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
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The results and conclusions in this report are based on a series of laboratory experiments. The conditions under which these experiments were carried out and the results have been reported with detail and 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.

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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.

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Practical Section for Growers

Commercial benefits of the project

It is hoped that the identification of bio-control products and micro-organisms with activity towards *Botrytis cinerea*, the cause of grey mould of tomato will enhance disease control strategies for UK tomato growers. A number of potential bio-control agents have been sourced and identified that have displayed antagonism *in vitro* against a range of *B. cinerea* isolates and some have been assessed for their activity on detached leaves and stem sections.

Background and objectives

UK tomato growers lead the world in achieving successful integrated pest management (IPM) strategies. However, this has focused attention on the continued use of fungicides for disease control, as several products disrupt pest/predator relationships. At the same time, there is concern by environmental and consumer groups, as the 'apparent' unabated use of pesticides on food crops continues to increase. Several leading growers have set in place a long-term objective of achieving a 'zero pesticide tolerance' for the UK tomato crop. This undoubtedly has set a significant challenge to the research community.

In a recent survey by the UK Tomato Growers Association (TGA), grey mould, caused by the fungus *Botrytis cinerea*, was identified as one of the most important problems affecting the industry. Previously, the pathogen caused extensive quality losses due to blemishes or 'ghost spotting' on the fruit. However, in recent years, ghost-spot has not been a significant problem (due primarily to improved environmental conditions in the glasshouse) and instead stem *Botrytis* has become more problematic causing plant losses due to girdling stem lesions (O'Neill, 1994). Most growers now incur considerable labour costs in an effort to minimise losses due to the disease.

Fungicides eg. benomyl, iprodione, have been used intensively in the past to improve control, though in recent years their efficacy has been compromised by the development of resistant and/or less sensitive strains of *B. cinerea* in the pathogen population. In the short-term, novel fungicides eg. pyrimethanil (Scala), azoxystrobin (Amistar) may alleviate the *Botrytis* problem. However, in the longer-term, the aim is to minimise and, hopefully, eliminate

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pesticide use from the protected crop environment and therefore alternative strategies must be sought.

There are already a few antagonists with claimed activity against *B. cinerea* either available commercially or are in the process of commercial development (eg Aspire, Biosave 100/110, Mycostop and Trichodex) though unfortunately not in the UK. It is also recognised in the literature that micro-organisms antagonistic to Botrytis occur naturally on the leaf and stem surfaces of various hosts (Church, 1992; Cohen *et al.*, 1996; Cook *et al.*, 1996; Elad *et al.*, 1994; Hausbeck & Pennypacker, 1991; Schmidt *et al.*, 1996; Sutton & Peng, 1993; Walter *et al.*, 1996; Whipps & McQuilken, 1993). It is possible that the widespread use of broad-spectrum fungicides on the tomato crop and, disinfectants during end of season clean-up procedures, in recent years may have reduced or eliminated many of the naturally occurring antagonists thereby exacerbating disease severity. The full potential of naturally-occurring antagonists in UK tomatoes has not been investigated in-depth previously, though studies on tomatoes at INRA, France (Nicot *et al.*, 1996) and O'Neill *et al.*, (1996) suggests it is an avenue of research worthy of further investigation.

It is, therefore, highly appropriate to reduce, if not eliminate, reliance on pesticides within this protected cropping environment. To develop a sustainable strategy that integrates effectively with IPM practices and to generate a broader understanding of the interactions between the pathogen and naturally occurring antagonists on the leaf/stem/truss surfaces and, where possible, harness those most effective for commercial exploitation. Such ecologically based strategies have been successful in developing several commercially available bio-control agents for other host-pathogen systems (Whipps, 1997).

The overall aims of this project are to:

(a) identify existing biological control agents with efficacy against *B. cinerea* and to determine their efficacy in relation to currently approved fungicides and

(b) investigate novel sources of biological control agents in situations where Botrytis stem rot does not appear to be problematic in tomatoes and to evaluate candidate organisms for their efficacy against *B. cinerea* compared to existing bio-control agents and fungicides.

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The **commercial objectives** that this project will address are an overall improvement in crop protection strategies by reducing labour, pesticide and other production costs, thereby increasing efficiency, improving the environmental and consumer acceptability of the harvested produce.

Summary of results and conclusions

- Eighteen bio-control products and isolates (BCAs), comprising 8 fungal and 10 bacterial, were collected from commercial companies and research organisations and placed in the reference collection.

- Attendance at the X11th International Botrytis Symposium, Reims, France allowed contact to be made with other researchers working on Botrytis and biological control of diseases and to provide an update on current research world-wide in these fields.

- A range of five isolates of *Botrytis cinerea* were sourced and isolated from commercial tomato crops and maintained in the laboratory for use in the BCAs screens.

- All the BCAs in the reference collection 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. Some of the BCAs exhibited large zones of inhibition of the growth of *B. cinerea* during these studies and the BCAs screened were ranked on their performance. Six products displayed large zones of inhibition and reduction in the radial growth of the pathogen.

- The BCAs showing antagonism in these laboratory screens, through changes in the growth patterns of *B. cinerea*, will be taken forward and assessed on tomato stem tissue. Some of the BCAs that did not perform well during these studies will also be assessed on plant tissue.

- A leaf and stem bioassay were assessed 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 bioassay, using conidial inocula, allowed the most realistic and reproducible conditions similar to infection development and pressure under commercial growing conditions.

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Action points for growers

From the work carried out in the first year of this project there are no specific recommendations to growers at this stage. A number of commercial products and isolates have been sourced and screened, however, further screening work is required to fully assess these BCAs. In addition, registration requirements for bio-control agents in the UK need to be considered carefully in respect to EU directives.

Anticipated practical and financial benefits

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 ca. £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.

This work should generate a better understanding of the microbial diversity in the tomato crop and, hopefully, generate an increased awareness of the potential for microbial antagonism against *Botrytis*. In addition, this work could, potentially, lead to new bio-control products effective against Botrytis. Although, further studies to support registration would be necessary before any novel bio-control products could be developed commercially. The potential UK market for any bio-control product is relatively small, assuming any bio-control products generated are specific to *B. cinerea* in tomato. The EU market on tomatoes however is significantly larger but because *B. cinerea* is unlikely to be non-host specific any effective antagonists identified could have a much broader appeal on other crops where the market size is vast in comparison eg. vines.

If effective control of this disease with zero or minimal use of fungicides could be achieved there would be a considerable impact on the industry. Apart from the immediate benefit to the consumer reaction to the reduced fungicide imput, pest control using IPM strategies would be improved and labour costs from managing the disease significantly reduced. There would also be considerable environmental benefits. Any reduction in pesticide use would be

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perceived favourably, particularly as *Botrytis,* alongside powdery mildew, is one of the primary targets for foliar applied fungicides. If pesticide inputs could be minimised whilst, at the same time, maintaining control of *Botrytis,* consumer reaction would be excellent.

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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, a laboratory bioassay for measuring the efficacy of these BCAs was developed using ‘live’ tomato plant tissue.

The main objectives for the first year of the project were to:

1. Attend the XIIth International Botrytis Symposium, Reims, France and produce a summary report on recent research progress on the bio-control of Botrytis.

2. Source reference isolates (up to 20) of commercially and other reported known micro-organisms with demonstrated antagonism to *B. cinerea*.

3. Collect a range of isolates of *B. cinerea* from commercial UK tomato crops to be used in the laboratory screening of these bio-control products.

4. Conduct challenge inoculations on agar of the selected bio-control products or micro-organisms with a range of *B. cinerea* isolates.

5. Evaluate a leaf bioassay and a stem bioassay to allow measurement of the antagonistic potential of the bio-control agents.

