Project title:	Tomatoes: development of biocontrol as a component of an integrated, sustainable strategy for the control of grey mould (*Botrytis cinerea)*
Project number:	PC 174
Report:	Year 3 annual report, June 2003
Previous reports:	Year 1 (2001) and Year 2 (2002) annual reports
Project leader:	Dr T M O'Neill ADAS Arthur Rickwood, Mepal, Ely, Cambs, CB6 2BA
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Project co-ordinator:	Dr Paul Challinor, Humber VHB
Date project commenced:	1 April 2000
Date completion due:	31 March 2004
Key words:	Tomato, grey mould, *Botrytis cinerea*, biological control, antagonists, bioassay

The results and conclusions in this report are based on a series of experiments conducted over one year. 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.

Use of pesticides

Only officially approved pesticides may be used in the UK. Approvals are normally granted only in relation to individual products and for specified uses. It is an offence to use non-approved products or to use approved products in a manner that does not comply with the statutory conditions of use except where the crop or situation is the subject of an off-label extension of use.

Before using all pesticides and herbicides check the approval status and conditions of use.

Read the label before use: use pesticides safely.

AUTHENTICATION

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

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GROWER SUMMARY

Headline

Biocontrol products and micro-organisms used preventatively were found to give control of tomato stem botrytis both in laboratory tests and in a glasshouse crop of cv. Espero. The degree of control with several treatments was equivalent to that of a fungicide (Scala). Temperature and humidity influenced biocontrol efficacy.

Background and expected deliverables

With increasing concern from retailers and consumer groups at the continued use of pesticides on food crops, several leading tomato growers have set in place a long-term objective of zero pesticide use. Currently fungicides remain a key component of effective disease management strategies, and grey mould, caused by the fungus *Botrytis cinerea*, is a principal target of these fungicide treatments. Stem botrytis is the most problematic phase of the disease.

The potential of biocontrol as a component of an integrated sustainable strategy for control of botrytis in tomato crops has been demonstrated elsewhere, for example in Israel and France. The potential under UK cropping conditions has not been thoroughly investigated.

The **expected deliverables** from this project are:

- Identification of existing biological control agents with efficacy against *B. cinerea;*
- Determination of the their efficacy in relation to currently approved fungicides;
- Investigation of potential novel sources of biological control agents (from nurseries where *Botrytis* stem rot does not appear to be a problem in tomatoes);
- Evaluation of candidate organisms for efficacy against *B. cinerea,* compared with fungicides.

Summary of the project and main conclusions

Sourcing existing biocontrol products and isolates

Twenty one bio-control products and isolates (BCAs) with reported antagonism against *B. cinerea* were collected from commercial companies and research organisations to form a project reference collection. Most originate from overseas. Agreements for experimental use of these products were negotiated. Some of these products (e.g. Gliomix, Stimagro) are currently sold in the UK for use as biological growth promoters, though not as bio-fungicides.

Obtaining micro-organisms from UK tomato crops

A total of 106 morphologically distinct micro-organisms were collected from UK tomato crops (including from organic crops), representing different sites, sampling times and plant tissues. Leaves generally yielded a wider range of species than stems or fruit trusses. The isolates comprised fungi, bacteria and yeasts. Isolates were cleaned and cultures put into long-term storage for future use in this project. Fifty isolates were selected for further study to determine their potential use in controlling *B. cinerea*.

Testing for biocontrol activity by agar plate challenge tests

All the candidate BCAs in the reference collection and 49 isolates obtained from UK tomato crops were successfully screened *in vitro* against a range of *B. cinerea* isolates. This was carried out by observing the growth of *B. cinerea* in the presence of individual BCAs in Petri dishes in the laboratory. Often, botrytis grew over the candidate BCA. Twelve of the BCAs however (6 from the reference collection and 6 from UK tomato crops) exhibited strong competition and grew over botrytis (Fig. 1).

Figure 1. Overgrowth of *Botrytis* by *Trichoderma* in an agar plate challenge test

Identification of new candidate biocontrol micro-organisms

The most promising isolates were identified as a *Trichoderma* sp. (01/14), *Scopulariopsis candida* (01/56) and *Geotrichum candidum* (01/62). These fungi are all common and have been found in a range of environments, including plant surfaces. *Geotrichum candidum* is sometimes found on fallen or split tomato fruit, causing sour rot. *S. candida* has been found associated with lesions on humans, so further work on this fungus was discontinued. Identification of the *Trichoderma* fungus to species level is in progress.

Devising a tomato tissue bioassay

Leaf and stem bioassays were devised and used in the laboratory to see which would

allow the most effective screening of the BCAs on tomato plant tissue. It was decided that the stem piece bioassay, using conidial inocula, allowed the most realistic and reproducible conditions similar to infection development under commercial growing conditions.

The stem piece bioassay gives reasonably consistent development of botrytis stem rot. Replicate tomato stem pieces (3 cm long) are inserted in moist, autoclaved vermiculite, and damaged stem ends are inoculated with the candidate biocontrol agent (100 µl) and then with 10^4 primed *B. cinerea* conidia (20 µl of 5 x 10^5 spores/ml). Pots of inoculated stem pieces are incubated at 15^0 C, 80% RH and low light intensity (16 h day/ 8 h night) for around 7 days and then assessed for extent of stem rotting and degree of botrytis sporulation.

Figure 2. Tomato stem piece bioassay, used for large-scale screening of micro-organisms for biocontrol activity

Testing for biocontrol activity using the stem piece bioassay

From the 21 micro-organisms in the reference collection, five were demonstrated to have the ability to reduce botrytis rot in tomato stem pieces:

Gliomix (fungal product)
Clonostachys roseum (fungal isolate)
Stimagro (Streptomycete product)
QRD 131 (bacterial product)
Yield Plus (yeast product)

From the 49 isolates from UK tomato crops, two (01/56 and 01/62) significantly reduced stem rotting.

Although no treatment gave a high level of control using this test procedure, the degree of control in a glasshouse crop, with living plants and natural inoculum dispersal, may be greater.

Effect of temperature and humidity on biocontrol activity

The effect of temperature and humidity on biocontrol efficacy was examined for

seven leading BCAs using the stem piece bioassay. Stimagro showed activity at all temperatures, reducing stem lesion length by at least half compared with the untreated control (Table 1). *Clonostachys* and Gliomix showed moderate activity at 15-20 ^{0}C and none at 25 ^{0}C. Yield Plus showed slight activity at all temperatures while QRD 131 appeared active at 15 and 25^0 C; the result at 20^0 C appears anomalous.

Table 1. Effect of temperature on the effectiveness of seven micro-organisms in controlling tomato stem rot caused by *B. cinerea*

Bioocontrol product or micro-organism	% control of stem rotting at:		
	15°C	20°C	25°C
Clonostachys	22.5	21.2	0.0
QRD 131	25.6	0.0	46.3
Stimagro	62.1	85.4	51.7
Gliomix	39.6	26.1	0.0
Yield Plus	19.4	6.6	18.3
01/62 (*Geotrichum*)	19.4	0.0	0.0
01/56 (*Scopulariopsis*)	2.4	0.0	0.0

Comparing humidities at a constant 20^0 C, Stimagro showed greater efficacy at high (85%) than at lower (70%) humidities. The other BCAs gave only slight control in this experiment and no treatment effects were discernible.

Testing for biocontrol activity on mature tomato plants

A 6-week trial was conducted on a mature crop of cv. Espero, in late autumn 2002. A single spray of each BCA, or a fungicide treatment (Scala), was applied to fresh de-leafing wounds prior to inoculation with botrytis. Although no stem lesions developed in the 6-week period between inoculation and crop termination, *B. cinerea* was readily recovered from stem pieces and internal stem browning was often visible. The extent of internal stem browning was used to assess biocontrol activity (Fig. 3). Four BCA products (Gliomix, QRD 131, Stimagro and YieldPlus) and five microorganisms (*Brevibacillus brevis, Clonostachys roseum, Geotrichum candidum, Trichoderma* sp. and *Ulocladium atrum*) gave statistically significant reductions of botrytis stem rot development. Preventative sprays of *Clonostachys roseum* and *Trichoderma* were equivalent in efficacy to a preventative spray of Scala.

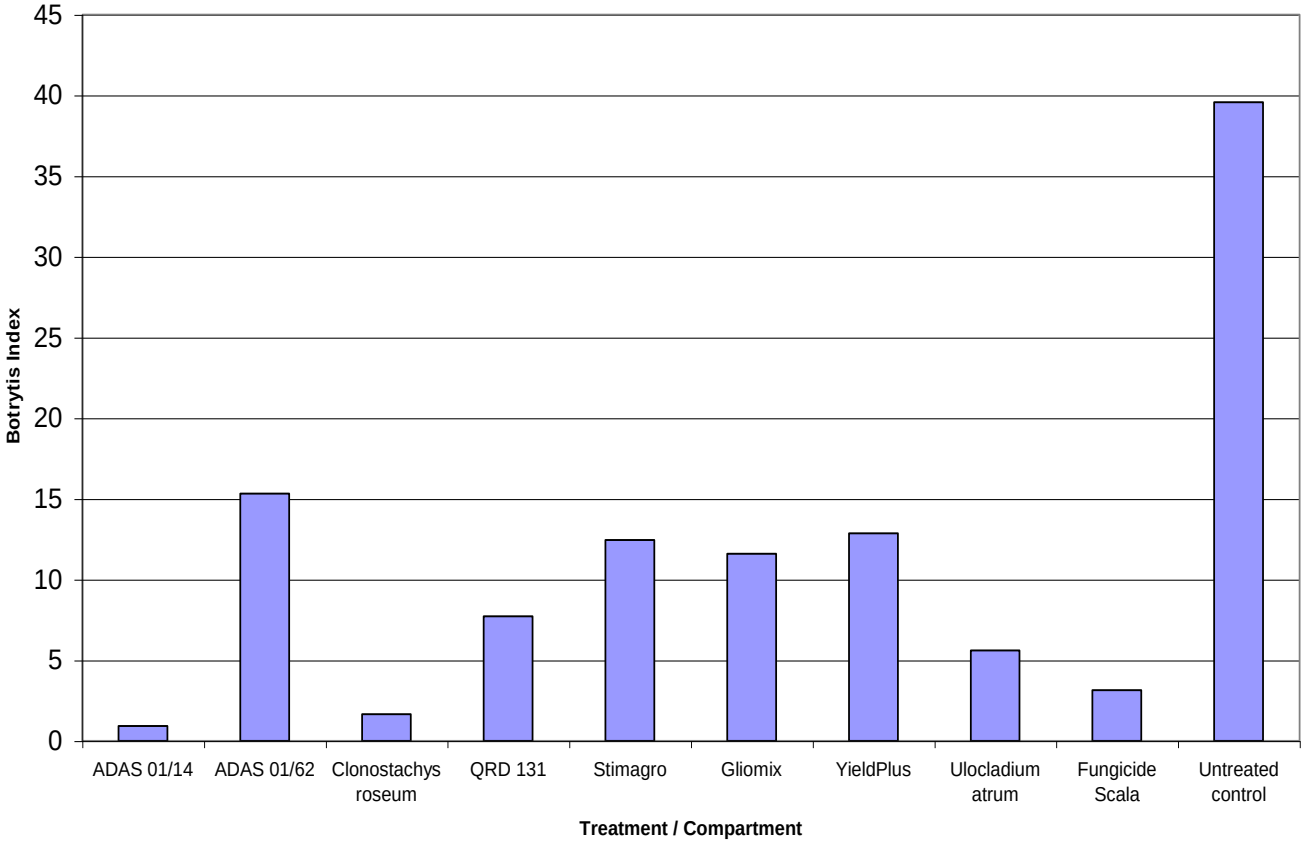

Figure 3. Effect of eight BCA treatments and a fungicide in controlling internal stem browning of tomato associated with botrytis (glasshouse trial, autumn 2002; 6-weeks after inoculation)

Financial benefits

None at present. Losses due to *Botrytis* have been estimated to be in the region of 5-10% per annum in recent years. With the farm gate value of the UK tomato industry at around £130M, losses as low as 1-2% are highly significant. Individual companies have reported losses due to botrytis ranging from £50,000 to £350,000 in a single season. Therefore, any measures, which can be taken to reduce botrytis, are likely to have a significant financial benefit both in terms of reducing plant losses and in minimising additional labour costs to the business.

