

3. Sampling and Analysis.

The first harvest was on the 8th June, allowing sufficient fruits to be picked for analysis. Subsequent harvests were on the 13th, 18th, 23rd and 30th June. All the ripe fruits were picked from each plot and stored in 2kg punnets. The fruits were removed from the plant with their stalks intact, which is the usual way of collecting the fruits. Malformed fruits were also collected (in a commercial field these would not normally be picked). Any fruits with symptoms of grey mould were recorded and discarded. Altogether there were 4 main picks, each approximately 6/7 days apart. Due to the size of the field trial commercial pickers were employed to harvest the plots.

Once the fruits were harvested they were immediately stored at 4°C until required. The maximum storage time in the cold room was 6 days, approximately the time required to complete a set of analyses. The following data was collected for each plot;

(i) The total number of fruits collected.

(ii) The weight of all the fruits collected.

(iii) The number of malformed fruits (Figure 3).

Once the data was collected any fruits with missing stalks or showing signs of disease were discarded. From the remaining fruits 30 were randomly selected, placed in 2x2kg punnets containing damp kitchen towel and maintained at 25°C (Figure 4). The strawberries were placed in the punnets so that there was no contact between neighbouring fruits, minimising the risk of cross contamination. The fruits were incubated for 5 days before being assessed for grey mould development (Figure 5) and placed in one of the following 4 categories (Figure 6);

(i) No symptoms visible.

(ii) Less than 25% infection.

(iii) 26%-50% infection.

(iv) 51%-100% infection.

Statistical Analysis.

Statistical computations were performed using Genstat 5 Release 1.3, Lawes Agricultural Trust, Rothamstead Experimental Station (1988). The data was examined using 'Orthogonal contrasts', so the sum of all the contrasts equalled the treatment sum of squares (Appendix 2&3). This system gave a general view of the field trial. A 'Least Significant Difference' test was performed to look at specific parameters (Appendix 4&5). A confidence level of 1% was used as recommended by 'Fisher' to ensure all the values showing significance were significant.

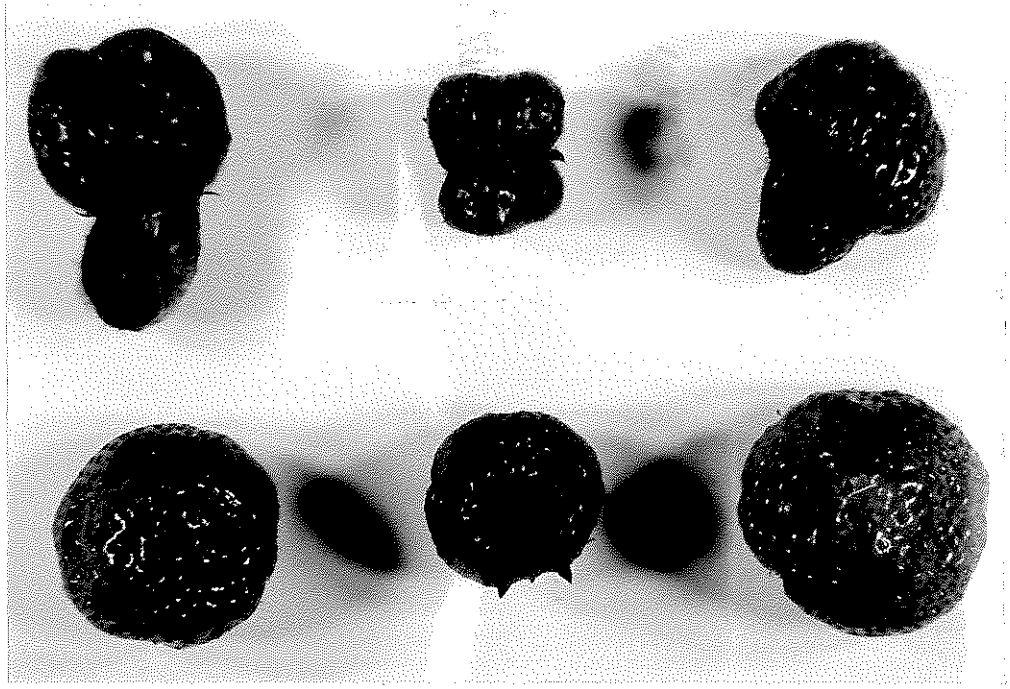


FIGURE 3. Different shapes of fruits categorised as being malformed.

FIGURE 4. A 2Kg punnet containing strawberry fruits ready for incubation at 25^0C.

FIGURE 5. A 2Kg punnet containing strawberry fruits infected with grey mould after incubating for 5 days.