6. To commence screening the range of bio-control products using the selected bioassay technique.

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1. Summary Report from XIIth International Botrytis Symposium

1.1 Introduction

Dr G M McPherson, HRI and Dr T M O'Neill, ADAS attended the XIIth International Botrytis Symposium, Reims, France, 3-7 July 2000 entitled, Progress with Biological Control. There were delegates from 22 different countries as far afield as the USA to Australia. At the symposium It was possible to draw from the benefit of numerous Botrytis research groups world-wide, spanning molecular techniques for sub-species differentiation through epidemiological studies on a variety of susceptible crops to control using both fungicides and bio-control agents.

There were a number of key points, of relevance to HDC Project PC 174, which are detailed below. Abstracts of relevant papers on biological control of *Botrytis cinerea* are contained in Appendix I.

1.2 Biological control – points of interest

- *Ulocladium atrum* tested and shown effective in control of botrytis on tomato, grapevine, cyclamen, pot roses and pelargonium stock plants. Application in tomato crops (i) to petiole stubs (ii) to leaf debris on the floor. No antibiotics are produced; action is by suppression of sporulation and preventing colonisation of necrotic tissue (L29). Successful mass production and formulation was reported. *Difficulty in finding and getting investment by a commercial partner* (but Koppert involved in this project)

- Novel mode of action with *Brevibacillus brevis*, involving a surfactant which reduces drying time of plant surface wetness. Also production of an antifungal secondary metabolite, gramicidin - S, bound to bacterial spore (L25). *Effect of temperature on B. brevis activity was not reported.*

- Possible novel form of bio-control by inoculating plants with L - form bacteria (e.g. *Pseudomonas syringae*) which then become systemic and induce chitinase activity (L26). L-form bacteria present in the cells (in Bean at least) and not in the vascular tissues.

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Seems they are capable of passing through 0.2micron filters. *Control slight; not shown in tomato; possible resistance by consumer to inoculated plants?*

- *Bacillus subtilis* QST-713 now registered in USA and Chile, as Serenade, for control of grey mould on grape vine (L27).

- Bio-control of Botrytis bunch rot of grapes in New Zealand examined using naturally occurring fungi and yeasts. The fungus *Ulocladium* was the best of 125 isolations (L28). *Ulocladium was not as effective as iprodione in field trials.*

- Two strains of the yeast *(Pichia guillermondii)* ex USA were shown to control botrytis on tomato stems. Pre-and co-inoculation more effective than post-inoculation with the BCA (L30). Mode of action believed to be nutrient competition at spore germination stage. Work now focused on the mechanism of action - believed to be induced resistance. *Weekly applications used; not as effective as tolylfluanid.*

- Saprophytic *Fusarium* sp. successfully used to protect tomato pruning wounds (L36). Species in *Microdochium* group. *Risk of mycotoxins?* *Dichlofluanid inhibited the BCA* (21 other chemicals did not).

- Studies with various yeasts, including *Cryptococcus* spp. and *T. harzianum* (T39) applied weekly to tomato, cucumber, cut flowers and potted plants reported up to 50-60% control of *Botrytis*. Some yeasts reported to be compatible with fungicide treatment e.g. iprodione (Rovral).

- Work on a protein bio-pesticide reported. The product harpin ('Messenger' Eden Biosciences, Washington, NT), an SAR inducer, is derived from the pathogenic bacterium *Erwinia amylovora*, cause of fireblight in fruit and other rosaceous hosts.

1.3 General technical points

- Development of resistance to BCAs producing antibiotics is considered possible. It is less likely for a BCA acting by competition for nutrients (e.g. yeasts, *Ulocladium*). No reports of allergenicity with *U. atrum.*

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- There is an increased interest now in yeasts as BCAs. They are less fastidious for nutrients, do not produce toxins, can be grown easily on media, are usually unaffected by fungicides and are tolerant of a wide temperature range.

- Need to add Tween when applying yeasts (e.g. *Cryptococcus*). It is therefore necessary to ensure Tween alone is also applied alone as a control in comparative experiments.

- Temperature reported to affect tomato stem lesion expansion with the greatest expansion at warm temperatures (20-25^{0}C). The incidence of stem wound infection is little influenced by temperature (10-30^{0}C) or RH (50-90 %).

- Guttation fluid recorded for up to 3 days after leaf removal. Extended periods (7-14h) of stem wound wetness may occur. Guttation fluid stimulates *B. cinerea* conidial germination. Consider using guttation duration (measure it) to predict botrytis risk.

- Resistance to tomato stem botrytis identified in *Lycopersicon hirsutum*.

- Methods for isolating *Bacillus* species from tomato and this may be particularly useful in Project PC 174 (P53).

- Methods for investigating effect of temperature and RH on *B. cinerea* - BCA interactions (P57).

- *Ulocladium* acts by reducing botrytis sporulation on necrotic tissue. Will it provide effective bio-control if there is a high external inoculum of *B. cinerea* conidia?

- One report of *Ulocladium* (species not known) in Almond orchards causing infection of the eye.

- Some *Gliocladium* and *Fusarium* spp. are toxin producers and this could influence the commercial potential of these species as bio-control agents.

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1.4 Contacts

Contacts were renewed or established with key researchers currently active in biological control of botrytis including:

Aleid Dik, Naaldwijk, Holland
Yigal Elad, Voljcani Centre, Israel
Jurgen Kohl, Wageningen, Holland
Barrie Seddon, Aberdeen, UK
Brian Williamson, SCRI, Scotland
Phillipe Nicot, INRA, Montfavet, France
Harry Epton, Manchester, UK

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2. Securing a Reference Collection of Commercial or Reported Antagonists

2.1 Introduction

HRI Stockbridge House and ADAS, Arthur Rickwood, worked together to secure a reference collection of bio-control products and microbial antagonists (BCAs) from around the world with demonstrated activity towards *B. cinerea*. The collection of 18 BCAs was finally assembled following 8 months of detailed and extensive investigations to source known isolates that were either commercially available or in the experimental stage of development and were known to be active against *B. cinerea*. The isolates were found through making contact with fellow researchers around the world with known expertise in this area, scientific colleagues, literature searching or by searching on the Internet. A number of the isolates required commercial agreements for the release and use of the micro-organisms in the project.

2.2 Reference collection secured

In total, 18 BCAs were secured in the reference collection. These have been coded A1 to A9 and H1 to H9, depending whether they were sourced by ADAS (A) or Stockbridge House (H). In order to obtain certain commercial products, experimentation agreements were developed and signed in consultation with ADAS and HRI legal advisers.

Isolate subcultures and product samples were maintained or stored at HRI Stockbridge House and ADAS Arthur Rickwood following product guidelines or scientific advice prior to use in subsequent experimental work.

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3. The Evaluation of Potential Antagonists in Agar Plate Tests

3.1 Introduction

Studies were undertaken to assess the potential for antagonism of the bio-control products and antagonistic micro-organisms (BCAs) *in vitro* through dual culture 'challenge inoculations'. Here the BCAs are cultured on agar media inside a Petri dish a few centimeters away from an isolate of *B.cinerea*. The antagonist and the *B. cinerea* grow across the plate and eventually they meet. If there is any level of inhibition exhibited by the antagonist then a 'zone of inhibition' will develop between the two colonies. The shape of the developing *B. cinerea* colony may also be distorted. These parameters can all be measured to allow the assessment of the efficacy of the BCAs. All the BCAs tested were ranked according to their antagonistic potential against the five isolates of *B. cinerea.*

3.2 Collection of *B. cinerea* isolates

Five isolates of *B.cinerea* were collected from commercial tomato crops and successfully cultured. Cultures of the five isolates were duplicated and split between ADAS, Arthur Rickwood and HRI, Stockbridge House. These isolates were then used in the agar plate and bioassay tests. The isolates were as follows:

Table 3.1: Five isolates of *Botrytis cinerea* obtained active infection in UK tomato crops

No.	Origins of Isolate	ADAS/HRI Code	Code Name
1	ADAS Plant Clinic ex Commercial crop in Sussex	2254	BC 01
2	Commercial tomato crop – Somerset	00/51	BC 02
3	ADAS Plant Clinic ex commercial crop - Monmouthshire	2847	BC 03
4	ADAS plant Clinic ex commercial tomato crop - Kent	3305	BC 04
5	Tomato crop at HRI Stockbridge House	M18(1)	BC 05

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3.3 Materials and methods

The treatments were comprised of the 18 microbial antagonists as given in Table 2.1. Each of the microbial treatments was cultured with each of the five *B.cinerea* isolates as given in Table 3.1. The challenge inoculations were replicated three times.

