Project title:	Sustainable management of *Mucor* and *Rhizopus* in strawberry
Project number:	SF 98
Project leader:	Dr David S Yohalem (Dr Angela M Berrie after 1/04/2010)
Report:	Annual report, April 2010
Previous report:	None
Key staff:	Dr David S Yohalem – Collection and identification of isolates Dr Angela M Berrie – Evaluation of anti-fungal compounds Tom Passey – Maintenance of cultures Karen Lower – Media preparation
Location of project:	East Malling Research New Road East Malling, Kent ME19 6BJ
Project co-ordinator:	Mr John Clark
Date project commenced:	1 April 2009
Date project completed (or expected completion date):	31 March 2011
Key words:	Strawberry, raspberry, *Mucor*, *Rhizopus*, fungicide, biocontrol agent

Disclaimer:

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

AUTHENTICATION

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

Dr A M Berrie
Research Leader
East Malling Research

Signature .... Date ....

Report authorised by:

Dr Christopher Atkinson
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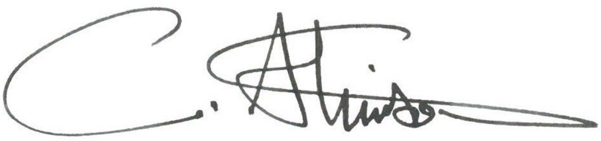

16 July 2010
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Grower Summary

Headline

- Lab based tests have identified chemical control agents which inhibit mycelial growth of *Mucor* and *Rhizopus* spp. in vitro.

Background and expected deliverables

Mucor and *Rhizopus* both cause soft rots in raspberries and strawberries and losses can be significant when conditions are favourable resulting in rapid spread of the fungi in harvested fruit. They are primarily post-harvest rots but also occur on ripe fruit in the field. Both fungi cause similar symptoms on fruit. Infected fruit are initially slightly discoloured, gradually turning pale brown. The tissue rapidly softens and collapses and, under humid conditions, the fruit is soon covered with a dense, fluffy white mycelium which bears stiff, black-headed sporangia (pin mould).

Both fungi survive in soil and crop debris between seasons and produce thick walled resting bodies (zygospores) which serve as long-term resting spores. Some circumstantial evidence suggests that the rots may be encouraged by green manures.
Mucor and *Rhizopus* are both wound pathogens and are favoured by wet, moderately warm conditions (around 18°C). The black spores are easily spread by wind, rain and pickers' hands. *Rhizopus* is inhibited by temperatures below 6°C. However *Mucor* can grow and infect fruit as well at 0°C as it can at higher temperatures. Essentially this means that *Rhizopus* can be controlled in soft fruit by cool chain management whereas *Mucor* will continue to develop during cold storage and marketing.

Neither fungus is well controlled by fungicides and there is evidence that the occurrence of these rots may be increased by the use of certain fungicides such as Rovral (iprodione) to control *Botrytis* rot. Control of these soft rots currently relies on cultural measures including the removal of all ripe fruit at harvest.

In developing improved methods of managing these rots to minimise losses, it is important first to establish the relative incidence of *Rhizopus* and *Mucor* in soft fruit crops.

Although fungicides appear to be relatively ineffective against these fungi, there may be alternative chemicals, such as those used in the food industry, that suppress *Mucor* or *Rhizopus* and could be used near to harvest.

This two year project aims to identify the relative incidence of *Mucor* and *Rhizopus* in soft fruit plantations. This will enable management systems to be developed which minimise losses. In addition laboratory studies will identify potential alternative chemicals offering activity against these rots.

Summary of the project and main conclusions

131 samples of soft rotted berries were received from fruit farms in England and Scotland. *Mucor* spp. was more frequently isolated from strawberry and raspberry soft rots than *Rhizopus*. 74% of isolates from strawberry and 88% from raspberry were identified as *Mucor* spp.

All except 6 samples (open field Elsanta) were obtained from tunnel covered crops, whether raspberry or strawberry. All isolates from the open field crop were *Mucor* spp. From this data it is not possible to draw any definitive conclusions on the effect of tunnels on the incidence of *Mucor* and *Rhizopus*. Very little information on management practices was collected from the growers that provided the rot samples, so it was not possible to investigate the relation between practice and rot incidence. This aspect of the project will be targeted in more detail in year two.

The efficacy of a range of chemicals against *Mucor* and *Rhizopus* was tested in the laboratory using potato dextrose agar (PDA) amended with the chemical under test at concentrations of 0, 1, 10, 100, 1000, 5000 ppm. Growth of mycelial plugs of the test fungus was measured after 1 or 2 days. Standard isolates of *Mucor piriformis, Mucor mucedo* and *Rhizopus stolonifera* were used as well as isolates of *Mucor* and *Rhizopus* obtained during the survey. Initial tests were conducted using Switch (cyprodonil + fludioxonil), Signum (pyraclostrobin + boscalid), potassium bicarbonate and potassium sorbate. All of the chemicals tested reduced or inhibited mycelial growth at the higher concentrations (1000, 5000 ppm). Switch and Signum also inhibited growth at lower concentrations. Switch was the most effective of the chemicals tested in inhibiting mycelial growth with most of the isolates failing to grow even on the lowest concentrations. Both potassium bicarbonate and potassium sorbate inhibited growth of the isolates at the highest concentration of 5000 ppm.