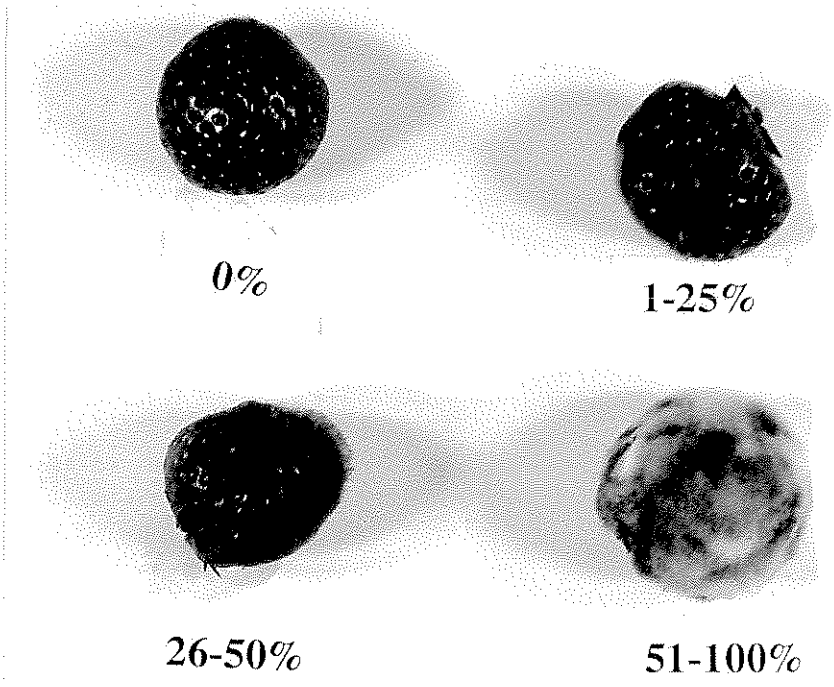


FIGURE 6. Disease index of grey mould infection on strawberry fruits.

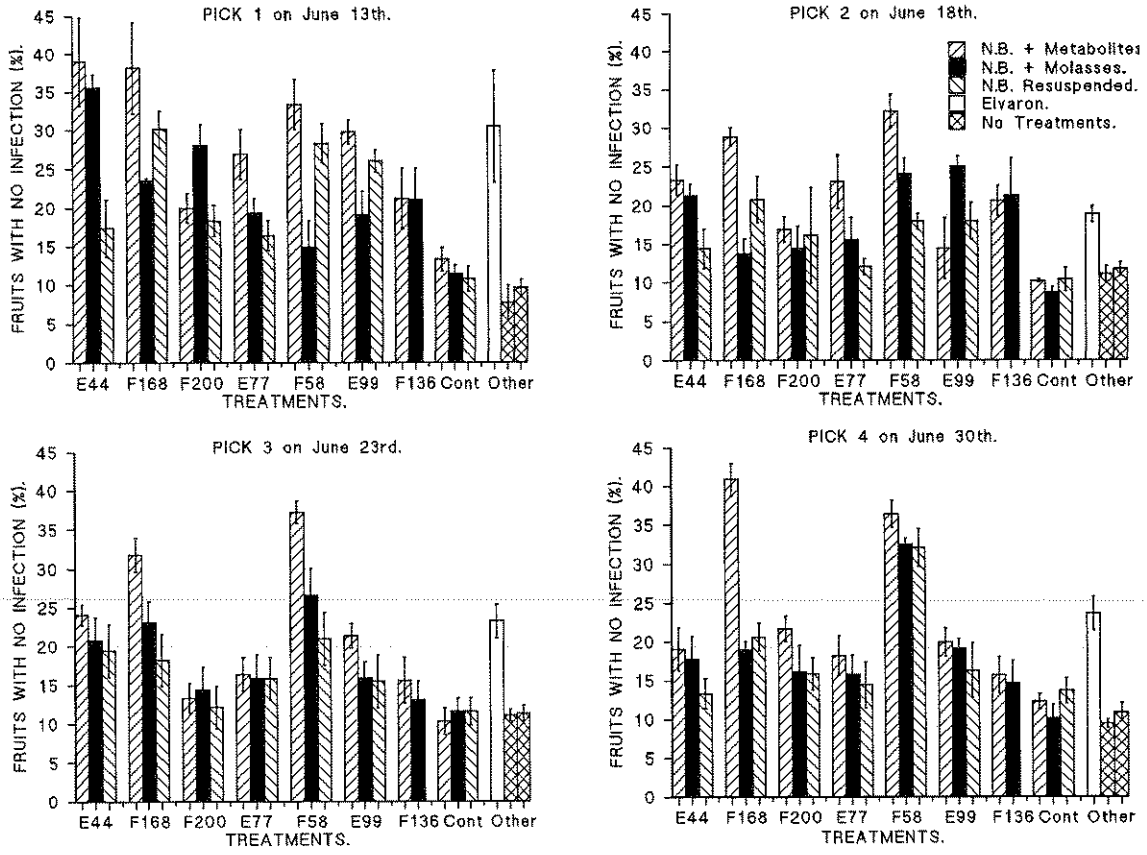
RESULTS.