Some of the microbial antagonists were supplied as living cultures in tubes or plates. While others were supplied as commercial products in dried powder form or as frozen granules. In each case the organism contained in the commercial product was extracted from the formulation by growing in pure culture before being used in the challenge inoculations. The method for testing bacterial and fungal antagonists varied with the two slightly different methods explained in the following two sections. Illustrations of the layout of the challenge inoculations for both bacterial and fungal antagonists are given in Appendix II.

A. Bacterial bio-control agents

The bacterial isolate was applied to the periphery of a petri dish containing PDA (Potato Dextrose Agar) as a thick band comprising of a streak 4 cm long of the test isolate. The *Botrytis cinerea* isolate was inoculated in the centre of the petri dish as a 5mm-agar plug taken from the edge of an actively growing colony of *B. cinerea.* In each instance the placement of the bacterial isolate and agar plug follows that of a standard template (see Figure 1 in Appendix II). Test cultures were replicated three times.

B. Fungal bio-control agents

The fungal isolate and *B.cinerea* were inoculated onto a petri dish containing PDA on opposite sides of the petri dish. Both the pathogen and the antagonist were taken from the edge of an actively growing colony as 5mm-agar plugs.

The placement of the two fungal isolates follows that of a standard template (see Figure 2 in Appendix II). Test cultures were replicated three times.

The challenge inoculation plates were incubated at 22^0C for 5-7 days after which measurements were made of the mycelial growth as given below in assessments.

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Assessments

The growth of *B. cinerea* on the challenge inoculation was assessed using two parameters for the growth of the *B. cinerea* isolates. These allowed discrimination between the inhibition of the growth of *B. cinerea* and the type of interaction, e.g. inhibition at a distance or by contact.

A. Bacterial Bio-Control Agents

A determination of the level of inhibition exhibited by the bio-control agents was calculated using the following parameters:

- A measurement of the width of the zone of inhibition (ZI),
- The radial growth of the *B.cinerea* colony as shown in the diagram in Figure 3 in Appendix III.

From the measurements of radial growth, a calculation of the percentage inhibition of growth was made using the following equation:

$$(100 \times \frac{(r_1 - r_2)}{r_1} = \text{\% inhibition of growth of } B.\ cinerea.$$

The mean results for (ZI) and % inhibition of growth were then plotted.

B. Fungal Bio-Control Agents - A determination of the level of inhibition exhibited by the bio-control agents is calculated using two parameters:

- A measurement of the width of the zone of inhibition (ZI),
- The radial growth of the *B. cinerea* colony as shown in the diagram in Figure 4 in Appendix III.

From the measurements of radial growth a calculation of the percentage inhibition of growth was made using the following equation:

$$100 \left[\left[\frac{(r2 + r3)}{2} - r1 \right] / \frac{(r2 + r3)}{2} \right]$$

The mean results for (ZI) and % inhibition of growth were then plotted.

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A range of characteristics other than zone of inhibition of growth and inhibition of pathogen mycelial growth can be recorded during the challenge inoculations including discoloration of the media, browning of mycelium and the development of sclerotia over the plate.

3.4 Results and discussion

The mean results for the challenge inoculations performed at HRI, Stockbridge House and ADAS, Arthur Rickwood on all of the reference collection are displayed in two graphs (Figures 3.1 and 3.2), with the mean data per *B. cinerea* isolates tabulated in Appendix III.

In general there are similarities between the performance of the antagonistic isolates screened at Stockbridge House and ADAS Arthur Rickwood when measuring the zone of inhibited growth and the percentage inhibition of radial growth of *B. cinerea.* Large variations between the BCAs screened from the reference collection were observed during the *in vitro* challenge inoculations. The performance of the BCAs has been ranked and is presented in Table 3.2. The experimental product, H9 performed best at all times with inhibition zones of between 6 and 13 mm and inhibition of radial growth between 41.4% and 70.8% *in vitro*. Variation in growth rates among candidate BCAs can affect the radial growth of the pathogen independent of its antagonistic actions, whereas the zone of inhibition remains unaffected. In this study the challenge inoculation tests were carried out until there was an equilibrium of growth when the results presented were recorded.

When the BCAs were characterised by the presence or absence of inhibition zones of the pathogen's growth *in vitro*, the products performed similarly in both laboratories. However, two products that gave the largest variation between the results recorded at Stockbridge House and ADAS Arthur Rickwood were two *Bacillus* isolates (H6 and A5). However, there was little variation with the screens carried out at each laboratory on the results recorded between the five Botrytis isolates (Appendix III).

With some BCAs, a negative inhibition zone was recorded ie the pathogen overgrew the test BCA colony. There are a range of other laboratory *in vitro* screening tests have been documented (Jackson *et al.*¸ 1994, 1997) although they are time consuming and do not always portray what would happen on plant material in a commercial cropping situation. Therefore

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the main difficulty in screening micro-organisms is the decision the disregard potential antagonists on the basis of failure to cause inhibition on agar (Fokkema, 1976).

The screening of micro-organisms *in vitro* is based on the candidate organism causing interference with pathogen growth or disease development. In general interference of growth was seen as some form of disruption or inhibition to the mycelium growth and development of *B. cinerea* mycelium *in vitro.* Measuring radial growth of *B. cinerea* alone does not take account of variation in growth rates among the different bacterial and fungal BCAs screened *in vitro*. However, isolates showing a zone of inhibition of zero or greater does indicate potential antagonism. Therefore failure of candidate to produce inhibition zones *in vitro* should not necessarily lead to rejection as some modes of antagonism are not detected in this way eg nutrient competition as nutrient levels in agar are not representative of the phyllosphere (Blakeman & Fokkema, 1982).

However, the use of *in vitro* techniques allows larger numbers of candidates to be screened reasonably quickly. Results in agar tests can be visually striking with zones of inhibited growth, being easy to conduct and interpret allowing environmental conditions to be controlled (Andrews, 1985). In addition, *in vivo* techniques can be too severe, rejecting potentially useful candidate BCAs. It has also been pointed out that experiments carried out *in vitro* can be poor predictors of *in vivo* performance with little correlation demonstrable between culture effectiveness in the filed (Andrews, 1985). Mechanisms, such as ability to grow on the plant surface as a saprophyte cannot be measured in Petri dishes screening *in vitro* (Elad, 1990).

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Table 3.2: BCAs ranked in order of antagonism demonstrated during *in vitro* screening[1]

ADAS	HRI
Zone of inhibition present	
H9	H9
A8	A8
H8	A1
A1	A7
A7	A3
A3	A2
H5	H5
H3	H8
A2	H7
H1	H3
	H1
	A5
No zone of inhibition (contact inhibition)	
H4	A4
A9	H4
A4	A9
Pathogen overgrew antagonist (no inhibition)	
A6	H2
A5	H6
H7	A6
H6	
H2	

[1] BCAs ranked in order of mean zone of inhibition measurement first followed by the mean percentage inhibition of radial growth.