Action points for growers

None at present. A number of commercial products and isolates have been obtained and screened, however, further screening work is required fully to assess the BCAs.

SCIENCE SECTION

Introduction

The work in the first year of the project focused on the collection of known microbial antagonists towards *Botrytis cinerea,* either commercialised bio-control products or antagonistic isolates (BCAs) from around the world. From this reference collection, a series of laboratory screens were carried out to examine the efficacy of the BCAs to affect the growth of *B. cinerea in vitro* during agar plate challenge inoculation tests. In addition to this work, laboratory bioassays for measuring the efficacy of these BCAs was developed using 'live' tomato plant tissue.

In the second year of the project we completed the project reference collection of commercial products and isolates with known activity against *B. cinerea,* undertook dual culture tests of potential antagonists against *B. cinerea* and presumptive identification of potential antagonists. The bioassay to screen micro-organisms was finalised and isolates were screened. Inoculation methods on tomato plants were compared and a suitable method was devised. Summary interim reports for the project consortium members were produced, in addition to the annual report (March 2002).

The key work areas this year were:

1. To complete the dual culture tests (milestone 3.2)
2. To confirm the identification of the most promising isolates revealed in bioassay (milestone 7.0)
3. To complete the laboratory bioassay of microorganisms (milestone 6.2)
4. To conduct a small scale glasshouse trial evaluating the efficacy of selected biocontrol products and isolates in comparison with a fungicide for control of tomato stem botrytis (milestone 8.3)
5. To assess the effect of environmental factors on the efficacy of antagonists (milestone 9.0)

Milestones (revised schedule as agreed October 2002)

Objective	Achievement Indicators	Target Date
1.1	Reference collection of commercial or reported antagonists secured	August 2000
1.2	Preparation of short summary report	September 2000
1.3	Further isolates sourced and secured	June 2001
2.0	Isolated with potential antagonism to *B. cinerea* collected	October 2001
3.1	All sourced isolates/products screened in dual culture plates	June 2001
3.2	All isolates (from tomato crops) screened in dual culture plates & short-listed	April 2002
4.0	Short-listed isolates identified (presumptive) and lodged in collection	April 2002
5.0	Laboratory bioassay to screen candidate micro-organisms developed	June 2001
6.1	Screen isolates/products in laboratory bioassay	April 2002
6.2	Screen micro-organisms (isolated from tomato crop) in laboratory bioassay	May 2002
7.0	Identify most promising isolates in bioassay (confirmatory)	September 2002
8.1	*In vivo* screening of products in comparison with fungicides completed	March 2003
8.2	*In vivo* screening of isolates (from tomato crops) in comparison with fungicides completed	March 2003
8.3	Small scale glasshouse trials	March 2003
9.0	Assessment of environmental factors on the efficacy of antagonists	September 2003
10.0	Evaluation of antagonists for commercial production	September 2003
11.1	Performance of most effective antagonists determined under commercial conditions in long-season tomato crop	December 2003
11.2	Performance of most effective antagonists on a commercial nursery site	December 2003
12.1	Preparation of Annual Report, year 1	March 2001
12.2	Preparation of Annual Report, year 2	March 2002
12.3	Preparation of Annual Report, year 3	March 2003
12.4	Preparation of Final Report, summarising 4 years work	March 2004
13.0	Reporting developments in biocontrol techniques from the XIIth International Botrytis Symposium as summary report in tandem with Objective 1.2	September 2000

1. Agar plate challenge screening tests (Objective 3.2)

Study Director: Dr Tim Pettitt
HRI Wellesbourne, Wellesbourne, Warwicks

Status of work: Completed

Period covered: April 2001 – March 2003

Introduction

After initial screens and tentative identifications, a collection of some 49 isolates of potential BCAs has been assembled from isolates from UK commercial tomato nurseries. These were tested against *B. cinerea* isolate B052 in the laboratory by agar plate challenge tests to help decide which were likely to be the most promising candidate BCAs for testing on whole tomato plants.

Materials and methods

All isolates selected for potential biological control activity against botrytis in tomatoes as well as *B. cinerea* isolate B052 were first assessed for their linear growth rates on potato dextrose agar (PDA) plates.

Two sets of agar plate challenge tests were carried out. In the first set, PDA plates were inoculated simultaneously with *B. cinerea* isolate B052 and prospective BCAs. Plates were inoculated by placing either a plug of actively growing mycelium taken from a PDA stock culture, or a single streak from a bacterial culture, 10 mm from the edge of the plate and on the opposite side of the Petri dish to a similarly placed plug of *B. cinerea* isolate B052. In the second set of tests, selected BCA isolates were plated in the same manner as above but inoculations with the BCA and the *B. cinerea* isolate were staggered to take into consideration their relative growth rates to try and ensure that the two would meet in the centre of the challenge plate.

The results of challenge tests were assessed by scoring for the presence/absence of zone of inhibition, evidence of competition, failure to inhibit (i.e. overgrowth of the BCA by botrytis) or botrytis growth up to but not over the BCA.

Results and discussion

Following the first set of challenge tests eight isolates were selected for the second set of tests (XHAPP, 01/14, XHAPP 01/52, XHAPP 01/56, XHAPP 01/62, XHAPP 01/71, XHAPP 01/81, XHAPP 01/94 and XHAPP 01/97). The results of the second tests were essentially the same as the first so all results are presented as one in Table 1.1. Several isolates showed good activity against botrytis in culture. Those showing zones of inhibition had to be excluded from further assessments because of the possibility of antibiotic production. Unfortunately this meant the exclusion of promising isolate XHAPP 01/56. Of the remaining promising isolates, which were XHAPP 01/14, XHAPP 01/52, XHAPP 01/62, XHAPP 01/71 and XHAPP 01/94, isolates XHAPP 01/14 and XHAPP 01/62 were selected for inclusion in the whole plant glasshouse assessment based on their competitive performance in these plate tests. The growth rates of the isolates tested are in Table 1.2.

Table 1.1. Results of dual culture plating onto PDA with Botrytis isolate B052 (isolates with good activity are shown in bold)

Isolation code	Results of dual culture with Botrytis isolate B052 after 10 days incubation at 20º C
XHAPP 01/3	Small zone of inhibition
XHAPP 01/5	Botrytis overgrown test culture
XHAPP 01/7	Small zone of inhibition
XHAPP 01/8	Botrytis growth up to (but not over) test culture
XHAPP 01/9	Small zone of inhibition
XHAPP 01/14	**Competition occurring**
XHAPP 01/15	Botrytis overgrown test culture
XHAPP 01/17	Botrytis overgrown test culture
XHAPP 01/19	Botrytis overgrown test culture
XHAPP 01/22	Botrytis overgrown test culture
XHAPP 01/25	Botrytis growth up to (but not over) test culture
XHAPP 01/27	Botrytis overgrown test culture
XHAPP 01/28	Botrytis growth up to (but not over) test culture
XHAPP 01/31	Small zone of inhibition
XHAPP 01/36	Botrytis growth up to (but not over) test culture
XHAPP 01/37	Botrytis growth up to (but not over) test culture
XHAPP 01/40	Botrytis growth up to (but not over) test culture
XHAPP 01/42	Botrytis growth up to (but not over) test culture
XHAPP 01/45	Small zone of inhibition
XHAPP 01/46	Botrytis overgrown test culture
XHAPP 01/47	Botrytis overgrown test culture
XHAPP 01/49	Botrytis growth up to (but not over) test culture
XHAPP 01/52	**Limited competition**
XHAPP 01/53	Botrytis overgrown test culture
XHAPP 01/56	**Large zone of inhibition**
XHAPP 01/57	Botrytis overgrown test culture
XHAPP 01/60	Botrytis overgrown test culture
XHAPP 01/62	**Competition occurring**
XHAPP 01/63	Large zone of inhibition
XHAPP 01/65	Botrytis growth up to (but not over) test culture
XHAPP 01/66	Zone of inhibition
XHAPP 01/71	**Competition occurring**
XHAPP 01/72	Botrytis overgrown test culture
XHAPP 01/75	Small zone of inhibition
XHAPP 01/77	Zone of inhibition
XHAPP 01/81	Botrytis growth up to (but not over) test culture
XHAPP 01/82	Botrytis growth up to (but not over) test culture
XHAPP 01/83	Botrytis growth up to (but not over) test culture
XHAPP 01/84	Botrytis growth up to (but not over) test culture
XHAPP 01/85	Botrytis growth up to (but not over) test culture
XHAPP 01/87	Botrytis growth up to (but not over) test culture
XHAPP 01/90	Zone of inhibition
XHAPP 01/93	Botrytis growth up to (but not over) test culture
XHAPP 01/94	**Botrytis growth up to (but not over) test culture and limited competition**
XHAPP 01/96	Botrytis growth up to (but not over) test culture
XHAPP 01/97	Small zone of inhibition and competition occurring
XHAPP 01/98	Botrytis growth up to (but not over) test culture
XHAPP 01/104	Botrytis growth up to (but not over) test culture
XHAPP 01/106	Botrytis growth up to (but not over) test culture

Table 1.2. Growth rates on PDA of potential BCA isolates assessed in challenge plate tests.

