

**EVALUATION OF SEED AND DRENCH TREATMENTS FOR MANAGEMENT OF  
DAMPING-OFF AND SEEDLING BLIGHT PATHOGENS OF SPINACH  
FOR ORGANIC PRODUCTION**

By  
JAIME ANNE CUMMINGS

A thesis submitted in partial fulfillment of  
the requirements for the degree of

MASTER OF SCIENCE IN PLANT PATHOLOGY

WASHINGTON STATE UNIVERSITY  
Department of Plant Pathology

DECEMBER 2007

To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of JAIME ANNE CUMMINGS find it satisfactory and recommend that it be accepted.

---

Chair

---

---

---

---

## ACKNOWLEDGEMENTS

### **Professional**

I would like to thank my advisor, Dr. Lindsey du Toit, for welcoming me into the Vegetable Seed Pathology Program at WSU Mount Vernon Northwestern Washington Research and Extension Center, and for her patience and enthusiasm in introducing me to the field of Plant Pathology. I am grateful for her advice and guidance. The experiences and skills gained while working with her are immeasurable. I also thank my committee members; Dr. Debra Inglis, Dr. Carol Miles, and Dr. Frank Dugan, for their collective and individual contributions which made this project a success. I would like to extend a special thank you to Dr. Inglis for her time, attention, and guidance; her contributions to the successful completion of this degree are greatly appreciated. I would like to thank the WSU Center for Sustaining Agriculture and Natural Resources Organic Research Program for funding this project and Dr. Miles for her part in securing the funding. Additionally, I would like to thank Dr. Dugan for his time and guidance while I was in Pullman and new to the department.

I would like to extend my gratitude to the entire WSU Mount Vernon NWREC faculty and staff for all their assistance and encouragement, and especially to the entire VSP team. Particularly Mike Derie for his expertise, patience, and assistance with many aspects of my project; Louise Brissey for all her expertise and the hours she contributed at the microscope and elsewhere, as well as for her undying encouragement; Barbara Holmes for all of her technical assistance with the greenhouse and field trials, and incredible willingness to do whatever needed to be done; and Kerri Brooks for her efficiency and accuracy with the germination assays and many other contributions, including her spectacular personality which made work fun. I would also like to acknowledge Carl Libbey for all his support and contributions as my 'go-to-man' at the station for so many uncountable things. Thank you to Ron Dralle, Dan Gorton, and Juan

Alonso for all they did to ensure that the facilities and fields were suitable for research, and to the office staff, Pat King, Kathy Gleissner, and Jeanne Burritt for answering all my questions and for processing all my paperwork. Additionally, I would like to thank the ladies in the office in Pullman, Cheryl Hagelganz and Mary Stormo, for all of their assistance.

**Personal**

I would like to thank Aaron Jacobsen for his support and encouragement, as well as his selfless tolerance and understanding throughout my time as a student. I would also like to thank my parents, Jim and Judy Cummings for their support and encouragement. I thank Moosie for being a constant and fantastic source of comfort and relaxation. Additionally, I would like to acknowledge and thank Leigh Ann Harrison for all her advice, guidance and support, and for being a terrific friend who always had an open ear.

**EVALUATION OF SEED AND DRENCH TREATMENTS FOR MANAGEMENT OF  
DAMPING-OFF AND SEEDLING BLIGHT PATHOGENS OF SPINACH**

**FOR ORGANIC PRODUCTION**

Abstract

by Jaime Anne Cummings, M.S.  
Washington State University  
December 2007

Chair: Lindsey du Toit

There are many concerns regarding losses to seedborne and soilborne pathogens in organic production systems because of limited effective options available for disease management that satisfy organic standards. Seed treatments can be inexpensive and effective, including those with biological control agents (BCAs). However, the reliability of BCAs for disease control is affected by crop species, whether a pathogen is seedborne or soilborne, and numerous abiotic factors.

The efficacy of a range of seed and drench treatments for control of soilborne seedling blight and damping-off pathogens was investigated using spinach as a model small-seeded vegetable. The seed and drench treatments included those that were EPA registered and approved for use in organic agriculture as defined by the National Organic Standards, and those that were being developed for registration and compliance with organic standards. Greenhouse inoculation rate trials were completed for each of three soilborne pathogens, *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, and *Rhizoctonia solani*. Results were then used to evaluate 14 seed and drench treatments in greenhouse trials against each pathogen. For *P. ultimum*, two experimental seed treatments, Experimental #1 and #2, provided equivalent control to that provided by a conventional fungicide seed treatment, Apron XL LS; while Natural II, Natural X, and Subtilex seed treatments each suppressed damping-off significantly in only one of

two trials. For *F. oxysporum* f. sp. *spinaciae*, drenches with a compost tea or Prestop, and seed treatment with Yield Shield suppressed post-emergence wilt significantly in one of two trials; but no treatment was highly effective. For *R. solani*, Experimental #1 and Natural II seed treatments reduced damping-off as effectively as a drench with the conventional fungicide Terraclor. Seed health assays revealed that treatments with Experimental #1, Experimental #2, or Mycostop Mix significantly reduced the incidence of seedborne *Verticillium* and *Alternaria*. Natural II and Natural X seed treatments significantly reduced early germination in seed germination assays.

Selected treatments were evaluated further under field conditions at three locations in western Washington. There was little consistency in results among field trials. However, Experimental #1 and #2 seed treatments consistently caused significantly earlier emergence than the other treatments. In contrast, the compost tea drench resulted in low total emergence and low spinach biomass, but also low post-emergence wilt in two of three trials.

# TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS .....	iii
ABSTRACT .....	v
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
CHAPTER	
1. Literature Review	
1.1. Soilborne fungal plant pathogens.....	1
1.2. Biological control of soilborne plant pathogens .....	4
1.3. Seed pathology and seed treatments .....	6
1.4. Organic production .....	10
1.5. Spinach	
1.5.1. Spinach and spinach seed production .....	13
1.5.2. Diseases affecting spinach and spinach seed production.....	15
1.6. Fusarium wilts	
1.6.1. Background and importance .....	17
1.6.2. <i>Fusarium oxysporum</i> .....	17
1.6.3. Management of Fusarium wilts .....	18
1.7. <i>Pythium</i> species as plant pathogens	
1.7.1. Background and importance .....	22
1.7.2. <i>Pythium ultimum</i> .....	23
1.7.3. Management of <i>Pythium</i> damping-off.....	23
1.8. <i>Rhizoctonia</i> species as plant pathogens	
1.8.1. Background and importance .....	25
1.8.2. <i>Rhizoctonia solani</i> .....	26
1.8.3. Management of <i>Rhizoctonia</i> damping-off and seedling blight.....	27
1.9. Conclusion and research needs .....	29
1.10. Literature cited.....	31
2. Greenhouse pathogen inoculation rate trials, and seed and drench treatment trials for organic production	
2.1. INTRODUCTION .....	42
2.2. MATERIALS AND METHODS	
2.2.1. Pathogen isolates.....	45
2.2.2. Preparation of inocula .....	48
2.2.3. Inoculation of potting mix for greenhouse inoculation rate trials.....	50

2.2.4. Seed health assay for inoculation rate trials.....	52
2.2.5. Inoculum application for seed and drench treatment trials .....	53
2.2.6. Seed and drench treatments.....	53
2.2.7. Disease assessment and trial maintenance .....	55
2.2.8. Potting mix pH .....	56
2.2.9. Seed health assays of treated seed.....	56
2.2.10. Germination assays of treated seed.....	56
2.2.11. Statistical analyses .....	57
2.3. RESULTS	
2.3.1. Pathogenicity tests .....	58
2.3.2. Seed health assays of non-treated seed .....	58
2.3.3. <i>Pythium ultimum</i> inoculation rate trials .....	59
2.3.4. <i>Rhizoctonia solani</i> inoculation rate trials.....	61
2.3.5. <i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i> inoculation rate trials .....	64
2.3.6. <i>Pythium ultimum</i> greenhouse seed and drench treatment trials.....	67
2.3.7. <i>Rhizoctonia solani</i> greenhouse seed and drench treatment trials.....	74
2.3.8. <i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i> greenhouse seed and drench treatment trials .....	79
2.3.9. Seed health assays of treated seed .....	84
2.3.10. Germination assays of treated seed.....	86
2.3.11. Potting mix pH from treatment trials .....	87
2.4. DISCUSSION .....	87
2.5. LITERATURE CITED .....	100
3. Field evaluations of seed and drench treatments for control of damping-off and seedling blight pathogens of spinach for organic production	
3.1. INTRODUCTION .....	151
3.2. MATERIALS AND METHODS	
3.2.1. Field trial locations and experimental designs.....	155
3.2.2. Quantification of pathogen populations in the soil at each location.....	156
3.2.3. Soil pH .....	157
3.2.4. Inoculum production.....	158
3.2.5. Seed and drench treatments .....	159
3.2.6. Disease rating and plot maintenance.....	162
3.2.7. Statistical analyses .....	163
3.3. RESULTS	
3.3.1. Pathogen populations in the soil and soil pH.....	164
3.3.2. Mount Vernon field site.....	164
3.3.3. Vancouver field site .....	167
3.3.4. Sequim field site .....	170
3.4. DISCUSSION.....	171
3.5. LITERATURE CITED .....	178

## LIST OF TABLES

	Page
Table 2.1. Seed and drench treatments evaluated in greenhouse trials for efficacy against damping-off and vascular wilt of spinach caused by <i>Pythium ultimum</i> , <i>Rhizoctonia solani</i> , and <i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i> .....	107
Table 2.2. Incidence of necrotrophic fungi on a seed lot of the spinach hybrid ‘Lazio’ used for greenhouse inoculation rate trials for damping-off and wilt pathogens.....	110
Table 2.3. Evaluation of seed and drench treatments for control of <i>Pythium ultimum</i> as a damping-off pathogen of spinach: First greenhouse trial.....	111
Table 2.4. Evaluation of seed and drench treatments for control of <i>Pythium ultimum</i> as a damping-off pathogen of spinach: Second greenhouse trial.....	113
Table 2.5. Evaluation of seed and drench treatments for control of <i>Rhizoctonia solani</i> as a damping-off pathogen of spinach: First greenhouse trial.....	115
Table 2.6. Evaluation of seed and drench treatments for control of <i>Rhizoctonia solani</i> as a damping-off pathogen of spinach: Second greenhouse trial.....	117
Table 2.7. Evaluation of seed and drench treatments for control of <i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i> as a wilt pathogen of spinach: First greenhouse trial.....	119
Table 2.8. Evaluation of seed and drench treatments for control of <i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i> as a wilt pathogen of spinach: Second greenhouse trial.....	121
Table 2.9. Results of a freeze-blotter seed health assay of spinach seed with seed treatments evaluated in greenhouse and field trials.....	123
Table 2.10. Results from germination assays of spinach seed with seed treatments evaluated in greenhouse and field trials.....	125
Table 3.1. Seed and drench treatments evaluated in field trials in Mount Vernon and Vancouver, WA for efficacy against damping-off and vascular wilt of spinach caused by <i>Pythium ultimum</i> , <i>Rhizoctonia solani</i> , and <i>Fusarium oxysporum</i> f. sp. <i>spinaciae</i> .....	182
Table 3.2. Seed and drench treatments evaluated in a field trial in Sequim, WA for efficacy against damping-off and vascular wilt of spinach.....	185

LIST OF FIGURES

	Page
Fig. 2.1. Greenhouse pathogenicity test of <i>Rhizoctonia solani</i> AG4 HGII isolates ‘VSP 05-01A’ and ‘VSP 05-01B’ on spinach seedlings. The non-inoculated control seedling (left), and seedlings inoculated with rye seed colonized by isolate ‘VSP 05-01A’ (top right), and isolate ‘VSP 05-01B’ (bottom right) were three weeks old at the time of inoculation. The photo was taken 4 weeks after inoculation. Refer to the text for details on the method of inoculation.....	127
Fig. 2.2. Emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off (pre- + post-emergence) caused by <i>Pythium ultimum</i> in greenhouse trials evaluating the effect of inoculation rate on spinach seedlings. Each trial was set up as a randomized complete block design with five replications of four or five rates of inoculation (measured in propagules/g, ppg). Trial 1 (A) was conducted in June to July 2006, and trial 2 (B) was conducted in January to February 2007, each at $25 \pm 5^{\circ}\text{C}$ . The durations of trials 1 and 2 were 32 and 35 days, respectively, with emergence and damping-off measured weekly. The 50 ppg rate was not included in trial 2 based on results of trial 1. Each data point shows the mean and standard error of five replications.....	128
Fig. 2.3. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC <sub>pre</sub> ), C) post-emergence damping-off (AUDPC <sub>post</sub> ), and D) total damping-off (AUDPC <sub>total</sub> ) for trial 1 of <i>Pythium ultimum</i> inoculation rate trials on spinach carried out in a greenhouse at $25 \pm 5^{\circ}\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of six rates of inoculation, and was conducted in June to July 2006. The duration of the trial was 32 days, with emergence and damping-off measured weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.....	129
Fig. 2.4. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC <sub>pre</sub> ), C) post-emergence damping-off (AUDPC <sub>post</sub> ), and D) total disease (AUDPC <sub>total</sub> ) for trial 2 of <i>Pythium ultimum</i> inoculation rate trials carried out in a greenhouse at $25 \pm 5^{\circ}\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation and was conducted in January to February 2007. The duration of the trial was 35 days, with emergence and damping-off measured weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.....	130
Fig. 2.5. Emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off (pre- + post-emergence) caused by <i>Rhizoctonia solani</i> in greenhouse trials evaluating the effect of inoculation rate on spinach seedlings. Each trial was set up as a randomized complete block design with five replications of five rates of inoculation (measured in propagules/g, ppg). Trial 1 was conducted July to August 2006, and trial 2 was conducted in February to March 2007, each at $25 \pm 5^{\circ}\text{C}$ . The duration of trial 1	

was 32 days, and trial 2 was 35 days, with emergence and damping-off measured weekly. The 5,000 and 100,000 ppg rates were not included in trial 2 based on results of trial 1. Each data point shows the mean and standard error of five replications.....131

Fig. 2.6. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence damping-off (AUDPC<sub>post</sub>), and D) total damping-off (AUDPC<sub>total</sub>) for trial 1 of *Rhizoctonia solani* inoculation rate trials carried out in a greenhouse at 25 ± 5°C. Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation and was conducted in July to August 2006. The duration of the trial was 32 days, with emergence and damping-off measured weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.....132

Fig. 2.7. Area under progress curves (AUEPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence damping-off (AUDPC<sub>post</sub>), and D) total damping-off (AUDPC<sub>total</sub>) for trial 2 of *Rhizoctonia solani* inoculation rate trials carried out in a greenhouse at 25 ± 5°C. Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation and was conducted in February to March 2007. The duration of the trial was 35 days, with emergence and damping-off measured weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.....133

Fig. 2.8. Emergence, pre-emergence damping-off, post-emergence wilt, and total damping-off or wilt (pre- + post-emergence) caused by *Fusarium oxysporum* f. sp. *spinaciae* in greenhouse trials evaluating the effect of inoculation rate on spinach seedlings. Each trial was set up as a randomized complete block design with five replications of five rates of inoculation (measured in propagules/g, ppg). Trial 1 (A) was conducted in July to August 2006, trial 2 (B) was conducted in February to March 2007, and trial 3 (C) was conducted July to September 2007, each at 25 or 28 ± 5°C. The duration of trials 1, 2 and 3 was 32, 56 and 56 days, respectively, with emergence and damping-off measured weekly. The 5,000 ppg and 100,000 ppg rates were not included in trials 2 or 3 based on results of trial 1. Each data point shows the mean and standard error of five replications.....134

Fig. 2.9. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 1 of *Fusarium oxysporum* f. sp. *spinaciae* inoculation rate trials carried out in a greenhouse at 25 ± 5°C. Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation, and was conducted in July to August 2006. The duration of the trial was 32 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.....136

- Fig. 2.10. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of *Fusarium oxysporum* f. sp. *spinaciae* inoculation rate trials carried out in a greenhouse at  $28 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation, and was conducted in February to March 2007. The duration of the trial was 56 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.....137
- Fig. 2.11. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 3 of *Fusarium oxysporum* f. sp. *spinaciae* inoculation rate trials carried out in a greenhouse at  $28 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation, and was conducted in July to September 2007. The duration of the trial was 56 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.....138
- Fig. 2.12. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 1 of seed and drench treatment trials carried out in a greenhouse at  $25 \pm 5^\circ\text{C}$  for control of *Pythium ultimum* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in February to April 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names, which appear in the same order as in this figure. The duration of the trial was 35 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.....139
- Fig. 2.13. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of seed and drench treatment trials carried out in a greenhouse at  $25 \pm 5^\circ\text{C}$  for control of *Pythium ultimum* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in April to May 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names, which appear in the same order as in this figure. The duration of the trial was 28 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.....141
- Fig. 2.14. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease

