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Biological Control and Biomass Evaluation of
Botrytis cinerea

A thesis presented in partial fulfilment of the requirements for the degree of Master of Applied Science at Massey University Palmerston North New Zealand

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Abstract

The efficacy of biocontrol agents is often judged by symptom development on inoculated plants. This process can involve long delays, as with Botrytis infection of kiwifruit and an alternative, quicker approach would be useful. When biocontrol is successful, then pathogen biomass is limited hence a means of measuring the biomass of a pathogen on/in a target substrate (plant material) could be used as a tool for rapid estimation of biocontrol efficiency.

Two yeast (Enterobacter agglomerans, Enterobacter aerogenes) and two bacteria (Candida sake, Trichosporon pullulans) with an already identified ability to attach to the surface of Botrytis cinerea and to reduce infection in tomato and kiwifruit, were evaluated for control of B. cinerea in bean, lettuce and rose in this study. Potential biological control and efficacy was assessed by measuring lesion size and percentage infection by B. cinerea.

An investigation of methods of conidial application of B. cinerea to these crops tissue showed that disease severity and incidence were increased by a high concentration of wet spore application to bean and dry spore application to lettuce and rose tissue. Each application technique was used as the standard technique for biocontrol experiments on the crop on which it was most efficient.

Three of the potential BCAs (Enterobacter agglomerans, Enterobacter aerogenes, Trichosporon pullulans) were found to reduce lesion size and percentage infection on all three crops at 20°C.

Biological control by bacterial BCAs, Enterobacter agglomerans and Enterobacter aerogenes, were demonstrated by applying them to bean tissue at the
time of inoculation with a suspension of $1 \times 10^8$ conidia per ml of *B. cinerea*. These two bacteria and the yeast, *Trichosporon pullulans*, showed biological control when applied to lettuce and rose tissue one or two days after inoculation with dry spores of *B. cinerea*.

A potential rapid assessment of biocontrol efficiency of microorganisms has been demonstrated using Laser Scanning Confocal Microscope. A clear image of the fungal hyphae in the host tissue was produced in confocal microscopy by using glutaraldehyde as a fluorescent stain for *B. cinerea* hyphae. Biomass of *B. cinerea* at an early stage of infection in bean and lettuce tissues was successfully measured by computer analysis before and after application of yeast and bacterial biocontrol agents. BCAs application in both tissues prevented development of a large biomass of *B. cinerea*. 
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Table of contents

ABSTRACT...........................................................................................................i
ACKNOWLEDGEMENTS.....................................................................................iii
TABLE OF CONTENTS.......................................................................................iv
TABLE OF FIGURES..........................................................................................ix
TABLE OF TABLES............................................................................................xiii

CHAPTER ONE

GENERAL INTRUCTION....................................................................................1

1.1 *Botrytis cinerea*.........................................................................................1
1.1.1 Economic losses due to *B. cinerea* infection..........................................1
1.1.2 Taxonomy and morphology.......................................................................2
1.1.3 General characteristics of *B. cinerea*....................................................2
1.1.4 Specific behaviour of *Botrytis cinerea* on plant surfaces.......................3
  1.1.4.1 Survival of conidia..............................................................................3
  1.1.4.2 Germination of conidia......................................................................3
    1.1.4.2.1 Spore concentration.................................................................3
    1.1.4.2.2 Relative humidity and free water..............................................4
    1.1.4.2.3 Temperature..............................................................................4
    1.1.4.2.4 Other factors influencing spore germination..............................5
1.2 Control of *Botrytis cinerea*.......................................................................6
  1.2.1 Non-Chemical control............................................................................6
  1.2.2 Chemical treatment...............................................................................6
  1.2.3 Biological control of *Botrytis cinerea*................................................7
    1.2.3.1 General view on Biological control..............................................7
    1.2.3.2 Attachment biocontrol agents.......................................................9
1.2.3.3 Utilisation of attachment biocontrol agents in disease control ................................................................. 10

1.3 Confocal microscopy ................................................................................................................................. 11
  1.3.1 Confocal microscope and computer image analysis .............................................................................. 11
  1.3.2 Staining for confocal microscopy ........................................................................................................ 12

1.4. Objectives of this studies .......................................................................................................................... 12

CHAPTER TWO

GENERAL MATERIALS AND METHODS .................................................................................................... 13

2.1 Botrytis cinerea isolates ........................................................................................................................... 13
  2.1.1 culture preparation .............................................................................................................................. 13
    2.1.1.1 Silica gel storage ......................................................................................................................... 13
  2.1.2 Preparation of spore suspensions ....................................................................................................... 14