Isolate number	Growth rate (mm/day)	Isolate number	Growth rate (mm/day)	Isolate number	Growth rate (mm/day)
XHAPP 01/3	0.82	XHAPP 01/42	0.57	XHAPP 01/77	0.42
XHAPP 01/5	0.78	XHAPP 01/45	1.17	XHAPP 01/81	4.64
XHAPP 01/7	0.96	XHAPP 01/46	0.03	XHAPP 01/82	0.63
XHAPP 01/8	0.22	XHAPP 01/47	0.64	XHAPP 01/83	0.63
XHAPP 01/9	1.04	XHAPP 01/49	1.89	XHAPP 01/84	3.15
XHAPP 01/14	1.94	XHAPP 01/52	2.74	XHAPP 01/85	0.64
XHAPP 01/15	0.04	XHAPP 01/53	5.72	XHAPP 01/87	0.32
XHAPP 01/17	0.19	XHAPP 01/56	0.11	XHAPP 01/90	2.81
XHAPP 01/19		XHAPP 01/57	3.19	XHAPP 01/93	3.54
XHAPP 01/22	0.03	XHAPP 01/60	0.24	XHAPP 01/94	6.00
XHAPP 01/25	0.83	XHAPP 01/62	3.08	XHAPP 01/96	0.43
XHAPP 01/27	0.11	XHAPP 01/63	2.25	XHAPP 01/97	3.08
XHAPP 01/28	0.25	XHAPP 01/65	0.92	XHAPP 01/98	3.33
XHAPP 01/31	0.14	XHAPP 01/66	1.79	XHAPP 01/104	0.93
XHAPP 01/36	0.86	XHAPP 01/71	3.72	XHAPP 01/106	0.54
XHAPP 01/37	0.51	XHAPP 01/72	0.13		
XHAPP 01/40	3.78	XHAPP 01/75	2.06		

2. Confirmatory identification of leading candidate BCAs (Objective 7.0)

Study Director: Professor J Whipps
HRI Wellesbourne, Wellesbourne, Warwicks

Status of work: Completed

Period covered: April 2002 – March 2003

Introduction

Fifty promising isolates from tomato crops were tentatively identified on the basis of colony morphology and microscopical characteristics following growth on potato dextrose agar. This enabled those that grew poorly or were obvious plant pathogens (e.g. *Botrytis* and *Sclerotinia*) to be excluded from the subsequent stem piece assay or agar plate challenge screening tests. During these screening tests, two isolates, XHAPP 01/56 and XHAPP 01/62, gave consistent inhibitory effects against *B. cinerea* and were considered leading isolates for subsequent glasshouse testing.

Materials and methods

XHAPP 01/56 and XHAPP 01/62 were plated onto potato dextrose agar (PDA) and examined microscopically. Both were clearly fungi and preliminary identifications were suggested as XHAPP 01/56 = *Acremonium* sp. and XHAPP 01/62 = Filamentous yeast (*Aureobasium* sp.). However, as both of these taxa are notoriously difficult to identify to species level, they were sent to CABI Biosciences identification services for confirmation.

Results and Discussion

The following are based on the reports received from CABI and additional information from CBS database in The Netherlands. Both are fungi.

XHAPP 01/56 (IMI 389400). *Scopulariopsis candida* (Guég.) Vuill. Members of this genus are mainly soil fungi but are found associated with lesions in humans, as is known for this species. There is no record in the CABI databases of this species causing disease in plants although *Scopulariopsis* species can occur on plant surfaces. It is an imperfect species of *Microascus.* Some *Scopulariopsis* species have *Acremonium* as a synonym.

XHAPP 01/62 (IMI 389401). *Geotrichum candidum* Link. This species is the conidial stage of *Galactomyces geotrichum* (Butler & Pedersen) Redhead & Malloch, a member of the ascomycete order Endomycetales (yeasts). Identification of this and related species is often problematic. *Geotrichum candidum* has been isolated from many different substrates in the past, including milk, soil, bulbs, plant tissue including tomato fruits, human and animal material, and industrial sources.

Both fungi are common and have previously been found in a range of environments including plant surfaces. *Geotrichum candidum* is particularly interesting as it has been recorded previously on tomato fruits and may be adapted to this plant. However, *Scopulariopsis candida* has been found associated with "lesions on humans" and so further work involving production and use of large amounts of this fungus in general glasshouse applications for *Botrytis* control cannot be recommended without extensive further risk assessment studies. This illustrates the value of accurate identification of potential biocontrol agents combined with the use of database information which may avoid wasting time on organisms that are unlikely ever to be registered on environmental or health risk grounds.

3. Bioassay of microorganisms for antagonistic activity against *Botrytis cinerea* (Objectives 6.1 and 6.2)

Study Director: Dr K R Green
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Site Manager: Ms A Shepherd
ADAS Arthur Rickwood, Mepal, Ely, Cambs. CB6 2BA

Status of work: Completed

Period covered: January – June 2002

Introduction

Products

Twenty-one potential biological control agents (BCAs), including commercially available products and isolates with reported activity against *B. cinerea*, have been collected for use in this project. A series of stem bioassays was undertaken to evaluate the potential of the BCAs for control of *B. cinerea* on tomato and results were detailed in last year's report (HDC, 2002). This report describes a repeat of one run of the bioassay that was carried out after the previous Annual Report was completed.

Microorganisms from commercial tomato crops

It is recognised in the literature that antagonists to botrytis occur naturally on the leaf and stem surfaces of various hosts. Of a total of 106 microorganisms isolated from leaves, stems and trusses of commercial tomato crops earlier in the project, fifty were selected for further screening. This report describes a series of stem bioassays that was undertaken to evaluate the potential of the microorganisms for control of *B. cinerea* on tomatoes.

Materials and methods

Products

Methods are reported in the previous Annual report. Treatments were:

1. Sterile distilled water
2. Gliomix
3. MBI 600
4. Bacillus pumulis 13374
5. *Pseudomonas fluorescens* 13373
6. *Bacillus subtilis* 39
7. *Bacillus subtilis* 83
8. Yield Plus

Microorganisms from commercial tomato crops

These are listed in the Table 3.1.

Table 3.1. Microbial antagonists tested in stem bioassays against *Botrytis cinerea*

Antagonist Code	Organism Type	Cfu Applied
Bioassay 1		
Sterile distilled water	-	-
XHAPP 01/65	Fungus	10^6
XHAPP 01/45	Fungus	10^6
XHAPP01/5	Fungus	10^6
XHAPP 01/15	Bacteria	10^8
XHAPP 01/17	Bacteria	10^8
XHAPP 01/19	Bacteria	10^8
XHAPP 01/31	Bacteria	10^8
Bioassay 2		
Sterile distilled water	-	-
XHAPP 01/71	Fungus	10^6
XHAPP 01/14	Fungus	10^6
XHAPP01/3	Fungus	10^6
XHAPP 01/7	Yeast	10^7
XHAPP 01/77	Fungus	10^6
XHAPP 01/27	Bacteria	10^8
XHAPP 01/22	Bacteria	10^8
Bioassay 3		
Sterile distilled water	-	-
XHAPP 01/63	Fungus	10^6
XHAPP 01/84	Fungus	10^6
XHAPP01/75	Fungus	10^6
XHAPP 01/83	Bacteria	10^8
XHAPP 01/46	Bacteria	10^8
XHAPP 01/72	Bacteria	10^8
XHAPP 01/60	Bacteria	10^8
Bioassay 4		
Sterile distilled water	-	-
XHAPP 01/42	Bacteria	10^8
XHAPP 01/47	Bacteria	10^8
XHAPP01/62	Bacteria	10^8
XHAPP 01/97	Fungus	10^6
XHAPP 01/82	Fungus	10^6
XHAPP 01/36	Fungus	10^6
XHAPP 01/56	Fungus	10^6

Bioassay 5

Sterile distilled water	-	-
XHAPP 01/9	Fungus	10^6
XHAPP 01/48	Yeast	10^7
XHAPP01/37	Yeast	10^7
XHAPP 01/85	Fungus	10^6
XHAPP 01/106	Yeast	10^7
XHAPP01/96	Yeast	10^7
XHAPP 01/25	Fungus	10^6

Bioassay 6

Sterile distilled water	-	-
XHAPP 01/81	Fungus	10^6
XHAPP 01/40	Fungus	10^6
XHAPP01/57	Yeast	10^7
XHAPP 01/94	Bacteria	10^8
XHAPP 01/49	Bacteria	10^8
XHAPP01/29	Bacteria	10^8
XHAPP 01/104	Fungus	10^6

Bioassay 7

Sterile distilled water	-	-
XHAPP 01/28	Bacteria	10^8
XHAPP 01/66	Bacteria	10^8
XHAPP01/90	Fungus	10^6
XHAPP 01/53	Fungus	10^6
XHAPP 01/93	Bacteria	10^8
XHAPP01/98	Fungus	10^6
XHAPP 01/52	Bacteria	10^8

Bioassay 8

Sterile distilled water	-	-
XHAPP 01/14	Fungus	10^6
XHAPP 01/94	Bacteria	10^8
XHAPP 01/81	Fungus	10^6
XHAPP 01/52	Bacteria	10^8
XHAPP 01/41	Bacteria	10^8

Bioassay 9

Sterile distilled water	-	-
XHAPP 01/72	Bacteria	10^8
XHAPP 01/47	Bacteria	10^8
XHAPP01/62	Bacteria	10^8
XHAPP 01/97	Fungus	10^6
XHAPP 01/56	Fungus	10^6
XHAPP01/37	Yeast	10^7
XHAPP 01/81	Fungus	10^6

Eight runs of the bioassay included seven BCA treatments and an untreated control

(Table 3.1). A final run of the bioassay to screen the most promising microorganisms included five treatments and an untreated control (bioassay 9). For each treatment, there were four replicates of ten stem pieces in a randomised block design. In addition, ten wounded stem pieces were monitored as an uninoculated check.

Treatments

Formulations of the following concentrations were prepared for the different microorganism types (Dik, 1999; J. Whipps, pers. comm.) and were applied at a rate of 80 µl per stem:

Fungi	1×10^6 spores/ml
Yeasts and actinomycetes	1×10^7 cfu/ml
Bacteria	1×10^8 cfu/ml

Preparation of plant material

Stem sections (3 cm length) were cut from side-shoots of tomato cv. Espero, avoiding the top 10 cm of the shoots. 320 stem pieces were required for each bioassay plus ten stem pieces for the uninoculated control. The stem pieces were rinsed in distilled water and wrapped in moist paper towel until required, ensuring that the upper end of the stem pieces could be subsequently identified. Plant pots (9-cm diameter) were filled with autoclaved vermiculite (121°C, 20 min) and wetted with distilled water (70 ml per pot). Ten stem pieces were vertically inserted into each pot with the top ends orientated upwards. Stem pieces were wounded prior to application of BCAs and inoculation with *B. cinerea* by gently crushing the end to be inoculated using a pair of pliers.

Inoculum preparation

A spore suspension (5×10^5 spores/ml) of *B. cinerea* was prepared 3 h before it was required for inoculation. A sporulating culture of *B. cinerea* (isolate code BC02), originally collected from stem lesions on tomato was used for all bioassays. The spore suspension was amended with 0.1 M glucose and 0.07 M potassium dihydrogen phosphate.