(AUDPC<sub>total</sub>) for trial 1 of seed and drench treatment trials carried out in a greenhouse at 25 ± 5°C for control of *Rhizoctonia solani* on spinach. The trial was set up as a randomized complete block design with four replications of 17 treatments, and was conducted in April to May 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names, which appear in the same order as in this figure. The duration of the trial was 28 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.....143

Fig. 2.15. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of seed and drench treatment trials carried out in a greenhouse at 25 ± 5°C for control of *Rhizoctonia solani* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in May to June 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names which appear in the same order as in this figure. The duration of the trial was 28 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.....145

Fig. 2.16. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 1 of seed and drench treatment trials carried out in a greenhouse at 28 ± 5°C for control of *Fusarium oxysporum* f. sp. *spinaciae* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in March to May 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names which appear in the table in the same order as in this figure. The duration of the trial was 42 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.....147

Fig. 2.17. Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of seed and drench treatment trials carried out in a greenhouse at 28 ± 5°C for control of *Fusarium oxysporum* f. sp. *spinaciae* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in April to June 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names which appear in the table in the same order as in this figure. The duration of the trial was 49 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.....149

Fig. 3.1. Area under progress curve (AUPC) values for A) emergence (AUEPC) and B) post-emergence disease (AUDPC<sub>post</sub>), and C) above-ground dry biomass for spinach plants in main plots at the Mount Vernon, WA field trial in which 12 seed and/or drench treatments were evaluated for control of seedling blight and damping-off of spinach. The trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. The main plot treatments included inoculation of the soil with *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, or *Rhizoctonia solani*, or non-inoculated soil for the control treatment. However, only results for *F. oxysporum* f. sp. *spinaciae*, *P. ultimum*, and the non-inoculated treatment are presented because of low emergence/excessive damping-off in the *R. solani*-inoculated plots (see text for explanation). Refer to Table 3.1 for details of the seed and drench treatments. The duration of the trial was 35 d. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications.....187

Fig. 3.2. Area under progress curve (AUPC) values for A) emergence (AUEPC) and B) post-emergence disease (AUDPC<sub>post</sub>), and C) above-ground dry biomass of spinach plants for the split-plot factor of seed or drench treatments for the Mount Vernon, WA field trial evaluating seed and/or drench treatments against seedling blight or damping-off pathogens of spinach. The trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. Main plots were inoculated with *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, or *Rhizoctonia solani*, or not inoculated. Split-plot treatments included 12 seed and/or drench treatments. Refer to Table 3.1 for details of the seed and drench treatments. The duration of the trial was 35 d. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the two control treatments (non-treated seed and a combination conventional fungicide seed and drench treatment).....189

Fig. 3.3. Area under progress curve (AUPC) values for A) emergence (AUEPC) and B) post-emergence disease (AUDPC<sub>post</sub>), and C) above-ground dry biomass of spinach plants in the main plots at the Vancouver, WA field trial in which 12 seed and/or drench treatments were evaluated for control of seedling blight and damping-off pathogens of spinach. The trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. The main plot treatments included inoculation of the soil with *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, or *Rhizoctonia solani*, or a non-inoculated soil for the control treatment. However, only results for *F. oxysporum* f. sp. *spinaciae*, *P. ultimum*, and the non-inoculated treatment are presented because of low emergence/excessive damping-off in the *R. solani*-inoculated plots (see text for explanation). Refer to Table 3.1 for details of the seed and drench treatments. The duration of the trial was 35 d. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications.....191

Fig. 3.4. Area under progress curve (AUPC) values for A) emergence (AUEPC) and B) post-emergence disease (AUDPC<sub>post</sub>), and C) above-ground dry biomass for spinach plants

for the split-plot factor of seed or drench treatments for the Vancouver, WA field trial evaluating seed and/or drench treatments against seedling blight and damping-off pathogens of spinach. The trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. Main plots were inoculated with *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, or *Rhizoctonia solani*, or not inoculated. The split-plot treatments included 12 seed and/or drench treatments. Refer to Table 3.1 for details of the seed and drench treatments. The duration of the trial was 35 d. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the two control treatments (non-treated seed and a combination conventional fungicide seed and drench treatment).....193

Fig. 3.5. A) Area under emergence progress curve (AUEPC) values and B) above-ground biomass of spinach plants for the Sequim, WA field trial evaluating seed and drench treatments for control of seedling blight and damping-off pathogens of spinach in a non-inoculated field. The trial was set up as a randomized complete block design with five replications of nine treatments, on a certified organic farm. The duration of the trial was 35 d. Refer to Table 3.2 for details of the seed and drench treatments, and to the text for the formula used to calculate the AUEPC values. Each bar shows the mean and standard error of five replications. The white bar represents the non-treated seed (control treatment).....195

## **Chapter 1**

### **Literature review**

#### **1.1. Soilborne fungal plant pathogens.**

Pathogenic microorganisms can be considered soilborne if any part of the life cycle is subterranean, ranging from those pathogens with survival propagules that contaminate the soil or fulfill a portion of the life cycle of the pathogen, to those that exist entirely within the soil (Bruehl, 1987). Compared to foliar fungal plant pathogens, soilborne fungal plant pathogens have simpler life cycles, with sporulation and dispersal of spores playing a minor role compared to foliar pathogens (Horsfall and Cowling, 1978). Growth, infection, and survival of pathogens in the soil system are less affected by atmospheric conditions, such as ultraviolet radiation or extreme fluctuations in temperatures, than those encountered by airborne pathogens. The survival of many airborne pathogens is measured in days or hours, whereas soilborne pathogen survival is usually measured in months or years (Horsfall and Cowling, 1978). Nonetheless, the soil habitat is complex and provides significant challenges to the research and management of soilborne pathogens and root diseases (Campbell and Neher, 1996; Hornby, 1985). Both the ecology of soilborne pathogens and the epidemiology of root diseases present challenges to researchers, because each pathosystem is unique (Campbell and Neher, 1996). Three specific challenges applicable to many root disease systems are difficulties in quantifying inoculum, assessing disease, and designing effective studies (Campbell and Neher, 1996).

Root pathogens must compete for survival against a vast diversity of soil microbes (Campbell and Neher, 1996). Competition for nutrients among microbes, especially nitrogen, can be intense. Plant roots provide significant amounts of such nutrients, making the roots

vulnerable to attack by soilborne pathogens. Germinating seeds and subterranean parts of plants release volatile and gaseous exudates into the surrounding soil environment, including carbon dioxide, alcohols, aldehydes, olefins, and volatile organic acids (Bowen, 1979; Catska, 1979). These exudates may stimulate or inhibit the germination of fungal spores. Catska (1979) found that exudates from germinating seeds usually inhibited spore germination in saprophytic fungi, while stimulating spore germination in phytopathogenic fungi.

Root diseases caused by soilborne pathogens cause extensive damage to numerous crops (Campbell and Neher, 1996). Soilborne pathogens vary greatly in host specificity, with some being highly host-specific, i.e., some forma speciales of *Fusarium oxysporum*, while others, such as *Rhizoctonia solani* and *Verticillium dahliae*, have a relatively wide host range (Henis, 1979). The inoculum potential of soilborne pathogens depends on the pathogens' competitive saprophytic ability, host range, ability to form dormant survival structures, tolerance to a variety of environmental conditions, and the susceptibility of survival propagules to biological factors (Mitchell, 1979). A wide host range, including an ability of the pathogen to colonize non-host tissue, increases the inoculum potential. Tolerance of the survival structures of the pathogen to a wide range of temperatures, water potentials, soil types, and nutrient availabilities also increases the pathogen inoculum potential. Furthermore, the susceptibility of such propagules to biological factors such as parasitism, predation, and mycostasis play important roles in the inoculum potential of the pathogen.

Soilborne plant pathogenic fungi have developed a variety of means to persist in the soil and infect a host (Henis, 1979). Garrett (1970) listed five methods by which fungi survive in the soil: 1) as competitive saprophytes on dead organic substrates; 2) by saprophytic survival on dead tissues of a host crop or of weeds infected during the parasitic phase; 3) by dormant

survival as resting propagules such as sexually produced oospores and other spores, asexually produced chlamydospores, and multicellular sclerotia; 4) by parasitic survival on living roots and other underground parts of hosts and ‘volunteer’ susceptible crop plants; and 5) by parasitic survival on living root systems that show no disease symptoms above ground. Few parasitic fungi, including *R. solani* and *Pythium ultimum*, are capable of more or less indefinite life as soil-inhabiting saprophytes (Coley-Smith, 1979). The majority of soilborne plant pathogenic fungi do not survive as vegetative mycelium, but rather invade living host tissues, rapidly degrade the tissues, then form various types of resting structures or dormant propagules (Coley-Smith, 1979). There is considerable variation in the ability of the different types of sclerotia and other dormant structures from different genera and species of fungi to survive for long periods in the soil (Coley-Smith, 1979; Henis, 1979).

Fungal wilt diseases are caused by a diverse group of soilborne microorganisms, with the greatest number of wilt diseases being caused by species from the genera *Fusarium* and *Verticillium* (Green, 1981). These organisms infect their hosts by entering the vascular system, and are transported within the conductive xylem tissue (Green, 1981). Fungal wilt pathogens show a variety of symptoms in their hosts. Common symptoms from wilt pathogens include vein clearing, epinasty, development of adventitious roots, unilateral development of wilt symptoms, stunting of the host, and even death of plants (Green, 1981). Dissemination of fungal wilt pathogens belonging to the genera *Fusarium* and *Verticillium* occurs principally by the movement of infected propagative materials, plant debris, or contaminated soil, as well as in or on seed, with little evidence of airborne inoculum (Green, 1981).

## 1.2. Biological control of soilborne plant pathogens.

Baker and Paulitz (1996) outlined three strategies for obtaining biological control of soilborne plant pathogens: 1) protection of infection courts, 2) reduction of inoculum potential in sites not necessarily associated with the infection court, and 3) induction of host resistance. They concluded that perhaps the most efficient of these strategies is the protection of a fixed infection court, such as seed, since the infection court remains stationary and does not encounter new inoculum over time. Therefore a single application of a biological control agent (BCA) may provide ample protection of the infection site. This is why a variety of BCAs have shown potential as seed treatments for protection against seed decay and seedling damping-off diseases (Baker and Paulitz, 1996). The activity expected from use of an antagonistic BCA applied to seed is short-term protection against damping-off pathogens, or longer-term protection of the root system through colonization of the roots and the rhizosphere of the host (Gindrat, 1979).

The mechanisms of fungal antagonism by fungal BCAs include antibiosis, exploitation, and competition (Gerhardson and Larsson, 1991). Some of the common BCAs include *Bacillus subtilis*, *Gliocladium virens*, *Pythium oligandrum*, and *Trichoderma* spp. (Baker and Paulitz, 1996). *B. subtilis* and *G. virens* utilize antibiosis as the main mechanism of antagonism, whereas *Trichoderma* spp. use mycoparasitism as the chief mechanism of antagonism (Baker and Paulitz, 1996). *Trichoderma* spp. have a worldwide distribution, are readily isolated from soils or other media, have strong antagonistic activities, fast growth rates, broad range of tolerances to a wide range of conditions, and ease of handling for large scale production and application. However, the results of many biocontrol trials with *Trichoderma* spp. have been ambiguous, negative, or only promising (Gerhardson and Larsson, 1991). Few biological methods of control have proven

successful enough to be used on a large scale in practice, due to difficulties in production, storage, and application (Alabouvette et al., 1979).

Several pseudomonads were approved by the U.S. Environmental Protection Agency (EPA) during the 1990's for use as biopesticides (Stockwell and Stack, 2007). Of the 94 named species of *Pseudomonas*, the species most commonly reported containing strains capable of suppressing plant diseases include *P. aureofaciens*, *P. chlororaphis*, *P. fluorescens*, *P. putida*, and non-pathogenic isolates of *P. syringae*. However, no strict relationship between biocontrol activity and phylogeny has been discovered (McSpadden Gardener, 2007). The successful interaction between the BCA bacterium and plant is dependent on numerous factors, including physical, chemical, and nutritional environment; requiring precise activation and repression of specific genes at the appropriate temporal and spatial regulations (Pierson and Pierson, 2007). Induced resistance occurs when a plant's ability to defend against biotic or chemical stimuli is enhanced (Van Loon et al., 1998). Induced systemic resistance (ISR) is a result of plant growth-promoting rhizobacteria (PGPR), including members of the fluorescent pseudomonads, suppressing diseases caused by both foliar and root-infecting pathogens (Van Peer et al., 1991). Systemic acquired resistance (SAR) results from the accumulation of salicylic acid in a plant as triggered by necrotizing pathogens (Sticher et al., 1997). Elicitation of ISR and SAR are widespread phenomena for a variety of nonpathogenic microorganisms and BCA's, and it has been suggested that the range of pathogens controlled might be extended when both ISR and SAR are combined (Bakker et al., 2007). However, commercial interest in producing *Pseudomonas* biocontrol products has been more limited than for spore-forming microbes, including *Bacillus* and *Trichoderma*, as a result of the limited viability of the *Pseudomonas* spp. (McSpadden Gardener, 2007).

Most reports of successful biocontrol by seed treatment with antagonists have been conducted under artificial conditions in laboratories or greenhouses (Baker and Paulitz, 1996; Gerhardson and Larsson, 1991). However, under field conditions the results often have not been as consistent or satisfactory as under greenhouse conditions, due to variable environmental conditions in the field and the complexity of the soil system (Baker and Paulitz, 1996; Gerhardson and Larsson, 1991; Harman, 1991). Thus, advancements in enhancing such strategies for efficacy under more realistic field conditions are needed. Most soilborne pathogens survive in the soil as dormant propagules or resting structures, such as sclerotia, which can be extremely long-lived in the soil, with virtually no effective long-term cultural control options. Therefore, there is increased interest in utilizing mycoparasitic BCAs of sclerotia and other survival structure-forming pathogens to effectively reduce inoculum potential (Baker and Paulitz, 1996; Whipps, 1991). One difficulty with this approach is that the pathogen propagules are distributed in a large volume of soil, with varying population densities. For a BCA to be effective, it must be situated close to the pathogen propagule, or have the ability to grow toward the propagules, resulting in the necessity of high application rates of BCAs to be effective (Baker and Paulitz, 1996). Pathogens involved in pre-emergence damping-off can infect the seedling stage of plant growth, as well as the seed itself, and BCAs used as seed treatments may not offer the same protection to seedlings as to the seed (Baker and Paulitz, 1996).

### **1.3. Seed pathology and seed treatments.**

Approximately 90% of food crops are cultivated through seed, and many plant pathogens are disseminated by seed (Agarwal and Sinclair, 1997; Neergaard, 1977). Seed pathology emerged as a science early in the 20<sup>th</sup> Century, and is considered a subset of the fields of seed

testing and plant pathology that includes examining the relationships of plant pathogens with propagative materials (Agarwal and Sinclair, 1997; McGee, 1997). Numerous scientific publications in the 1920's and 1930's were based on the analysis of crop seeds for identification and detection of seedborne pathogens. The International Seed Testing Association (ISTA) was founded in the early 1900's, with the primary purpose of developing, adopting, and publishing standardized procedures for evaluating seeds involved in international trade, and to promote additional research in all aspects of seed science and technology (Agarwal and Sinclair, 1997). In 1958, the Plant Disease Committee (PDC) was formed by the ISTA to develop seed health test methods, and many methods developed at that time are still used today (McGee, 1997). The first record of seedborne pathogens as important factors affecting seed quality was by Neergaard (1977), and since then seed health testing has become an important part of seed quality analysis (McGee, 1997).

As estimated by Agarwal and Sinclair (1997), there is about a 12% global loss of production from plant diseases. However, losses from seedborne diseases have been difficult to quantify, with the extent of economic losses from seedborne pathogens depending on numerous factors, including various aspects of the seed production and distribution industry such as restrictions related to tolerances for certifiable seed, the difficulty in obtaining pathogen-free seed, and the costs associated with seed treatments (Agarwal and Sinclair, 1997). The majority of seed transmitted plant pathogens are fungal, causing considerable impacts by reducing seed quality, yield, and vigor (Neergaard, 1977). Environmental conditions greatly influence the type and amount of inoculum produced by seedborne pathogens, as well as the significance of seedborne inoculum relative to other sources of infection (Neergaard, 1977). Many plant

pathogens survive in seeds, and seeds can be an important factor in the perpetuation of some plant pathogens (Agarwal and Sinclair, 1997).