2.2 Biological Control Agents (BCA); bacteria and yeast ............................................................................. 14
  2.2.1 Culture preparation ........................................................................................................................... 14
  2.2.2 Preparation of cell suspension ......................................................................................................... 15
  2.2.3 Cells counts ..................................................................................................................................... 15

2.3 Source of test materials ........................................................................................................................... 15
  2.3.1 Bean (Phaseolus vulgarus L) ........................................................................................................... 15
  2.3.2 Lettuce (Lactuca sativa L) ............................................................................................................... 15
  2.3.3 Rose (Rosa hybrida L) ..................................................................................................................... 16

2.4 Fluorescent treatment ................................................................................................................................. 16
  2.4.1 KH4 antibody fluorescent dye ............................................................................................................. 16
  2.4.2 Trypan blue fluorescent dye .............................................................................................................. 16
  2.4.3 Glutaraldehyde fluorescent dye ........................................................................................................ 17
    2.4.3.1 Glutaraldehyde fluid (GA) preparation ....................................................................................... 17
2.4.3.2 Buffered sucrose solution preparation ........................................ 17
2.4.3.3 Procedure of glutaraldehyde fixation ........................................ 17

2.5 Confocal microscopy ........................................................................ 18
2.6 Computer Image Analysis ............................................................... 18
2.7 Assessment of Botrytis cinerea infection ........................................... 19
   2.7.1 Data collection ........................................................................ 19
   2.7.2 Observation and recording ......................................................... 19
2.8 Experimental designs and statistical analysis ...................................... 19

CHAPTER THREE

INFECTION OF DETACHED PLANT MATERIALS BY
BOTRYTIS CINEREA ........................................................................ 20

3.1 Introduction ....................................................................................... 20
3.2 Objective .......................................................................................... 21
3.3 Materials and Methods .................................................................... 22
   3.3.1 B. cinerea infection on bean and lettuce leaves and rose petals .... 22
3.4 Results .............................................................................................. 25
3.5 Discussion ......................................................................................... 37

CHAPTER FOUR

BIOLOGICAL CONTROL OF INFECTION OF ROSE, BEAN AND
LETTUCE BY BOTRYTIS CINEREA .................................................. 39

4.1 Introduction ....................................................................................... 39
4.2 Objective .......................................................................................... 40
4.3 Materials and Methods ..................................................................... 41
4.4 Results ............................................................... 45
4.5 Discussion ............................................................. 57

CHAPTER FIVE

ATTACHMENT OF BACTERIA AND YEAST TO B. CINEREA HYphae AS A BIOCONTROL MECHANISM ................. 60

5.1 Introduction ............................................................ 60
5.2 Objectives ............................................................... 61
5.3 Materials and Methods ............................................... 61
  5.3.1 In vitro bioassay for attachment BCAs ..................... 61
    5.3.1.1 B. cinerea and BCA preparation ......................... 61
    5.3.1.2 Media preparation ........................................... 62
    5.3.1.3 Inoculation and incubation .............................. 62
    5.3.1.4 Dark field microscopy ..................................... 62
  5.3.2 In vivo bioassay for attachment BCAs ....................... 63
5.4 Results ................................................................. 63
  5.4.1 Biocontrol and BCAs attachment assay – in vitro .......... 63
    5.4.1.1 Biocontrol of B. cinerea ................................. 63
    5.4.1.2 Dark field microscopy on attachment assay ........... 64
  5.4.2 Biocontrol and BCAs attachment assay – in vivo ........... 64
5.5 Discussion ............................................................. 70

CHAPTER SIX

BIOMASS MEASUREMENT OF BOTRYTIS CINEREA USING LASER SCANNING CONFOCAL MICROSCOPE ................. 72

6.1 Introduction ........................................................... 72
6.2 Objective...........................................................................................................74

6.3 Materials and Methods.....................................................................................75
  6.3.1 \textit{B. cinerea} inoculation and BCAs application...........................................75
  6.3.2 Fluorescent treatment.....................................................................................75
  6.3.3 Confocal microscopic examination.............................................................75
  6.3.4 Image analysis...............................................................................................76

6.4 Results...............................................................................................................76
  6.4.1 Fluorescent treatment.....................................................................................76
  6.4.2 Image analysis...............................................................................................77

6.5 Discussion..........................................................................................................84