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Figure 3.1: HRI, SH - Mean Level of Inhibition shown by 18 Microbial Antagonists to Five Isolates of *Botrytis cinerea*

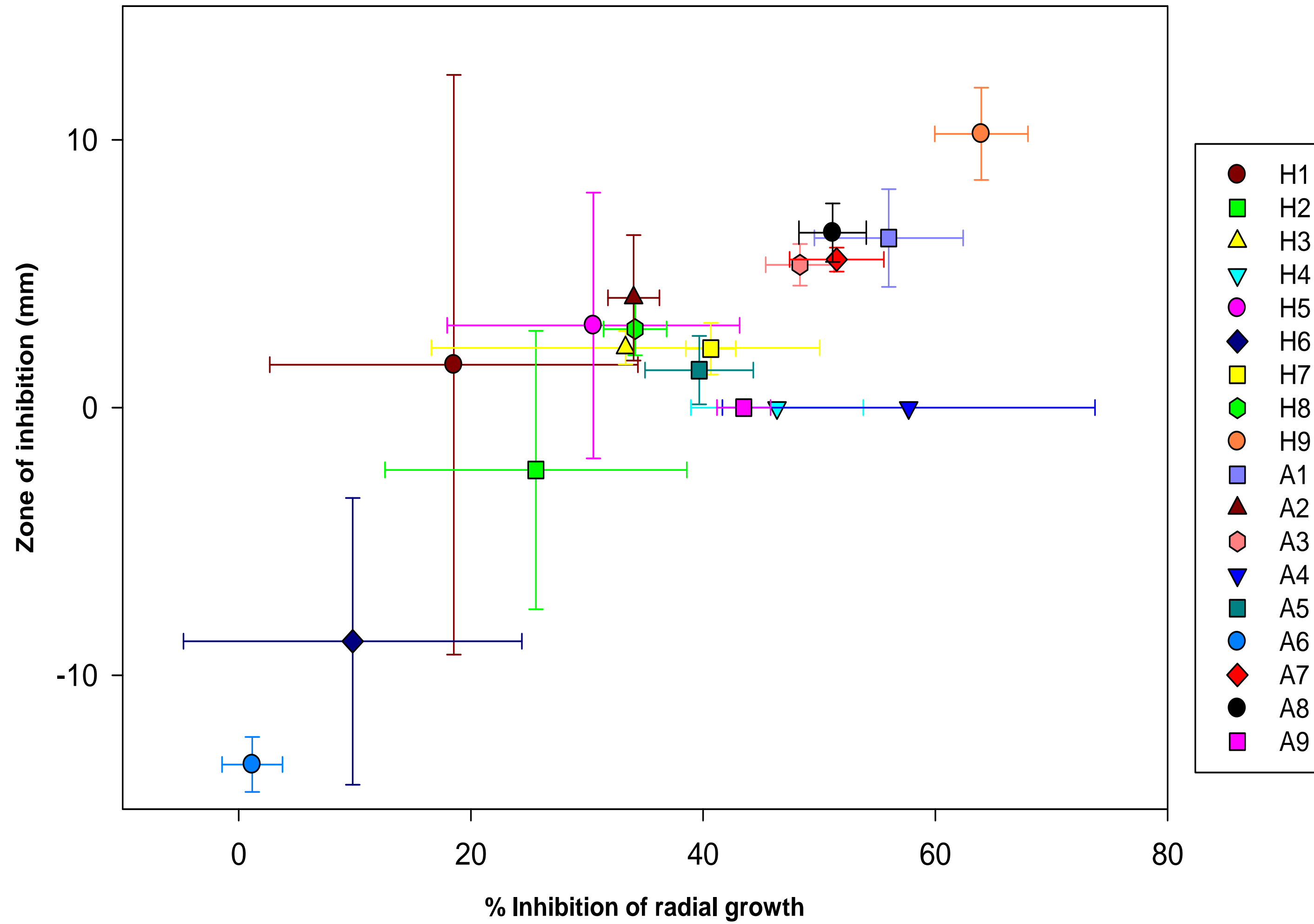

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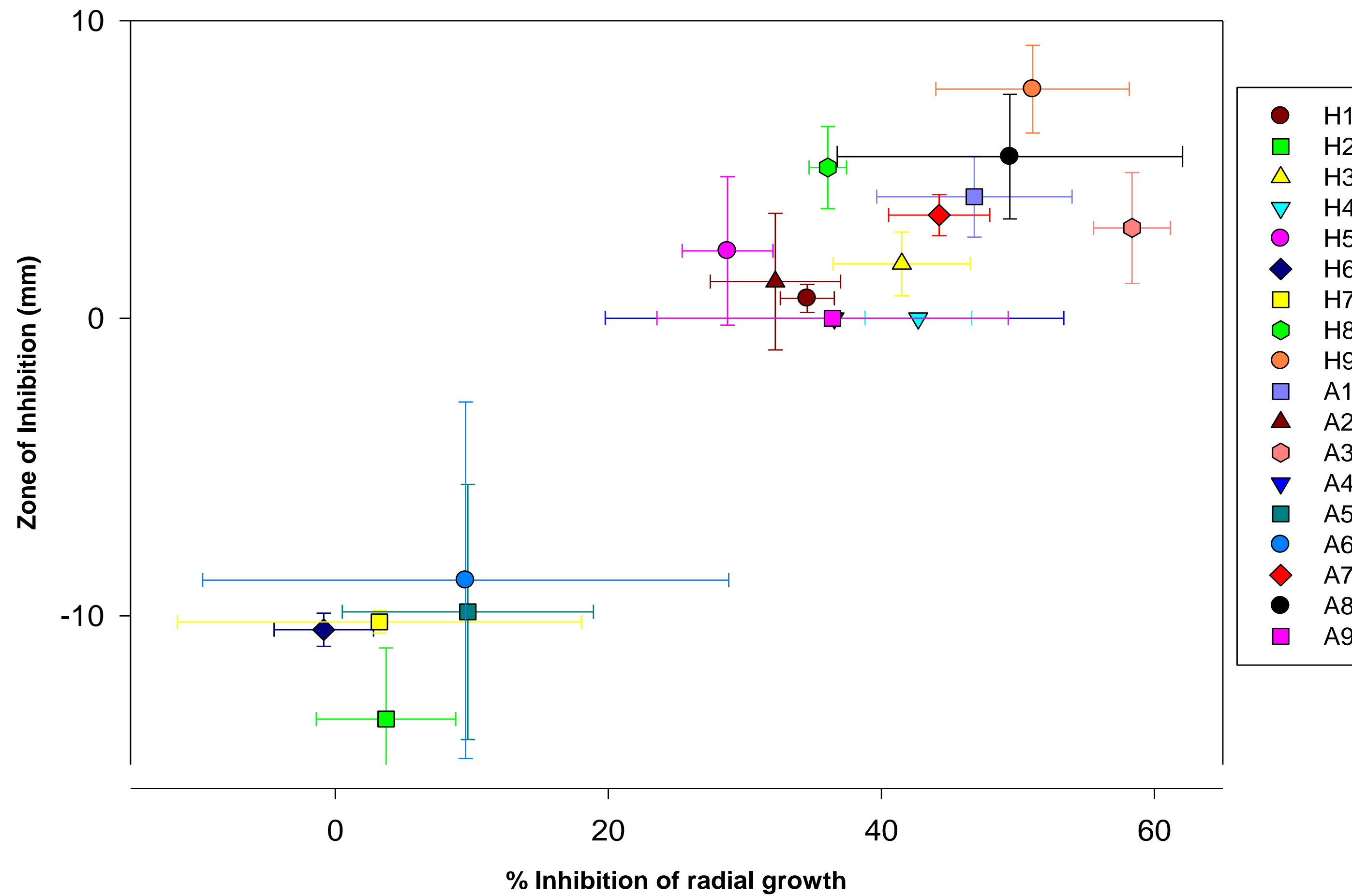
Figure 3.2: ADAS, AR - Mean Level of Inhibition shown by 18 Microbial Antagonists to Five Isolates of *Botrytis cinerea*



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3.5 Conclusions

1. A range of five isolates of *Botrytis cinerea* were isolated from commercial tomato crops and maintained *in vitro* for use in laboratory screening experiments.