In the second year of the project, further tests will be conducted using a range of fungicides and alternative chemicals including biocontrol agents..

Financial benefits

Soft rots caused by *Mucor* and *Rhizopus* are currently managed by cultural methods only or not at all, and are often overlooked as problems. The incidence of these rots has recently increased, particularly during the last two seasons when the weather has been particularly wet and favourable for spread, especially after harvest during marketing, when rot spread in punnets can be particularly unacceptable to the retailers. Once the disease is present in harvested fruit, spread can be very rapid, especially of *Mucor,* which continues to grow at the temperatures used in cold storage and cool chain marketing.

For developing strategies to control these rots it is important to know the relative incidence of *Mucor* and *Rhizopus* in soft fruit crops. If *Mucor* were the predominant species then additional control measures would need to be considered pre-harvest to control the problem. Such measures would not be needed for *Rhizopus,* which would be suppressed by the temperatures used in cool chain marketing.

This project will establish the relative incidence of the two rots in soft fruit plantations, identify practices that may increase the risk of these pathogens and identify chemicals or biocontrol agents that could be used to control the problem. From the information generated a sustainable management system will be developed, leading to a reduction in the incidence of *Mucor* and *Rhizopus* and a corresponding increase in marketable yields and returns.

Action points for growers

- The project is at an early stage and there are no action points to report at present.

Science Section

Introduction

Mucor and *Rhizopus* both cause soft rots in raspberries and strawberries and losses can be significant when conditions are favourable, resulting in rapid spread of the fungi in harvested fruit. They are primarily post-harvest rots but also occur on ripe fruit in the field. Both fungi cause similar symptoms on fruit. Infected fruit are initially slightly discoloured, gradually turning pale brown. The tissue rapidly softens and collapses and, under humid conditions, the fruit is soon covered with a dense, fluffy white mycelium which bears stiff, black-headed sporangia (pin mould). Both fungi survive in soil and crop debris between seasons and produce thick walled resting bodies (zygospores) which serve as long-term resting spores. Some circumstantial evidence suggests that the rots may be encouraged by green manures.

Mucor and *Rhizopus* are both wound pathogens and favoured by wet, moderately warm conditions (around 18^{o}C). The black spores are easily spread by wind, rain and pickers hands. *Rhizopus* is inhibited by temperatures below 6^{o}C whereas *Mucor* can grow and infect fruit at 0^{o}C as well as it can at higher temperatures. This means that the former can be controlled in soft fruit by cool chain management whereas *Mucor* will continue to develop during cold storage and marketing.

Neither fungus is well controlled by fungicides and there is evidence that the occurrence of these rots may be increased by the use of certain fungicides such as Rovral (iprodione) to control *Botrytis* rot. Currently control of these soft rots is based on cultural measures, including removal of all ripe fruit at harvest.

In order to develop improved methods of managing these rots to minimise losses it is important first to establish the relative incidence of *Rhizopus* and *Mucor* in soft fruit crops. Although fungicides appear to be relatively ineffective against these fungi there may be alternative chemicals such as those used in the food industry that suppress *Mucor* or *Rhizopus* and could be used near harvest.

Over two years, this project will identify the relative incidence of *Mucor* and *Rhizopus* in soft fruit plantations. This will enable management systems for minimising losses to be developed. In addition laboratory studies will identify potential alternative chemicals that are active against these rots.

Overall aim of project

Development of a set of 'best practices' that minimize the risk of *Mucor* and *Rhizopus* in raspberry and strawberry

Specific objectives

1. Establish the relative incidence and importance of *Mucor* and *Rhizopus* in soft fruit production
2. Identification of management practices that may increase the risk of the diseases
3. Laboratory evaluation of alternative chemicals, fungicides and biological products for management of the diseases
4. Identification of the importance of fruit flies in the spread and incidence of the diseases

Materials and methods

Objective 1 – Incidence and importance of *Mucor* and *Rhizopus*

Collection of samples

A sampling protocol and kit (see Appendix B) was sent to FAST, KG Growers and ADAS consultants. Appendix B presents the protocol and questionnaire. Individual berries of both strawberry and raspberry were collected and transported to East Malling Research in 50 ml sterile centrifuge tubes from sites in East Anglia, Kent, the Midlands and Scotland.