The seven microorganisms screened suppressed *B.cinerea* on strawberries to various extents ranging from 12% to 40% depending upon the medium they were applied in and the time they were picked (Figure 7). Isolate F58 was the most effective antagonist, controlling grey mould significantly for all the picks, with as much as 37% of the fruit showing no infection when the antagonist was applied in the nutrient broth plus metabolite formulation. F58 also showed significant control, in 3 of the 4 picks when applied in the resuspended and molasses treatments (32% of the fruits showing no grey mould in the final pick). Isolate F168 was the next most effective and showed significant control of grey mould when applied with its metabolites, especially in the final pick where 41% of the fruits remained uninfected. F168 in the other media was effective only 50% of the time.

The third effective antagonist was E44, which controlled the disease when applied in the molasses and with its metabolites over the first three picks but was ineffective on the last pick. Isolates, F200, E77 and F136 showed no ability to control *B.cinerea* on the fruits in any media. The remaining isolate was inconsistent over the four picks controlling grey mould 50% of the time when applied in the molasses and nutrient broth plus metabolites form, and only on the first pick when resuspended. The three forms of media alone or the no treatment control all resulted in a similar number of diseased fruits. The fungicide Elvaron, when compared with the no treatment controls significantly controlled the amount of infection on the fruits, except after the second harvest, at the 1% confidence level.

Isolate F58 is the only antagonist which significantly reduced the total fruit yield. This isolate produced the lowest yield of 2.5Kg, when applied in the nutrient broth plus metabolites and 2.9Kg in the molasses treatment. However the resuspended F58 produced a significantly higher yield of fruit (3.6Kg). In the absence of bacteria the type of medium had no significant effect on fruit yield, producing on average approximately 3.5Kg, which was higher than the no treatment control (2.9Kg), but did not show significance (Figure 8).

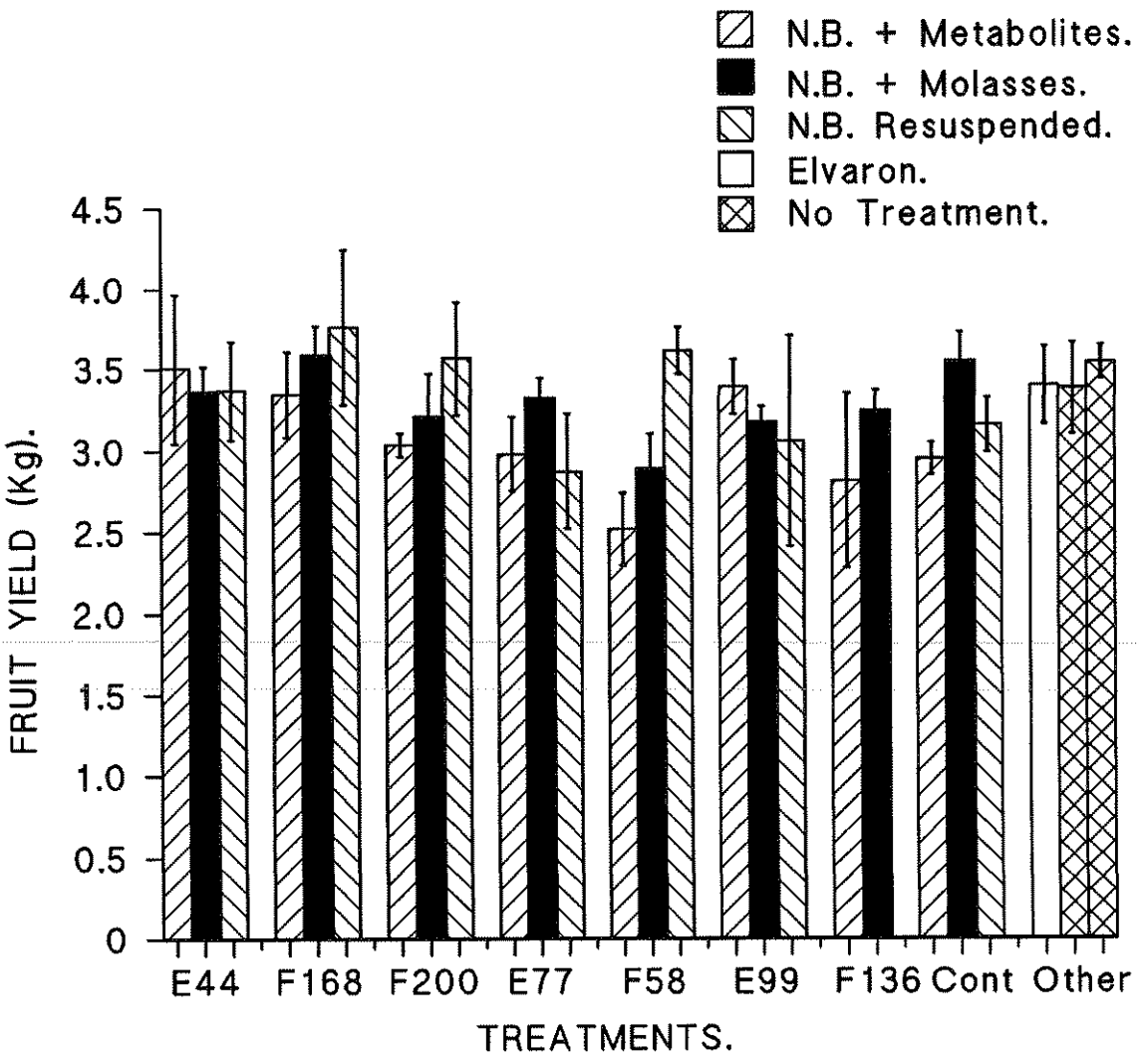
The antagonists had no significant effect on the average fruit weight,with the smallest fruit weighing 12g and the largest 14g. However when comparing the overall effect of all the