Seedborne fungi can be either saprophytic or pathogenic (Neergaard, 1977). In addition, seeds can either be infected or infested with seedborne pathogens, i.e., infected seeds carry the pathogens “internally”, and infested seeds carry the pathogens on the seed surface (Agarwal and Sinclair, 1997). Systemic infection of a plant can occur as a result of seed transmission of certain pathogens from infected or infested seed, and results in movement of the pathogen throughout the plant, with subsequent or concomitant development of symptoms on the plants. Non-systemic transmission of seedborne pathogens leads to localized infection and development of symptoms during pre- or post-emergence of seedlings. The method and rate of seed transmission of pathogens depends on a number of factors, including the crop species or cultivar, environmental conditions, seed quality, and amount and type of inoculum (Agarwal and Sinclair, 1997).

Some important and effective means of controlling seedborne diseases include seed treatments and seed certification (Agarwal and Sinclair, 1997; Neergaard, 1977). The first documented seed treatment was the accidental brine treatment of wheat (*Triticum aestivum*) seeds in 1660 (Agrios, 1988). Intentional use of seed treatments originates as far back as the mid-1700’s, when Tillet discovered seed dusting treatments for bunt of wheat in 1755, followed by Prevost discovering Bordeaux mixture with copper sulfate for managing bunt in 1873, and hot water treatments in 1888 by J.L. Jensen for cereal smut (Agrios, 1988; Neergaard, 1977). Seed treatments with organic mercury compounds emerged in the early 1900’s, and were routinely used until the 1960’s, when they were removed from the market due to their toxicity and detrimental environmental effects (Agrios, 1988). Fungicide seed treatments became important

tools in the early 1980's for disease management. In addition to chemical fungicide seed treatments, advances have been made in the development of biopesticide seed treatments as the mechanisms of action of biocontrol agents have been clarified and manipulated for this purpose (McGee, 1997). A multitude of seed treatments have since been developed, including anaerobic water, dry heat, solar heat, aerated steam, aerobic fermentation, dust, slurry, wet, pelleting, and fumigation treatments, depending on the crop seed and the targeted pathogen (Neergaard, 1977). Often, combinations of seed treatments can be more effective than a single treatment, with synergistic effects (Neergaard, 1977).

Seed treatments can be inexpensive and very effective forms of plant disease control (Taylor and Harman, 1990). The main objectives are to prevent rotting of planted seeds and/or infection of the developing seedlings, either by killing the pathogens directly in or on the seed or by protecting the developing seedling from infection by soilborne pathogens; or directly improving plant growth through application of nutrients or microorganisms that improve nutrient uptake of the seedlings. The reliability of seed treatments at meeting these objectives varies among crop species, seed treatment products, seedborne or soilborne diseases, soil types and conditions, etc. (Taylor and Harman, 1990). Seed treatments can provide curative and/or protective control of seedborne as well as soilborne pathogens (Neergaard, 1977; Taylor and Harman, 1990). Seed treatment technologies exploit the seed as the vehicle for delivering treatment materials in an economical fashion, because a much smaller amount of the treatment material is applied per hectare compared to conventional foliar or soil applications in the developing crops, and the seed treatment is in direct contact with the seed and surrounding soil (Taylor and Harman, 1990).

#### **1.4. Organic production.**

The need for more sustainable agricultural practices is one of the driving forces behind organic farming (Groot et al., 2004). The National Organic Standards Board (NOSB) of the U.S. Department of Agriculture (USDA) defines organic agriculture as “an ecological production management system that promotes and enhances biodiversity, biological cycles, and soil biological activity” (USDA NOSB, 1995). Organic agriculture is typically more environmentally-sound than intensive conventional agriculture, by minimizing inputs of synthetic pesticides and fertilizers, and focusing on farming practices and philosophies that emphasize sustainable systems with ecologically-sound practices (Bengtsson et al., 2005; Groot et al., 2004; Koenig and Baker, 2002; Vogl et al., 2005). Organic agricultural practices can reduce leaching of nutrients, improve carbon storage in the soil, minimize erosion, and lower the concentrations of pesticides in water systems (Bengtsson et al., 2005).

Conventional, high-input agricultural systems have resulted in a decrease in biodiversity throughout agricultural landscapes (Bengtsson et al., 2005). Organic agricultural systems have typically been developed to enhance biodiversity in agricultural landscapes, with organic systems averaging 30% higher species richness and 50% greater abundance of organisms compared to conventional agricultural systems (Bengtsson et al., 2005). For example, Bengtsson et al. (2005) demonstrated that populations of beneficial, predatory organisms, such as spiders and carabid beetles were higher in the organic systems, whereas non-predatory insect pests were not found to be any more abundant in organic systems than in conventional systems, suggesting that natural beneficial insects were more negatively affected by conventional management practices than insect pests. In addition, the densities of soil-inhabiting animals were usually higher in organic systems, especially earthworms that were favored by higher organic matter in organically

managed soils (Bengtsson et al., 2005). However, concerns for food-borne illnesses incidences from organic production have increased, especially since the outbreaks of *Escherichia coli* infections from consumption of organic spinach produced in California in September-October, 2006 (<http://www.cdc.gov/ecoli/2006/september/>; Brandl, 2006).

Organic agriculture in the U.S. has evolved from a small number of farmers to a multibillion-dollar agricultural sector that is involved in domestic and international trade (Koenig and Baker, 2002). Since 1990, there has been approximately a 20% annual growth rate in the organic sector, with organic food sales reaching \$9.3 billion in 2002 (Koenig and Baker, 2002). In 1990, Congress passed the Organic Foods Production Act (OFPA) in the Farm Bill, establishing consistent organic production standards nationwide, by implementing federally mandated organic standards. As a result, the USDA created the National Organic Program (NOP) as a part of the Agriculture Marketing Service (AMS). The NOP operates under the National Organic Production Standards (NOPS), which were implemented in October 2002, and require that all participants in organic production, including growers, handlers, and processors, be certified by a USDA accredited certification organization. These standards, based on input from all sectors of the organic production community and government, set consistent standards for consumers (Koenig and Baker, 2002). The Final Rule, authorized under the OFPA of 1990, established the NOP under the direction of the AMS, with the purpose of facilitating domestic and international standards for the production, marketing, and labeling of organic food through a national-level accreditation program for production and handling operations ([www.ams.usda.gov/nop/NOP/standards/FullText.pdf](http://www.ams.usda.gov/nop/NOP/standards/FullText.pdf)).

Although organic agriculture relies primarily on a systems approach that considers all aspects of the agricultural system with ecologically sound practices, a limited number of

synthetic substances are allowed under the NOPS

([www.ams.usda.gov.nop/NOP/standards/FullText.pdf](http://www.ams.usda.gov.nop/NOP/standards/FullText.pdf)). The OFPA requires that the Secretary of Agriculture establish a National List of Allowed and Prohibited Substances which identifies synthetic substances that may, or may not, be used in certified organic production systems. Individuals may petition the NOSB to add or remove substances to or from the list, and the OFPA requires that the NOSB and Secretary of Agriculture review all listed substances every five years. The EPA works with the NOP to establish a labeling system for products on the list (Koenig and Baker, 2002).

Organic agriculture is an issue of public concern, as well as becoming a significant industry that is regulated by government standards (Vogl et al., 2005). Organic agriculture can contribute to socio-economic and ecologically sustainable development, but there are issues of concern regarding global harmonization through legal production standard mandates, and the local adoption of standards in various countries (Vogl et al., 2005). Over the last decade, many countries in North America, the E.U., Latin America, Africa, Asia, and Oceania have reported significant increases in the numbers of certified organic farms, with approximately 23 million hectares currently managed organically worldwide and approximately 90 developing countries exporting certified organic products commercially. According to the International Trade Center (Geneva, Switzerland), annual sales for organic products grew from \$17.5 billion in 2000 to \$21 billion in 2001, with growth rate estimates of 5 to 15% for 2003 to 2005 (Vogl et al., 2005).

According to the Organic Foods Production Act of 1990, Section 2109 (a), Seed, Seedlings and Planting Practices: “For a farm to be certified under this title, producers on such farm shall not apply materials to, or engage in practices on, seeds or seedlings that are contrary to, or inconsistent with the applicable organic certification program”

([www.ams.usda.gov/nop/NOP/NOPhome.html](http://www.ams.usda.gov/nop/NOP/NOPhome.html)). This means that producers must use organically grown seeds, annual seedlings, and planting stock, unless otherwise not available ([www.ams.usda.gov/nop/NOP/standards/FullText.pdf](http://www.ams.usda.gov/nop/NOP/standards/FullText.pdf)). The demand for organically produced seed has increased since the rules of the USDA NOP have required the use of organic seed in organic production (L. du Toit, *personal communication*). However, concern for losses due to seedborne and soilborne pathogens has also increased because of the limited effective options available for seed treatments that satisfy organic standards (du Toit et al., 2005b). For some crops, organically produced seeds are more readily available, but for many crop species it is very difficult to produce organic seeds of the same quality as conventionally produced seed, especially for biennial seed crops that have higher risks of disease outbreaks over the two seasons needed for seed production (Groot et al., 2004). Therefore, research is necessary to aid seed companies at improving organic seed production, e.g., through development and refinement of organic disease management tools (Groot et al., 2004). Although the organic sector is among the fastest growing sectors of agriculture, funding for research in this area prior to 2002 was minimal (Koenig and Baker, 2002).

## **1.5. Spinach.**

**1.5.1. Spinach and spinach seed production.** Spinach (*Spinacia oleracea* L.), in the Chenopodiaceae, is a dicotyledonous, cold-hardy crop that is thought to be native to central Asia, where it has been cultivated for more than 1300 years (Correll et al., 1994; Mills, 2005; Sanders, 2001). Spinach was introduced to Europe during the Middle Ages, and by 1806 was listed in American seed catalogs (Mills, 2005). Prized for its nutritional value because of high concentrations of vitamins and minerals (including vitamin A, calcium, phosphorus, iron,

potassium, and vegetable protein) spinach has gained popularity both for processing and fresh markets (Correll et al., 1994; Mills, 2005; Sanders, 2001).

Spinach is an economically important leafy vegetable crop in many countries, with approximately 1400 ha produced in the U.S, where the major spinach producing states include Arizona, California, Colorado, Maryland, New Jersey, Oklahoma, Texas, and Virginia (Correll et al., 1994). The crop is valued at approximately \$70 million annually. Spinach can be grown as a fall, winter, or spring crop, but is also grown year-round in California (Correll et al., 1994). Spinach is an annual, direct-seeded crop that does best in fertile, sandy loam soils that are high in organic materials and have a pH of  $\geq 6$ , and at temperatures of 20 to 25°C. Spinach crops require abundant moisture and high levels of fertility, especially nitrogen (Sanders, 2001; Sumner et al., 1976). A variety of cultivars are grown for fresh markets or for processing uses, including smooth leaf, savoy leaf, and semi-savoy leaf, with the smooth and savoy types used mainly for processing, and the smooth and semi-savoy types for fresh markets (Correll et al., 1994; Mills, 2005). Spinach reaches edible maturity within 37 to 45 days (Sanders, 2003).

The Pacific Northwest (PNW) is the primary area for spinach seed production in the U.S., primarily Skagit, Island and Whatcom Counties of Washington state, where up to 3000 acres are grown annually (Foss and Jones, 2005). Additionally, >500 acres of spinach seed are produced in the Willamette Valley of Oregon (Rackham, 2002). Spinach is typically the most economically important small-seeded vegetable seed crop grown in western Washington (Foss and Jones, 2005; Thomas et al, 1997). Washington State seed growers produce up to 50% of the U.S. supply and up to 20% of the world supply of spinach seed, with an annual market value of seed sold to commercial growers of approximately \$24 million dollars. Production costs for spinach seed growers average \$1000 to \$1200 per acre (Foss and Jones, 2005). The main first

and third party seed companies producing spinach seed in Washington include Ag Alternatives, Alf Christianson Seed Company, Bejo Seeds, D&D Seeds, Sakata Seeds America, Schafer Ag Services, Seminis Vegetable Seeds, Skagit Seed Services, Sorensen Seed Co., and Syngenta Seeds (Western Washington Small Seed Advisory Committee, *personal communication*).

Spinach seed crops in the PNW are grown as annuals that are direct-seeded between late March and mid May, and harvested in July through September (Foss and Jones, 2005). The crops are wind-pollinated. Fields planted to spinach seed crops undergo 6 to 15 year rotation periods, depending on susceptibility of the cultivars grown to soilborne diseases (Foss and Jones, 2005; Thomas et al., 1997).

**1.5.2. Diseases affecting spinach and spinach seed production.** The most widespread and potentially destructive disease of spinach globally is downy mildew, or blue mold, caused by *Peronospora farinosa* (Fr.:Fr.) Fr. f. sp. *spinaciae* Byford (= *P. effusa* (Grev.) Ces.) (Correll et al., 1994). Another pathogen that can have significant negative impacts on spinach production is *Albugo occidentalis* G.W. Wils., which causes white rust in areas east of the Rocky Mountains in the U.S. (Correll et al., 1994). Other important diseases include those caused by *Alternaria*, *Cladosporium*, *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, and *Stemphylium* species (Correll et al., 1994). *Aphanomyces cochlioides*, *F. oxysporum* f. sp. *spinaciae*, *Pythium* species, *Phytophthora cryptogea*, and *R. solani* have been identified as significant pathogens causing soilborne diseases of spinach in Japan, the U.S., Canada, Australia, and Sweden (Larsson and Gerhardson, 1992).

*Fusarium* wilt can be a major problem for spinach at any stage of growth, and is the most economically important disease of spinach seed crops in the U.S. (Foss and Jones, 2005). The pathogen is able to persist for many years in the soil, even in the absence of its host, and is also

seed transmissible. The disease is favored by warm, acidic soils, and control measures have included crop rotation, use of resistant cultivars (where available for specific market needs) and benomyl as a fungicide seed treatment. However, benomyl is no longer registered for any use in the U.S., resulting in the need for alternative fungicide seed treatments for managing *Fusarium* wilt in spinach (Foss and Jones, 2005).

*Verticillium* wilt is a growing issue of concern in spinach production as the pathogen is seedborne, seed-transmitted, and highly systemic in spinach (du Toit et al., 2005a). *V. dahliae* is very persistent in soils (Beckman, 1987). Long-term rotation with crops that are not susceptible to *Verticillium* spp. is employed as the primary method of disease control (Foss and Jones, 2005).

Both pre- and post-emergence damping-off, caused by soilborne *Fusarium*, *Pythium*, *Rhizoctonia*, and *Aphanomyces* species, are important worldwide, with the severity of these spinach diseases influenced by soil moisture and temperature, cultivar, and amount of inoculum of the pathogens (Correll et al., 1994). Severity of damping-off diseases is exacerbated in wet soils that are frequently cropped to spinach. *Pythium* spp. and *R. solani* can persist in soils indefinitely, and are favored by cool, wet weather, and saturated or compacted soils (Hendrix and Campbell, 1973; Naiki, 1985). Conditions favorable to these pathogens can result in up to 100% mortality and yield loss if non-treated seeds are planted into infested soils. Control measures include planting spinach seed in field sites that are suppressive to the pathogens but have favorable growing conditions for spinach, and use of fungicide seed treatments such as metalaxyl (Bayer CropScience, Research Triangle Park, NC), which is effective at controlling *Pythium* spp. but not *R. solani*. Seed treatment with fungicides has been the primary control measure against many damping-off pathogens of spinach (Foss and Jones, 2005).

## 1.6. *Fusarium* wilts.

**1.6.1. Background and importance.** *Fusarium* species are ubiquitous, being found in temperate, tropical, arctic, and desert regions of the world (Nelson, 1981). The genus *Fusarium* consists of numerous plant parasitic species and formae speciales that cause disease in a variety of fruit, vegetable, grain and ornamental crops (Nelson, 1981). The Fusaria are widely distributed in the soil, on both aerial and subterranean plant parts, and other debris in the soil (Burgess, 1981). *Fusarium* wilts are generally more severe in warm soil, favored at temperatures near 28°C (Bruehl, 1987). Members of the genus *Fusarium* have the ability to undergo extensive mycelial growth, with quick response to nutrients and efficient use of ephemeral substrates as they become available to maintain their inoculum potential (Mitchell, 1979). Members of this genus can persist as resistant dormant hyphae in plant residues both parasitically and saprophytically, or as chlamydospores and resistant conidia (Burgess, 1981). Survival of chlamydospores in the soil varies among species and formae speciales, and also depends on climate and soil characteristics (Schippers and van Eck, 1981). Some *Fusarium* species can produce both macro- and microconidia from conidiophores in sporodochia on infected plant parts, or on aerial mycelia (Nelson, 1981). Macroconidia are multiseptate, boat-shaped structures, whereas microconidia are typically unicellular and spherical or oval (Alexopoulos et al., 1996). Reproductive propagules are able to germinate and increase mycelial biomass, then form relatively resistant propagules during saprophytic growth. Prolific sporulation can occur quickly even as the fungus continues colonization of the substrate (Mitchell, 1979).