Culturing and identification of Mucorales order pin mould fungi

Isolation of fungi from fruits was made onto potato dextrose agar (PDA) or malt extract agar (MEA: 7.5 g malt extract, 15 g agar, 1 L distilled water), both of which permit prolific growth of the fungi. Primary isolations were conducted and incubated at ambient temperatures. Subcultures were transferred to potato-carrot agar (PCA: 20 g grated potato and 20 g grated carrot boiled for 30 minutes in 1 L distilled water, filtered through cheesecloth, brought back to 1 L volume with 15 g agar) (Atlas 2004). The PCA permits growth of more diffuse cultures, which enhances distinguishing character visualisation. Cultures of the fungi were incubated at 15 ºC. Sporulating cultures were observed under the dissecting microscope for characteristic structures and appearance of the two genera. *Rhizopus* spp. develop rihizoids, which appear as basal root-like structure on sporangiophores, which are generally darkly pigmented. On PCA, sporangiophores frequently arise from the termini of aerial hyphae, called stolons. *Mucor* spp. generally have longer sporangiophores, do not form rhizoids, or stolons and, if pigmented, are less so than *Rhizopus* spp. *M. piriformis* and *M. mucedo* can be distinguished by the size and shape of the terminal sporangium, but such distinction was outside the scope of this work.

Authentic cultures of *R. stolonifer, M. mucedo* and *M. piriformis* were purchased from CABI: *Rhizopus stolonifer* var. stolonifer isolate 340068; *Mucor mucedo isolate* 133298; and *M. piriformis* isolate 190877 for purposes of identification and authentification. They were maintained on PDA, MEA and PCA. These cultures were also used for evaluation of the fungicides, chemical and non-chemical agents.

Objective 2 – Management practices and disease risk

At the time of the rot survey, information was collected on factors likely to increase the risk of *Mucor* or *Rhizopus*. Likely factors include soil sterilisation method, fungicide programme applied, cultivation practice (raised beds, plastic cover, straw cover, tunnel venting) frequency of picking, harvest practice (e.g. removal of rotted fruit), location of fruit dump, cool chain marketing. Factors identified will be related to rot incidence. This was an observational exercise to determine if growers are compliant with 'best' cultural practice and, if not whether any bad practices relate to higher *Mucor* / *Rhizopus* incidence.

In addition, weather data in relation to soft rot incidence was also collected.

Objective 3 – Laboratory evaluation of efficacy of fungicides and alternative products

Production of fungal cultures

For evaluation of effects of treatment on mycelial growth isolates of *Mucor* and *Rhizopus* from different origins were selected including standard isolates obtained from CABI (Table 1). The isolates were grown on PDA and mycelial plugs for testing obtained from one-day-old cultures (*Rhizopus*) or two-day-old cultures (*Mucor*).

Table 1. Isolates used in laboratory evaluations

Isolate	Origin
Mucor piriformis (IMI190877)	CABI
Mucor mucedo (IMI133298)	CABI
Rhizopus stolonifera var. *stolonifera* (IMI340068)	CABI
Mucor spp. (SC195-106)	EMR, Kent
Rhizopus spp. (Fav2)	Faversham, Kent
Rhizopus spp. (K1)	Wickhambreaux, Kent

Preparation of test chemical media

Stock chemical solutions A (1000 ppm) and B (100,000 ppm) were prepared for each of the test chemicals as in the Tables below. Chemical stock solutions were freshly prepared for each test. 250 ml quantities of PDA were autoclaved at 121^{o}C for 15 min and allowed to cool to 55^{o}C. The appropriate quantities of stock suspension or solution were added to give the required chemical concentration as in the Tables 2-4. After thorough mixing 16 ml (+ or – 1 ml) quantities of agar were poured into labeled sterile plastic Petri dishes.

Table 2. Stock solution preparation for Signum

Amount of Signum (33.4% ai) product per 100 ml water	Concentration of fungicide ai in stock solution	Volume of stock solution (ml) to be added to 250 ml PDA	Final concentration of fungicide (ai) in agar (ppm)
0.294 g	1,000 (A)	0.25 ml	1
		2.5 ml	10
29.42 g	100,000 (B)	0.25	100
		2.5	1,000
		12.5	5,000

Table 3. Stock solution preparation for Switch

Amount of Switch (62.5% ai) product per 100 ml water	Concentration of fungicide ai in stock solution	Volume of stock solution (ml) to be added to 250 ml PDA	Final concentration of fungicide (ai) in agar (ppm)
0.16 g	1,000 (A)	0.25 ml	1
		2.5 ml	10
16.0 g	100,000 (B)	0.25	100
		2.5	1,000
		12.5	5,000

Table 4. Stock solution preparation for Potassium bicarbonate and potassium sorbate (100% ai)

Amount of Chemical (100% ai)+ product per 100 ml water	Concentration of fungicide ai in stock solution	Volume of stock solution (ml) to be added to 250 ml PDA	Final concentration of fungicide (ai) in agar (ppm)
0.1g	1,000 (A)	0.25 ml	1
		2.5 ml	10
10 g	100,000 (B)	0.25	100
		2.5	1,000
		12.5	5,000

<u>Inhibition of mycelial growth</u>

PDA plates were amended with six concentrations of the test chemicals (0, 1, 10, 100, 1000, 5000 ppm). Plates were inoculated with a 5 mm diameter plug from one or two-day old cultures of selected isolates of *Mucor* and *Rhizopus*. Colony diameters were measured after 1-2 days incubation at 20^{o}C. Two replicate plates were included for each isolate/chemical concentration.