Least significant difference at the 99% confidence level for each pick is;

PICK 1 - 12.3%
PICK 2 - 9.9%
PICK 3 - 8.8%
PICK 4 - 8.0%

FIGURE 7. Effect of 7 bacterial isolates on controlling *B.cinerea* on ripe fruits after 4 harvests.

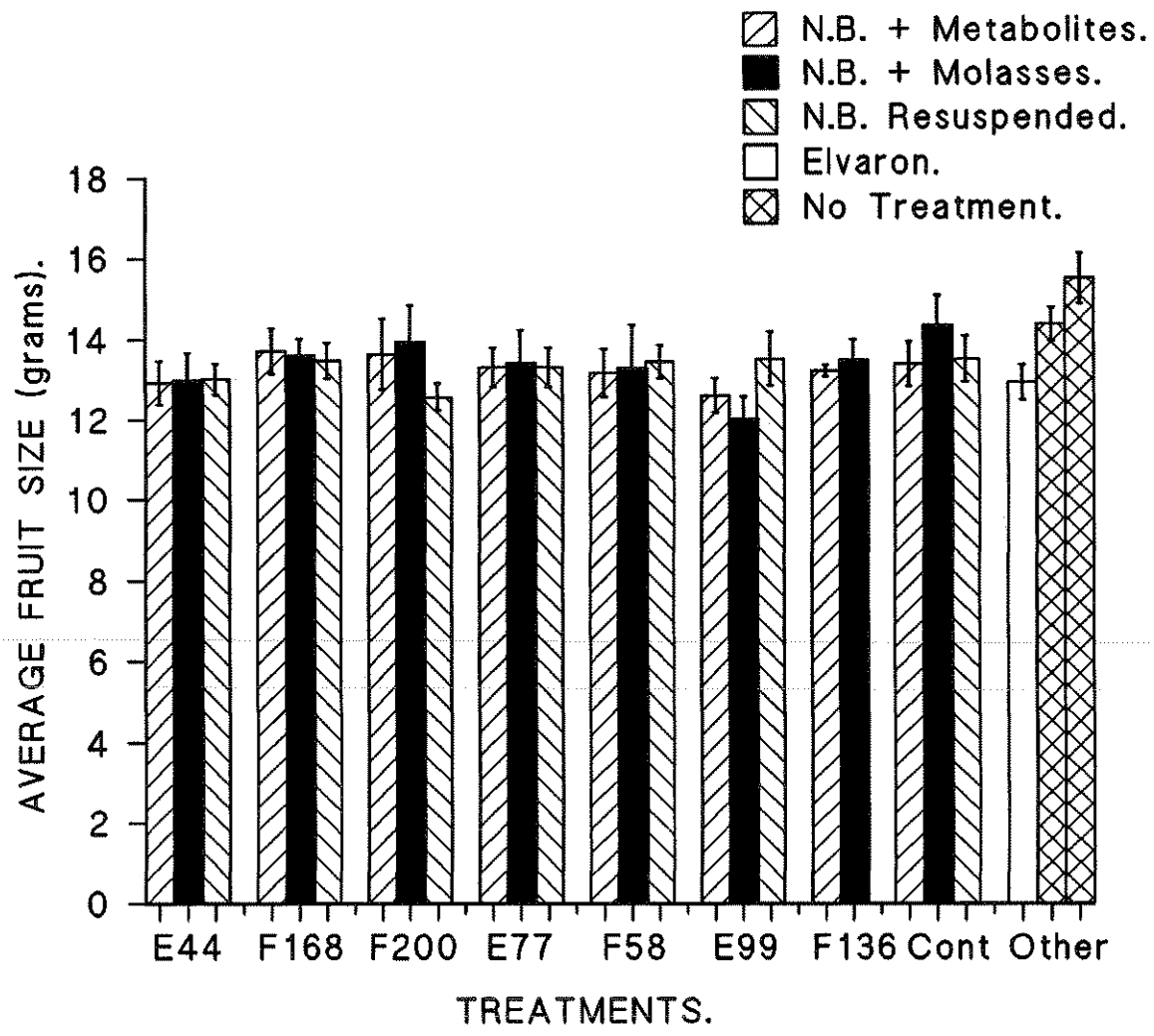


(L.S.D. at the 99% Confidence Level is 1.016).