**1.6.2. *Fusarium oxysporum*.** *F. oxysporum*, which consists of many forma speciales that have high levels of host specificity, can cause wilting of the host plants when the mycelia and conidia of the fungi invade and block xylem vessels of the vascular tissue (Alexopoulos et

al., 1996; Burgess, 1981; Nelson, 1981). This restricts the translocation of water throughout the plants. In addition, production of toxins by the fungi affects cellular metabolism of the host plants, resulting in wilt symptoms. *F. oxysporum* is a persistent and important crop pathogen with worldwide distribution (Nelson, 1981). This species is one of the most common soilborne members of the genus (Burgess, 1981). Many of these fungi can be seed transmitted, and many necessitate crop rotations of 10 to 12 years or longer of the specific host to eliminate the pathogen or at least significantly reduce inoculum levels (Fravel et al., 2003; Neergaard, 1977). Although these fungi are predominantly soilborne pathogens, seed transmission of *Fusarium* species even at low incidences can be a significant source of inoculum when the pathogens are introduced to non-infested soils (Neergaard, 1977).

*F. oxysporum* Schlect. f. sp. *spinaciae* (Sherb.) Snyder and Hans. was first described as the causal agent of Fusarium wilt of spinach by C. W. Hungerford in 1923 (Bassi and Goode, 1978). Research into the seedborne nature of *F. oxysporum* f. sp. *spinaciae* following a widespread outbreak of Fusarium wilt in the U.S.A. in the 1960's showed that the pathogen is internally seedborne (Bassi and Goode, 1978). Seed transmission occurs when conidia or chlamydospores are carried on the seed coat, or in plant debris remaining with the seed (Nelson, 1981). Pathogenic isolates of *F. oxysporum* f. sp. *spinaciae* were obtained from spinach seed that had been surface-sterilized, offering proof of the internal seedborne nature of the pathogen (Bassi and Goode, 1978).

**1.6.3. Management of Fusarium wilts.** Conventional control measures for Fusarium wilt include crop rotation, seed treatments, soil fumigation, and the use of resistant cultivars (Fravel et al., 2003). There are also Fusarium wilt-suppressive soils, in which microbial activity, and physical and chemical properties of the soil prevent the development of wilts induced by

various formae speciales of the genus (Alabouvette et al., 1979; Louvet et al., 1981). The suppressiveness of such soils can be destroyed by biocidal treatments aimed at managing *Fusarium* wilts, and the suppression can be transferred to conducive soils by mixing suppressive soils with conducive soils. Soil fumigation has been used to eliminate *Fusarium* spp. from the soil; however, some fumigants, especially methyl bromide, have detrimental environmental effects and will no longer be available for commercial use in the U.S.A. in the future ([http://www.epa.gov/oppsrrd1/reregistration/methyl\\_bromide/index.htm](http://www.epa.gov/oppsrrd1/reregistration/methyl_bromide/index.htm)). Newer, biological fumigants, such as Muscodor (*Muscodor albus*, AgraQuest, Inc., Davis, CA), may be promising replacements for conventional fumigants ([http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet\\_006503.htm](http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_006503.htm)). The most environmentally-sound and cost effective method of managing *Fusarium* wilt is through the use of resistant cultivars, when available (Fravel et al., 2003).

Many conventional chemical seed treatments have been evaluated for management of *Fusarium* wilt in a variety of crop species. Three fungicide seed treatments, captan (Gustafson Inc., Plano, TX), difenoconazole (Syngenta Crop Protection, Greensboro, NC), and fludioxonil (Syngenta Crop Protection), were evaluated against six *Fusarium* species pathogenic to maize (*Zea mays*) seedlings (Munkvold and O'Mara, 2002). All three fungicides were effective in the laboratory and under growth chamber conditions. However, captan was less effective than the other fungicides, and the efficacy of the treatments varied among the species of *Fusarium* (Munkvold and O'Mara, 2002). Inglis (2004) suggested that seed treatment with the fungicides thiram and captan were effective at minimizing *Fusarium* damping-off in vegetable crops, by forming a protective barrier on the seed against the resting structures of the pathogen. PlantPro 45 (iodine, Ajay North America, Powder Springs, GA), an iodine-based compound, was

determined to be a successful seed treatment against Fusarium wilt of basil (*Ocimum basilicum*) when applied at rates of 800 to 1000 mg/liter, and significantly increased seed germination and transplant growth (Adams et al., 2003). Galperin et al. (2003) demonstrated that up to 50% of *F. moniliforme* infection of corn seedlings was due to systemic seedborne inoculum, thus emphasizing the need for effective seed treatments. Prochloraz (Bayer CropScience) proved to be an effective seed treatment for eliminating the pathogen from corn seeds.

A number of bacterial strains have shown potential effectiveness for the management of Fusarium wilt when applied as seed treatments. Treatment of radish (*Raphanus sativus*) seed with *Pseudomonas fluorescens* WCS374 proved effective against Fusarium wilt caused by *F. oxysporum* f. sp. *raphani*, with an average reduction in incidence of diseased plants by >40% and an increase in yield of almost 45% (Leeman et al., 1995). The bacterial strain was also found to remain in the soil and colonize the successive radish crop (Leeman et al., 1995). Application of *Pseudomonas putida* WCS358 and *P. fluorescens* WCS374 to root tips was also evaluated for control of Fusarium wilt of radish (Raaijmakers et al., 1995). *P. putida* suppressed the pathogen through siderophore-mediated competition for iron, whereas *P. fluorescens* induced systemic resistance against *F. oxysporum* f. sp. *raphani*. The efficacies of the mechanisms of suppression were highly dependent on disease incidence, and rhizosphere population densities of the bacteria were important in the efficacy of the strains for suppression of Fusarium wilt of radish (Raaijmakers et al., 1995).

Arbuscular mycorrhizal fungi, alone or in combination with bacterial BCA's, have also been found to be effective at managing Fusarium wilt in a number of crop species. The management of Fusarium wilt of tomato (*Lycopersicon esculentum*), caused by *F. oxysporum* f. sp. *lycopersici*, was achieved through inoculation of seedlings with the arbuscular mycorrhizal

fungus *Glomus intraradices*, and some rhizobacteria, including *P. fluorescens*, *P. putida*, and *Enterobacter cloacae* (Akkopru and Demir, 2005). Protection was achieved by each of the BCA's, as well as by additive, synergistic effects of combinations of the treatments. The BCA's controlled the pathogen through mechanisms of competition, antibiosis, and induced resistance. The mutual establishment of these BCA's enhanced plant growth through improved plant rooting and uptake of nutrients from the soil (Akkopru and Demir, 2005). Inoculation of common bean (*Phaseolus vulgaris*) seedling roots with the vesicular arbuscular mycorrhizal fungus *G. mosseae*, either alone or in combination with the bacterium *Rhizobium leguminosarum*, improved plant growth, nutrient uptake, and induced resistance to *Fusarium* spp. by reducing root rot by 34 to 77% (Hassan Dar et al., 1997).

Hot water seed treatments are another option for management of seedborne *Fusarium*. *F. moniliforme* was eliminated from corn seed through hot water treatments not exceeding 60°C, in order to maintain acceptable germination rates (Daniels, 1983). Compared with traditional NaOCl surface-sterilant treatments for corn seed, the hot water treatment eliminated the pathogen both internally and externally (Daniels, 1983). Galperin et al. (2003) suggested the use of hot water seed treatments for controlling *F. moniliforme* infection of corn seedlings as an alternative to chemical seed treatments (Galperin et al., 2003). du Toit and Hernandez-Perez (2005) demonstrated that hot water treatment of spinach seed significantly reduced the mean incidence of *Fusarium* spp. compared to non-treated seed.

Control of *Fusarium* wilt of a number of host plants was attained by using non-pathogenic species of *Fusarium*, through direct antagonism of the non-pathogenic species on the pathogenic species, as well as indirect antagonism mediated through the host plant (Fravel et al., 2003). The non-pathogenic strains competed with the pathogenic isolates for nutrients in the soil

and rhizosphere, as well as for infection sites on and within the root system of the host plant, and they induced systemic resistance in the host plant to infection by the non-pathogenic strains (Fravel et al., 2003).

## **1.7. *Pythium* species as plant pathogens.**

**1.7.1. Background and importance.** The genus *Pythium*, belonging to the Pythiaceae, contains over 120 species that are widespread, soil-inhabiting organisms, consistently associated with root diseases (Alexopoulos et al., 1996; Hendrix and Campbell, 1973; Neergaard, 1977). Most species primarily infect juvenile or succulent tissues of seedlings, or feeder roots or root tips of older plants (Hendrix and Campbell, 1973). Some of these species are commonly problematic in cultivated soils, posing major problems for a variety of agronomically important crops by causing pre- and post-emergence damping-off and seed rots. Pythiaceae species have coenocytic hyphae, and their taxonomy is based on morphological characters of swimming zoospores and the sexual structures, oogonia and antheridia, as well as differences in growth at a range of temperatures, lighting, and on various nutrient media (Alexopoulos et al., 1996; Hendrix and Campbell, 1973). These pathogens are not vigorous competitors. They are opportunistic, and can survive as saprophytes on readily available energy sources from dead plant debris, where the pathogens can efficiently maintain an inoculum potential indefinitely without dependence on the presence of a host plant (Alexopoulos et al., 1996; Hendrix and Campbell, 1973; Mitchell, 1979; Neergaard, 1977). Soil moisture is important for saprophytic growth of some species, but survival by resistant resting structures is more important than saprophytic growth (Hendrix and Campbell, 1973). Members of the genus have a mycelial stage which collapses in the absence of available energy sources, but form metabolically inactive sporangia, chlamydozoospores, and/or

oospores, which serve as dormant survival structures in the soil (Hendrix and Campbell, 1973; Mitchell, 1979).

**1.7.2. *Pythium ultimum*.** *P.ultimum* germinates and grows rapidly in wet soils in the presence of host exudates (Bruehl, 1987; Hendrix and Campbell, 1973). *P. ultimum* invades seeds in the food-rich environment of the seeds, which exude nutrients into the surrounding wet soil during germination (Bruehl, 1987; Hendrix and Campbell, 1973; Mitchell, 1979). The factors that influence infection include inoculum density, soil moisture, soil pH, soil cation composition, light intensity, and presence of competitive microorganisms (Hendrix and Campbell, 1973).

**1.7.3. Management of *Pythium* damping-off.** Use of resistant cultivars can reduce disease caused by *Pythium* spp. (Hendrix and Campbell, 1973). However, breeding for resistance to this genus is difficult, and is not effective for all crops (Hendrix and Campbell, 1973). Crop rotation may reduce populations of *Pythium* spp. (Hendrix and Campbell, 1973). However, due to the wide host ranges of many Pythiaceous species, crop rotation is not usually effective unless a crop with low moisture requirements is used to alter the conditions of the soil, thus influencing the conditions *Pythium* spp. require for optimum growth (Hendrix and Campbell, 1973).

The resting structures of *Pythium* spp., once widely established in a soil, are virtually impossible to eliminate (Hendrix and Campbell, 1973). Wide-spectrum soil fumigants, including chloropicrin or methyl bromide, or the combination of the two, have been the only effective means of eradicating the pathogen from soils, but this method of disease management can be difficult and expensive (Hendrix and Campbell, 1973). On a smaller scale, the pathogens can be

eliminated from soil by heating, specifically steam treatment or pasteurization (Hendrix and Campbell, 1973).

Some conventional fungicides have been effective at managing damping-off diseases caused by *Pythium* spp. For example, seed treatments with the fungicides captan and thiram minimized damping-off caused by *Pythium* in vegetable crops (Inglis, 2004). Damping-off caused by some *Pythium* spp. has been managed with the use of specific bacterial strains. Treatment of cotton (*Gossypium hirsutum*) seed with strains of *Enterobacter cloacae* and *Erwinia herbicola* were effective against seed rot and pre-emergence damping-off caused by *Pythium* spp. under growth chamber conditions, but had variable results in the field, depending on temperature (Nelson, 1988). The results suggested that development of more cold-tolerant strains of the bacteria may have provided more consistent control of damping-off of cotton. Seed treatment of chickpea (*Cicer arietinum*) with *P. fluorescens* proved to be as effective as chemical seed treatment with metalaxyl plus thiabendazole (Apron T69, Syngenta Crop Protection) in field studies against *P. ultimum* (Trapero-Casas and Ingram, 1990). Seed treatment with a variety of strains of *Trichoderma* spp. effectively reduced pre-emergence damping-off of cotton caused by *P. ultimum* in a study by Howell (2007). A study by Huang and Erickson (2007) demonstrated that treatment of pea and lentil with *Rhizobium leguminosarum* bv. *viceae* effectively controlled damping-off by *Pythium* spp. compared to the non-treated control, but that the efficacy of control was strain specific with strain R21 effective for pea and strain R12 for lentil.

Soil populations of *P. ultimum* are significantly influenced by physical factors and content of plant organic matter in the soil (Hancock, 1979). Hancock (1979) discovered soils suppressive to *P. ultimum* in California's San Joaquin Valley in 1977, and realized the

suppressiveness could be transmitted to conducive soils of the same texture. Such soils were characteristically finely textured, but depended on amounts and types of organic matter.

Other, non-conventional methods of managing *Pythium* damping-off have been tested. Soil amendments such as sawdust, bark, green manures, and other crop residues have been implemented, with limited control (Hendrix and Campbell, 1973). Treatment of sugar beet (*Beta vulgaris* L.) seed with specific crop straw powders, alone or in combination with the bacterium *P. fluorescens*, proved to be effective against *Pythium* damping-off as a result of the production of volatile ammonia that suppressed growth of *Pythium* (Bardin et al., 2004). Scheuerell and Mahaffee (2004) determined that a compost tea supplemented with kelp and humic acids effectively reduced damping-off in cucumber caused by *P. ultimum* in a soilless peat-based container medium that was naturally conducive to the disease.

## **1.8. *Rhizoctonia* species as plant pathogens.**

**1.8.1. Background and importance.** *Rhizoctonia* spp., members of the Hyphomycetes, are identified, in part, by their septate hyphae with multinucleate compartments, and hyphal branching at approximately 90° angles (Alexopoulos et al., 1996). These fungi are problematic soilborne pathogens worldwide for a multitude of crop species, with long-term saprophytic survival in the absence of a host (Alexopoulos et al., 1996; Naiki, 1985; Neergaard, 1977). Taxonomy of this group was difficult prior to the use of molecular tools. The classification systems include anastomosis groups (AG), which are based on isolates that can undergo hyphal fusion, and intraspecific groups (ISGs), which are based on morphology and pathology (Alexopoulos et al., 1996; Sneh et al., 1991).

**1.8.2. *Rhizoctonia solani*.** *R. solani* Kuhn is a basidiomycete anamorph of *Thanatephorus cucumeris* (Frank) Donk, and is the most studied species in the genus (Sneh et al., 1991). This species is identified based on the presence of brown hyphal pigmentation, branching near the distal septum of cells in young vegetative hyphae, constriction of hyphae and formation of septa near hyphal branches, dolipore septa, formation of monilioid cells and/or sclerotia, and multinucleate cells in young vegetative hyphae (Sneh et al., 1991). The fungus is a ubiquitous plant pathogen that is primarily soilborne but can be seedborne, causing a range of diseases in a multitude of crops worldwide (Alexopoulos et al., 1996; Naiki, 1985; Neergaard, 1977). The pathogen rapidly increases its biomass in the soil when energy sources are available, increasing inoculum potential and expanding through the soil away from a food source (Mitchell, 1979). *R. solani* can survive in soil for long periods in the absence of a host, either as thick-walled sclerotia or as thick-walled, melanized hyphae in plant debris (Naiki, 1985; Henis, 1979; Mitchell, 1979; Sneh et al., 1991). These propagules reside in the upper 15 to 20 cm of soil, and are unevenly distributed in aggregated pockets in the soil as a result of pathogenic or saprophytic colonization of substrates within the soil (Sneh et al., 1991). The number of survival units and the distribution of those units in the soil are important in the epidemiology of diseases caused by the fungus (Naiki, 1985). Symptoms of *R. solani* infection of plants include seed and root rots and damping-off (Alexopoulos et al., 1996; Neergaard, 1977).

*R. solani* can be divided into 11 or 12 AGs based on hyphal fusion, pathology, and morphology (Ogoshi and Ui, 1985; Sneh et al., 1991). Anastomosis is useful for identification of AGs within *R. solani*, but tells us little about the behavior of the fungi. Various AGs of *R. solani* are found worldwide, but distribution of AGs within locations depends on the particular crops cultivated (Ogoshi and Ui, 1985). The AG2-2 and AG4 types have mostly been isolated from

members of the Chenopodiaceae. Isolates of anastomosis group AG4 were demonstrated to be more pathogenic on spinach than isolates of AG1, AG2, and AG5, which were also pathogenic on spinach (Naiki and Kanoh, 1978). *R. solani* was found to occur more frequently on spinach plants in early seedling stages of growth, compared with later stages of plant growth (Naiki and Kanoh, 1978).