Application of BCAs and *Botrytis* inoculum

For each microorganism treatment in a bioassay, 80 µl was applied to each stem end (4 pots of 10 stems). Once the microorganism had been absorbed by the stem tissue, each stem end was inoculated with 20 ul of the spore suspension of *B. cinerea* to give 10^4 spores per stem.

Incubation

Pots were placed within individual polythene bags (left open), in plastic trays containing water to a depth of approximately 1 cm. The individual bags were used to minimise cross contamination between treatments. Each tray was completely enclosed in an autoclave bag and transferred to a controlled environment (CE) cabinet for incubation at 15ºC and 80% RH, with a 16 h day/8 h night light regime (low light intensity). The stems were misted regularly (e.g. twice daily) to maintain high relative humidity, but the autoclave bags were left open periodically to prevent build-up of excess condensation.

For each bioassay, one pot containing ten wounded stem pieces for the uninoculated

check was incubated in an individual polythene bag in the CE cabinet. 80 µl of sterile distilled water was applied to each stem piece.

Assessments

The severity of stem infection was assessed 7-10 days after inoculation by measuring the lesion length on each stem. A sporulation index for each stem was recorded as follows:

0=no sporulation
1=sporulation on stem end only
2=sporulation on up to 25% of stem length
3=sporulation on 25-50% of stem length
4=sporulation on 50-75% of stem length
5=sporulation >75% of stem length

Data were subjected to analysis of variance.

Results and discussion

Products

Although treatment effects were non-significant, there was apparent reduction in lesion length due to Yield Plus and a reduced sporulation index with Yield Plus and Gliomix (standard BCA included in all bioassays). The results with Yield Plus are in agreement with those reported in the original run of the bioassay.

Table 3.1. Lesion development and sporulation on the outside of tomato stems after inoculation with *B. cinerea* spore suspension and seven microbial antagonists (BCAs): repeat of Bioassay 5

Treatment	Code	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	SDW	10.0	18.6	9.5	1.3
Gliomix (standard)	A2	10.0	16.9	3.0	0.3
MBI 600 (standard)	A3	10.0	17.7	4.8	0.6
Bacillus pumulis	A5	9.8	17.9	6.5	0.8
Pseudomonas fluorescens	A6	10.0	15.9	6.0	0.8
Bacillus subtilis 39	A7	10.0	16.1	6.8	1.0
Bacillus subtilis 83	A8	10.0	16.2	7.8	1.1
Yield Plus	A11	8.8	12.1	3.5	0.4
SED			2.327	1.995	0.330
df			21	21	21
Significance		(Skewed)	0.236	0.060	0.087

Microorganisms from commercial tomato crops

In all eight runs of the bioassay, there was good development of botrytis lesions on the untreated control stems. Although some microorganisms apparently reduced lesion length and/or sporulation index in some bioassays, there were no significant treatment effects in comparison with the untreated control (Tables 3.2 – 3.9). Microorganisms that gave apparent reduction in lesion length were compared in a final bioassay together with one microorganism (01/81) that was considered as having potential as an antagonist based on preliminary identification (J. Whipps, HRI, pers. comm.). There was a significant treatment effect in the final bioassay, with microorganisms 01/56 and 01/62 leading to a reduction in stem lesion length compared with the untreated control treatment.

None of the treatments gave a high level of control. Possibly this reflects the severe challenge of the stem piece bioassay where a large and fresh wound site is inoculated with *B. cinerea* conidia primed in nutrients. Earlier work in this project indicated that this inoculation procedure was necessary in order to obtain consistently high incidences of rotting in the botrytis only treatment.

Table 3.2. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and seven microorganisms. Bioassay 1

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	10.0	23.8	5.8	0.7
01/65	10.0	25.1	5.3	0.6
01/45	10.0	24.4	3.5	0.5
01/5	10.0	25.5	5.5	0.8
01/15	10.0	21.4	4.5	0.7
01/17	10.0	23.4	3.8	0.5
01/19	10.0	21.7	5.0	0.8
01/31	10.0	24.9	3.8	0.4
SED		2.518	1.157	0.185
Df		21	21	21
Significance	-	0.648	0.373	0.295

Table 3.3. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and seven microorganisms. Bioassay 2

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	9.8	13.2	6.3	0.7
01/71	10.0	16.1	5.3	0.6
01/14	10.0	13.8	7.5	0.8
01/3	10.0	16.8	9.0	1.1
01/7	10.0	17.2	7.0	1.1
01/77	10.0	16.3	5.8	0.7
01/27	10.0	15.4	4.0	0.4
01/22	10.0	14.5	6.8	0.8
SED		1.707	1.483	0.177
Df		21	21	21
Significance	-	0.243	0.072	0.016

Table 3.4. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and seven microorganisms. Bioassay 3

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	10	25.4	5.8	0.9
01/63	10	22.3	5.5	0.8
01/84	10	23.7	6.0	0.7
01/75	10	21.4	5.8	0.8
01/83	10	22.2	5.5	0.8
01/46	10	23.7	5.8	0.7
01/72	10	18.4	3.0	0.3
01/60	10	21.0	5.5	0.7
SED		2.399	1.051	0.162
Df		21	21	21
Significance	-	0.205	0.169	0.086

Table 3.5. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and seven microorganisms. Bioassay 4

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	10	26.3	8.8	1.2
01/42	10	24.9	6.5	0.9
01/47	10	22.5	6.8	1.0
01/62	10	22.2	6.3	0.9
01/97	10	22.9	7.5	1.0
01/82	10	24.5	8.0	1.3
01/36	10	23.7	6.5	1.0
01/56	10	22.5	7.3	0.9
SED		1.645	1.353	0.251
Df		21	21	21
Significance	-	0.200	0.585	0.775

Table 3.6. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and seven microorganisms. Bioassay 5

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	9.8	16.5	0.0	0.0
01/9	9.5	15.7	1.3	0.2
01/48	9.8	16.2	0.5	0.1
01/37	9.5	14.5	0.0	0.0
01/85	9.8	18.1	0.8	0.1
01/106	9.8	15.4	1.0	0.1
01/96	9.8	20.4	0.3	0.1
01/25	9.8	17.6	0.8	0.1
SED	0.984	0.208	0.459	0.493
Df	21	21	21	21
Significance	0.374	2.122	0.648 (Skewed)	0.075 (Skewed)

Table 3.7. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and seven microorganisms. Bioassay 6

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	10	20.3	0.3	0.1
01/81	9.8	18.0	0.3	0.1
01/40	9.8	18.7	0.3	0.1
01/57	10	20.4	0.0	0.0
01/94	9.8	19.7	0.0	0.0
01/49	10	21.8	0.0	0.0
01/29	10	23.2	0.3	0.1
01/104	9.5	20.5	0.5	0.1
SED	0.323	2.348	0.211	0.021
Df	21	21	21	21
Significance	0.496 (Skewed)	0.469	0.257 (Skewed)	0.257 (Skewed)

Table 3.8. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and seven microorganisms. Bioassay 7

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	9.8	21.3	0.0	0.0
01/28	10	22.3	0.0	0.0
01/66	10	22.5	0.3	0.1
01/90	10	23.3	0.3	0.1
01/53	10	22.7	0.0	0.0
01/93	10	21.5	0.3	0.1
01/98	10	21.6	0.3	0.1
01/52	10	23.2	0.0	0.0
SED		2.947	0.232	0.023
Df		21	21	21
Significance	-	0.994	0.698 (Skewed)	0.698 (Skewed)

Table 3.9. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and five microorganisms. Bioassay 8

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	10	17.8	0.0	0.0
01/14	10	16.4	0.5	0.1
01/94	9.8	16.4	0.0	0.0
01/81	10	17.3	7.0	0.7
01/52	10	16.6	0.0	0.0
01/41	10	16.2	0.8	0.1
SED		3.007	0.781	0.078
Df		15	15	15
Significance	-	0.994	<0.001 (Skewed)	<0.001 (Skewed)

Table 3.10. Lesion development and sporulation on tomato stems after inoculation with *B. cinerea* spore suspension and seven microorganisms. Bioassay 9

Treatment	Lesion incidence (out of 10)	Mean stem lesion length (mm)	Mean no. sporulating stems (out of 10)	Mean sporulation index
Sterile distilled water	10	12.5	9.8	1.6
01/72	10	13.6	10	1.7
01/47	10	13.6	10	1.6
01/62	9.8	9.9	9.5	1.0
01/37	10	13.2	10	1.7
01/97	9.8	13.0	10	1.5
01/56	10	9.5	9.3	1.1
01/81	10	13.2	9.5	1.5
SED	0.181	1.422	0.354	0.243
Df	21	21	21	21
Significance	0.583 (Skewed)	0.037	0.246 (Skewed)	0.096

Conclusions

Based on results from previous work (HDC, 2002) and bioassays reported here, the following products and microorganisms were short-listed for further studies:

BCA	Organism	Organism type
Clonostachys roseum	*Clonostachys roseum*	Fungus
QRD 131	*Bacillus subtilis*	Bacteria
Stimagro	*Streptomyces griseovirides*	Actinomycete
Gliomix	*Gliocladium catenulatum*	Fungus
YieldPlus	*Cryptococcus albidus*	Yeast
XHAPP 01/56*	*Scopulariopsis candida*	Fungus
XHAPP 01/62	*Geotrichum* sp.	Fungus

*Members of the genus are mainly soil fungi but occasionally found associated with lesions in humans. The fungus is not known to be a plant pathogen but can occur on plant surfaces. Based on this information, 01/56 was subsequently replaced by 01/14 (a *Trichoderma* sp. which performed well in laboratory bioassays) for glasshouse studies.

References

Dik AJ, Koning G & Kohl J (1999). Evaluation of microbial antagonists for biological control of *Botrytis cinerea* stem infection in cucumber and tomato. *European Journal of Plant Pathology* **105**, 115-122.

4. Environmental factors and biocontrol efficacy (Objective 9.0)

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Status of work: Ongoing

Period covered: August 2002 – March 2003

Introduction

Short-listed products and isolates are being studied in detail to determine the effect of environmental conditions and potential use pattern (protectant or eradicant) on their level of effectiveness. This report describes studies undertaken to date to determine the effects of temperature and relative humidity on the efficacy of selected BCAs against *B. cinerea* on tomato stems.

Materials and methods

Experiment 1 – Relative humidity

Experimental design

The experiment was a completely randomised split plot design with relative humidity treatments as main plots and BCA treatments as sub-plots. There were three replicates of each relative humidity treatment. Plots comprised ten stems per pot.