2. The active micro-organisms from the range of BCAs sourced for the reference collection were successfully screened *in vitro* in replicated challenge inoculations.

3. A number of BCAs consistently developed large zones of inhibited growth of *B. cinerea* mycelial growth *in vitro*. These included (in performance order) H9, A1, A7, A8, A3, H5, H8, H3 and H1. In addition, there were large variation within the measurements of percentage inhibition of *B. cinerea* mycelial growth among the BCAs tested.

4. Three isolates (H4, A9 and A4) did not form a zone of inhibited growth of *B. cinerea* but stopped further development of mycelial growth of Botrytis across the candidate BCA in the Petri dish.

5. H2 and A6 were overrun by mycelium of the *B. cinerea* isolates screened in the challenge inoculations. The performance of two BCAs, H6 and A5 was variable during screening experiments at Stockbridge House and Arthur Rickwood.

6. Of all the candidate BCAs screened *in vitro*, at both laboratories (Stockbridge House and Arthur Rickwood) similar results were obtained when the BCAs were ranked according to inhibition zones and inhibition of radial growth in the Petri dishes.

7. A number of BCAs did not exhibit zones of inhibition or large percentages of inhibition of growth, where the pathogen mycelium overgrew the candidate BCA. The lack of symptoms of antagonism *in vitro* will not necessarily lead to rejection from further screening on plant material.

8. The results from this initial screen gave an indication of potential performance for further screening to be carried out during the course of this experiment.

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4. The Development of a Bioassay to Measure the Potential of Microbial Antagonists

4.1 Introduction

Studies were undertaken to develop a leaf bioassay and a stem bioassay for measurement of the antagonistic potential of bio-control products and isolates (BCAs) collected during the project. Our objective was to devise a bioassay in which: i) conditions are close to the natural environment (ie the use of tomato tissue which is regularly infected by *B. cinerea*, and inoculation and incubation conditions which are similar to those occurring in commercial greenhouses); ii) inoculation and incubation conditions can be controlled; iii) the bioassay is compact, so that a large number of candidate BCAs can be screened at one time; iv) the bioassay is relatively quick to run (1-2 weeks); v) the degree of tissue rotting can be quantified and the candidate BCA products/isolates thereby differentiated on their bio-control efficacy. Ideally, a final screen bioassay will involve whole plants rather than detached tissues.

A leaf bioassay involving modification of a standard technique was developed at HRI Stockbridge House. Stem bioassays were developed and evaluated at ADAS Arthur Rickwood. Stem bioassay experiments 1-3 were conducted using stem pieces. A modified bioassay was then developed which allowed the use of intact young plants, and further experiments were undertaken utilising this system.

4.2 Materials and methods

B. cinerea isolate BC02, originally isolated from a tomato stem lesion, was used for all the experiments described in this section. Four BCAs, comprising four different fungal or bacterial species, were selected for testing during bioassay development. The products/isolates were used at the following rates (according to recommendations from manufacturers or scientific literature) unless stated otherwise:

H9	3 g in 1 litre water (10^{10} cfu per g of product)
A2	0.4 g in 1 litre water (minimum of 10^{7} cfu per g of product)
H1	10^{7} spores/ml
A3	0.5 g/l (minimum of 10^{11} cfu per g of product)

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Leaf bioassay

Experiment 1 Aim: To test the effect of BCAs applied to leaves 2 h prior to inoculation with mycelial plugs of *B. cinerea.*

Each test bio-control agent was sprayed onto separate tomato plants, in a propagation glasshouse with a hand-held lance sprayer. After each spray was dry (2 h), three replicate samples of ten leaves representing a range of age categories were harvested. In the laboratory, leaflets were excised from the leaves and placed inside humid chambers containing moist blotting paper.

A 5 mm-diameter agar plug from a 7-day old culture of *B. cinerea* was inoculated into the centre of each of the incubated leaflets. The inoculation site immediately below the agar plug was wounded by inserting a sterile needle through the leaf surface to create a puncture to aid infection. The inoculated leaflets were kept in the humid chambers and out of direct light at ambient room temperature for 7 days. The diameter of the resulting leaf lesions was measured after 5 and 7 days. When lesions were irregular in shape, two measurements were used to calculate the mean diameter. Measurements are presented as the lesion diameter minus the width of the agar plug (5 mm).

Experiment 2 Aim: To test the effect of BCAs applied to leaves 24 h prior to inoculation with a spore suspension of *B. cinerea.*

Whole tomato plants were sprayed with bio-control products as described in Experiment 1. The leaves were harvested after 24 h. Leaflets were excised then wounded and inoculated with a 100 µl droplet of a spore suspension of *B. cinerea* (10^4 spores/ml) in sterile distilled water (SDW). After 5 days of incubation, no symptoms were visible and the leaflets remained healthy. The leaflets were subsequently inoculated with 5-mm agar plugs of *B. cinerea*, then assessed for lesion diameter after 5 days of incubation.

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Stem bioassay

Experiment 1   Aim: To test the effect of BCAs applied to stem pieces 1 h prior to inoculation with mycelial plugs of *B. cinerea.*

Stem pieces of 4-5 cm in length were cut from the side shoots of glasshouse-grown tomato plants (cv. Espero). The top 10 cm of stem were discarded. The stem pieces were rinsed in sterile distilled water and wrapped in moist tissue paper until required. Plant pots (9-cm diameter) were filled with autoclaved vermiculite moistened with distilled water. Five stem pieces were vertically inserted into each pot, representing a plot. The stems were inoculated to the point of run-off with the BCAs or distilled water (control) using a hand-held mister. H1 was used at 2.5 x 10^4 spores/ml because insufficient spores were present on the isolates. Distilled water was used as a control treatment. Each treatment was replicated four times.

One hour after application of the BCAs, each stem end was inoculated with a 5-mm plug from the leading edge of a 7-day old culture of *B. cinerea*. The pots were placed within individual perforated polythene bags to prevent cross-contamination. The stem pieces were incubated in a controlled environment (CE) cabinet at 20°C, 70 % RH, 16 h day/8 h night and were misted daily. Illumination was approximately 250 µmol.m^{-1}. Stem lesion length and sporulation intensity (0-4 index) were assessed after 4 days.

Experiment 2   Aim: To test the effect of BCAs applied to stem pieces 24 h prior to inoculation with a spore suspension of *B. cinerea.*

Stem pieces were prepared and inoculated with the BCAs or distilled water as described in Experiment 1. After 24 h, each stem end was inoculated with 100 µl of a spore suspension of *B. cinerea* (10^5 spores/ml). Incubation and disease assessments were as in Experiment 1.

Experiment 3   Aim: To test the effect of one bio-control agent applied to stem pieces at different time intervals prior to inoculation with mycelial plugs of *B. cinerea.*

Stem pieces were prepared as in Experiment 1 and sprayed with A3 or distilled water (control). The stem pieces were incubated as previously and inoculated with 4 mm plugs of *B.*

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cinerea (7-day-old culture) after 24 h, 48 h or 72 h. Disease assessments were undertaken 6 days after inoculation with *B. cinerea* as in Experiment 1.

<u>Experiment 4</u> Aim: To test the effect of 10% V8 juice and glucose (0.1M) plus potassium dihydrogen phosphate (0.07M) on germination of *B. cinerea* conidia.