CHAPTER SEVEN
GENERAL DISCUSSION.........................................................................................86
  Introduction..........................................................................................................86
  \textit{B. cinerea} infection on plant tissue.................................................................86
  Application of BCAs............................................................................................87
  BCA’s attachment and biocontrol.........................................................................88
  Potential biocontrol agents....................................................................................88
  Biomass measurement..........................................................................................90

CHAPTER EIGHT
CONCLUSION AND RECOMMENDATION FOR FUTURE STUDIES.............91
REFERENCES...........................................................................................................93
Table of figures

Fig 3-1. Lesion size and percentage infection of wounded and unwounded tissue of bean, lettuce and rose. Means accompanied by same letter are not significantly different at P<0.05. ........................................ 27

Fig 3-2. Lesion size and percentage infection by dry and wet (1x10^6 and 1x10^6 spores per ml.) spore inoculation on bean, lettuce and rose tissue. Means accompanied by same letter are not significantly different at P<0.05... 28

Fig 3-3. Lesion size and percentage infection by B. cinerea on bean leaves inoculated with different spore concentration wound and unwounded tissue. Wet1: 1x10^6 spores per ml. Wet2: 1x10^8 spores per ml. Dry: Dry spores. Means accompanied by same letter are not significantly different at P<0.05. ...... 29

Fig 3-4. Lesion size and percentage infection by B. cinerea on lettuce leaves inoculated with different spore concentration on wound and unwounded tissue. Wet1: 1x10^6 spores per ml. Wet2: 1x10^8 spores per ml. Dry: Dry spores. Means accompanied by same letters are not significantly different at P<0.05. ........................................ 30

Fig 3-5. Lesion size and percentage infection by B. cinerea on rose petals inoculated with different spore concentration on wound and unwounded tissue. Wet1: 1x10^6 spores per ml. Wet2: 1x10^8 spores per ml. Dry: Dry spores. Means accompanied by same letters are not significantly different at P<0.05. ........................................ 31

Fig 3-6. Differences of lesion size and percentage infection between wounded and unwounded tissues of bean, lettuce and rose ...................... 32

Fig 3-7. Lesion size measured 48 h and 96 h after inoculation of B. cinerea on bean leaves. Dark = leaves left 24 h in dark before inoculation; W= Spores in water suspension; T= Infected tissue with spores .................. 33

Fig 3-8. Lesion size measured at 48 h and 96 h after B. cinerea inoculation on different age and position bean leaves. Coty = cotyledon .................... 34

Fig 3-9. Percentage infection measured after different B. cinerea isolates were inoculated on bean and lettuce leaves and rose petals ............. 35
Fig 3-10. Lesion development over time. A) Rose petals inoculated only with *B. cinerea*. Size of lesions after 24 h (1-3), 48 h (4-6) and 72 h (7-9) incubation. B) Lettuce leaves after 72 h incubation.

Fig 4-1. BCAs added at 0h (A), 24h (B) and 48 h (C) after inoculation of rose petals by *B. cinerea*. 1) Control 1: no BCAs or *B. cinerea* inoculated 2) Control2: only *B. cinerea* was inoculated 3) OX2 4) OX8a 5) 561 6) 622b.

Fig 4-2. Lesion size (A) and percentage infection (B) measured every day from 3rd to 9th day (except 7th day) of inoculation on bean leaf tissue. BCAs added at 0h after *B. cinerea* inoculation. Bars with same letter in a day is not significantly different (P<0.05).

Fig 4-3. Lesion size (A) and percentage infection (B) measured every day from 3rd to 9th day (except 7th day) of inoculation on bean leaf tissue. BCAs added at 24h after *B. cinerea* inoculation. Bars with same letter in a day is not significantly different (P<0.05).

Fig 4-4. Lesion size (A) and percentage infection (B) measured every day from 3rd to 9th day (except 7th day) of inoculation on bean leaf tissue. BCAs added at 48h after *B. cinerea* inoculation. Bars with same letter in a day is not significantly different (P<0.05).

Fig 4-5. Effect on lesion size (A) and percentage infection (B) of *B. cinerea* by different time of application of bacterial isolate OX2 over time on bean leaf tissue. Bars with same letter in a day is not significantly different (P<0.05).

Fig 4-6. Effect on lesion size (A) and percentage infection (B) of *B. cinerea* by different time of application of bacterial isolate OX8a over time on bean leaf tissue. Bars with same letter in a day is not significantly different (P<0.05).