The effects of relative humidity on the efficacy of the following BCAs was tested:

Treatment	Code	Min. product concentration (cfu/g product)	Formulation in SDW	Cfu applied to each stem piece
1. Sterile distilled water	SDW	-	-	-
2. *Clonostachys roseum*	H1	-	-	10^6
3. QRD 131	H10	10^9	10 ml in 90 ml	10^8
4. Stimagro	A1	10^8	10 g in 100 ml	10^7
5. Gliomix	A2	10^7	10 g in 100 ml	10^6
6. YieldPlus	A11	10^8	1.5 g in 1 litre	10^8
7. XHAPP 01/62	01/62	-	-	10^8
8. XHAPP 01/56	01/56	-	-	10^6

BCAs were applied at higher concentrations than *B. cinerea*, to simulate prior colonisation of stem tissue. The application rates in the table above (10^6 spores per stem for fungal BCAs, 10^7 spores per stem for actinomycetes, 10^8 spores per stem for bacterial BCAs) follow advice from J. Whipps (pers. comm.) and previous research, (Dik *et al*., 1999). The yeast application rate (A11) was determined from previous bioassays and follows the commercial rate.

The following relative humidity treatments were tested: 75 %, 85 % and 100 %

Preparation of plant material

Stem sections (3-cm length) were cut from side-shoots of tomato cv. Espero, avoiding the top 10-cm of the plant. 720 stem pieces were required for the main experiment plus 30 stem pieces for the uninoculated control. The stem pieces were rinsed in distilled water and wrapped in moist paper until required, ensuring that the upper end of the stem pieces could be identified later. Plant pots (9-cm diameter) were filled with autoclaved vermiculite (121^{o}C, 20 min) and wetted with distilled water (70 ml per pot). Stem pieces were wounded prior to application of BCAs and inoculation with *B. cinerea*, by gently crushing the end to be inoculated using a pair of pliers.

Inoculum preparation

A spore suspension (5 x 10^5 spores/ml) of *B. cinerea* was prepared 3 h before it was required for inoculation. A sporulating culture of *B. cinerea* (isolate BC02), originally collected from stem lesions on tomato was used. The spore suspension was amended with 0.1 M glucose and 0.07 M potassium dihydrogen phosphate.

Application of BCAs and Botrytis inoculum

For each treatment, 90 stem pieces were dipped into the BCA formulation ensuring that the stem pieces were completely covered with the BCA. The formulation was agitated as necessary before dipping to ensure the BCA was evenly distributed. For each treatment, 10 stem pieces were vertically inserted into replicate pots containing sterile vermiculite, with the top ends orientated upwards. Once the stem tissue had absorbed the excess BCA, each stem end was inoculated with 20 ul of the spore suspension of *B. cinerea*, to give 10^4 spores per stem.

Humidity regimes

Different RHs were produced by means of saturated salt solutions in distilled water (Winston and Bates, 1960). The solutions were prepared by adding salt to the solution until no further salt dissolved and was visible at the bottom of the container. Eight pots of stem pieces (one of each BCA treatment) were placed within individual perforated polythene bags (left open) in plastic Petri dishes, positioned in large white plastic trays. The trays (but not the Petri dishes) were flooded to a depth of 5 mm with distilled water (100 % RH), KCl (85 % RH) or NaCl (75 % RH). Each tray was completely enclosed in a large polythene bag, ensuring that the bag did not come into contact with the stem pieces. The trays were transferred to a controlled environment growth room for incubation at 20^{o}C in the dark for 7 days.

An uninoculated check was set up for each humidity regime, using one pot of ten stems placed in a Petri dish positioned in a small tray of the appropriate salt solution, all contained in an individual bag, within the growth room.

Every 1-2 d, the saturated salt solutions were checked and replenished as necessary to ensure that excess (undissolved) salt was visible at the bottom of the tray.

Assessments

The severity of stem infection was assessed 13 days after inoculation by measuring length of lesion on each stem. A sporulation index for each stem was recorded as

follows:

0 = no sporulation
1 = sporulation on stem end only
2 = sporulation on up to 25 % of stem length
3 = sporulation on 25-50 % of stem length
4 = sporulation on 50-75 % of stem length
5 = sporulation on >75 % of stem length

Data were subjected to analysis of variance.

Experiment 2 - Temperature

Experimental design
The experiment was a counter-balanced split-plot incomplete block design, with four replicates of each temperature treatment. Each week, pair-wise comparisons of temperature treatments were made, using two controlled environment (CE) cabinets running simultaneously. In each cabinet, there was one plot of each BCA treatment (1 pot with 10 stems) with the arrangement of BCA treatments randomised. Runs in weeks 4, 5 and 6 were intended to enable the effects of time and cabinet, if any, to be identified:

Week	Cabinet 1	Cabinet 2
1	15	20
2	20	25
3	25	15
4	20	15
5	25	20
6	15	25

Treatments
BCA treatments evaluated were the same as in Experiment 1. The following temperature treatments were tested:
15, 20 and 25ºC

For preparation of plant material, wounding, preparation of inoculum and application of BCAs and Botrytis inoculum, the techniques described in Experiment 1 were used.

Two CE cabinets were run simultaneously each week. For each cabinet, eight pots of stem pieces (one of each BCA treatment) was placed within individual perforated polythene bags (left open) in plastic Petri dishes, positioned in a large white plastic tray. The trays were flooded to a depth of 5 mm with a saturated NaCl solution (75 % RH). The tray was completely enclosed in a large polythene bag, ensuring that the bag did not come into contact with the stem pieces. The tray was transferred to a controlled environment cabinet for incubation at one of the test temperatures in the dark for 7 days.

An uninoculated check was set up for each temperature treatment, using one pot of ten stems placed in a Petri dish in a small tray of the appropriate salt solution, all contained in an individual bag, within the controlled environment cabinet.

Results and discussion

Experiment 1 – Relative humidity

There was no effect of the humidity treatments on stem lesion length. However data loggers placed under equivalent conditions, running in parallel with the main experiment indicated that the relative humidity for each treatment did not reach the desired level. The relative humidity for the NaCl, KCl and distilled water treatments were recorded as 69.5 %, 70.5 % and 85.5 %, respectively after 10-11 h. It is possible that the required humidity levels were not achieved because the volume of the bags was too high.

There was a significant effect of BCA treatments on the development of botrytis on tomato stems, with Stimagro leading to a reduction in lesion length (Table 4.1). The mean sporulation index was low (<1) for all treatments.

Table 4.1. Effect of humidity and BCA treatment on lesion length on tomato stems after inoculation with *Botrytis cinerea* spore suspension

	Mean stem lesion length (mm)			
BCA Treatment	NaCl	KCl	Distilled water	Mean
Sterile distilled water	13.3	14.3	12.2	13.3
Clonostachys roseum	18.3	19.4	14.7	17.5
QRD 131	15.4	14.5	12.9	14.3
Stimagro	10.0	9.1	5.7	8.3
Gliomix	17.9	22.6	18.4	19.6
YieldPlus	10.4	17.0	10.3	12.6
XHAPP 01/62	8.4	14.5	11.2	11.4
XHAPP 01/56	15.6	16.2	18.2	16.6
SED (BCA)				2.465
Df				42
Significance				0.001

Experiment 2 - Temperature

The experimental design outlined above was followed until week 6 when only cabinet 2 was available. A run at 15ºC was carried out in week 6 and a run at 25ºC was subsequently carried out in cabinet 2 in week 7. Statistical analyses indicated that there were no experimental effects due to conducting the experiment over time or to use of two cabinets. The results of run 4 at 20ºC should be treated with caution as botrytis failed to develop in the inoculated control treatment.

There was a significant effect of BCA treatment on stem lesion length ($P<0.001$) with Stimagro showing the best antagonistic activity meaned over the three temperatures (Table 4.3). The effect of BCAs on stem lesion length varied between runs carried out at individual temperatures, such that there was no significant BCA treatment/temperature interaction effect. However, some trends in temperature effects emerged (Table 4.2 and 4.3). Stimagro was effective at all temperatures, reducing mean stem lesion length by at least half compared with the untreated control, but showed best antagonistic activity at 20ºC. Gliomix was most effective at 15ºC, although this was not consistent across all runs at this temperature. At 25ºC, QRD 131 and Stimagro appeared to have the best antagonistic activity.

Table 4.2. Effect of BCAs on lesion length on tomato stems after inoculation with *Botrytis cinerea* spore suspension, at three temperatures

15°C	Lesion length (mm)				
Treatment	Run 1	Run 2	Run 3	Run 4	Mean
Sterile distilled water	21.1	12.1	10.9	7.3	12.9
Clonostachys roseum	10.9	10.8	11.6	6.6	10.0
QRD 131	11.2	10.0	10.9	6.1	9.6
Stimagro	3.0	10.1	4.5	1.9	4.9
Gliomix	12.0	5.9	9.4	3.8	7.8
YieldPlus	15.1	7.3	14.6	4.5	10.4
XHAPP 01/62	13.7	10.5	9.8	7.6	10.4
XHAPP 01/56	24.8	11.0	10.5	4.2	12.6

20°C	Lesion length (mm)				
Treatment	Run 1	Run 2	Run 3	Run 4	Mean
Sterile distilled water	17.7	18.0	13.4	0.0	12.3
Clonostachys roseum	17.1	6.1	12.5	3.0	9.7
QRD 131	13.1	9.8	15.9	10.9	12.4
Stimagro	0.0	0.0	6.0	1.0	1.8
Gliomix	10.9	4.3	15.1	6.2	9.1
YieldPlus	22.3	9.0	10.8	3.9	11.5
XHAPP 01/62	20.1	19.4	10.3	3.0	13.2
XHAPP 01/56	19.9	13.9	12.1	4.0	12.5

25°C	Lesion length (mm)				
Treatment	Run 1	Run 2	Run 3	Run 4	Mean
Sterile distilled water	13.4	10.0	5.8	7.8	9.3
Clonostachys roseum	10.5	12.1	4.9	16.7	11.1
QRD 131	0.0	6.5	3.0	10.3	5.0
Stimagro	0.0	5.0	2.2	10.6	4.5
Gliomix	14.6	14.4	3.7	5.4	9.5
YieldPlus	13.3	9.1	2.8	5.2	7.6
XHAPP 01/62	15.1	0.9	7.1	18.7	10.5
XHAPP 01/56	19.0	7.7	3.7	10.3	10.2

Table 4.3 Mean effect of temperature on effectiveness of seven BCAs in controlling tomato stem rot.

Treatment	Lesion length (mm) at incubation temperature:						
		15°C		20°C		25°C	Mean
SDW	12.9	-	12.3	-	9.3	-	11.5
C. roseum	10.0	(22.5)	9.7	(21.2)	11.1	(0.0)	10.3
QRD 131	9.6	(25.6)	12.4	(0)	5.0	(46.3)	9.0
Stimagro	4.9	(62.1)	1.8	(85.4)	4.5	(51.7)	3.7
Gliomix	7.8	(39.6)	9.1	(26.1)	9.5	(0.0)	8.8
YieldPlus	10.4	(19.4)	11.5	(6.6)	7.6	(18.3)	9.8
01/62	10.4	(19.4)	13.2	(0)	10.5	(0.0)	11.4
01/56	12.6	(2.4)	12.5	(0)	10.2	(0.0)	11.8
SED (BCA)							1.743
Df							63
Significance							$P<0.001$

( ) - % control

Conclusions

- Methods for the relative humidity experiment may need to be re-considered, perhaps using the controlled environment cabinets to provide humidity conditions instead of saturated salt solutions.