**1.8.3. Management of Rhizoctonia damping-off and seedling blight.** Damping-off and root rot caused by *R. solani* can be managed with a variety of methods, including cultural practices and crop rotations, as well as using bacterial BCAs (Leach and Garber, 1970). Kasuya et al. (2006) successfully reduced damping-off of sugar beet (*Beta vulgaris*) caused by *R. solani* through soil amendment with plant residues from specific cultivars of *Brassica rapa*. Many cruciferous plants are known to produce isothiocyanates, a natural biofumigant by-product, when the glucosinolates contained in the plant are hydrolyzed by the enzyme myrosinase during residue incorporation into the soil (Manici et al., 1997; Manici et al., 2000).

Bare-root dips of tomato seedlings, and soil drenches with both *Pseudomonas aeruginosa* and *P. fluorescens* were most effective at suppressing damping-off caused by *R. solani* at low levels of inoculum of the pathogen compared to higher inoculum levels (Siddiqui and Shaukat, 2002). Induced systemic resistance to the pathogen was achieved by coating tomato seeds with high numbers of the bacterial antagonists or by adding suspensions of the bacteria to the soil at seeding or at transplanting. Three antagonistic strains of ecto- and endophytic bacteria, *P. fluorescens* B1, *P. fluorescens* B2, and *Serratia plymuthica* B4, were evaluated for their control of *R. solani* damping-off by adding the bacterial suspensions to infested soil in growth chambers and under field conditions (Grosch et al., 2005). *P. fluorescens* B1 was the most effective of the three bacteria as a candidate for commercial development as a BCA (Grosch et al., 2005). Soil

amendments in both greenhouse and field trials using Ca-alginate beads containing a combination of *Pseudomonas boreopolis*, brassica seed pomace, and glycerin (PBGG) proved effective at controlling damping-off of Chinese cabbage (*Brassica pekinensis*) caused by *R. solani*, whether applied alone or in combination with specific antagonistic strains of *Streptomyces* spp. (Chung et al., 2005).

Some *Trichoderma* spp. are mycoparasites of *R. solani*, causing suppression of damping-off caused by this fungus (Baker and Paulitz, 1996). Seed treatment with *T. harzianum*, in combination with planting into acidic soils, was effective at reducing damping-off of snap bean (*Phaseolus vulgaris*) caused by *R. solani* (Marshall, 1982). The acid soil conditions in that study reduced growth of the pathogen while favoring growth and antagonistic properties of *T. harzianum* (Marshall, 1982). A combination of the pathogen-specific fungicide, benodanil (BASF Agricultural Products, Limburgerhof, Germany), and *T. harzianum* as seed treatments for control of damping-off of radish caused by *R. solani* was more effective than seed treatment with the fungicide alone (Lifshitz et al., 1985). The combination treatment was also effective in alkaline soils, in which *T. harzianum* was not effective when used alone. The control provided by the combination seed treatment was determined statistically to be additive, not synergistic (Lifshitz et al., 1985). Comparison of mycelial and conidial preparations of *Trichoderma* spp. and *Gliocladium* spp. showed that mycelial preparations were more effective at reducing *Rhizoctonia* activity and survival in soil, but that conidial preparations could be used more effectively as seed treatments because conidia can be produced and handled more easily than mycelium of these BCAs (Lewis and Papavizas, 1985).

Two isolates, BNR1 and BNR2, of a binucleate *Rhizoctonia* sp. produced on wheat bran substrate, and slurries of the bacterium *Bacillus amyloliquefaciens*, as well as two isolates of *P.*

*putida*, were evaluated as amendments to potting media for control of damping-off caused by *R. solani* on bell pepper (*Capsicum annuum*) under a range of conditions (Harris and Adkins, 1999). Control of *R. solani* by the two binucleate *Rhizoctonia* isolates was effective at low doses of the isolates, low temperatures, and in various potting media, demonstrating their potential for commercial development (Harris and Adkins, 1999).

Treatments of cotton seed with myclobutanil (Dow AgroSciences, Indianapolis, IN), alone and in combination with metalaxyl, suppressed damping-off caused by *Rhizoctonia*, and improved stand establishment in field trials on a variety of soil types, at a range of levels of inoculum of the pathogen, and a range of air temperatures (Davis et al., 1997). Seed treatment with myclobutanil alone displayed specific activity against *Rhizoctonia* spp., but also increased susceptibility of cotton seedlings to attack by *Pythium* spp. The combination of the two fungicides was effective against both *Rhizoctonia* and *Pythium* (Davis et al., 1997).

## **1.9. Conclusion and research needs.**

Organic agriculture faces many challenges, including seed- and soilborne pathogens. There is an obvious need for seed or drench treatments approved for use in organic production systems that are effective against the diversity of these pathogens. A variety of products has been claimed or demonstrated to have efficacy against these pathogens, but results have often been highly variable, and many have been generated under artificial (greenhouse, growth chamber, or laboratory) conditions. Such products require more extensive evaluation under a range of field conditions. In addition, product formulations that have the potential for application in organic production need to be evaluated. Since spinach is susceptible to many of the most prevalent soilborne pathogens that cause seedling blights and damping-off, and is a

popular crop for organic production, spinach is an ideal model of a small-seeded vegetable crop for investigating the efficacy of EPA registered seed and drench treatments and products with potential for registration and use in certified organic production for control of soilborne pathogens. In consideration of these research needs, the objectives of this thesis project are to:

1. Obtain and confirm pathogenicity of isolates of *P. ultimum*, *R. solani*, and *F. oxysporum* f. sp. *spinaciae* for use in greenhouse and field trials;
2. Determine effective means of inoculum production, inoculation of a greenhouse growth substrate, and rates of inoculation for all three pathogens that achieve approximately 50% damping-off or seedling mortality under greenhouse conditions to optimize differentiation of the efficacy of seed and drench treatments evaluated;
3. Evaluate selected seed and drench treatment products in a greenhouse against each of the three pathogens separately, at rates of inoculation determined by the inoculation rate trials;
4. Determine if any of the selected seed treatments effectively reduce the incidence of seedborne necrotrophic fungi present on each seed lot;
5. Determine if any of the selected seed treatments have an effect on germination of the treated seed;
6. Determine effective means of inoculum production, and inoculation of field plots;
7. Evaluate seed and drench treatment products that proved most efficacious under greenhouse conditions in field trials at three locations in the western region of Washington State.

### 1.10. Literature cited.

- Adams, P. D., Kokalis-Burelle, N., and Basinger, W. H. 2003. Efficacy of Plantpro 45 as a seed and soil treatment for managing Fusarium wilt of basil. HortTech. 13:77-80.
- Agarwal, V. K., and Sinclair, J. B. 1997. Principles of Seed Pathology, Second Edition. CRC Press, Inc., Boca Raton, FL.
- Agrios, G. N. 1988. Plant Pathology, Third Edition. Academic Press, Inc., San Diego, CA.
- Akkopru, A., and Demir, S. 2005. Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some Rhizobacteria. J. Phytopathology 153:544-550.
- Alabouvette, C., Rouxel, F., and Louvet, J. 1979. Characteristics of Fusarium wilt-suppressive soils and prospects for their utilization in biological control. Pages 165-182, in: Soil-borne Plant Pathogens. B. Schippers, and W. Gams, editors. Academic Press, New York, NY.
- Alexopoulos, C. J., Mims, C. W., and Blackwell, M. 1996. Introductory Mycology. John Wiley & Sons, Inc., New York, NY.
- Baker, R., and Paulitz, T. C. 1996. Theoretical basis for microbial interaction leading to biological control of soilborne plant pathogens. Pages 50-79, in: Principles and Practice of Managing Soilborne Plant Pathogens. R. Hall, editor. APS Press, St. Paul, MN.
- Bakker, P. A. H. M., Pieterse, C. M. J., and van Loon, L. C. 2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology 97:239-243.
- Bardin, S. D., Huang, H. C., and Moyer, J. R. 2004. Control of *Pythium* damping-off of sugar beet by seed treatment with crop straw powders and biocontrol agents. Biological Control 29:453-460.

- Bassi, A., Jr., and Goode, M. J. 1978. *Fusarium oxysporum* f. sp. *spinaciae* seedborne in spinach. *Plant Dis. Rep.* 62:203-205.
- Beckman, C. H. 1987. *The Nature of Wilt Diseases of Plants*. APS Press, St. Paul, MN.
- Bengtsson, J., Ahnstrom, J., Weibull, A. 2005. The effects of organic agriculture on biodiversity and abundance: A meta-analysis. *J. Applied Ecology* 42:261-269.
- Bowen, G. D. 1979. Integrated and experimental approaches to the study of growth of organisms around roots. Pages 209-227, in: *Soil-borne Plant Pathogens*. B. Schippers, and W. Gams, editors. Academic Press, New York, NY.
- Brandl, M. 2006. Fitness of human enteric pathogens on plants and implications for food safety. *Annu. Rev. Phytopath.* 44:367-392.
- Bruehl, G. W. 1987. *Soilborne Plant Pathogens*. Macmillan Publishing Co., New York, NY.
- Burgess, L. W. 1981. General ecology of the Fusaria. Pages 225-235, in: *Fusarium: Diseases, Biology, and Taxonomy*. P. Nelson, T. Toussoun, and R. Cook, editors. The Pennsylvania State University Press, University Park, PA.
- Campbell, C. L., and Neher, D. A. 1996. Challenges, opportunities, and obligations in root disease epidemiology and management. Pages 20-49, in: *Principles and Practice of Managing Soilborne Plant Pathogens*. R. Hall, editor. APS Press, St. Paul, MN.
- Catska, V. 1979. Volatile and gaseous metabolites of germinating seeds and their role in fungus-plant interactions. Pages 241-245, in: *Soil-borne Plant Pathogens*. B. Schippers, and W. Gams, editors. Academic Press, New York, NY.
- Centers for Disease Control. Multi-State Outbreak of *E. coli* O157:H7 Infections From Spinach. <http://www.cdc.gov/ecoli/2006/september/>.

- Chung, W. C., Huang, J. W., and Huang, H. C. 2005. Formulation of a soil biofungicide for control of damping-off of Chinese cabbage (*Brassica chinensis*) caused by *Rhizoctonia solani*. *Biological Control* 32:287-294.
- Coley-Smith, J. R. 1979. Survival of plant-pathogenic fungi in soil in the absence of host plants. Pages 39-57, in: *Soil-borne Plant Pathogens*. B. Schippers, and W. Gams, editors. Academic Press, New York, NY.
- Correll, J. C., Morelock, T. E., Black, M. C., Koike, S. T., Brandenberger, L. P., and Dainello, F. J. 1994. Economically important diseases of spinach. *Plant Dis.* 78:653-660.
- Daniels, B. A. 1983. Elimination of *Fusarium moniliforme* from corn seed. *Plant Dis.* 67:609-611.
- Davis, R. M., Nunez, J. J., and Subbarao, K. V. 1997. Benefits of cotton seed treatments for the control of seedling diseases in relation to inoculum densities of *Pythium* species and *Rhizoctonia solani*. *Plant Dis.* 81:766-768.
- du Toit, L. J., Derie, M. L., Hernandez-Perez, P. 2005a. Verticillium wilt in spinach seed production. *Plant Dis.* 89:4-11.
- du Toit, L. J., and Hernandez-Perez, P. 2005. Efficacy of hot water and chlorine for eradication of *Cladosporium variabile*, *Stemphylium botryosum*, and *Verticillium dahliae* from spinach seed. *Plant Dis.* 89:1305-1308.
- du Toit, L. J., Inglis, D. A., and Miles, C. M. 2005b. USDA Organic Cropping Research for the Northwest: Organic Seed Treatments: 2005 Progress Report. Washington State University-NWREC, Mount Vernon, WA.
- Foss, C. R., and Jones, L. J. 2005. USDA Crop Profile for Spinach Seed in Washington. USDA Pest Management Centers. <http://cipm.ncsu.edu/cropprofiles/docs/waspinachseed.html>.

- Fravel, D., Olivain, C., and Alabouvette, C. 2003. *Fusarium oxysporum* and its biocontrol. *New Phytologist* 157:493-502.
- Galperin, M., Graf, S., and Kenigsbuch, D. 2003. Seed treatment prevents vertical transmission of *Fusarium moniliforme*, making a significant contribution to disease control. *Phytoparasitica* 31:344-352.
- Garrett, S. D. 1970. *Pathogenic Root-infecting Fungi*. Cambridge University Press, New York, NY.
- Gerhardson, B., and Larsson, M. 1991. Effects of *Trichoderma* and other fungal antagonists on the incidence of fungal pathogens. Pages 121-128, in: *Biotic Interaction and Soilborne Diseases*. A. B. R. Beemster, G. J. Bollen, M. Gerlagh, M. A. Ruissen, B. Schippers, and A. Tempel, editors. Elsevier Science Publishing Co. Inc., New York, NY.
- Gindrat, D. 1979. Side effects of pesticides: Concluding remarks. Pages 531-534, in: *Soilborne Plant Pathogens*. B. Schippers and W. Gams, editors. Academic Press, New York, NY.
- Green, R. J., Jr. 1981. An Overview. Pages 1-24, in: *Fungal Wilt Diseases of Plants*. M. Mace, A. Bell, and C. Beckman, editors. Academic Press, NY, New York.
- Groot, S., van der Wolf, J. M., Jalink, H., Langerak, C. J., and van den Bulk, R. W. 2004. Challenges for the production of high quality organic seeds. *Seed Testing International ISTA News Bulletin* No. 127:12-15.
- Grosch, R., Franziska, F., Lottmann, J., Kofoet, A., and Berg, G. 2005. Effectiveness of three antagonistic bacterial isolates to control *Rhizoctonia solani* Kuhn on lettuce and potato. *Can. J. Microbiol.* 51:345-353.

- Hancock, J. G. 1979. Occurrence of soils suppressive to *Pythium ultimum*. Pages 183-189, in: Soil-borne Plant Pathogens. B. Schippers, and W. Gams, editors. Academic Press, New York, NY.
- Harman, G. E. 1991. Seed treatments for biological control of plant disease. Crop Protection 10:166-171.
- Harris, A. R., and Adkins, P. G. 1999. Versatility of fungal and bacterial isolates for biological control of damping-off disease caused by *Rhizoctonia solani* and *Pythium* species. Biological Control 15:10-18.
- Hassan Dar, G., Zargar, M. Y., and Beigh, G. M. 1997. Biocontrol of Fusarium root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mossea* and *Rhizobium leguminosarum*. Microbial Ecology 34:74-80.
- Hendrix, F. F., and Campbell, W. A. 1973. Pythiums as plant pathogens. Annu. Rev. Phytopath. 11:77-98.
- Henis, Y. 1979. Relationship between metabolism, morphogenesis, survival and parasitism in sclerotial soil-borne pathogenic fungi. Pages 59-71, in: Soil-borne Plant Pathogens. B. Schippers, and W. Gams, editors. Academic Press, New York, NY.
- Hornby, D. 1985. The study of soilborne plant pathogens: Changing outlook or more of the same? Pages 3-6, in: Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Fovira, K. J. Moore, and P. T. W. Wong, editors. APS Press, St. Paul, MN.
- Horsfall, J. G., and Cowling, E. B. 1978. Plant Disease: An Advanced Treatise. Volume II. Academic Press, New York, NY.
- Howell, C. R. 2007. Effect of seed quality and combination fungicide-*Trichoderma* spp. seed treatments on pre- and post-emergence damping-off in cotton. Phytopathology 97:66-71.

- Huang, H. C., and Erickson, R. S. 2007. Effect of seed treatment with *Rhizobium leguminosarum* on Pythium damping-off, seedling height, root nodulation, root biomass, shoot biomass, and seed yield of pea and lentil. *J. Phytopathology* 155:31-37.
- Inglis, D. A. 2004. Management of fungal and oomycete diseases: Vegetable crops. Pages 681-683, in: *Encyclopedia of Plant and Crop Science*. R. M. Goodman, editor. Marcel Dekker, New York, NY.
- Kasuya, M., Olivier, A. R., Ota, Y., Tojo, M., Honjo, H., and Fukui, R. 2006. Induction of soil suppressiveness against *Rhizoctonia solani* by incorporation of dried plant residues into soil. *Phytopathology* 96:1372-1379.
- Koenig, R. L., and Baker, B. 2002. U.S. National Organic Program Standards: Implications for researchers. APSnet <http://www.apsnet.org/online/feature/organic/>
- Larsson, M., and Gerhardson, B. 1992. Disease progression and yield losses from root diseases caused by soilborne pathogens of spinach. *Phytopathology* 82:403-406.
- Leach, L. D., and Garber, R. H. 1970. Control of *Rhizoctonia*. Pages 189-198, in: *Rhizoctonia solani*, Biology and Pathology. J. R. Parmeter, Jr., editor. University of California Press, Berkeley and Los Angeles, CA.
- Leeman, M., van Pelt, J. A., Hendrickx, M. J., Scheffer, R. J., Bakker, P. A. H. M., and Schippers, B. 1995. Biocontrol of Fusarium wilt of radish in commercial greenhouse trials by seed treatment with *Pseudomonas fluorescens* WCS374. *Phytopathology* 85:1301-1305.
- Lewis, J. A., and Papavizas, G. C. 1985. Effect of mycelial preparations of *Trichoderma* and *Gliocladium* on populations of *Rhizoctonia solani* and the incidence of damping-off. *Phytopathology* 75:812-817.