Fig 4-7. Effect on lesion size (A) and percentage infection (B) of *B. cinerea* by different time of application of bacterial isolate 622b over time on bean leaf tissue. Bars with same letter in a day is not significantly different (P<0.05).
Fig 4-8. Lesion size and percentage infection measured at 5 days after inoculation on lettuce leaf tissue. BCAs added at 0 h after B. cinerea inoculation. Means accompanied by same letter are not significantly different at $P<0.05$.

Fig 4-9. Lesion size and percentage infection measured at 5 days after inoculation on lettuce leaf tissue. BCAs added at 24 h after B. cinerea inoculation. Means accompanied by same letter are not significantly different at $P<0.05$.

Fig 4-10. Lesion size (A) and percentage infection (B) measured at 5 days after inoculation on lettuce leaf tissue. BCAs added at 0 h, 24h and 48 h after B. cinerea inoculation. Means accompanied by same letter are not significantly different at $P<0.05$.

Fig 4-11. Lesion size (A) and percentage infection (B) measured at 4th and 5th days after inoculation on rose peta tissue. BCAs added at 48 h after B. cinerea inoculation. Bars with same letter in an inoculation time is not significantly different ($P<0.05$).

Fig 5-1. Bacterial isolates OX 8a (A) and OX2 (B) attached to B. cinerea hyphae after 72 h incubation in NB medium at 20°C and observed under dark field microscopy. Bacteria added to the medium 24 h after B. cinerea spores. ab: Bacteria attached to the mycelial cell wall, nb: Non-attached bacteria scattered in the medium, bh: B. cinerea hyphae washed with SDW before mounted on slide, ae: amorphous extra-cellular material. (bar 20 µm).

Fig 5-2. Yeast isolate 561 (A) and 622b (B) attached to B. cinerea hyphae after 72 h incubation in NB medium at 20°C and observed under dark field microscopy. Yeast added to the medium 24 h after B. cinerea spores. ay: Yeast attached with the mycelial cell wall, bh: B. cinerea hyphae washed with SDW before mounted on slide. (bar 20 µm).

Fig 5-3. Confocal micrograph of bacteria isolate OX8a attached to hyphae of B. cinerea on lettuce leaf tissue treated with Glutaraldehyde. Bacteria applied 24 h after the tissue was inoculated with B. cinerea. ab: Bacteria attached with the mycelial cell wall, nb: Non-attached bacteria scattered on the surface of the leaf tissue, bh: Glutaraldehyde treated B. cinerea hyphae.
Fig 5-4. Confocal micrograph of Yeast isolate 561 (A) and 622b (B) attached to hyphae of B. cinerea on bean (B) and lettuce (A) leaf tissue treated with Glutaraldehyde. Yeast applied 24 h after the tissue was inoculated with B. cinerea. ay: Yeast attached with the mycelial cell wall, ny: Non-attached yeast scattered on the surface of the leaf tissue, bh: Glutaraldehyde treated B. cinerea hyphae......................69

Fig 6-1. Confocal micrograph of B. cinerea inoculated onto a wounded lettuce leaf surface and incubated for 72 h. Specimen treated with glutaraldehyde. A) Hyphal development and penetration into the tissue (bh). B) A portion of the mycelium from the advance margin of the infection........80

Fig 6-2. Confocal micrograph of B. cinerea hyphal development following addition of bacterial isolates OX2 (A) and OX8a (B) application to the surface of bean leaf tissue. BCAs applied 24 h after B. cinerea inoculation. Specimen treated with glutaraldehyde.............................................81

Fig 6-3. Confocal micrograph of B. cinerea hyphal development following addition of yeast isolate 561 (A) and 622b (B) to the surface of lettuce leaf tissue. BCAs applied 24 h after B. cinerea inoculation. Specimen treated with glutaraldehyde.............................................82

Fig 6-4. Confocal micrograph of B. cinerea hyphae (A) before and (B) after removal of fluorescing background plant cells..................................................83
Table of tables

Table 5-1. Observation of BCA attachment to hyphae and other interaction at 0 h and 24 h application of BCAs after *B. cinerea* inoculation in vitro........65

Table 6-1. Hyphal volume of *B. cinerea* per unit area of inoculated bean leaf tissue measured by LSCM and image analysis. Bacterial BCAs OX2 and OX8a were applied 12 or 24 h after inoculation. All tissues were fixed with glutaraldehyde 24-36 h after *B. cinerea* inoculation..............................79

Table 6-2. Hyphal volume of *B. cinerea* per unit area measured by LSCM and image analysis. Yeast BCAs 561 and 662b applied 12 or 24 h after inoculation. All tissues were treated with glutaraldehyde 24-36 h after *B. cinerea* inoculation..............................................................79