- Results from the temperature experiments suggested that there were differences in temperature optima for antagonist activity of individual BCAs. Some BCAs showed antagonism at a single temperature (e.g. QRD 131), while others were effective at a range of temperatures (Stimagro).

- The superior antagonistic activity shown by Stimagro at 25°C, compared with Gliomix, was in agreement with experimental findings from the manufacturer of these two products (Kemira, Finland, pers. comm.)

References

Dik, AJ, Koning, F & Köhl, J. 1999. Evaluation of microbial antagonists for biological control of Botrytis cinerea stem infection in cucumber and tomato. *European Journal of Plant Pathology* **105**: 115-122.

Winston PW & Bates DH. 1960. Saturated solutions for the control of humidity in biological research. *Ecology* **41**: 232-237.

5. Evaluation of potential BCAs in a short-term glasshouse trial (Objective 8.3)

Study Director: Dr Tim Pettitt
HRI Wellesbourne, Wellesbourne, Warwicks

Site manager: Mike Wainwright, HRI Efford

Status of work: Completed

Period covered: April 2002 – March 2003

Introduction

A short-list of nine BCA products and isolates was made based on bioassay results and dual culture evaluation. These were all tested for their activity in reducing botrytis stem rot on whole tomato plants in a glasshouse trial at HRI Efford.

Materials and methods

A glasshouse trial was conducted from October 2002 to January 2003 on mature long season tomato plants (cv. Espero) at HRI Efford. These plants had previously been used as uninoculated control plants in a DEFRA Pepino Mosaic Virus trial, and had been tested frequently to ensure absence of PepMV contamination. The performance of selected BCAs was compared against both inoculated and uninoculated controls, and a standard commercial fungicide (Scala). The BCAs were selected from the isolates and products found to be most promising in the stem bioassay by ADAS, with the exception of isolate XAPP 01/56 which was excluded as it showed antibiotic production characteristics likely to prevent registration for commercial use on tomato crops. This was determined due to the presence of a large zone of inhibition in the dual culture plate tests (see section 1).

The trial comprised 2 compartments of M Block (M2 and M7) at HRI Efford, each with 192 treated plants (see Figure 5.1 and Table 5.1). Each plant was given 5 inoculation sites by removing lower leaves at the normal deleafing height, (between 50 and 100 cm above floor level, this was equivalent to approximately 8 m from the stem base) and making crosshatched scalpel cuts in the resulting wound. The BCA treatments were then applied immediately to the wound. Wounding and BCA inoculations were carried out in the morning. In the evening of the same day botrytis conidial suspensions were applied to the wounds on appropriate plants. Inoculations of both BCA treatments and botrytis conidia were conducted using a hand-held De Vilbis sprayer with a plastic shield to prevent cross-contamination and collect run-off (see Appendix 2 for spray calibration).

The botrytis conidia were collected dry and then primed by suspension in 0.1M glucose and 0.07M potassium dihydrogen-phosphate for 3 hours prior to inoculation, at a concentration of $5x10^5$/ml. The guard rows on either side of the inoculated rows were used for the uninoculated control treatment plants, to prevent the risk of cross contamination with botrytis inoculum, as the stems were layered closely together. For operational reasons (due to the time input necessary on the day of inoculation) it

was not possible to inoculate both compartments simultaneously. Inoculation of compartment M2 took place on 30th October and M7 on 12th November.
Glasshouse conditions were changed to favour botrytis infection in each compartment on the day of inoculation. This comprised reduction of minimum pipe temperature settings and reducing venting. (See Appendix 1 for daily mean temperatures and humidities.)

Table 5.1. Treatments used in the glasshouse trials at HRI Efford

Treatment		Isolate/Preparation	Concentration applied / ml	Co-inoculated with *Botrytis*
1	BCA 1	*Trichoderma* sp. (01/14)	10^6	YES
2	BCA 2	Geotrichum candidum (01/62)	10^6	YES
3	BCA 3	*Clonostachys roseum*	10^7	YES
4	BCA 4	QRD 131	10^8	YES
5	BCA 5	Stimagro	10^6	YES
6	BCA 6	Gliomix	10^6	YES
7	BCA 7	Yield Plus	10^8	YES
8	BCA8	*Ulocladium atrum*	10^6	YES
9	BCA 9	*Brevibacillus brevis*	10^8	YES
10	Fungicide	Pyrimethanil (Scala)	-	YES
11	Control 1	Distilled water	-	YES
12	Control 2	Distilled water	-	NO

Figure 5.1. Glasshouse trial plan and treatment layout

M 2																							
Block 1	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6	Plot 7	Plot 8	Plot 9	Plot 10	Plot 11	Block 5	Plot 45	Plot 46	Plot 47	Plot 48	Plot 49	Plot 50	Plot 51	Plot 52	Plot 53	Plot 54	Plot 55
	3	10	7	6	11	8	1	9	2	4	5		9	4	8	3	2	11	6	7	10	1	5
Block 2	Plot 12	Plot 13	Plot 14	Plot 15	Plot 16	Plot 17	Plot 18	Plot 19	Plot 20	Plot 21	Plot 22	Block 6	Plot 56	Plot 57	Plot 58	Plot 59	Plot 60	Plot 61	Plot 62	Plot 63	Plot 64	Plot 65	Plot 66
	2	1	7	9	10	11	5	3	6	4	8		4	1	8	11	2	10	6	7	3	9	5
Block 3	Plot 23	Plot 24	Plot 25	Plot 26	Plot 27	Plot 28	Plot 29	Plot 30	Plot 31	Plot 32	Plot 33	Block 7	Plot 67	Plot 68	Plot 69	Plot 70	Plot 71	Plot 72	Plot 73	Plot 74	Plot 75	Plot 76	Plot 77
	5	4	9	11	8	10	2	3	1	6	7		10	6	7	4	3	11	8	9	5	2	1
Block 4	Plot 34	Plot 35	Plot 36	Plot 37	Plot 38	Plot 39	Plot 40	Plot 41	Plot 42	Plot 43	Plot 44	Block 8	Plot 78	Plot 79	Plot 80	Plot 81	Plot 82	Plot 83	Plot 84	Plot 85	Plot 86	Plot 87	Plot 88
	2	8	1	7	11	10	5	9	3	4	6		9	5	2	8	3	6	4	7	10	1	11

M 7																							
Block 9	Plot 89	Plot 90	Plot 91	Plot 92	Plot 93	Plot 94	Plot 95	Plot 96	Plot 97	Plot 98	Plot 99	Block 13	Plot 133	Plot 134	Plot 135	Plot 136	Plot 137	Plot 138	Plot 139	Plot 140	Plot 141	Plot 142	Plot 143
	2	10	1	4	3	7	11	9	6	8	5		9	7	5	2	6	1	10	3	8	11	4
Block 10	Plot 100	Plot 101	Plot 102	Plot 103	Plot 104	Plot 105	Plot 106	Plot 107	Plot 108	Plot 109	Plot 110	Block 14	Plot 144	Plot 145	Plot 146	Plot 147	Plot 148	Plot 149	Plot 150	Plot 151	Plot 152	Plot 153	Plot 154
	5	10	2	1	3	9	11	7	8	6	4		7	5	11	9	6	4	8	2	10	3	1
Block 11	Plot 111	Plot 112	Plot 113	Plot 114	Plot 115	Plot 116	Plot 117	Plot 118	Plot 119	Plot 120	Plot 121	Block 15	Plot 155	Plot 156	Plot 157	Plot 158	Plot 159	Plot 160	Plot 161	Plot 162	Plot 163	Plot 164	Plot 165
	9	3	7	11	1	8	5	10	2	4	6		3	7	2	4	10	6	11	9	5	1	8
Block 12	Plot 122	Plot 123	Plot 124	Plot 125	Plot 126	Plot 127	Plot 128	Plot 129	Plot 130	Plot 131	Plot 132	Block 16	Plot 166	Plot 167	Plot 168	Plot 169	Plot 170	Plot 171	Plot 172	Plot 173	Plot 174	Plot 175	Plot 176
	2	7	8	6	1	3	11	9	4	10	5		10	5	6	2	4	7	8	1	11	3	9

Results and discussion

Examination of the glasshouse climate records (Appendix 1) show that conditions considered conducive to development of botrytis were achieved in both compartments for much of the 6-week periods. Temperatures were around 15°C and mean RH consistently above 85%. Very high mean humidities (>90%) were recorded in compartment M2 in the 14 days after inoculation. Despite these apparently favourable conditions, six weeks after inoculation there were no aggressive or sporulating botrytis lesions developing on any of the inoculation sites in either compartment. However, plating tests showed that viable botrytis was present at inoculation sites. The change in glasshouse environmental conditions designed to favour botrytis development resulted in an outbreak of lower stem botrytis lesions on uninoculated sites near the stem base, leading to a number of plant deaths, particularly affecting compartment M2. These appeared mainly to be at deleafing sites. After a meeting between the experimenters on 4th December 2002, it was decided that a destructive sample of the whole crop should be taken to gain the greatest possible level of information from the trial. It was also evident from plating stem sections at the inoculation sites that botrytis infection had led to internal stem browning not visible on the outer stem. By measuring (mm) the extent of this internal browning, a disease spread index score was developed as follows:

$$\text{Disease Spread Index} = \frac{\text{Disease spread length} - \text{Wound site diameter} \times 100}{\text{Wound site diameter}}$$

A value above zero indicates spread beyond the inoculation site. The greatest spread at an individual site was 18 mm, from a wound site of 10 mm. It was notable for the uninoculated control that there was no browning even at the wound site.

Stem sections from all wound sites were surface sterilised in 10% sodium hypochlorite for 1 minute, and both plated onto Petri dishes of Potato Dextrose Agar, and placed in damp chambers. The extent of botrytis growing from the wound sites was assessed on a 0-5 scale. The results of these assessments are presented in Table 5.2 and Figure 5.2 which show that most of the BCA treatments had some impact on botrytis disease. The best BCA treatments were BCA1 (XHAPP 01/14, *Trichoderma* sp.) and BCA 3 (*Clonostachys roseum*) both of which gave comparable suppression of botrytis to the fungicide treatment (Scala).

A statistical comparison of treatments is shown in Fig 5.3. Comparisons of untransformed stem browning scores resulted in too great a non-homogeneity of the variance, with increasing variability as the amount of browning increased. Log transformations were therefore used to stabilise the variance. In order to account for the impact of variations in the initial wound size on the subsequent expression of stem browning, the relationship between log browning score and log intitial wound size was fitted by covariate regression analysis. Using this a covariate adjustment was made in the analysis of browning scores to a standardised initial wound size. Least significant differences (LSDs) are shown on the Figure.