Objective 4 – Fruit flies and *Mucor*/*Rhizopus* risk

Work on this objective will be done in year 2.

Results and discussion

Objective 1 – Incidence and importance of *Mucor* and *Rhizopus*

Isolations were made from 131 berries (Table 5). Of these, 68 were positively identified as *Mucor* spp. and 19 as *Rhizopus* spp. The remainder were either not identified as members of the Mucorales order or failed to grow in culture. Twenty two of the *Mucor* spp. isolates were recovered from raspberry, while only three of the *Rhizopus* isolates were associated with raspberry.

Strawberry leak is a post-harvest disease of cold-stored strawberry fruit with various fungal causal agents. 46 of the strawberry leak disease associated fungal samples found were *Mucor* spp. and 16 were identified as *Rhizopus.* That is, 74% of the strawberries with symptoms of leak disease had *Mucor* spp. as the causal agent and 88% of the soft-rotted raspberries were infected by *Mucor* spp. These results are consistent with past surveys of leak disease in Europe (Maas, 1998; Ellis *et al.* 1991), which suggest that *Mucor* is the more common cause of the disease on this side of Atlantic, while *Rhizopus* is more common in the Americas.

All raspberry samples came from the same farm in Kent and mainly from one cultivar - Tulameen.

Two sites - one in Scotland, the other in Kent – grew the strawberry everbearer cultivar Camarillo. At both of these sites the distribution of *Mucor* spp. and *Rhizopus* spp. was closer to 50-50 and not different from each other (($\chi^2 = 0.00$, $P < 1.00$ and $\chi^2 = 0.025$, $P = 0.853$, for the two sites.).

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Table 5. Details of origin of Fruit samples, numbers and identification of isolates in 2009

Source Location	Grower	Date collected	Crop	Cultivar	Cultivation method	No. of isolations	No of Mucor	No. of Rhizopus
Hugh Lowe Farms, Kent	Regan	5 Aug 09	Raspberry	Tulameen	Tunnel	15	10	2
Hugh Lowe Farms, Kent	Regan	5 Aug 09	Raspberry	Tulameen	Tunnel	14	11	1
Hugh Lowe Farms, Kent	Regan	7 Aug 09	Strawberry	Jubilee	Raised beds, tunnel	6	1	1
Hugh Lowe Farms, Kent	Regan	7 Aug 09	Raspberry	Maravilla	Tunnel	2	1	0
Worcester	Singh		Strawberry			2	0	0
EMR – SC195, Kent	Berrie	20 Aug 09	Strawberry	Elsanta	Raised beds, tunnel	10	8	0
Home Farm, Ardleigh, Colchester, Essex	Peake Fruit	1 Sep 09	Strawberry	Malling Pearl	Tunnel	8	3	1
Wickhambreaux, Canterbury, Kent	Kelsey Farms	3 Sep 09	Strawberry	Elsinore	Table top, tunnel	12	0	4
Wickhambreaux, Canterbury, Kent	Kelsey Farms	3 Sep 09	Strawberry	Jubilee	Table top, tunnels	4	3	1
Staffordshire	Reule Farm		Strawberry	Camarillo	?	1	0	0
EMR – SC195, Kent	Berrie	9 Sep 09	Strawberry	Elsanta	Raised beds, tunnel	7	4	1
EMR – SC196, Kent	Berrie	25 Aug 09	Strawberry	Elsanta	Raised beds, open Field	6	2	0
EMR – DM182, Kent	Farm	24 Sep 09	Strawberry	Elsanta	Table top, tunnel	6	4	2
Maltmas Farm, Norfolk	Duncalfe	26 Sep 09	Strawberry	Malling Pearl	Raised beds, tunnel	10	8	0
Manor Farm, Ightham, Kent	Chesson	30 Sep 09	Strawberry	Evie II	Raised beds, tunnel	13	6	0
Blairgowrie, Scotland	Thomson	19 Oct 09	Strawberry	Camarillo	Raised beds, tunnel	7	3	2
Faversham, Kent	Brooks	2 Oct 09	Strawberry	Camarillo	Raised beds, tunnel	8	4	4

Objective 2 – Management practices and disease risk

All samples except for six (open field Elsanta) were obtained from tunneled crops whether raspberry or strawberry. All isolates from the open field crop were *Mucor* spp. From these data it is not possible to draw any conclusions on the effect of tunnels on the incidence of *Mucor* and *Rhizopus*.

Very little information on management practices was collected from the growers that provided the rot samples so it was not possible to investigate the relation between practice and rot incidence. This aspect of the project will be targeted in year two.