The effect of 10% V8 juice and glucose (0.1M) plus potassium dihydrogen phosphate (0.07M) on germination of *B. cinerea* conidia on glass slides was compared after 24 h, using three spore concentrations.

<u>Experiment 5</u> Aim: To establish a technique for infection of whole young plants with *B. cinerea.*

Tomato plants raised in rockwool blocks were used (4-8 weeks old, 25-30 cm in height). Petioles from the side of the stems were removed but top leaves were left intact. The plants were removed from the rockwool blocks, placed with their roots in plastic vials and laid horizontally on tissue paper in trays. Wounds were made at 4-5 cm intervals along the plant stem, with four wound sites per stem. Wounding treatments were either a prick with a sterile needle or a shallow scalpel scrape (5 mm length). The wound sites were inoculated with *B. cinerea* (isolate BC02) either as a plug (4 mm diameter) or as a 20 µl droplet of spore suspension (4.4 x 10^5 spores/ml). The spore suspension included 10% V8 juice because results from Experiment 4 showed that this improved percentage spore germination. The spore suspension was applied either immediately after it had been made or after 24 h. A stem with four inoculation sites represented a plot and each treatment combination was replicated three times.

The stems were left on damp tissue paper in trays covered with polythene bags for 24 h, to allow the spore droplets to dry. The plants with their plastic vials filled with water were then transferred to a CE cabinet, for incubation at 20°C, 60 % RH and 16 h day/8 h night with daily misting. Illumination was approximately 875 µmol.m^{-}1. The incidence of lesion development was recorded 7 days after inoculation. All of the plants were enclosed within a polythene tent to prevent tissues drying out rapidly.

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Experiment 6 Aim: To test the effect of one bio-control agent applied to whole young plants 44 h prior to inoculation with *B. cinerea* with different inoculum types and wounding techniques.

Plants were prepared as described in Experiment 5. The treatments tested are listed below and the experimental design was as in Experiment 5:

	B. cinerea inoculum	Wound type	Bio-control agent
1	Spore suspension	Scalpel scrape	nil
2	Mycelial plug	Needle prick	nil
3	Mycelial plug	Scalpel scrape	nil
4	Spore suspension	Scalpel scrape	A3
5	Mycelial plug	Needle prick	A3
6	Mycelial plug	Scalpel scrape	A3

The spore suspension (9 x 10^5 spores/ml) containing 10% V8 juice, was prepared 24 h prior to inoculation and was applied as 20 µl droplets. The mycelial plugs were 4 mm in diameter from a 7 day-old culture. A3 was used at 0.4 g/litre and was sprayed onto the plants 44 h prior to inoculation with *B. cinerea.* After spraying with A3, the plants were maintained in the CE cabinet (conditions as in Experiment 5) until inoculation with *B. cinerea*, then returned to the cabinets after 1 h. Lesion length was measured 5 and 9 days after inoculation.

Experiment 7 Aim: To establish a technique to infect stems of whole young plants with a spore suspension of *B. cinerea.*

Plants were prepared as described in Experiment 5. The treatments tested are listed below and the experimental design was as in Experiment 5:

Wound type	Spore suspension nutrient	Time before transfer to CE cabinet
Scalpel scrape	V8	1 h
Scalpel scrape	V8	24 h
Scalpel scrape	glucose and phosphate	1 h
Scalpel scrape	glucose and phosphate	24 h
Needle prick	V8	1h
Needle prick	V8	24 h
Needle prick	glucose and phosphate	1h
Needle prick	glucose and phosphate	24 h

A spore suspension of *B. cinerea* was prepared (5 x 10^5 spores/ml) and amended with either 10% V8 juice or glucose (0.1 M) and potassium dihydrogen phosphate (0.07 M), to improve

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spore germination. The spore suspensions were left for 3 h prior to inoculation. Wounds were made as in Experiment 4, except that needle pricks were widened to make a slightly larger wound site. Spore suspension droplets of 20 μl were applied to each wound site. For half of the treatments, spore droplets were allowed to dry for 1 h before the plants were placed in module trays and transferred to the CE cabinet. The remaining plants were maintained under conditions of high humidity (on moist tissue paper in trays enclosed in polythene bags) for 24 h before transfer to the CE cabinet. Incubation conditions were as in Experiment 5. Lesion length was assessed 9 days after inoculation.

4.3 Results and discussion

Leaf bioassay

The technique used for the leaf bioassay in Experiment 1 (inoculation with mycelial plugs) was successful in consistently initiating *Botrytis* lesion development on leaves. However, none of the BCAs reduced lesion development on the leaves when they were applied just before inoculation with *B. cinerea* (Table 4.1). Factors preventing poor performance of the BCAs could have been i) insufficient time for establishment of the bio-control organisms on the leaf surface prior to inoculation with the pathogen, and ii) unrealistically high inoculum pressure resulting from the use of mycelial plugs.

Table 4.1: Lesion development on tomato leaves inoculated with mycelial plugs of *B. cinerea* approximately 1 h after treatment with four BCAs.

Bio-control agent	Mean lesion measurement (mm) after 5 days	Mean lesion measurement (mm) after 7 days
Control	19.4 (1.63)[a]	39.4 (2.64)
H5	24.2 (2.27)	47.0 (3.33)
A2	20.0 (2.67)	36.8 (3.13)
H1	19.7 (2.37)	36.5 (3.42)
A3	21.7 (3.13)	40.0 (3.54)

[a] Standard errors in parenthesis

In Experiment 2, there was no lesion development on any leaflets 5 days after inoculation with a spore suspension. Five days after leaf re-inoculation with mycelial plugs, lesion development had occurred and there was an apparent reduction in lesion diameter on leaflets treated with BCAs compared with the control treatment, particularly for A2, H1 and A3

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(Table 4.2). This result suggested that a period of several days between BCA treatment and Botrytis infection might improve the efficacy of a BCA.

Although the leaf bioassay used in Experiment 2 was found to be an efficient system and was successful in evaluation BCA performance, it was not repeated since it was considered that a stem bioassay could provide a more accurate representation of the development of stem Botrytis in a glasshouse.

Table 4.2: Lesion development on tomato leaves inoculated with mycelial plugs of *B. cinerea* 6 days after treatment with four BCAs.

Bio-control agent	Mean lesion diameter (mm) 5 days after inoculation
Control	11.1 (4.10)[a]
H5	8.2 (4.35)
A2	0.0 (0)
H1	1.4 (1.11)
A3	0.0 (0)

[a] Standard errors in parenthesis

Stem bioassay

Sporulating stem lesions developed consistently on incubated stem pieces inoculated with mycelial plugs of *B. cinerea*. However, application of BCAs 1 h prior to pathogen inoculation did not reduce stem lesion development in Experiment 1 (Table 4.3). As in the leaf bioassay, it was considered that inoculum pressure may have been too high or that there was insufficient time for BCA establishment prior to inoculation.

Table 4.3: Lesion development on tomato stems inoculated with *B. cinerea* 1 h after treatment with four BCAs (stem experiment 1).

Bio-control agent	Mean stem lesion length (mm) after 4 days	Mean sporulation index[a] after 4 days
Control	26.0 (0.84)[b]	2.9 (0.08)
H5	29.7 (0.73)	2.9 (0.08)
A2	29.6 (0.90)	2.9 (0.08)
H1	27.4 (1.01)	2.6 (0.15)
A3	26.3 (1.25)	3.0 (0.05)

[a]Sporulation index:
0 = no sporulation; 1= sporulation on stem end only; 2=sporulation on up to 25% stem length; 3=sporulation on 25-75% stem length; 4=sporulation on >75% stem length.