FIGURE 8. Effect of the treatments on fruit production.

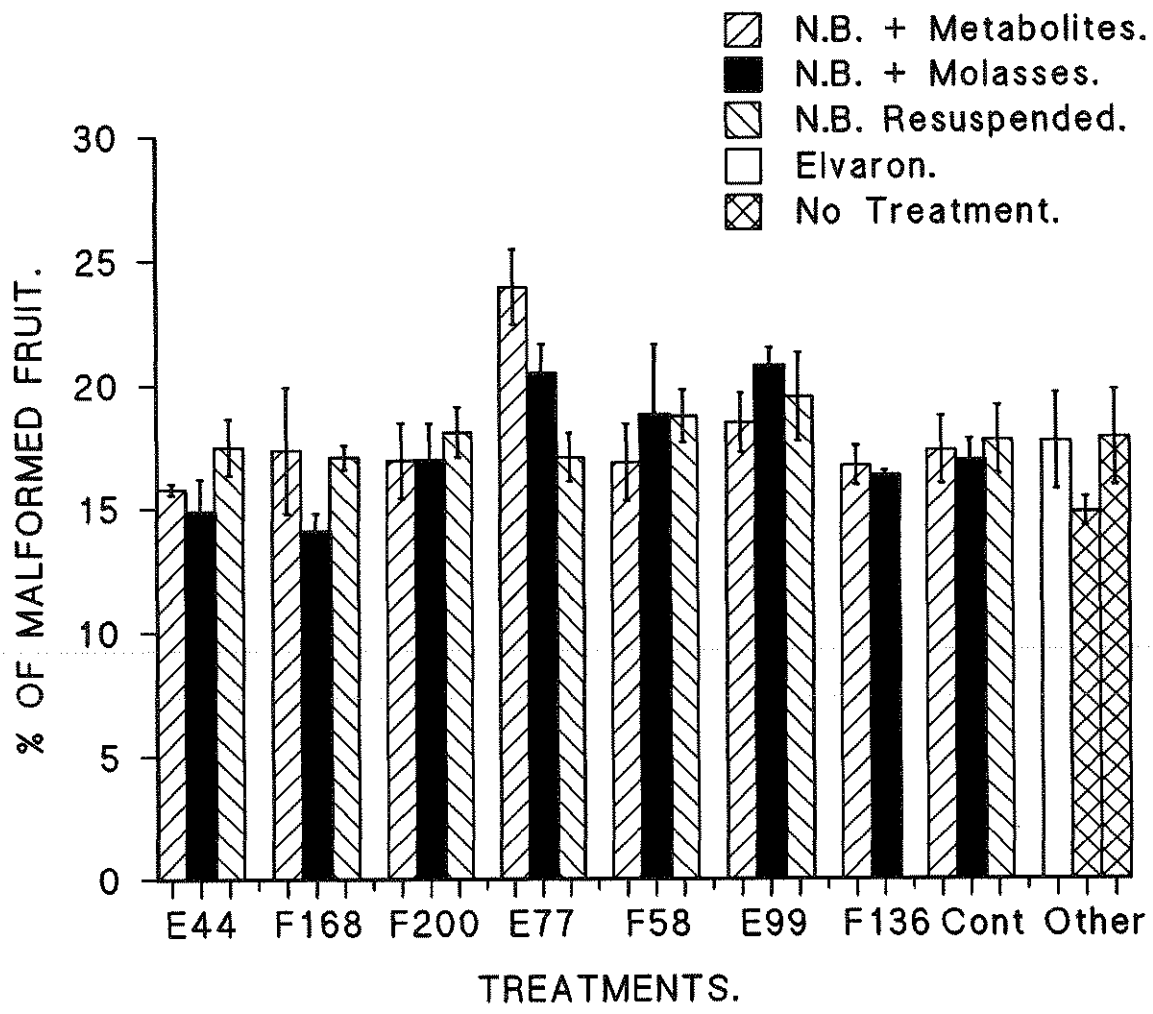
bacteria and the Elvaron treatments with the no treatment control, the no treatment control resulted in significantly larger fruits (averaging 14.5g and 15.5g in the respective plots). The different media had no significant effect on the average fruit weight (Figure 9).

The antagonists isolated from the leaves compared with those isolated from the flowers had a much greater effect on producing malformed fruits. The E44 treatments had the lowest quantity of malformed fruits, with E77 having the highest at 24% in the nutrient broth plus metabolite treatments. The treatment with the highest number of perfectly formed fruits was the no treatment control which had 14.8% malformation in one of the plots and 17.8% in the other. The type of media the antagonists were applied in had no significant effect on fruit shape (Figure 10).



(L.S.D. at the 99% confidence level is 1.92g).

FIGURE 9. Effect of the treatments on the average size of harvested fruits.



(L.S.D. at the 99% confidence level is 5.27).