The incidence of *Mucor* spp. in fungicide untreated plots was recorded at harvest and post-harvest following 7 days incubation at ambient temperature in two strawberry crops cv. Elsanta located at East Malling Research. One crop was covered and the other open field. During the harvest period (17 August-2 September) the maximum out door temperature ranged from 19-29°C and the minimum from 8-13°C. Only trace levels of rain were recorded (see Appendix A). Samples of soft rot taken from this were all identified as *Mucor* spp. The incidence of *Mucor* at harvest (Table 6) in both crops was negligible. However, *Mucor* incidence steadily increased with harvest date in the post-harvest tests in both crops. There appeared to be no obvious relationship between rot incidence and temperature and rainfall, although relative humidity, which is reported to influence *Mucor* / *Rhizopus*, was not recorded. Possibly the main factor influencing rot incidence in these trials was the build up of rotting fruit in the adjacent rows which were left unharvested. This suggests that removing rotted fruit from the crop may be an important cultural control method.

Objective 3 – Laboratory evaluation of efficacy of fungicides and alternative products

Colony diameters on the amended PDA were measured after 1-2 days. The measurement included the original mycelial plug (5 mm diameter) so measurements of 5 mm indicate no fungal growth. The results are shown in Table 7. *Rhizopus* spp. in general grew more rapidly than *Mucor* spp. and appeared to be less sensitive to the chemical products tested than the *Mucor* spp. All of the chemicals tested reduced or inhibited mycelial growth at the higher concentrations (1000, 5000 ppm). Switch (cyprodonil + fludioxonil) and Signum (pyraclostrobin + boscalid) also inhibited growth at lower concentrations. Switch was the most effective of the chemicals tested in inhibiting mycelial growth with most of the isolates failing to grow even on the lowest concentrations. Both potassium bicarbonate and potassium sorbate inhibited growth of the isolates at the highest concentration of 5000 ppm. Both these chemicals would be used in practice at these high concentrations.

Further chemical tests will be conducted in the second year including tests on inoculated strawberry fruit. Other products to be tested include Amistar (azoxystrobin), sulphur, ascorbic acid, sodium bicarbonate, Wetcit (a natural wetter based on citrus extract), Chitoplant (chitosan) and the biocontrol agents Shemer (antagonistic yeast) and Trichodex WP (*Trichoderma*).

Table 6. Percentage of fruit infected with *Mucor* recorded in fungicide untreated plots of strawberry cv. Elsanta at harvest or post-harvest after 7 days incubation at ambient temperature in adjacent crops covered or open field in 2009

Harvest date	Tunnel crop		Outdoor crop	
	Harvest	Post-harvest	Harvest	Post-harvest
17 Aug 09	0	9.6		
18 Aug 09			0.25	15.1
20 Aug 09	0.25	28.4		
21 Aug 09			0	27.3
24 Aug 09	0	24.5		
25 Aug 09			0.25	66.4
27 Aug 09	0	80.9		
28 Aug 09			0	72.9
2 Sep 09	2.0	91.8	0.75	82.1

Table 7. Mean colony diameter (mm) of various isolates of *Mucor* or *Rhizopus* on PDA amended with various concentrations (ppm) of Signum (pyraclostrobin + boscalid), Switch (cyprodonil + fludioxonil), potassium bicarbonate and potassium sorbate tested in March 2010

Isolate	Concentration ppm	Signum	Switch	Potassium bicarbonate	Potassium sorbate
Mucor piriformis Isolate IMI190877	0	29.5	29.5	29.5	29.5
	1	19	14.5	30	32
	10	10.5	15.5	27	30
	100	8.5	6.5	29.5	24
	1,000	5*	5	13.5	6.5
	5,000	5	5	5	5.5
Rhizopus stolonifera Isolate IMI340068	0	80	80	80	80
	1	44.5	6.5	78.5	77
	10	19.5	5	77.5	76
	100	14.5	5	61	50
	1,000	6	5	30.5	12.5
	5,000	5	5	8	5
Mucor mucedo Isolate IMI133298	0	22	22	22	22
	1	9	15.5	24.5	24.5
	10	5	8	25	25
	100	5	5	18.5	22.3
	1,000	5	5	11.5	12
	5,000	5	5	6	5
Mucor spp SC195-106	0	62.3	62.3	62.3	62.3
	1	41	6	55.8	64.5
	10	23.5	5	53	67
	100	13.5	5	51.8	51
	1,000	6	5	8.5	5
	5,000	5.5	5	5.5	5
Rhizopus spp-Fav2	0	82	82	82	82
	1	42.5	6.5	74	83
	10	16.3	5	78.3	81.8
	100	10.5	5	82	5
	1,000	7.8	5	52	5
	5,000	5	5	10	5
Rhizopus spp – K1	0	47.3	47.3	47.3	47.3
	1	34.5	6.5	61	60.8
	10	11.5	5	49.5	52.5
	100	5	5	54.5	35.3
	1,000	5	5	33	5
	5,000	5	5	6	5