- Lifshitz, R., Lifshitz, S., and Baker, R. 1985. Decrease in incidence of *Rhizoctonia* preemergence damping-off by use of integrated chemical and biological controls. *Plant Dis.* 69:431-434.
- Louvet, C., Alabouvette, C, and Rouxel, F. 1981. Microbiological suppressiveness of some soils to *Fusarium* wilts. Pages 261-275, in: *Fusarium: Diseases, Biology, and Taxonomy*. P. Nelson, T. Toussoun, and R. Cook, editors. The Pennsylvania State University Press, University Park, PA.
- Manici, L. M., Lasserri, L., Baruzzi, G., Leoni, O., Galletti, S., and Palmieri, S. 2000. Suppressiveness activity of some glucosinolate enzyme degradation products on *Pythium irregulare* and *Rhizoctonia solani* in sterile soil. *Pest Manage. Sci.* 56:921-926.
- Manici, L. M., Lazzari, L., and Palmieri, S. 1997. In vitro fungitoxic activity of some glucosinolates and their enzyme-derived products toward plant pathogenic fungi. *J. Agric. Food Chem.* 45:2768-2773.
- Marshall, D. S. 1982. Effect of *Trichoderma harzianum* seed treatment and *Rhizoctonia solani* inoculum concentration on damping-off of snap bean in acidic soils. *Plant Dis.* 66:788-789.
- McGee, D. C. 1997. Relevance of seed pathology research priorities to worldwide movement of seed. Pages 1-16, in: *Plant Pathogens and the Worldwide Movement of Seeds*. APS Press, St. Paul, MN.
- McSpadden Gardener, B. B. 2007. Diversity and ecology of biocontrol *Pseudomonas* spp. in agricultural systems. *Phytopathology* 97:221-226.
- Mills, H. A. 2001. Vegetable Crops. Spinach. University of Georgia, Athens, GA.  
<http://www.uge.edu/vegetable/spinach.html>.

- Mitchell, J. E. 1979. The dynamics of the inoculum potential of populations of soil-borne plant pathogens in the soil ecosystem. Pages 3-20, in: Soil-borne Plant Pathogens. B. Schippers and W. Gams, editors. Academic Press, New York, NY.
- Munkvold, G. P., and O'Mara, J. K. 2002. Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedling blight caused by *Fusarium* species. Plant Dis. 86:143-150.
- Naiki, T. 1985. Population and survival of sclerotia of *Rhizoctonia solani* in soil. Pages 51-53, in: Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Fovira, K. J. Moore, and P. T. W. Wong, editors. APS Press, St. Paul, MN.
- Naiki, T., and Kanoh, M. 1978. Grouping of *Rhizoctonia solani* Kuhn causing root diseases of spinach in plastic house cropping. Ann. Phytopath. Soc. Japan 44:554-560.
- National Organic Program. 2005. Organic Foods Production Act of 1990. Section 2109: Prohibited Crop Production Practices and Materials.  
<http://www.ams.usda.gov/nop/NOP/NOPhome.html>
- Neergaard, P. 1977. Seed Pathology. John Wiley & Sons, Inc., New York, NY.
- Nelson, E. B. 1988. Biological control of *Pythium* seed rot and preemergence damping-off of cotton with *Enterobacter cloacae* and *Erwinia herbicola* applied as seed treatments. Plant Dis. 72:140-142.
- Nelson, P. 1981. Life cycle and epidemiology of *Fusarium oxysporum*. Pages 51-80, in: Fungal Wilt Diseases of Plants. M. Mace, A. Bell, and C. Beckman, editors. Academic Press, New York, NY.

- Ogoshi, A., and Ui, T. 1985. Anastomosis groups of *Rhizoctonia solani* and binucleate *Rhizoctonia*. Pages 57-58, in: Ecology and Management of Soilborne Plant Pathogens. C. A. Parker, A. D. Fovira, K. J. Moore, and P. T. W. Wong, editors. APS Press, St. Paul, MN.
- Pierson, L. S. III, and Pierson, E. A. 2007. Roles of diffusible signals in communication among plant-associated bacteria. *Phytopathology* 97:227-232.
- Raaijmakers, J. M., Leeman, M., van Oorschot, M. M. P., van der Sluis, I., Schippers, B., and Bakker, P. A. H. M. 1995. Dose-response relationships in biological control of Fusarium wilt of radish by *Pseudomonas* spp. *Phytopathology* 85:1075-1081.
- Rackham, R. L. 2002. History of Specialty Seed Crop Production in the Pacific Northwest. Willamette Valley Specialty Seed Crops Association, Corvallis, OR.
- Sanders, D. C. 2003. Spinach. North Carolina State University Horticultural Information Leaflets. <http://www.ces.ncsu.edu/depts/hort/hil/hil-17.html>.
- Scheuerell, S. J., and Mahaffee, W. F. 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. *Phytopathology* 94:1156-1163.
- Schippers, B., and van Eck, W. H. 1981. Formation and survival of chlamydospores in *Fusarium*. Pages 250-260, in: *Fusarium: Diseases, Biology, and Taxonomy*. P. Nelson, T. Toussoun, and R. Cook, editors. The Pennsylvania State University Press, University Park, PA.
- Siddiqui, I. A., and Shaukat, S. S. 2002. Resistance against the damping-off fungus *Rhizoctonia solani* systemically induced by the plant-growth-promoting rhizobacteria *Pseudomonas aeruginosa* (IE-6S+) and *P. fluorescens* (CHA0). *J. Phytopathology* 150:500-506.

- Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* Species. APS Press, St. Paul, MN.
- Sticher, L., Mauch-Mani, B., and Metraux, J. P. 1997. Systemic acquired resistance. *Annu. Rev. Phytopathology* 35:235-270.
- Stockwell, V. O., and Stack, J. P. 2007. Using *Pseudomonas* spp. for integrated biological control. *Phytopathology* 97:244-249.
- Sumner, D. R., Kays, S. J., and Johnson, A. W. 1976. Etiology and control of root diseases of spinach. *Phytopathology* 66:1267-1273.
- Taylor, A. G., and Harman G. E. 1990. Concepts and technologies of selected seed treatments. *Annu. Rev. Phytopathology* 28:321-339.
- Thomas, J., Schreiber, A., Pelter, G., and Havens, D. 1997. Washington's Small-Seeded Vegetable Seed Industry. Washington State University Extension Bulletin No. 1829. Pullman, WA.
- Trapero-Casas, A., and Ingram, D. M. 1990. Control of *Pythium* seed rot and preemergence damping-off of chickpea in the U.S., Pacific Northwest and Spain. *Plant Dis.* 74:563-569.
- United States Department of Agriculture Agricultural Marketing Service. Summary of the NOP Final Rule. Page 1. Seeds and Planting Stock Practice Standard. Pages 374-375.  
<http://www.ams.usda.gov.nop/NOP/standards.html>
- United States Department of Agriculture National Organic Standards Board. 1995. Definitions of "Organic". United States Department of Agriculture, Washington, D.C.
- United States Environmental Protection Agency. 2006. Methyl Bromide.  
[http://www.epa.gov/oppsrrd1/reregistration/methyl\\_bromide/index.htm](http://www.epa.gov/oppsrrd1/reregistration/methyl_bromide/index.htm)

- United States Environmental Protection Agency. 2005. *Muscodora albus* QST 20799 (006503) Fact sheet.
- [http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet\\_006503.htm](http://www.epa.gov/pesticides/biopesticides/ingredients/factsheets/factsheet_006503.htm)
- van Loon, L. C., Bakker, P. A. H. M., and Pieterse, C. M. J. 1998. Systemic resistance induced by rhizosphere bacteria. *Annu. Rev. Phytopathology* 36:453-483.
- van Peer, R., Niemann, G. J., and Schippers, B. 1991. Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS417r. *Phytopathology* 81:728-734.
- Vogl, C. R., Kilcher, L., and Schmidt, H. 2005. Are standards and regulations of organic farming moving away from small farmers' knowledge? *J. Sustainable Agriculture* 26:5-26.
- Whipps, J. M. 1991. Effects of mycoparasites on sclerotia-forming fungi. Pages 129-140, in: *Biotic Interaction and Soilborne Diseases*. A. B. R. Beemster, G. J. Bollen, M. Gerlagh, M. A. Ruissen, B. Schippers, and A. Tempel, editors. Elsevier Science Publishing Co. Inc., New York, NY.

## Chapter 2

### Greenhouse Pathogen Inoculation Rate Trials, and Seed and Drench Treatment Trials for Organic Production

#### 2.1. INTRODUCTION

Soilborne pathogens, including *Pythium* spp., *Fusarium* spp., and *Rhizoctonia* spp., cause extensive damage to numerous crops worldwide (Campbell and Neher, 1996). Both the ecology of soilborne pathogens and the epidemiology of root diseases present challenges to researchers, because each pathosystem is unique. Three specific challenges applicable to many root disease systems include difficulties in quantifying inoculum, assessing disease, and designing effective studies to assess specific objectives (Campbell and Neher, 1996).

Disease management in organic production systems is especially challenging because organic producers do not have the option to utilize all of the conventional disease control methods such as synthetic chemical treatments or fumigation. Thus, organic producers must rely on methods of disease control such as crop rotation, cover cropping, and approved organic pesticides, including approved formulations of biological control organisms (Koenig and Baker, 2002). In 1990, Congress passed the Organic Foods Production Act (OFPA) in the Farm Bill, establishing consistent organic production standards nationwide by implementing federally mandated organic standards. As a result, the USDA created the National Organic Program (NOP) as a part of the Agriculture Marketing Service (AMS). According to the Organic Foods Production Act of 1990, Section 2109 (a), Seed, Seedlings and Planting Practices: “For a farm to be certified under this title, producers on such farm shall not apply materials to, or engage in practices on, seeds or seedlings that are contrary to, or inconsistent with the applicable organic certification program” ([www.ams.usda.gov/nop/NOP/NOPhome.html](http://www.ams.usda.gov/nop/NOP/NOPhome.html)).

The demand for organically produced seed has increased since the rules of the USDA NOP have required the use of organic seed in organic production (Groot et al., 2004). However, concern over losses due to seedborne and soilborne pathogens has also increased because of the limited effective options available for seed treatments that satisfy organic standards (du Toit et al., 2005b). There is an obvious need for seed treatments that can be approved for use in organic production that are effective against the diversity of soilborne pathogens. A variety of products have been developed for which the registrants claim efficacy against such pathogens, but results of independent studies evaluating these claims have often been highly variable (Harman, 1991). Therefore, research is necessary to aid seed companies and growers at improving organic seed production and seed treatment options, e.g., through development and refinement of organic disease management tools (Groot et al., 2004).

Seed treatments can be inexpensive and very effective forms of plant disease control (Taylor and Harman, 1990). The main objectives of seed treatments are to prevent rotting of planted seeds and/or infection of the developing seedlings, either by killing pathogens directly in or on the seed, or by protecting the developing seedling from infection by soilborne pathogens; or directly improving plant growth through application of nutrients or microorganisms that improve nutrient uptake of the seedlings (Taylor and Harman, 1990). The reliability of seed treatments at meeting these objectives varies among crop species, seed treatment products, seedborne or soilborne diseases, soil types and conditions, etc. (Taylor and Harman, 1990). Many biological seed and drench treatments have been developed to protect against soilborne plant pathogens. Baker and Paulitz (1996) outlined three strategies for obtaining biological control of soilborne plant pathogens: 1) protection of infection courts, 2) reduction of inoculum potential in sites not necessarily associated with the infection court, and 3) induction of host

resistance. They concluded that perhaps the most efficient of these strategies is the protection of a fixed infection court, such as seed, since the infection court remains stationary and does not encounter new inoculum over time. Therefore, a single application of a biological control agent (BCA) may provide ample protection of a fixed infection site. This is why a variety of BCAs have shown potential as seed treatments for protection against seed decay and seedling damping-off diseases (Baker and Paulitz, 1996). The activity expected from an antagonistic BCA applied to seed is short-term protection against damping-off pathogens, or longer-term protection of the root system through colonization of the roots and the rhizosphere of the host (Gindrat, 1979).

The purpose of this research was to provide an objective evaluation of seed and drench treatment products with EPA registrations, and products that have the potential for formulations approved for use in organic production, for control of soilborne seedling blight or damping-off diseases. Three pathogens from three phyla were selected for evaluating seed and drench treatments based on the individual and collective impacts these pathogens have on the seedling blight and damping-off complex for many small-seeded vegetables: *Rhizoctonia solani* Kühn, a basidiomycete anamorph of *Thanatephorus cucumeris* (Frank) Donk well-known for causing seed rot and damping-off (Sneh et al., 1991); *Fusarium oxysporum* Schlecht. f. sp. *spinaciae* (Sherb.) Snyd. and Hans., an ascomycete anamorph that causes seedling blight and a vascular wilt of spinach (Bassi and Goode, 1978); and *Pythium ultimum* Trow, an oomycete responsible for causing severe losses from both pre- and post-emergence damping-off of seedlings (Hendrix and Campbell, 1973). Since spinach is susceptible to many of the most prevalent soilborne pathogens that cause seedling blights and damping-off, and is a popular crop for organic production, spinach is an ideal model of a small-seeded vegetable crop for investigating the efficacy of EPA registered seed and drench treatments and products with potential for

registration for use in organic production systems for control of soilborne pathogens. The specific objectives of this study were to:

1. Determine effective rates of inoculation for each of the three pathogens to achieve approximately 50% damping-off or seedling mortality under greenhouse conditions;
2. Evaluate selected seed and drench treatments in a greenhouse against each of the three pathogens separately;
3. Determine if any of the selected seed treatments reduce the incidence of necrotrophic fungi present on spinach seed; and
4. Determine if any of the selected seed treatments affect germination of spinach seed.

## **2.2. MATERIALS AND METHODS**

**2.2.1. Pathogen isolates.** Two isolates of *R. solani*, VSP 05-01A and VSP 05-01B were obtained by the vegetable seed pathology program at the Washington State University (WSU) Mount Vernon Northwestern Washington Research and Extension Center (NWREC) from blemished onion bulbs of the cv. ‘Stuttgart’ grown in the Columbia Basin of Washington in 2005. The isolates were identified as belonging to anastomosis group 4 (AG4) and hyphal group II (HGII) by C. Pagani at North Carolina State University (Raleigh, NC), based on morphological characterization; nuclear condition; anastomosis grouping; and DNA sequencing of the internal transcribed spacer (ITS) regions 1 and 2, and 5.8S rDNA region, followed by comparisons through GenBank Blast search, and phylogenetic tree analysis with several *Rhizoctonia* AG groups (Sneh et al., 1991). Following the species, AG, and HG verification, pathogenicity tests of each isolate on spinach were carried out in the greenhouse in August 2005 using three 3 week-old seedlings of the spinach inbred ‘9420.553’ (Alf Christianson Seed Co.,

Mount Vernon, WA) per fungal isolate. This inbred was used because of partial tolerance of the inbred to thrips feeding, which can cause significant damage to spinach (Oparaocha and Okigbo, 2003). The seedlings were grown in RediEarth Starter Medium (Sun Gro Horticulture, Vancouver, British Columbia, Canada) in 72 cell flats. To produce inoculum of the pathogen, 250 g rye seed was soaked in 0.5 liter deionized water in a 1 liter flask for 24 h, autoclaved at 120°C and 15 psi for 90 min, cooled for 24 h, and autoclaved a second time for 90 min at 120°C and 15 psi. Ten plugs (each 5 mm in diameter) were taken from the edge of actively growing colonies of the appropriate *R. solani* isolate growing on potato dextrose agar (PDA) in Petri dishes, added to the rye seed, and left on the lab bench for 2 weeks for the fungus to colonize the rye seed. The flasks were shaken intermittently to promote more uniform colonization of the rye seed. The colonized rye seed was then dried on paper toweling for 1 week in a fume hood, and stored in a seed storage bag in a refrigerator.