FIGURE 10. Effect of the treatments on the shape of harvested fruits.

DISCUSSION.

The purpose of this field trial was to establish whether the bacteria selected from an intensive *in vitro* and *in vivo* screening system, have the potential to control grey mould disease on strawberries in a commercial environment. The results from the field trial showed that three bacteria, F58, F168 and E44, all produced a significantly higher yield of undiseased fruits than the controls.

Isolate F58 was the most promising of all the antagonists tested. This isolate was shown to be successful in reducing grey mould on strawberries in all of the treatments. F58 also surpassed the control exhibited by the dichlofluanid fungicide 'Elvaron', when applied in the nutrient broth plus metabolites formulation. More importantly it showed the ability to control grey mould equally well as the fungicide when it was resuspended in nutrient broth.

F58 was identified as a dual culture of one gram positive and one gram negative bacteria. Further *in vitro* work identified the gram positive bacterium was responsible for the control observed. During later trials the possibility of a synergistic effect will be investigated. This isolate was identified as being the only gram positive, spore forming bacterium in the isolates tested. It is a *Bacillus* species. Further work is planned to identify to species level. The advantage of this isolate is it produces spores enabling it to be more easily formulated and stored, and also to be applied. The production of resistant spores may be responsible for the success observed in its ability to control grey mould, especially when it is applied in the resuspended form.

F58 has not only managed to control grey mould better than the controls but has also shown to be more effective than the fungicide 'Elvaron'. This factor alone should encourage further studies on this antagonist. The overall control may only be around 35-40% at best, but with further work on media and identifying the mode and site of action, this control can be significantly improved. The antagonist has the added bonus of producing spores, enabling a cheap and effective way of storing and applying it. F58 has also shown that it has the ability to inhibit grey mould development in the field when applied as a fresh culture to the flowers. Isolate F168 was effective at controlling grey mould when applied in the nutrient broth plus

metabolites form, with the presence of its metabolites proving significant. The resuspended treatment showed variable results, only inhibiting over the first two picks. This variability could be due to the weather conditions. The survival of the bacteria in the nutrient broth will depend upon the ability of the isolate to withstand both the varying temperatures, harmful U.V. and varying humidities. If the conditions are favourable after the spray application then the antagonist will survive longer and exhibit better control. The bacteria were applied as close to sunset as possible ensuring that they had the best chance to establish on the flower parts.

Isolate E44 was effective in controlling grey mould over the first three picks, when applied with its metabolites either with or without the molasses. The resuspended treatment had no significant effect on control. This suggests that antibiotic production is important and that either the bacteria were destroyed soon after their application or their inhibitory effects observed in earlier screens could not be reproduced in the field.

The inhibition occurring in the nutrient broth plus metabolites is probably a result of antibiotics being produced prior to application. This explains why E44 failed to inhibit in the resuspended form, yet showed significant control in the presence of its metabolites. The molasses treatment had similar results, probably due to the molasses protecting either the survival of the bacteria or breakdown of the inhibitory metabolites. The beneficial effects of the molasses were probably counteracted by the detrimental effects of improving fungal growth. This explains why the U.V. protectant, despite being applied in an acceptable concentration, did not show significant control over the other treatments. The evaporation of the water from the media ingredients would also have the effect of increasing the relative concentration of the molasses, resulting in the antagonists inhibitory effects becoming significantly reduced.

The results also showed that isolates F200, F136 and E77 were ineffective at controlling grey mould disease when applied in any of the three formulations. Isolate E99 was unpredictable, therefore making it an unreliable proposition as an alternative means of controlling *B.cinerea*. It is not surprising that some of the isolates screened, despite being effective in the *in vitro* and *in vivo* studies, are ineffective in a constantly changing external environment. Baker and

Cook (1974) outlined some of the reasons why there may be differences between the antagonists behaviour in the external environment and in artificially controlled conditions;

1. There are other organisms present that the antagonist has to compete with.
2. Conditions differ between the screening and the external environment.
3. If the mode of antagonism is via the production of antibiotics, then these may only be produced under certain conditions, such as when in a rich nutrient source.
4. The conditions in the screens may favour the antagonist rather than the pathogen.

This field trial was designed to select antagonists which were effective at controlling *B.cinerea* if applied during the flowering stage i.e. a substitute for 'Elvaron'. It did not take into account any infection which may have occurred during fruiting where no disease management system was used. This means that the isolates may exhibit better control at flowering than the results depict. Since one of the isolates, F58, has been identified as *Bacillus*, there is a good possibility that it will not be pathogenic to humans and can therefore be used as a short term spray during fruiting. It could also be used in an integrated management programme with Elvaron to maximise control.

The technique used to assess grey mould development on strawberries was designed to maximise infection. The fruits were incubated at high humidities for 5 days ensuring any infection occurred. Also due to the time it took to process the fruits, they were stored at 4^0C for a approximately 5 days. This would have increased infection levels. It is also possible that during this storage in the cold room, some of the fruits may have become infected. This helps explain the high level of infection observed in the controls during the field trial and why the Elvaron treatment was not very effective. It also means that the antagonists would probably exhibit much better control under normal processing and storage conditions.