Table 5.2. Glasshouse trials at HRI Efford – results of destructive sampling

	Treatment	Disease spread Index	Botrytis Score (0-5)	Botrytis on damp chambers (0-5)
1	*Trichoderma*	0.92	0.17	0.21
2	*Geotrichum*	15.32	1.87	2.72
3	*Clonostachys*	1.65	0.36	0.65
4	QRD 131	7.72	1.13	1.63
5	Stimagro	12.44	1.81	2.68
6	Gliomix	11.59	1.51	2.19
7	Yield Plus	12.86	1.77	2.48
8	*Ulocladium*	5.61	0.69	1.02
9	*Brevibacillus*	19.33	2.19	2.89
10	Scala	3.14	0.40	0.55
11	Water	39.58	4.05	4.72
12	Control	0.00	0.00	0.00

Conclusions

- The results of this trial must be viewed with caution because plating stem sections may not give efficacy results equivalent to the incidence or severity of aggressive sporulating lesions that might eventually develop on whole plants.

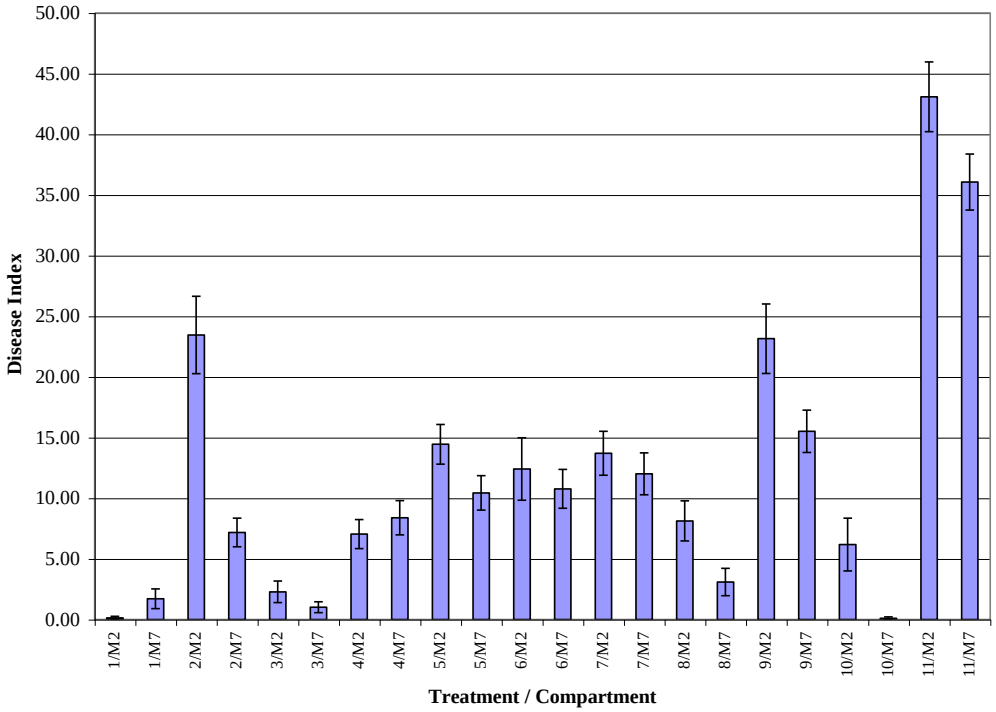
- Nevertheless, the extent of internal stem browning and botrytis development on assessed stem sections were consistently greatest in the inoculated control while there were no signs of disease in the uninoculated control. This indicates that the destructive assessment of wound sites is a useful method to measure disease potential and the efficacy of BCA treatments.

- All treatments reduced botrytis compared with the inoculated controls, therefore all the BCAs tested showed some degree of biocontrol ability.

- BCAs 1 (*Trichoderma*) and 3 (*Clonostachys*) showed great promise with comparable efficacy to the Scala fungicide treatment. BCA 8 (*Ulocladium*) also showed some potential, but was less effective and consistent than BCAs 1 and 3. These three BCAs merit further investigation (as well as possibly BCA 4 [QRD 131]).

- Efficacy of BCA may also be linked to the prevailing environmental conditions during the experiment. However, this is a very complex area and would require considerable extra work fully to elucidate.

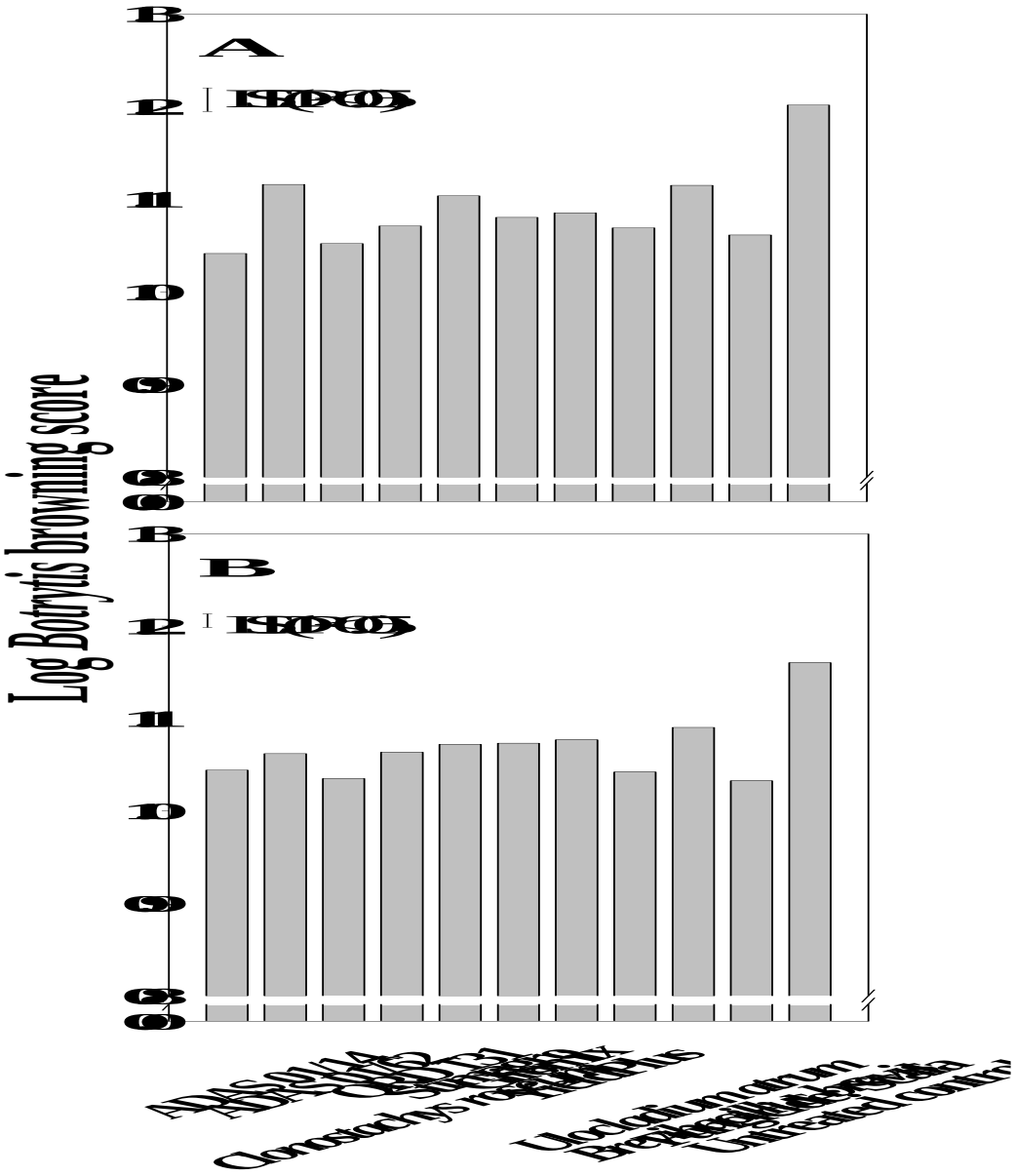
Figure 5.2. Effects of BCA treatments on botrytis as determined by disease index (2 determinations per BCA treatment, M2 and M7 are different compartments in M block at HRI Efford; bars are standard errors).



Key

1. *Trichoderma* (01/14)
2. *Geotrichum* (01/62)
3. *Clonostachys*
4. QRO 131
5. Stimagro
6. Gliomix
7. Yield Plus
8. *Ulocladium*
9. *Brevibacillus*
10. Scala
11. Water

Figure 5.3. Effects of biocontrol treatments on the spread of stem browning caused by *Botrytis* (expressed as log browning score), compared with a fungicide treatment (Scala) and untreated controls.



6. Overall conclusions

1. Six out of 49 microorganisms isolated from UK tomato crops were shown by agar plate challenge tests to have useful activity against *B. cinerea*. All isolates have been placed in long-term storage at HRI Wellesbourne. Two were selected for evaluation as preventative spray treatments in a glasshouse trial (see 9 below).

2. A tomato stem piece bioassay was used to assess biocontrol products and microorganisms. Five showed useful levels of activity (*Clonostachys roseum*, Gliomix, QRD 131, Stimagro and Yield Plus). These five, plus two microorganisms (*Brevibacillus brevis* and *Ulocladium atrum*) previously shown to have good activity, were selected for inclusion in the glasshouse trial (see 9 below).

3. The isolates identified as showing useful activity against *B. cinerea* by *in vitro* and *in vivo* tests were not identical. Two isolates (01/56 and 01/62) showed activity in both tests.

4. CE cabinet bioassay studies on tomato stem pieces showed that Stimagro gave control at all temperatures tested being most effective at 20°C. *Clonostachys* and Gliomix showed activity at 15-20°C and none at 25°C. YieldPlus showed slight activity at all temperatures.

5. At 20°C, Stimagro exhibited greater efficacy in reducing stem rot at 85% RH than at 70% RH.

6. Although no stem lesions had developed 6 weeks after inoculation of deleafing sites on a mature crop of cv. Espero, the fungus was readily recovered from internal stem tissue at the inoculation sites. Assuming such infections eventually develop into stem lesions, this suggests there may be a latent period of at least 6 weeks between infection of wound sites by *B. cinerea* conidia and the occurrence of visible stems lesions.

7. Assessment of biocontrol efficacy in a tomato crop, judged by extent of internal stem browning beyond the inoculation site, indicated that four biocontrol products and five microorganisms reduced disease. Particularly effective were a *Trichoderma* sp. and *Clonostachys roseum*, equal in effectiveness to the fungicide Scala when used as a preventative spray.

7. Technology transfer

Article

Green K, O'Neill TM & Pettitt T (2003). Progress in biocontrol of tomato stem botrytis. *HDC News* (in press).