For each isolate of *R. solani*, 25 colonized rye seed were placed around the root plug of each spinach seedling that was transplanted into Sunshine Mix #1 potting mix (Sun Gro Horticulture) in a 10 cm-diameter pot. Additional potting mix was placed over the inoculum and root plug. For the control treatment, non-colonized rye seed was placed around the root plug of three seedlings as described above. The seedlings were monitored for disease symptoms for one month, i.e., stunting, chlorosis, wilting, necrotic lesions on the hypocotyl or stem, and death of the seedling. Isolations were carried out 4 weeks after inoculation by surface-sterilizing (10% NaOCl for 30 to 60 s) sections of roots from symptomatic plants, and plating them onto PDA and water agar (WA) in Petri dishes to re-isolate *R. solani* and fulfill Koch's postulates.

The colonized rye seed was ground using a coffee grinder (Braun, Kronberg, Germany) and stored at 4°C for future use. Additionally, the isolates were stored on colonized

filter disks at -20°C. To do so, four 1.5 cm-diameter sterile filter disks (VWR Scientific Products, West Chester, PA) were arranged on a 6 cm-diameter Petri dish of PDA to which a colonized plug (5 mm in diameter) taken from the edge of an actively growing PDA culture of the appropriate isolate was placed in the middle of the four filter disks. Once the filter disks were covered with a mycelial mat of the fungus, the filter disks were removed, placed in sterile coin envelopes (5.7 cm x 8.9 cm) (Westvaco Envelope Division, Springfield, MA), dried overnight in a laminar flow hood, and stored with desiccant at -20°C (Peever et al., 1999).

An isolate of each of *P. ultimum* '030141' and *P. irregulare* '0900101' were obtained from T. Paulitz (USDA ARS scientist at WSU, Pullman, WA) and tested for pathogenicity on spinach. About 10 plugs (each approximately 5 mm in diameter) were taken from the edge of an actively growing colony of the appropriate isolate on PDA, and placed around the root plug of each of three 3- to 4-week-old spinach seedlings of the inbred '9420.553', as described for the *R. solani* pathogenicity test. Non-colonized PDA plugs were used as a control treatment for comparison. Seedlings were monitored for symptoms of seedling blight for 4 weeks and isolations were completed, as described for *R. solani*. Each isolate was transferred to PDA and WA slants in test tubes and stored at 4°C. The isolates were transferred to new slants every six months to maintain viable cultures.

Isolate '001' of *F. oxysporum* f. sp. *spinaciae* was obtained from a wilted spinach plant in a spinach seed crop trial at the WSU Mount Vernon NWREC in Mount Vernon, WA in 2001. Pathogenicity of the isolate on spinach was re-confirmed by dipping the root plugs of three spinach seedlings for 60 s into a microconidial suspension of the fungus prepared in Kerr's broth (Kerr, 1963), as described below. For the control treatment, seedlings were dipped into Kerr's broth that was not inoculated with the fungus. Seedlings were monitored for symptoms of wilt,

and isolations from symptomatic seedlings were carried out 4 weeks after inoculation, as described for *R. solani*, to fulfill Koch's postulates. Following confirmation of pathogenicity, the isolate of *F. oxysporum* f. sp. *spinaciae* was grown on PDA in a Petri dish, and a 1 mm<sup>3</sup> plug was used to inoculate a 1 liter flask containing 50 ml Kerr's broth. The inoculated flask was placed on a shaker for 5 days to enhance production of microconidia. Three milliliters of the microconidial suspension was dispensed into a 20 ml vial containing 15 g sterile soil/sand mixture (1:1 ratio), and shaken vigorously to disperse the spores in the soil. The vial was stored at 4°C for future use. Additionally, the isolate was stored on filter disks as described for *R. solani*.

**2.2.2. Preparation of inocula.** For greenhouse inoculation rate trials, non-sterile Puget silt loam field soil (Klungland and McArthur, 1989) from the WSU Mount Vernon NWREC was passed through a 1 mm sieve to remove plant debris, and then placed on butcher paper to air dry for two days on a greenhouse bench. Ground oatmeal (Quaker, Chicago, IL) was added (1% by weight) to the dried, sieved soil and mixed thoroughly in a PK Blendmaster soil blender (Paterson-Kelley Co. division of Harsco Corp., East Stroudsburg, PA) for 10 min. During the last 5 min of mixing, deionized water (15% w/w) was added to the soil/oatmeal mix through the funnel and hose on the soil blender. Next, 500 g of this mixture was added to a 0.95 liter Mason jar (Kerr regular Mason jar, Jarden Corp., Muncie, IN). The jar was topped with an autoclavable plastic lid typically used for mushroom spawning (Fungi Perfecti, Olympia, WA), with 1.27 cm diameter holes drilled into the lid, and a 70 mm synthetic filter disk (Fungi Perfecti) placed beneath the lid. The lid was then covered with two layers of aluminum foil, and the jar was autoclaved for 50 min at 120°C and 15 psi. The jars were cooled overnight at room temperature, and then autoclaved a second time approximately 24 h later for an additional 50 min at 120°C

and 15 psi to kill any bacteria that may have germinated from dormant endospores following the first autoclave cycle. The jars were stored at room temperature until they were used for producing inoculum of the respective pathogens.

Three to four day-old cultures of *P. ultimum* and *R. solani* grown on PDA were used to inoculate the soil/oatmeal mix. Four jars were inoculated for each pathogen. Five 1 mm<sup>3</sup> agar plugs were taken from the edge of an actively growing culture of the appropriate pathogen, and placed in each jar. The jars were then shaken manually to partially bury the agar plugs in the soil/oatmeal mix. The pathogenic isolate of *F. oxysporum* f. sp. *spinaciae* was grown on PDA, and used to inoculate 1 liter flasks containing 50 ml Kerr's broth. The inoculated flasks were placed on a rotating platform shaker (Innova 2100, New Brunswick Scientific, Edison, NJ) for 5 days to produce microconidia. One milliliter of the liquid inoculum was then used to inoculate each jar of the soil/oatmeal mix. The jars were then set in a dark cabinet at room temperature for 4 to 6 weeks, and were shaken intermittently by hand to promote growth and colonization of the fungus throughout the soil/oatmeal mix. When thorough colonization of each jar was observed, i.e., mycelia were visible throughout the medium, the jars were stored at 4°C until needed.

The jars of inoculum were quantified using soil-dilution plating. Each jar was shaken vigorously by hand to mix the inoculum. Ten grams of inoculum was added to a 200 ml flask containing 90 ml 0.1% WA (sloppy agar) to aid suspension of the inoculum, and placed on a rotating platform shaker for 12 min. Ten-fold dilutions of each suspension were carried out to 10<sup>-4</sup> using 0.1% WA. *F. oxysporum* f. sp. *spinaciae* inoculum dilutions were plated onto Komada's agar medium (Komada, 1975), and incubated at room temperature on a laboratory bench. *F. oxysporum* colonies were counted 4 days after incubation of the plates to determine the number of propagules of the pathogen/g inoculum (ppg). The *P. ultimum* inoculum dilution

series was plated similarly, but onto a *Pythium*-selective medium (PSM) (Mircetich and Kraft, 1973). The plates were then incubated in the dark at room temperature, because the rose bengal and rifampicin ingredients of the medium are light sensitive. Since *P. ultimum* is a fast-growing species, colonies on the dilution plates were counted after 40 to 48 h of incubation to calculate ppg. The *R. solani* inoculum dilution series was plated onto WA and incubated at room temperature on a lab bench. *R. solani* colonies grew very rapidly and were therefore counted 24 h after plating to determine ppg.

**2.2.3. Inoculation of potting mix for greenhouse inoculation rate trials.** A small greenhouse trial was conducted to determine an appropriate potting mix approved for use in organic production and watering regime to use for the inoculation rate trials. Sunshine Growers Organic potting mix (Sun Gro Horticulture) was selected as the growth medium for greenhouse trials. The potting mix was moistened with tap water to attain an appropriate moisture content for seed imbibition and germination. Three 30.5 cm x 30.5 cm x 6.4 cm tall open growing flats (Jiffy Products of America Inc., Norwalk, OH) were filled with the wetted potting mix to within 1.3 cm of the top of the flat. Each flat was then weighed, and an average weight of 1,200 g potting mix was determined to be the appropriate volume/flat to use for greenhouse trials. This weight was used to calculate the amount of inoculum to add for the inoculation rate trials.

The objective of the inoculation rate trials was to determine the amount of inoculum needed for each pathogen to accomplish approximately 50% total damping-off or wilt of the spinach seedlings, to facilitate differentiation of the efficacy of the various seed and drench treatment products to be evaluated for control of damping-off and seedling blight pathogens. Initially, rates of 0, 500, 1,000, 5,000, and 10,000 ppg potting medium were evaluated for damping-off caused by each of the three pathogens. However, these inoculation rates proved too

low for *F. oxysporum* f. sp. *spinaciae* and *R. solani*, so the trials were repeated for these pathogens at rates of 0, 5,000, 10,000, 50,000, and 100,000 ppg, and additionally 500,000 ppg for *F. oxysporum* f. sp. *spinaciae*. The 50 ppg rate of inoculation used in the first *P. ultimum* trial was not included in the second trial based on the low percentage of damping-off at this rate of inoculation in trial 1. Similarly, the 100,000 and 500,000 ppg rates of inoculation used in the first *F. oxysporum* f. sp. *spinaciae* trial were not included in subsequent trials due to the high percentage of damping-off at these rates of inoculation. For each pathogen, the potting medium was mixed with the appropriate volume of the soil/oatmeal inoculum using a Gustafson Batch Lab Treater (Gustafson Equipment, Bayer CropScience, Shakopee, MN) for 5 min to achieve thorough mixing of the inoculum throughout the potting medium at the desired rate. The inoculated potting mix was then portioned into the flats (1200 g/flat). A separate trial was carried out for each pathogen in a greenhouse at approximately  $25 \pm 5^\circ\text{C}$ , using a randomized complete block design with five replications of the five or six rates of inoculum. Each trial was repeated. Additionally, a separate *P. ultimum* inoculation rate trial was conducted in Percival growth chambers (Percival Scientific, Inc., Perry, IA) set at  $15 \pm 2^\circ\text{C}$ , to assess the effect of temperature on the inoculation rate trial for this pathogen. The hybrid spinach cultivar ‘Lazio’ (Pop Vriend Seeds BV, Andijk, the Netherlands) was used, with 36 seeds planted per flat (6 rows of 6 seeds planted at a 5 cm spacing within and between rows). Lazio was selected due to the fresh market popularity of this hybrid, susceptibility of the hybrid to damping-off pathogens, and resistance of the hybrid to the 10 known races of the spinach downy mildew pathogen (Irish et al., 2007).

The incidence of seedlings emerged and the incidence of post-emergence damping-off or wilt of seedlings were counted in each flat at 3- to 4-day intervals for 32 to 56 days, depending

on how rapidly symptoms progressed. Flats were fertilized weekly with an OMRI-listed organic fertilizer (Alaska Fish Fertilizer, 5:1:1, Lilly Miller Brands, Walnut Creek, CA) at 7.9 ml/liter water, using a plastic watering can. The OMRI-listed insecticides Entrust (Spinosad, Dow AgroSciences, Indianapolis, IN) applied at 0.17 g/liter water and AzaDirect (Azadirachtin, Gowan Company, Yuma, AZ) applied at 1.6 ml/liter water, were applied to the spinach seedlings using a Viton spray bottle (U.S. Plastics Corp., Lima, OH) as necessary for thrips and aphid management, respectively. Each trial was repeated, and the rating frequency was reduced to once every seven days. All seedlings in the repeat inoculation rate trials were cut at the soil line at the final rating for biomass samples. The foliage of the seedlings was then dried at 32°C (Model 1370F forced air oven, VWR Scientific Products, West Chester, PA) for 3 to 5 days and the dry weights measured.

Isolations were carried out on randomly selected, damped-off seedlings from each trial to confirm the pathogen responsible for mortality of the seedlings. Five symptomatic and three asymptomatic seedlings, including root systems, were used in each trial for isolations. Seedlings were rinsed under running tap water, and foliage above the crown was removed. The crown and root systems were surface-sterilized by soaking in 10% NaOCl for 60 s, then triple-rinsed in sterile water and placed on a sterile paper towel to dry for five min. The crown and root systems were then cut into pieces and plated onto PDA and WA on a lab bench for three to five days. Fungi growing out of the seedling pieces were identified microscopically to genus.

**2.2.4. Seed health assay for inoculation rate trials.** A modified freeze-blotter seed health assay was carried out in May 2006 to determine the incidence of seed of the ‘Lazio’ spinach seed lot that were infected with various necrotrophic fungi, as described by du Toit et al. (2005a). For each the surface-sterilized and non-surface-sterilized seed assays, 400 seeds (100

seed/replication) were assayed. For the surface-sterilized seed assay, seed were placed in a mesh tea leaf strainer (Model 101, Venalicia Tea, Neuss, Germany) and agitated by hand in 1.2% NaOCl in a beaker for 60 s, then triple-rinsed with sterile water and dried under a laminar flow hood. The non-surface-sterilized seed was not rinsed in NaOCl or triple-rinsed. The dried seeds were arranged onto sterile Steel blue germination blotters (8.25 cm diameter, Anchor Paper Co., St. Paul, MN) moistened with 5 ml sterile water in 10 cm diameter plastic Petri plates, sealed with Parafilm, and allowed to imbibe for 24 h. The plates were then moved to a -20°C freezer for 24 h to kill the imbibed embryos, then thawed at room temperature for approximately 30 min and placed in an incubator for 14 days (Model I30BLL, Percival Scientific, Perry, IA). The seeds were examined microscopically at 10 to 100 x magnification 5, 9, and 14 days after plating, and the necrotrophic fungi developing on the seed were identified and recorded.

**2.2.5. Inoculum application for seed and drench treatment trials.** Inoculum for each of the three pathogens was produced and quantified using the soil/oatmeal method described above for the inoculation rate trials. Based on results of the inoculation rate trials for each pathogen, the rate of inoculum that resulted in approximately 50% mortality of spinach seedlings 5 to 7 weeks after planting was selected for evaluating the seed and drench treatments. A rate of 1,000 ppg was used for *P. ultimum* trials, 10,000 ppg for the *F. oxysporum* f. sp. *spinaciae* trials, and 50,000 ppg for the first *R. solani* treatment trial. However, the first *R. solani* trial resulted in a very high incidence of pre-emergence damping-off across all treatments, so the rate of inoculum was reduced to 25,000 ppg for the second *R. solani* trial.

**2.2.6. Seed and drench treatments.** Fourteen biological and/or organic seed or drench treatments, a conventional fungicide seed or drench treatment, and non-treated seed planted into each of inoculated and non-inoculated potting medium (control treatments) were selected for

evaluation under greenhouse conditions against each of the three pathogens evaluated in the inoculation rate trials, i.e., *R. solani*, *F. oxysporum* f. sp. *spinaciae*, and *P. ultimum*. Details of the treatments are shown in Table 2.1. The treatments included products that were OMRI-listed in 2006 and 2007, products with the potential for formulations approved for use in organic production, or experimental products being developed for use in organic production. The two control treatments included non-treated seed planted into inoculated potting mix (non-treated, inoculated control), or non-treated seed planted into non-inoculated potting mix (non-treated, non-inoculated control). Seed of the hybrid spinach cultivar ‘Lazio’ was used for all the trials. Each trial was set up as a randomized complete block design with five replications of the 17 treatments. For each experimental unit, six rows of six seed were planted into a 30.5 cm x 30.5 cm x 6.4 cm flat at a 5 cm spacing within and between rows. Each trial for each pathogen was conducted twice.

Each seed and drench treatment was applied at the highest appropriate rate recommended by the label or the manufacturer. Experimental #1, Experimental #2, Natural II, and Natural X seed treatments were each applied by the respective companies, and the treated seed was returned to the WSU Mount Vernon NWREC. Kodiak Concentrate Biological Fungicide, Micro 108, Mycostop Mix, PGPR Galaxy, Prestop, SoilGard 12-G, Subtilex, T-22 Planter Box, Yield Shield, Apron XL LS, Mertect 340F, and Terraclor 75% WP were applied at the WSU Mount Vernon NWREC at the rates described in Table 2.1. The compost tea was brewed by Catherine Crosby at the WSU Crop and Soil Sciences Department, Pullman, WA. Crosby’s M.S. thesis project was in progress at the time this study was carried out, and involved developing, characterizing, and evaluating compost tea communities for suppression of *Xanthomonas campestris* in cabbage seed production. This compost tea was developed specifically for high

bacterial diversity, with ingredients including vermicompost, seaweed powder, liquid humic acids, and azomite rock dust (Scheuerell and Mahaffee, 2004). The compost tea was brewed the day before planting each trial, and was shipped over-night to the WSU Mount Vernon NWREC.