The trial also examined the possible detrimental effects the antagonists might have on fruit

yield, size and shape. For example, the yield would be affected if the antagonists killed the flowers. The analysis showed that isolate F58 caused a lower yield than any of the other bacteria. This effect was only apparent in the nutrient broth plus metabolites and molasses treatments. The resuspended application had no effect on the fruit yield. This would suggest that the metabolites in the sprays maybe responsible for damaging some of the flowers.
Fruit size was not significantly affected by any of the bacteria. Untreated flowers, however produced significantly larger fruits. Taking into account the degree of control, type of antagonist and other effects on fruit yield, size and shape, isolates F58 and F168 were selected for further work. Isolate F58 was chosen because of its good control in all of the treatments and because of its ability to produce spores.

F168 was selected because of its ability to control the disease over all four picks (unlike the third candidate E44). It also showed some ability in controlling grey mould in the resuspended and molasses formulation. In addition, F168 was originally isolated from the strawberry flowers whereas E44 came from the leaves.

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APPENDIX 1. Field Trial design at Ratling Court Farm.

F200(Nom)		N.B.+ Mol		E77 (Mol)
F136(Nom)		F168(Res)		F58 (Nom)
No.Treat.		Elvaron		E99 (Res)
E99 (Res)		E77 (Mol)		F136(Mol)
F200(Mol)		E99 (Nom)		F200(Nom)
N.B.+ Mol	G	E44 (Res)	G	F58 (Mol)
F200(Res)		E44 (Mol)		E99 (Mol)
E77 (Mol)	U	N.B.	U	No Treat.
No.Treat.		F136(Nom)		N.B.+ Mol
F58 (Mol)	A	No.Treat.	A	E44 (Mol)
E77 (Res)		F200(Nom)		N.B
N.B.	R	E44 (Nom)	R	E77 (Nom)
F168(Mol)		F168(Nom)		E99 (Nom)
F168(Res)	D	F58 (Nom)	D	F200(Res)
E99 (Nom)		E99 (Mol)		F58 (Res)
F136(Mol)		F168(Res)		No.Treat.
E44 (Nom)	B	F58 (Res)	B	E44 (Res)
E77 (Nom)		No.Treat.		F200(Mol)
Elvaron	E	F136(Mol)	E	N.B.
F58 (Res)		N.B.		E44 (Nom)
N.B.	D	F200(Res)	D	F136(Nom)
F168(Nom)		E77 (Nom)		E77 (Res)
E44 (Mol)		F58 (Mol)		F168(Mol)
F58 (nom)		E99 (Res)		Elvaron
E44 (Res)		E77 (Res)		F168(Nom)
E99 (Mol)		F200(Mol)		F168(Res)

Key

Res - Resuspended.
Nom - Normal.
Mol - Molasses.
N.B. - Nutrient Broth.
No.Treat - No Treatment.
N.B.+ Mol- Nutrient Broth & Molasses.

APPENDIX 2. Statistical program written on 'Genstat' for calculating contrasts and analysis of variance.

```
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text [n=1] t
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        'f168n','f168m','f168r','f200n','f200m','f200r',\
        'e77n','e77m','e77r','f58n','f58m','f58r',\
        'e99n','e99m','e99r','contn','contm','contr',\
        'elv','nota','notb','7n','7m'\
fact [lev=3] block ; val=!((1,2,3)26)
fact [lev=26;lab=ta] treatmnt ; val=!(3(1...26))
matrix [rows=5 ; col=26] m1 ; val=!(3(1,-1,-1,1,-1,1),8(0),\
        3(-2,0,0,1,0,1),8(0),3(0,,0,0,-1,0,1),8(0),\
        3(0,1,1,0,-2,0),8(0),3(0,-1,1,0,0,0),8(0))
matrix [rows=6 ; col 26] m2 ; val=!(1,0,-1,23(0),1,-2,1,23(0),\
        3(0),1,0,-1,20,(0),3(0),1,-2,1,20(0),\
        6(0),1,0,-1,17(0),6(0),1,-2,1,17(0))
matrix [rows=6 ; col=26] m3 ; val=!(9(0),1,0,-1,14(0),\
        9(0),1,-2,1,14(0),12(0),1,0,-1,11(0),\
        12(0),1,-2,1,11(0),15(0),1,0,-1,8(0),15(0),1,-2,1,8(0))
matrix [rows=4 : col=26] m4 ; val=!(18(1),3(0),-18,4(0),\
        18(1),3(0),2,2(-10),2(0),22(0),1,-1,2(0),\
        18(1),3(-7),3(!),2(0))
matrix [rows=4 ; col=26] m5 ; val=!(18(0),1,0,-1,5(0),\
        18(0),1,-2,1,5(0),24(1),2(-12),24(0),1,-1))
block block
open 'file name'; chan=2
for x=pick
read [chan=2]
read [chan=2;serial=y;print=data] x
print treatmnt,block,x;dec=0,0,1
print t
treat reg(treatmnt ; 5 ; m1)
anova [print=aov,m,%cv; cont+5 ; fprob=y] x
treat reg(treatmnt ; 6 ; m2)
anova [print=aov,m,%cv; cont=6 ; fprob=y] x
treat reg(treatmnt ; 6 ; m3)
anova [print=aov,m,%cv; cont=6 ; fprob=y] x
treat reg(treatmnt ; 4 ; m4)
anova [print=aov,m,%cv; cont=4 ; fprob=y] x
treat reg(treatmnt ; 4 ; m5)
anova [print=aov,m,%cv; cont=4 ; fprob=y] x
endfor
```