Summary progress reports to consortium members

1. Report 4, August 2002
2. Report 5, January 2003

Scientific progress meetings

Project progress meeting, HRI Efford, 4 December 2002 (TMO, MW)
Project progress meeting, HRI Efford, 24 March 2003 (TMO, MW)
Project review meeting, ADAS Arthur Rickwood, 11 April 2003

Presentation

Project overview and progress report to TGA Technical Committee, September 2002 at FEC, Stonleigh (TMO).

8. Acknowledgements

We thank John Overvoorde, John Drew, Paul Challinor, Philip Pearson and Gerry Hayman for their support and advice in helping to steer the project. In addition, we thank all of the companies and researchers who have given product samples or antagonistic isolates as well as technical information for use in this project. We also thank Rodney Edmonson, Biometrics Department, HRI Wellesbourne and Doug Wilson, ADAS statistician for their support in experimental design and statistical analyses.

Appendix 1a. Compartment M2 Temperature and humidity data (recorded by the Priva glasshouse control computer) (see section 5 in the report)

M2	Temperature			Humidity		
Date	Max	Min	Average	Max	Min	Average
29/10/2002	20	15.8	17.9	92.9	84	89.9
30/10/2002	19.3	16.5	17.8	94.9	91.4	92.6
31/10/2002	19.4	16.3	18.0	94.2	90.4	93.0
01/11/2002	20	16.1	17.9	98.6	90.7	94.8
02/11/2002	19.3	15.6	17.2	98.8	96.5	97.8
03/11/2002	22.2	14.6	16.9	98.3	72.3	91.9
04/11/2002	22.2	14.5	17.2	94	75.6	88.8
05/11/2002	19.6	14.7	16.8	98	94.6	96.5
06/11/2002	18.7	14.2	16.2	98.3	89.1	95.4
07/11/2002	21.1	14.2	16.6	92.3	65.8	85.1
08/11/2002	18.5	14.7	16.1	98	88.9	94.0
09/11/2002	20.5	14.5	16.8	98	87.8	95.2
10/11/2002	20.2	14.3	16.4	98.3	92.8	96.6
11/11/2002	21.2	14.4	16.7	96.3	73.8	90.8
12/11/2002	20	14.3	16.0	96.8	89.9	94.9
13/11/2002	20.9	14.6	16.5	97	86	93.5
14/11/2002	20.3	14.7	16.2	96.3	81.7	92.0
15/11/2002	21.2	15.1	17.2	93.8	72	88.9
16/11/2002	21.7	15.3	17.2	93.1	77.5	90.3
17/11/2002	20.9	14.5	16.9	92.8	82.8	89.3
18/11/2002	22.2	14.9	17.1	89.8	74.3	85.1
19/11/2002	21.1	15.3	17.2	89	71.1	86.1
20/11/2002	20.5	15.3	16.8	89.1	83.2	87.1
21/11/2002	19.9	14.5	16.2	91.5	83.3	87.2
22/11/2002	20.1	14.6	15.9	88.5	78.3	86.5
23/11/2002	20.1	14.6	16.1	90.5	82.6	86.3
24/11/2002	20.1	14.5	16.1	90.1	80.1	85.7
25/11/2002	18.2	14.5	15.6	88.7	77.1	82.0
26/11/2002	20.4	14.5	16.3	87.7	71.8	82.4
27/11/2002	19.7	14.8	15.9	89.8	81.1	84.7
28/11/2002	20.1	14.6	16.6	89.5	82.6	88.0

Appendix 1a. Compartment M2 Temperature and humidity data contd.

M2	Temperature			Humidity		
Date	Max	Min	Average	Max	Min	Average
29/11/2002	20.4	14.7	16.6	89.8	74.6	84.2
30/11/2002	18.6	14.7	15.8	91.2	75.8	84.9
01/12/2002	20.6	14.3	16.2	92	73.5	85.1
02/12/2002	20	14.2	16.0	84.1	72.4	79.6
03/12/2002	21.2	14.6	16.5	85.4	59	77.9
04/12/2002	20.6	14.4	15.9	86.3	66.8	79.4
05/12/2002	20.4	14.3	16.2	82.8	65.8	72.1
06/12/2002	19	14.4	15.7	84.6	70.8	78.8
07/12/2002	17.3	14.6	15.6	77.4	68.8	73.4
08/12/2002	17.5	14.6	15.6	73.6	66.4	70.5
09/12/2002	18.2	14.6	15.6	72	59.3	66.0
10/12/2002	17.2	14.5	15.6	70.8	57.3	63.6
11/12/2002	17.6	14.7	15.6	72.4	63.6	67.4
12/12/2002	17.6	14.4	15.6	77.7	64.7	70.8
13/12/2002	17.4	14.5	15.5	83.3	72.6	77.2
14/12/2002	17.2	14.5	15.5	80.7	72	76.2
15/12/2002	17.5	14.5	15.6	74.8	69.7	72.4
16/12/2002	18.3	14.7	15.7	81.8	73.2	77.6
17/12/2002	20.4	14.7	15.9	76.2	59.4	71.4
18/12/2002	18.4	14.4	15.8	71.6	60	64.3
19/12/2002	20.5	11.4	14.9	81.5	54.3	66.1
20/12/2002	15.4	10.8	12.5	88.9	82	84.5
21/12/2002	15.7	12.9	13.9	89.5	84.8	87.4
22/12/2002	16.6	12.9	14.3	90.3	82.3	87.2
23/12/2002	16.5	13	14.1	90.9	82.6	87.1
24/12/2002	18.1	12.6	14.3	89.9	78.1	85.1
25/12/2002	19.1	10.9	13.9	87.8	70.7	82.8
26/12/2002	17.6	12.3	13.8	89	77.2	85.1
27/12/2002	15.6	12.1	13.3	88.9	80.3	85.8
28/12/2002	20.5	10.2	13.2	86.3	62.9	78.9
29/12/2002	15.2	11.8	13.2	90.3	82.8	88.2
30/12/2002	19.3	13	14.4	90.4	74.5	85.4
31/12/2002	13.2	10.5	11.5	85.4	78.8	82.1
01/01/2003	18.7	11.5	13.5	90.4	77.1	86.7

Appendix 1b. Compartment M7 Temperature and humidity data (see section 5 in the report)

M7	Temperature			Humidity		
Date	**Max**	**Min**	**Average**	**Max**	**Min**	**Average**
11/11/2002	21.4	15.6	17.8	85.7	71.2	82.3
12/11/2002	20.4	16.2	17.4	88.4	83.4	85.1
13/11/2002	21.1	16.6	18.1	87.3	79.9	84.5
14/11/2002	20.4	14.6	16.6	86.8	74.5	83.7
15/11/2002	20.9	14.6	16.6	92.2	75.7	86.8
16/11/2002	21.1	14.5	16.4	92.4	79.6	88.4
17/11/2002	20.5	14.3	16.2	91.6	79.3	87.6
18/11/2002	21.4	14.8	17.0	86.5	76.8	82.8
19/11/2002	21.2	15.3	17.3	87.8	70	84.1
20/11/2002	20.4	15.4	16.9	89.1	83.1	86.1
21/11/2002	20.1	14.6	16.3	90.9	83.3	87.0
22/11/2002	20.2	14.5	15.9	88.9	81.3	86.5
23/11/2002	20.3	14.6	16.1	90	83.5	86.4
24/11/2002	20.1	14.5	16.2	89.2	79.6	85.7
25/11/2002	18.2	14.5	15.6	90.1	77.6	83.2
26/11/2002	20.5	14.6	16.4	89.3	76	84.3
27/11/2002	19.5	14.7	16.0	90.1	83.1	86.8
28/11/2002	20.1	14.5	16.5	90.5	85.8	89.4
29/11/2002	20.3	14.6	16.5	91.7	80.9	87.4
30/11/2002	18.7	14.7	15.8	92.1	79.3	87.6
01/12/2002	20.5	14.2	16.1	93	81.4	87.7
02/12/2002	20	14.3	16.0	88.2	80.7	83.0
03/12/2002	21.2	14.5	16.4	86.6	65.6	80.6
04/12/2002	20.4	14.3	15.9	87.9	73.3	82.4
05/12/2002	20.2	14.3	16.1	85.6	72.2	76.1
06/12/2002	18.7	14.4	15.7	87.9	72.2	81.2

Appendix 1b. Compartment M7 Temperature and humidity data contd.

M7	Temperature			Humidity		
Date	Max	Min	Average	Max	Min	Average
07/12/2002	17.6	14.7	15.6	81.5	73.6	77.5
08/12/2002	17.8	14.5	15.6	79.2	72.2	74.4
09/12/2002	18	14.6	15.6	77.8	62	70.0
10/12/2002	17	14.6	15.5	73.9	60.8	67.9
11/12/2002	17.6	14.7	15.6	77.5	66.6	71.2
12/12/2002	18	14.3	15.5	81.5	67.3	74.2
13/12/2002	17.4	14.5	15.5	85.7	74	79.4
14/12/2002	17.3	14.5	15.5	82	74	78.4
15/12/2002	17.6	14.5	15.6	78.4	73	75.4
16/12/2002	19.1	14.7	15.8	84	76.6	80.1
17/12/2002	21.7	14.7	16.0	82.5	64.8	75.5
18/12/2002	18.6	14.4	15.7	77.8	63	68.5
19/12/2002	20.5	14.6	15.9	77.5	62.8	70.0
20/12/2002	17.3	14.8	15.5	88.3	76.9	81.8
21/12/2002	17.8	14.7	15.6	89.4	85	87.0
22/12/2002	17.6	14.7	15.7	89.9	85.4	87.4
23/12/2002	17.5	14.7	15.6	90	85	87.5
24/12/2002	19.5	14.6	15.9	88.7	82.8	85.6
25/12/2002	19.9	14.7	16.0	88.2	78.9	83.6
26/12/2002	18.8	14.6	15.7	86.2	80.8	83.9
27/12/2002	17.4	14.6	15.6	87.7	82	84.1
28/12/2002	20.4	14.2	16.1	83.4	70.7	78.1
29/12/2002	17.2	14.7	15.6	87.2	80.1	83.7
30/12/2002	20.2	14.6	15.9	87.8	78.3	84.7
31/12/2002	17.3	14.5	15.6	79.7	74	76.9
01/01/2003	19.6	14.8	15.8	84.9	78	81.8

Appendix 2. Spray calibration (see section 5 in the report):

Average weight of sprayer before one application:	128.80g
Average weight of sprayer after one application:	128.42g
Difference (weight applied in one application)	0.38g
Average weight of stem section before one application:	19.67g
Average weight of stem section after one application:	19.82g
Difference (weight applied in one application)	0.15g

Appendix 3. Glasshouse trials at HRI Efford 2002/3 (M block)– Summary Crop Diary (see section 5)

Date	Action
22nd Oct 2001	Seeds sown (J Block HRI Efford)
10th Dec 2001	Slab contact made
30th Oct 2002	Inoculation of compartment M2
12th Nov 2002	Inoculation of compartment M7
10th Dec 2002	Commencement of destructive sampling of compartment M2
2nd Jan 2003	Commencement of destructive sampling of compartment M7