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[b]Standard errors in parentheses

In Experiment 2, there was no stem lesion development following inoculation of stems with spore suspension. This might have been due to wound healing (reduced susceptibility) in the 24 hours between stem preparation and inoculation with spores. Later experiments on whole plants investigated, inoculation immediately after wounding, the addition of nutrients to the spore suspension and preparation of the suspension several hours prior to inoculation, in order to improve the infection rate.

Experiment 3 again showed consistent development of lesions on stems inoculated with mycelial plugs of *B. cinerea*. There was no effect of A3 (shown in the leaf bioassay and agar challenge tests to have good antagonistic potential) on lesion development, even when it was left for 3 days on the plant to colonise before pathogen inoculation (Table 4.4). It was considered that inoculum pressure was too high.

Table 4.4: Lesion development on tomato stems after inoculation with *B. cinerea* at three time intervals after treatment with A3 (stem experiment 3).

Treatment	Mean stem lesion length (mm) after 6 days	Mean sporulation index after 6 days
Control + BC02 after 24h	24.6 (1.34)[a]	2.4 (0.13)
A3 + BC02 after 24 h	25.4 (1.24)	2.0 (0.14)
Control + BC02 after 48 h	23.9 (1.52)	2.3 (0.15)
A3 + BC02 after 48 h	19.3 (1.88)	2.1 (0.15)
Control + BC02 after 72 h	23.5 (1.96)	2.2 (0.14)
A3 + BC02 after 72 h	23.7 (2.07)	2.3 (0.14)

[a]Standard errors in parentheses

Cut stems provided a convenient bioassay system that was economical in the amount of stem material needed and space required. Subsequent experiments were, however, conducted using whole young plants as it was felt that a growing plant with slight stem wounding would provide more resistance to infection than cut stem pieces, thus allowing BCA performance to be differentiated.

In Experiment 4, both 10% V8 juice and glucose (0.1M) plus potassium dihydrogen phosphate (0.07M) greatly increased the incidence of spore germination after 24 hours, compared with sterile de-ionised water (Table 4.5). V8 juice resulted in extensive germination and germ tube growth with abundant hyphal branching. Glucose and phosphate

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resulted in good germination, with germ tubes up to 15 x spore length and less branching than with V8 juice. In de-ionised water, those spores that had germinated produced germ tubes barely longer than the spore itself.

Table 4.5: Effect of added nutrients on germination of *B. cinerea* conidia on glass slides.

Treatment	Spore concentration (no. conidia/ml)	Mean no. germinating spores (24 hours)
10% V8 juice	1×10^4	100
10% V8 juice	1×10^5	100
10% V8 juice	1×10^6	100
Glucose + phosphate	1×10^4	28
Glucose + phosphate	1×10^5	98
Glucose + phosphate	1×10^6	100
SDW	1×10^4	2
SDW	1×10^5	0
SDW	1×10^6	1

In Experiment 5, lesions developed on all wound sites inoculated with mycelial plugs (Table 4.6). There was no lesion development on wounds inoculated with spore suspension in V8 juice used immediately after preparation, irrespective of wounding technique. The infection rate increased when the spore suspension was made 24 h before inoculation, with 75 % lesion development on scalpel wounds and 17 % lesion development on needle prick wounds.

Table 4.6: Stem lesion development on tomato plants after inoculation with different *B. cinerea* inoculum types and wounding techniques (stem experiment 5).

Inoculum	Wound type	*Botrytis* lesions per stem (out of 4)		
		Block 1	Block 2	Block 3
Spore suspension (after 24 h)	Scalpel scrape	4	3	2
Spore suspension (after 24 h)	Needle prick	2	0	0
Spore suspension (0 h)	Scalpel scrape	0	0	0
Spore suspension (0 h)	Needle prick	0	0	0
Mycelial plug	Scalpel scrape	4	4	4
Mycelial plug	Needle prick	4	4	4

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In Experiment 6, there was no lesion development on stems inoculated with spore suspension in V8 juice, while the majority of wound sites (scalpel scrape and needle prick) inoculated with mycelial plugs developed lesions (Table 4.7). There was no consistent effect of A3 on lesion development.

Table 4.7: Stem lesion development on tomato plants after inoculation with different *B. cinerea* inocula and wounding techniques, 44 h after treatment with A3 (stem experiment 6).

Inoculum	Wound type	Bio-control agent	Mean no. lesions per stem (out of 4)	Mean stem lesion length (mm) (5 days)[a]
Spore suspension	Scalpel scrape	Nil	0.0	0.0 (0.00)
Mycelial plug	Needle prick	Nil	3.7	6.8 (2.74)
Mycelial plug	Scalpel scrape	Nil	4.0	31.0 (3.40)
Spore suspension	Scalpel scrape	A3	0.0	0.0 (0.00)
Mycelial plug	Needle prick	A3	4.0	13.7 (3.59)
Mycelial plug	Scalpel scrape	A3	4.0	26.8 (2.83)

[a]Standard errors in parentheses

In Experiment 7, the use of glucose and phosphate as a nutrient amendment in the spore suspension followed by incubation for 24 h under conditions of high relative humidity before transfer to the CE cabinet, resulted in lesion incidence ranging from 68 – 82%, depending on wound type (Table 4.8). Although not all wound sites developed lesions under these conditions, it is possible that this level of infection may be more appropriate for evaluation of BCAs, rather than use of inoculation conditions tested in previous experiments which resulted in development of severe lesions on all wound sites.

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Table 4.8: Stem lesion development on tomato plants following inoculation with nutrient-amended spore suspension of *B. cinerea* and different incubation conditions (stem experiment 7).

Wound type	Spore suspension nutrient	Time before transfer to CE cabinet (h)	Mean no. lesions per stem (out of 4)	Mean stem lesion length[a] (mm)
Scalpel scrape	V8	1	0.3	0.4 (0.42)
Scalpel scrape	V8	24	0.0	0.0 (0.00)
Scalpel scrape	Glucose and phosphate	1	2.3	4.7 (1.28)
Scalpel scrape	Glucose and phosphate	24	3.3	14.7 (3.78)
Needle prick	V8	1	0.0	0.0 (0.00)
Needle prick	V8	24	0.0	0.0 (0.00)
Needle prick	Glucose and phosphate	1	0.7	0.8 (0.56)
Needle prick	Glucose and phosphate	24	2.7	13.3 (4.83)

[a]Standard errors in parentheses

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4.4 Conclusions

1. Inoculation of detached wounded tomato leaves with mycelial plugs of *B. cinerea* consistently resulted in spreading lesions. However, this method appears to be too severe a challenge to allow differentiation of micro-organisms with bio-control activity.

2. Inoculation of detached wounded tomato leaves with conidial inocula of *B. cinerea* in SDW failed to cause lesions within 5 days.

3. Inoculation of the cut end of detached tomato stem pieces with mycelial plugs of *B. cinerea* on agar consistently resulted in stem rotting. However, as with the leaf bioassay, this method appeared too severe a challenge to allow the selection of micro-organisms with bio-control activity.

4. Inoculation of the cut end of detached tomato stem pieces with *B. cinerea* conidia in SDW, followed by incubation at 20°C and 70% RH, failed to cause lesions within 7 days. Possibly the RH was insufficiently high to allow infection to occur.

5. Application of BCAs to the cut end of detached tomato stem pieces 24-72 hours prior to inoculation with *B. cinerea* on agar plugs, failed to demonstrate any bio-control activity. Possibly the inoculum was still too high (see 3 above).

6. Addition of 10% V8 juice, and glucose (0.1M) plus potassium dihydrogen phosphate (0.07M), to *B. cinerea* conidia on glass slides greatly increased the rate of germination, compared with de-ionised water. V8 juice appeared the more effective at low spore concentrations, while the two nutrients were equally effective at higher spore concentrations.

7. Inoculation of fresh wound sites (needle prick or scalpel scrape) on the stems of young tomato plants with mycelial plugs of *B. cinerea* consistently resulted in spreading lesions after 7 days.