* Mycelial plug diameter = 5 mm. Colony measurements of 5 mm = no growth

Conclusions

- *Mucor* spp. were more frequently isolated from strawberry and raspberry soft rots than *Rhizopus*. 74% of isolates from strawberry and 88% from raspberry were *Mucor* spp.
- All of the chemicals tested reduced or inhibited mycelial growth at the higher concentrations (1,000 and 5,000 ppm)
- Switch (cyprodonil + fludioxonil) and Signum (pyraclostrobin + boscalid) also inhibited growth at lower concentrations
- Switch was the most effective of the chemicals tested in inhibiting mycelial growth with most of the isolates failing to grow even on the lowest concentrations
- Both potassium bicarbonate and potassium sorbate inhibited growth of the isolates at the highest concentration of 5,000 ppm

Technology transfer

There have been no technology transfer events to date for this project.

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Glossary

Mucorales: an order of the Zygomycotina that contains the family Mucoraceae, which includes the genera *Mucor* and *Rhizopus*

Rhizoid: a group of cells at the base of a sporangiophore that appears root-like.

Sporangium: a structure at the end of a sporangiophore that contains the asexual spores of zygomycetes

Sporangiophore: the elongate cell that bears the sporangia of zygomycetes at its terminus

Acknowledgements

I am most grateful to Tom Passey and Karen Lower for culturing the rots and conducting the chemical tests and for soft fruit growers and consultants from FAST Ltd, ADAS and KG Growers for sending in the rot samples.

Appendix A

Weather data for East Malling (1 March – 30 September 2009)

WEATHER DATE	TEMP ºC MAX	TEMP ºC MIN	RAINFALL mm
01/03/2009	9.4	1	0.2
02/03/2009	10.9	5.7	0
03/03/2009	9	3.9	14.2
04/03/2009	7.2	-1.3	1.2
05/03/2009	8	-0.6	0.2
06/03/2009	10.2	0.7	0
07/03/2009	12.1	5.9	0.4
08/03/2009	10.5	1.7	2.6
09/03/2009	10.5	6.2	6.2
10/03/2009	12.5	-0.2	2
11/03/2009	12	6.1	0
12/03/2009	12.9	3.9	0
13/03/2009	11.4	6	0
14/03/2009	13.2	2.6	0
15/03/2009	14.3	1.5	0
16/03/2009	15	1.5	0.2
17/03/2009	12.8	0.4	0
18/03/2009	15.3	-0.5	0.2
19/03/2009	9.9	-0.2	0
20/03/2009	11.4	-3.4	0
21/03/2009	14.7	5.6	0
22/03/2009	14.8	5.8	0
23/03/2009	13.3	2.3	3.4
24/03/2009	10.9	5.7	1.6
25/03/2009	11.7	6.8	2
26/03/2009	13.1	3.9	2.8
27/03/2009	10.4	1.1	0.8
28/03/2009	9.1	0.4	3.2
29/03/2009	9.6	-1.7	0
30/03/2009	13.3	6.3	0
31/03/2009	14.3	2.4	0
01/04/2009	13.5	7	0
02/04/2009	13.9	5	0
03/04/2009	12.3	5.4	0
04/04/2009	17.1	2.7	0
05/04/2009	15.3	6.8	0
06/04/2009	17.3	8	0
07/04/2009	14.5	7.3	2.2
08/04/2009	15.9	4.6	0.2
09/04/2009	15.3	8.3	0
10/04/2009	17	9.8	2.6
11/04/2009	14.8	9.8	1
12/04/2009	14.2	10.8	0.2
13/04/2009	16.4	5.3	0
14/04/2009	17.1	9.2	3.2
15/04/2009	22.1	10.7	0.4
16/04/2009	15	8.4	9.2
17/04/2009	10.4	6	5.2