APPENDIX 3. Key for the contrasts performed using the statistical programme in 'Appendix 1'.

'M1'.

1. Isolates E v isolates F
2. E44 v E77,E99
3. E77 v E99
4. F58 v F168, F200
5. F168 v F200

'M2'.

6. Normal(Nor) v Resuspended ((Res)E44)).
7. Molasses(Mol) v Nor., Res. (E44).
8. Nor. v Res. (F168).
9. Mol. v Nor., Res. (F168).
10. Nor. v Res. (F200).
11. Mol. v Nor., Res. (F200)

'M3'.

12. Nor. v Res. (E77).
13. Mol. v Nor., Res. (E77).
14. Nor. v Res. (F58).
15. Mol. v Nor., Res. (F58).
16. Nor. v Res. (E99).
17. Mol. v Nor., Res. (E99).

'M4'.

18. Elvaron v Bacteria isolates.
19. NOTa,b v Elvaron, bacteria isolates.
20. NOTa v NOTb
21. Controls v Bacteria, Elvaron, NOT's

'M5'.

22. Cont.Nor. v Cont. Res.
23. Cont.Mol. v Cont. Nor., Res.
24. Nor.,Mol. (F136) v Rest.
25. Nor. (F136) v Res. (F136).

APPENDIX 4. Significant differences between the treatments and their relevant controls in controlling *Botrytis cinerea* of strawberries.

	PICK 1	PICK 2	PICK 3	PICK 4
E44 (Nom)	+++	+++	+++	+
E44 (Mol)	+++	++	++	+
E44 (Res)	-	-	+	-
F168(Nom)	+++	+++	+++	+++
F168(Mol)	+	-	+++	++
F168(Res)	+++	++	+	+
F200(Nom)	-	-	-	++
F200(Mol)	+++	-	-	-
F200(Res)	-	-	-	-
E77 (Nom)	++	+++	-	-
E77 (Mol)	-	-	-	-
E77 (Res)	-	-	-	-
F58 (Nom)	+++	+++	+++	+++
F58 (Mol)	-	+++	+++	+++
F58 (Res)	+++	+	++	+++
E99 (Nom)	+++	-	++	+
E99 (Res)	-	+++	-	-
E99 (Mol)	++	+	-	-
F136(Nom)	-	++	-	-
F136(Mol)	+	++	-	-
Elvaron	+++	+	+++	+++
N.B.(Res)	-	-	-	-
N.B.(Nom)	-	-	-	-
N.B.+ Mol	-	-	-	-

Confidence Levels are; - No Significant Difference. ++ 99%
+ 95% +++ 99.9%

(N.B. Fischer recommends confidence levels greater than 99% should be taken as significantly different).

APPENDIX 5. Significant Differences of the Treatments Controlling Grey Mould Disease of Strawberries Compared with the Fungicide Elvaron.

	PICK 1	PICK 2	PICK 3	PICK 4
E44 (Nom)	-	-	-	-
E44 (Mol)	-	-	-	-
E44 (Res)	o	-	-	**oo**
F168(Nom)	-	++	++	+++
F168(Mol)	-	-	-	-
F168(Res)	-	-	-	-
F200(Nom)	-	-	**oo**	-
F200(Mol)	-	-	**oo**	o
F200(Res)	o	-	**oo**	o
E77 (Nom)	-	-	o	-
E77 (Mol)	o	-	o	o
E77 (Res)	**oo**	-	o	**oo**
F58 (Nom)	-	+++	+++	+++
F58 (Mol)	**oo**	-	-	++
F58 (Res)	-	-	-	++
E99 (Nom)	-	-	-	-
E99 (Res)	o	-	o	-
E99 (Mol)	-	-	o	o
F136(Nom)	o	-	o	o
F136(Mol)	o	-	**oo**	**oo**

Confidence Levels are;

-	No Significant difference.
+ / o	95%
++ / **oo**	99%
+++ / **ooo**	99.9%

Key.

'+' Represents confidence levels where the control of grey mould is greatest in the isolate treatments.

'o' Represents confidence levels where the control of grey mould is greatest with the Elvaron treatments.