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8. Inoculation of fresh wound sites (needle prick or scalpel scrape) on the stems of young tomato plants with 24h pre-germinated conidia of *B. cinerea* in SDW, but not ungerminated conidia, resulted in stem lesions at 17-75% of inoculation sites.

9. The biocontrol product A3 applied to fresh wounds on stems of young tomato plants 44 hours prior to inoculation with mycelial plugs of *B. cinerea* failed to reduce infection or lesion development.

10. Inoculation of fresh stem wounds on young tomato plants with *B. cinerea* conidia in glucose and phosphate (prepared 3 h prior to inoculation) consistently resulted in stem tissue rotting. Incubation of inoculated stems at a low VPD (high RH) for 24 hours prior to transfer to a CE cabinet (maintained around 60% RH) resulted in a greater incidence and severity of tissue rotting than on tissue transferred after 1 hour. This result suggests a prolonged period of low VPD after inoculation is required for consistent infection of wounded tomato stems from *B. cinerea* conidia.

4.5 Recommended bioassays for testing bio-control products and organisms isolated from tomato tissues

Stem pieces to be inoculated with *B. cinerea* conidia in SDW amended with glucose and phosphate and prepared 3 h prior to use. With this treatment, the spores are primed but remained ungerminated at the time of inoculation. Incubate the stems at a low VPD after inoculation.

Investigate effect of crush wounding stem ends on 1) the duration of time susceptibility to infection from conidia, and 2) the need for nutrients.

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7. Overall Conclusions

1. Eighteen bio-control products and isolates (BCAs), comprising 8 fungal and 10 bacterial, were collected from commercial companies and research organisations and placed in the reference collection.

2. Attendance at the X11th International Botrytis Symposium, Reims, France allowed contact to be made with other researchers working on Botrytis and biological control of diseases and to provide an update on current research in these fields.

3. All the bio-control agents in the reference collection were successfully screened *in vitro* against a range of *B. cinerea* isolates. Some of the BCAs exhibited large zones of inhibition of the pathogen's growth *in vitro* and the products screened were ranked on their performance. These products displaying the largest zones of inhibition and reduction in the radial growth of the pathogen include; H9, A8, A1, A7, A3 and H5.

4. The BCAs showing antagonism in the challenge inoculations *in vitro* will be taken forward and assessed on tomato stem tissue. Some of the BCAs that did not perform well in the *in vitro* screening will also be assess on plant tissue.

5. A leaf and stem bioassay were assessed in the laboratory to see which would allow the most effective screening of the BCAs. It was decided that the stem bioassay, using conidial inocula, allowed the most realistic and reproducible conditions similar to infection development and pressure under commercial growing conditions.

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6. Technology Transfer

Presentations

- Southern Tomato Growers Seminar Day. HRI Stockbridge House, 13 June 2000.

Scientific meetings

- Startup project meeting. HRI Wellesbourne, 14 March 2000.
- Bioassay progress and laboratory methodolgy. ADAS Arthur Rickwood, 28 November 2000.

Review meeting

- Annual Review Meeting/Steering Group. 6 March 2000.

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Appendix I Selected abstracts from XIIth International Botrytis Symposium

Appendix II Layout of Petri dishes in the challenge inoculations

Appendix III Challenge inoculation plate tests – tables of results

Results from the challenge inoculation results from *in vitro* screening (Stockbridge House)

Bio-control Products	Isolate of *Botrytis cinerea*									
	BC 01		BC 02		BC 03		BC 04		BC 05	
	ZI	% INH	ZI	% INH	ZI	% INH	ZI	% INH	ZI	% INH
H1	0	28.93	0	29.83	-11.00	1.77	0	31.47	19	0.63
H2	0	37.50	-11	7.50	-3.00	27.70	0	37.50	2.33	17.78
H3	2.67	4.67	1.17	38.33	2.33	45.00	2.67	45.00	5.33	33.53
H4	0	43.27	0	40.70	0	39.43	0	56.20	0	52.23
H5	0	41.43	0	31.47	3.00	29.27	0.67	40.43	11.67	10.15
H6	-12.00	0	-12.00	0	-8.00	16.60	-12.00	0	0.33	32.50
H7	3.67	43.30	2.00	40.83	2.00	40.00	1.00	37.50	2.33	41.67
H8	4.67	30.30	2.33	36.29	2.67	32.24	2.67	35.56	2.33	36.27
H9	13.00	70.83	10.30	63.97	10.00	60.83	9.50	62.50	8.33	61.67
A1 (plug)	15.67	25.70	-	-	12.00	36.40	10.67	37.57	11.33	36.60
A1 (Streak)	9.33	65.80	6.67	59.17	4.67	52.50	5.33	51.67	5.67	50.80
A2	4.33	34.90	3.00	32.27	1.67	31.77	1.17	33.83	5.33	37.30
A3	6.33	50.00	5.00	46.67	4.67	47.50	6.00	52.50	4.67	45.00
A4	0	54.30	0	47.43	0	46.47	0	54.73	0	85.60
A5	1.33	35.00	2.33	39.17	0	47.50	0.33	38.30	3.00	38.33
A6	-11.67	5.83	-14.00	0	-14.00	0	-14.00	0	-13.00	0
A7	7.33	55.00	5.33	47.50	5.33	49.17	6.33	56.67	5.33	49.17
A8	6.33	50.80	5.67	49.17	5.67	50.83	6.33	55.83	6.67	51.67
A9	0	43.83	0	40.95	0	41.58	0	44.43	0	46.69

Results from the challenge inoculation results from *in vitro* screening (ADAS, Arthur Rickwood)

Bio-control Products	Isolate of *Botrytis cinerea*									
	BC 01		BC 02		BC 03		BC 04		BC 05	
	ZI	% INH	ZI	% INH	ZI	% INH	ZI	% INH	ZI	% INH
H1	1.33	35.66	0.67	34.24	0.67	35.37	0	31.3	0.67	36.3
H2	-15.3	1.57	-13.7	2.22	-14.3	2.38	-9.3	12.65	-14.7	-0.24
H3	0	33.31	2.00	42.19	2.00	41.50	2.67	43.49	2.50	46.95
H4	0	47.93	0	42.03	0	44.80	0	37.57	0	41.17
H5	6.67	23.12	0.83	28.70	1.00	31.72	1.00	29.73	1.83	30.37
H6	-9.67	0.77	-11.0	1.16	-11.0	-6.75	-10.33	2.40	-10.3	-1.81
H7	-10.3	-5.26	-10.7	-3.29	-9.67	1.43	-10.00	-5.92	-10.3	29.19
H8	6.33	37.86	5.00	36.14	6.33	34.14	3.00	35.57	4.67	36.66
H9	7.67	52.42	9.00	61.47	6.00	49.63	6.50	50.15	9.33	41.70
A1 (plug)	2.67	56.24	3.75	41.23	3.67	40.24	6.33	52.50	4.00	43.82
A2	0.17	35.9	0.33	33.82	0	34.69	0.33	32.81	5.33	23.95
A3	1.67	55.11	1.00	57.01	4.33	61.66	5.50	57.06	2.67	60.94
A4	0	43.81	0	30.60	0	10.80	0	54.98	0	42.63
A5	-13.3	1.51	-14.3	-0.83	-10.7	10.71	-4.33	17.82	-6.67	19.36
A6	-9.67	9.23	-14.0	-5.00	-13.7	-7.85	-7.33	10.66	0.67	40.68
A7	3.33	41.76	4.33	50.35	2.67	41.46	3.00	42.50	4.00	45.08
A8	5.33	51.91	5.00	54.59	2.33	27.61	6.50	52.44	8.00	60.49
A9	0	30.59	0	28.64	0	22.81	0	52.46	0	47.61

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