WEATHER DATE	TEMP ºC MAX	TEMP ºC MIN	RAINFALL mm
18/04/2009	13.3	4.8	0
19/04/2009	14.7	7.4	0
20/04/2009	17.5	2.2	0.2
21/04/2009	19.8	6.7	0
22/04/2009	19	4.4	0
23/04/2009	17.3	8.1	0
24/04/2009	17	10.1	0
25/04/2009	14.6	1.6	0
26/04/2009	17.3	4.5	0.2
27/04/2009	11.1	1.5	4.4
28/04/2009	12.8	1.4	6
29/04/2009	16.6	4.3	0
30/04/2009	17.5	5.5	0
01/05/2009	19.7	8.9	0
02/05/2009	18.6	8.3	0.2
03/05/2009	15.3	3.1	0
04/05/2009	14.5	10.5	0
05/05/2009	19.3	10.9	0
06/05/2009	20	8	0
07/05/2009	14.8	7.9	1.2
08/05/2009	15.9	4.2	1.2
09/05/2009	17	4	0
10/05/2009	17.7	9.4	0
11/05/2009	12.8	8.8	0
12/05/2009	14.2	11.1	0.2
13/05/2009	14.9	10.7	0
14/05/2009	17.8	11.4	2.2
15/05/2009	15.4	6.7	2.2
16/05/2009	16.2	8.1	0
17/05/2009	15.3	9	4.6
18/05/2009	15.7	8.8	3.2
19/05/2009	16.9	6.1	0
20/05/2009	18.6	6.9	0
21/05/2009	19.2	5.5	0
22/05/2009	18.5	5.7	0
23/05/2009	19.3	6	0.2
24/05/2009	21.1	9	0
25/05/2009	23.6	11	8
26/05/2009	17.2	7.2	0.2
27/05/2009	17.3	11.2	0.6
28/05/2009	22.3	10.3	0.2
29/05/2009	22	10	0
30/05/2009	20.5	11	0
31/05/2009	21.6	11.9	0
01/06/2009	22.9	11	0
02/06/2009	23.4	9.6	0
03/06/2009	16.8	5.3	0
04/06/2009	16.8	7	0
05/06/2009	13.3	9.6	0.4
06/06/2009	14.9	8.8	7.2
07/06/2009	17.1	5.3	0.2
08/06/2009	16.7	10.4	14.4
09/06/2009	16.2	9.2	2.2

WEATHER DATE	TEMP ºC MAX	TEMP ºC MIN	RAINFALL mm
10/06/2009	17.7	8.6	2.2
11/06/2009	19.7	5.7	0
12/06/2009	19.6	12.4	0
13/06/2009	22.3	13.7	0
14/06/2009	23.8	13.7	0
15/06/2009	21.9	11	0
16/06/2009	21.6	8.1	0
17/06/2009	22.1	10.3	0
18/06/2009	18.9	10.2	0
19/06/2009	19.3	10.8	0
20/06/2009	18.9	11.9	0.2
21/06/2009	21.2	8.4	0
22/06/2009	21.1	12.3	0
23/06/2009	21.2	12.9	0
24/06/2009	21.1	14.3	0
25/06/2009	23.4	15.4	0
26/06/2009	24.2	15.8	0
27/06/2009	26	11.3	0.4
28/06/2009	25.9	12.5	0
29/06/2009	29	13.3	0
30/06/2009	27	14	0
01/07/2009	25.3	12.9	0
02/07/2009	27.6	18.1	1.6
03/07/2009	25.1	12.5	0
04/07/2009	25.3	15.6	0.8
05/07/2009	23.6	12.6	1
06/07/2009	20	13.8	4.4
07/07/2009	21.1	12.1	13.4
08/07/2009	20	11.9	1.4
09/07/2009	19.5	10.5	0
10/07/2009	21.3	14	0
11/07/2009	19.1	14.7	3.8
12/07/2009	22.5	10.7	0
13/07/2009	22.6	14.1	0
14/07/2009	22.9	14	1
15/07/2009	21	11.9	0.4
16/07/2009	23.7	15.1	3
17/07/2009	19.9	13.6	1
18/07/2009	21.4	10.6	0
19/07/2009	20.4	12.2	0.2
20/07/2009	20.8	14.7	0
21/07/2009	24.6	14.7	1
22/07/2009	21.7	13	0.2
23/07/2009	22.2	13.3	2.6
24/07/2009	19.1	11.2	7.4
25/07/2009	23.1	10.8	0.2
26/07/2009	22.1	12.8	4.4
27/07/2009	20.2	11	1.2
28/07/2009	20.6	12.6	0.4
29/07/2009	21.9	11.7	7.4
30/07/2009	19.9	10.1	3.2
31/07/2009	21.1	15.3	0
01/08/2009	21.5	12.5	4.8

WEATHER DATE	TEMP ºC MAX	TEMP ºC MIN	RAINFALL mm
02/08/2009	21.8	9.4	0
03/08/2009	21.8	14.6	0
04/08/2009	23.1	13.5	0
05/08/2009	27.7	14.6	0
06/08/2009	29.2	16.7	6.4
07/08/2009	21.4	10.9	0.2
08/08/2009	24.4	12.1	0
09/08/2009	23.8	11.6	0
10/08/2009	22.7	17.1	0
11/08/2009	25.2	14.6	0
12/08/2009	23.8	15.1	5.6
13/08/2009	23.5	12.6	0
14/08/2009	21.4	13.4	0
15/08/2009	22.9	14.5	0.2
16/08/2009	24.7	12.3	0
17/08/2009	23.3	11.1	0
18/08/2009	24.2	10.7	0
19/08/2009	28.8	13.3	0
20/08/2009	23.7	11.7	0
21/08/2009	21.5	8.7	2.4
22/08/2009	22.7	12.1	0.2
23/08/2009	26.8	11	0
24/08/2009	24.6	9.3	0
25/08/2009	21.3	12.4	0.4
26/08/2009	22.7	16.1	0.2
27/08/2009	22	11.4	0.2
28/08/2009	20.2	10.1	0
29/08/2009	20.7	8.8	0
30/08/2009	20.8	16.3	0.2
31/08/2009	25.5	12.4	0
01/09/2009	20.3	12.9	0.2
02/09/2009	18.8	13.3	8.6
03/09/2009	18.3	9.9	0.4
04/09/2009	19.1	9.4	0
05/09/2009	20	12.8	0
06/09/2009	20.1	11.3	0
07/09/2009	24.4	15.4	0
08/09/2009	27.4	14.7	0
09/09/2009	20.7	10.8	0
10/09/2009	20.2	11.8	0
11/09/2009	20.3	13.3	0
12/09/2009	20.5	6.7	0
13/09/2009	17.9	12.9	0
14/09/2009	19.6	13.8	5.4
15/09/2009	16.3	14.2	11
16/09/2009	18.8	12.8	0
17/09/2009	17.7	8.7	0
18/09/2009	18.9	12	0
19/09/2009	22.4	12.9	0
20/09/2009	19.6	11	0
21/09/2009	21.4	11	0
22/09/2009	21.6	15.2	0
23/09/2009	20	8.6	0.2

WEATHER DATE	TEMP ºC MAX	TEMP ºC MIN	RAINFALL mm
24/09/2009	20	6.6	0.2
25/09/2009	20.6	5.2	0
26/09/2009	20.5	5.7	0
27/09/2009	21	7.2	0.2
28/09/2009	18.5	9.4	0
29/09/2009	21.4	9.5	0.2
30/09/2009	18	13.5	0

Appendix B

Sampling *Mucor* and *Rhizopus* – Instructions supplied with sampling kit

1. Select fruit with symptoms of leak disease from different tunnels and/or fields. Photos have been appended, below, of the common appearance of strawberries and raspberries that have been infected with leak-causing fungi (*Mucor* spp. and *Rhizopus* spp.) Please avoid sending fruit with grey mould or anthracnose, which can both be mistaken for strawberry leak.

2. Place an individual fruit into one of the 50 ml capacity screw capped centrifuge tubes. Be careful to avoid cross contamination between samples. This may be accomplished by washing hands in soap and water between taking samples.

3. Ten tubes with strawberries and ten with raspberries should be collected, preferably before fruit are completely decayed. Please include data on cultivar, management practices (tunnel or open, ventilation, fungicide treatments), planting and picking dates for each sample (tube).

4. Tubes may be stored under refrigeration and mailed in bulk or, alternatively (better), sent at time of collection to:

Leak disease data sheet

Sample number: (farm indicator and sample #):
Date of collection:
Location of collection (farm; field):
Crop/Variety:
Growing conditions (*e.g.* tunnels, raised beds, tables, disease management treatments:

Fig 1. *Rhizopus stolonifer* on strawberry

Fig. 2 *Mucor mucedo* on strawberry

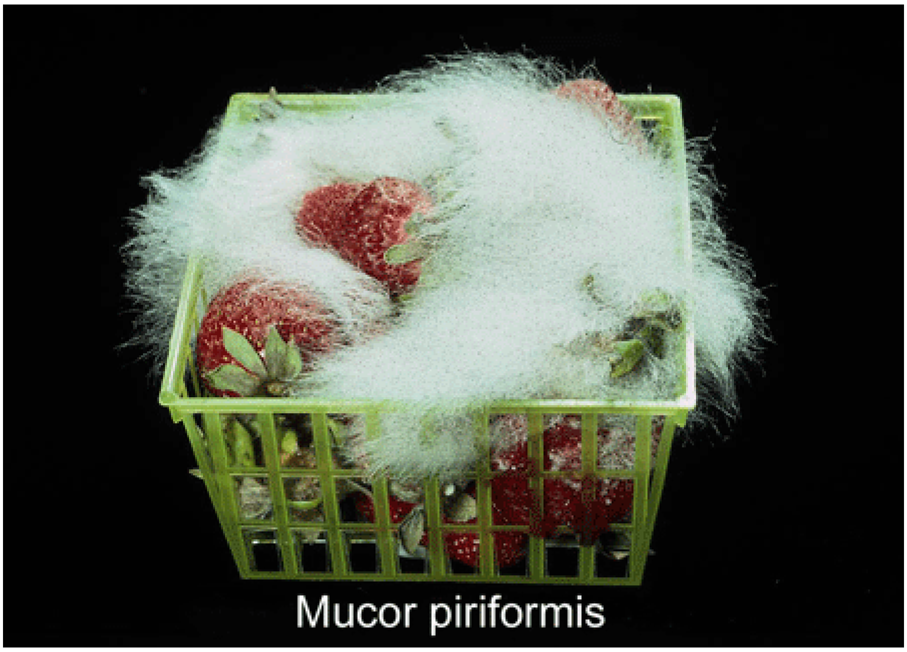

Fig. 3 *Mucor piriformis* on strawberry

Fig. 4 *Mucor* spp. on raspberry