**2.2.7. Disease assessment and trial maintenance.** The number of seedlings emerged, and the number of damped-off or wilted seedlings were counted weekly starting 7 days after planting, for 4 to 7 weeks depending on the pathogen. The *P. ultimum* and *R. solani* trials were terminated 4 to 5 weeks after planting due to the nature of the diseases caused by the pathogens. Both *P. ultimum* and *R. solani* caused significant pre-emergence damping-off, followed by post-emergence damping-off of seedlings within three weeks after emergence. The *F. oxysporum* f. sp. *spinaciae* trials were continued for 6 to 7 weeks after planting, as the isolate of this pathogen primarily caused post-emergence wilt 4 to 7 weeks after planting. The *P. ultimum* and *R. solani* trials were carried out in a greenhouse set at  $25 \pm 5^\circ\text{C}$ , whereas the *F. oxysporum* f. sp. *spinaciae* trials were in a greenhouse set at  $28 \pm 3^\circ\text{C}$  because the higher temperatures enhanced expression of vascular wilt caused by this pathogen as a result of increased transpirational demand of the seedlings at the higher temperatures.

The plants in each trial were fertilized weekly using the same rate of the OMRI-listed organic fish fertilizer used for the inoculation rate trials, as well as an additional seaweed extract fertilizer (Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada) that was mixed with the fish fertilizer at a rate of 2.5 g/liter of water using a plastic watering can. All seedlings in each trial were cut at the soil line at the final rating for biomass samples, as described for the inoculation rate trials. Isolations from five symptomatic and three asymptomatic seedlings randomly selected from each trial were conducted to verify the causal agent of damping-off or wilting, as described for the inoculation rate trials.

**2.2.8. Potting mix pH.** The same organic potting mix used for the inoculation rate trials was used for the seed and drench treatment trials. Due to the biological nature of many of the products evaluated, the pH of the potting medium could have an impact on the function or efficacy of the biological control organisms in the products (Harman, 1991). In addition, the aggressiveness of the pathogens evaluated could be affected by pH of the medium (du Toit et al., 2006). The pH of Sunshine Organic Growers Mix was measured after the potting mix was moistened immediately before each of the second *P. ultimum* and second *F. oxysporum* f. sp. *spinaciae* seed and drench treatment trials were planted, and again on the last day of the second *F. oxysporum* f. sp. *spinaciae* seed and drench treatment trial. For each trial, three 10 g samples of potting mix were sampled randomly and each added to 30 ml deionized water. Each sample was stirred for 30 s, then left for 10 min, and the process was repeated two more times, after which the pH was measured using a VWR Symphony pH meter (VWR).

**2.2.9. Seed health assays of treated seed.** The same freeze-blotter seed health assay described above was used for the seed treated with each product, with four replications of 100 seed assayed for each treatment. All seed treatment products were included in the seed health assays, including each of the two conventional seed treatments (Apron XL LS and Mertect 340F), and non-treated seed. The seed health assay was repeated with a different commercial seed lot of ‘Lazio’ than in the first assay, as the second seed lot was also used in the second set of greenhouse treatment trials for each pathogen.

**2.2.10. Germination assays of treated seed.** For each of the two ‘Lazio’ spinach seed lots used in the greenhouse trials, 100 seed treated with each of the seed treatment products, including the two conventional seed treatments (Apron XL LX and Mertect 340F) and the non-treated seed, were subjected to a germination seed assay based on the Association of Official

Seed Analysts (AOSA) protocol (Yaklich, 1985). For each treatment, 50 seed were placed between two layers of Anchor seed germination blotters (25.4 cm x 38.1 cm, 38# regular weight; Anchor Paper Co.) moistened with deionized water. Each set of blotters containing 50 seed was rolled up in a single sheet of wax paper (61 cm x 91.4 cm, Anchor Paper Co.), and placed upright in a plastic bag in a seed germinator (Stults Scientific Engineering Corp., Springfield, IL) at 15°C with no light. The incidence of seed that had germinated, had abnormal germination, or was rotten was counted after 7, 14, and 21 days of incubation for both assays. In addition, a 5 day reading for the second assay was included to determine if any of the seed treatments induced significantly earlier seed germination than the non-treated seed at 5 days.

**2.2.11. Statistical analyses.** Analyses of variance (ANOVAs), and means comparisons using Fisher's protected least significant difference (LSD at  $P < 0.05$ ) were carried out using PROC GLM of SAS (Version 9.1, SAS Institute, Cary, NC) on each of the dependent variables in each seed health assay, germination assay, greenhouse inoculation rate trial, and greenhouse seed and drench treatment trial. Friedman's non-parametric rank test was used when the original data and transformations of the data (logarithmic, square root, or arcsin square root) did not meet assumptions for parametric analyses, i.e., normally distributed data with homogeneous variances (Steele and Torrie, 1980). For each greenhouse trial, the percentage pre-emergence damping-off in each flat was calculated by subtracting the percentage non-emerged seedlings in the non-inoculated, non-treated control treatment [= 36 - (number of emerged seedlings)] from the percentage non-emerged seedlings in that flat. Post-emergence damping-off or wilt was calculated as the percentage of emerged seedlings that damped-off or developed vascular wilt symptoms typical for each pathogen. Additionally, total above-ground biomass in each flat was determined as described above, as well as the area under emergence progress curve (AUEPC),

area under pre-emergence damping-off progress curve (AUDPC<sub>pre</sub>), area under post-emergence damping-off (or wilt) progress curve (AUDPC<sub>post</sub>), and area under total damping-off (or wilt) progress curve (AUDPC<sub>total</sub>). The area under emergence and disease progress curves is a cumulative measurement calculated as an average of emergence or disease ratings over time:  $[(\Sigma(y_i + y_{i+1})/2)(t_i - t_{i+1})]$ , where  $y_i$  = the number of emerged or diseased seedlings at the  $i^{\text{th}}$  rating,  $y_{i+1}$  = the number of emerged or diseased seedlings at the (i+1) rating,  $t_i$  = the number of days at the  $i^{\text{th}}$  rating, and  $t_{i+1}$  = the number of days at the (i+1) rating (Shaner and Finney, 1977).

## 2.3. RESULTS

**2.3.1. Pathogenicity tests.** Pathogenicity of each isolate of *R. solani* AG4 HGII on spinach was verified. Each of the seedlings inoculated with rye seed colonized by one of the isolates died, whereas the seedlings inoculated with the non-colonized rye seed remained healthy (Fig. 2.1). Following confirmation of pathogenicity, isolate VSP 05-01B was selected randomly from the two isolates for use in subsequent trials. Similarly, both the *P. ultimum* and *P. irregulare* isolates proved pathogenic on spinach. The *P. ultimum* 030141 isolate was selected for use in subsequent trials based on the slightly broader host range of *P. ultimum* versus *P. irregulare* (Farr et al., 2007). Pathogenicity of the *F. oxysporum* f. sp. *spinaciae* 001 isolate on spinach was also confirmed.

**2.3.2. Seed health assays of non-treated seed.** Results from the non-surface-sterilized seed health assay revealed an incidence of  $4.8 \pm 1.7\%$  infestation with *Fusarium* spp. (Table 2.2). Additionally, *Verticillium* spp. were identified in the seed lot at a high incidence of  $42.0 \pm 4.3\%$ . Other necrotrophic fungi observed on the seed included *Stemphylium botryosum* ( $35.0 \pm 2.4\%$ ), *Cladosporium variabile* ( $2.5 \pm 1.0\%$ ), other *Cladosporium* spp. ( $33.5 \pm 6.0\%$ ), and *Alternaria*

spp. ( $42.0 \pm 4.3\%$ ) (Table 2.2). Surface-sterilization for 60 s eliminated the *Fusarium* spp., and reduced the incidence of all other fungal species significantly with the exception of *S. botryosum*, demonstrating that a majority of these seedborne necrotrophic fungi were present primarily on or in the pericarp of the seed (not deep-seated infections) (Table 2.2). However, only non-surface sterilized seed was used in the greenhouse trials.

**2.3.3. *Pythium ultimum* inoculation rate trials.** Results of the inoculation rate trials indicated that the soil/oatmeal inoculation method used for *P. ultimum* effectively produced damping-off of spinach seedlings compared to seedlings in non-inoculated flats. Based on the ANOVAs, the rate of inoculation did have a significant effect on emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off in each trial for all weekly ratings [7, 14, 21, 28, and 32 or 35 days after planting (dap)], with the exception of post-emergence wilt at 7 dap in trials 1 and 2. Additionally, based on the ANOVAs, the rate of inoculation did have a significant effect on the AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub> and AUDPC<sub>total</sub> in each trial, and the biomass in trial 2 at 35 dap. Both pre-and post-emergence damping-off contributed similarly (approximately 50% each) to the negative impacts of *P. ultimum* on the spinach seedlings. All isolations conducted from symptomatic seedlings in each trial demonstrated the presence of *Pythium* and *Trichoderma* spp. Only *Trichoderma* spp. were isolated from asymptomatic seedlings (*data not shown*).

Results from the first greenhouse and the growth chamber inoculation rate trials for *P. ultimum* were similar (*data not shown*). Therefore, only results from the greenhouse trial are presented, since all subsequent *P. ultimum* trials were conducted in the greenhouse. For the first greenhouse inoculation rate trial, rates of inoculation from 100 to 5,000 ppg resulted in total damped-off seedlings (pre- plus post-emergence damping-off) ranging from 30 to 65% at 32 dap

(Fig. 2.2A). However, the percentages of total damping-off, pre-emergence damping-off, and post-emergence damping-off from the 500, 1,000, and 5,000 ppg treatments were not statistically different at 32 dap (Fig. 2.2A and Fisher's protected LSDs). Total damping-off at the 50 ppg rate was only  $8.7 \pm 2.5\%$  at 32 dap (Fig. 2.2A). Total emergence for the non-inoculated control treatment was  $92.3 \pm 2.4\%$  by 32 dap, but emergence in the inoculated flats was only  $60.8 \pm 3.2$ ,  $68.3 \pm 4.5$ , and  $64.5 \pm 4.3\%$  at rates of inoculation of 500, 1,000, and 5,000 ppg, respectively (Fig. 2.2A). In trial 1, post-emergence damping-off was first observed 7 dap. Emergence for the 500, 1,000, or 5,000 ppg rates of inoculation was not significantly different at each weekly rating. Results of the statistical analyses for AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub>, and AUDPC<sub>total</sub> were similar to the 32 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 1 (Fig. 2.3).

For the second trial, total damping-off for each rate of inoculation (500, 1,000, and 5,000 ppg) was not significantly different at 35 dap based on Fisher's protected LSD, and ranged from  $50.8 \pm 4.7$  to  $74.2 \pm 5.1\%$  (Fig. 2.2B). The percentage total damping-off, pre-emergence damping-off, and post-emergence damping-off observed for the 500, 1,000, and 5,000 ppg treatments was not statistically different for each of these dependent variables (Fig. 2.2B). Total emergence at 35 dap for the non-inoculated control treatment was  $91.7 \pm 2.5\%$ , but in the inoculated flats was only  $69.5 \pm 2.5\%$  for the 100 ppg rate, and ranged from  $56.1 \pm 3.2\%$  to  $65.0 \pm 6.6\%$  for the 500 to 5,000 ppg rates (Fig. 2.2B). In trial 2, post-emergence damping-off was first observed 8 dap. Emergence in the non-inoculated flats was significantly higher compared to all rates of inoculation at each weekly rating. Pre-emergence damping-off was significantly higher for the 5,000 ppg rate compared to all other rates of inoculation at 21, 28, and 35 dap. Results of the statistical analyses for the AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub>, and AUDPC<sub>total</sub> were

similar to the 35 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 2 (Fig. 2.4). Biomass of the seedlings declined significantly with increasing rate of inoculation from 0 to 500 ppg, but biomass was not significantly different at the 500, 1,000, and 5,000 ppg rates of inoculation. Biomass measured for the non-inoculated control treatment was  $2.38 \pm 0.24$  g, and for the inoculated treatments was  $1.12 \pm 0.10$ ,  $0.65 \pm 0.10$ ,  $0.62 \pm 0.07$ , and  $0.46 \pm 0.07$  g for rates of inoculation of 100, 500, 1,000, and 5,000 ppg, respectively.

The decision for which inoculation rate to use in subsequent greenhouse seed and drench treatment trials was made based on results from the first greenhouse trial, as the second inoculation rate trial was not completed when the first seed and drench treatment trial was set up. Because total percentage damping-off at inoculation rates of 500, 1,000, and 5,000 ppg in trial 1 were not significantly different, the inoculation rate of 1,000 ppg for *P. ultimum* was selected for use in the greenhouse seed and drench treatment trials. Total damping-off at the 1,000 ppg rate for trial 1 was  $43.5 \pm 6.4\%$ , and was  $71.9 \pm 8.4\%$  for trial 2.

**2.3.4. *Rhizoctonia solani* inoculation rate trials.** Results of the inoculation rate trials indicated that the soil/oatmeal inoculation method used for *R. solani* effectively produced damping-off of spinach seedlings compared to seedlings in non-inoculated flats (Fig. 2.5A and 2.5B). Based on the ANOVAs, the rate of inoculation did have a significant effect on emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off in each trial for all weekly ratings (7, 14, 21, 28, and 32 or 35 dap), with the exception of post-emergence wilt 7 dap in each trial. Additionally, based on the ANOVAs, the rate of inoculation did have a significant effect on the AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub> and AUDPC<sub>total</sub> in each trial, and the biomass in trial 2 at 35 dap. Pre-emergence damping-off contributed more to the

negative impact of the *R. solani* on the spinach seedlings than post-emergence damping-off, unlike *P. ultimum*. Biomass was not measured for the first trial. All isolations conducted from symptomatic seedlings in each trial demonstrated the presence of *Rhizoctonia* and *Trichoderma* spp. Only *Trichoderma* spp. were isolated from asymptomatic seedlings (*data not shown*).

The initial inoculation rates evaluated (0, 50, 100, 500, 1,000, and 5,000 ppg) proved too low for achieving approximately 50% total damping-off for *R. solani* (*data not shown*).

Therefore, the trial was repeated at higher rates of inoculation (0, 5,000, 10,000, 50,000, and 100,000 ppg), for which the incidence of damping-off was significantly higher. Total damping-off for trial 1 at 32 dap for rates of 5,000 to 100,000 ppg ranged from 7 to 88% at 32 dap (Fig. 2.5A). Total damping-off at the 5,000 ppg rate was only  $7.0 \pm 3.7\%$  at 32 dap, which was not significantly different than that for the non-inoculated flats ( $1.3 \pm 0.8\%$ ). The total percentage damped-off seedlings was  $40.5 \pm 9.9\%$  at the rate of 50,000 ppg, which was not significantly different from the 10,000 ppg rate ( $30.8 \pm 17.1\%$ ) but was significantly lower than that of the 100,000 ppg rate ( $88.4 \pm 7.6$ ) (Fig. 2.5A). The total incidence of emerged seedlings for the non-inoculated control treatment reached  $80.6 \pm 6.0\%$  by 32 dap, but emergence in the inoculated flats was  $85.6 \pm 3.3$ ,  $70.6 \pm 14.9$ ,  $57.8 \pm 5.4$ , and  $12.8 \pm 2.1\%$  at rates of inoculation of 5,000, 10,000, 50,000, and 100,000 ppg, respectively (Fig. 2.5A). In trial 1, post-emergence damping-off was first observed 7 dap. Percentage emergence at the 100,000 ppg rate was significantly lower at each weekly rating, and percentage total damping-off at this rate of inoculation was significantly higher at 7, 14, and 21 dap compared with all other rates. Pre-emergence and post-emergence damping-off was significantly higher for the 100,000 ppg rate of inoculation ( $67.8 \pm 7.2$ , and  $34.0 \pm 7.1\%$ , respectively) compared to that of all other rates of inoculation. Results of the statistical analyses for the AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub>, and AUDPC<sub>total</sub> were similar to

the 32 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 1 (Fig. 2.6).

For the second trial, the highest rate of inoculum was reduced to 75,000 ppg rather than 100,000 ppg, due to the very high incidence of damped-off seedlings at the 100,000 ppg rate in trial 1. Total damping-off at 35 dap was not significantly different between the 25,000 and 50,000 ppg rates of inoculation, reaching  $35.2 \pm 4.2$  and  $43.0 \pm 4.5\%$ , respectively, but was significantly higher ( $69.5 \pm 3.2\%$ ) at the 75,000 ppg rate (Fig. 2.5B). Total, pre-emergence, and post-emergence damping-off in the non-inoculated flats averaged  $1.1 \pm 0.7$ ,  $1.1 \pm 0.7$ , and  $0\%$ , respectively (Fig. 2.5B). Percentage pre-emergence damping-off at 35 dap was not significantly different for inoculation rates of 10,000, 25,000, and 50,000 ppg, ranging from  $16.1 \pm 2.7$  to  $22.8 \pm 4.5\%$ , but was significantly lower than that observed at 75,000 ppg ( $51.1 \pm 4.5\%$ ) (Fig. 2.5B). In contrast, the percentage post-emergence damping-off was not significantly different for inoculation rates of 25,000, 50,000, and 75,000, ranging from  $18.4 \pm 3.7$  to  $20.2 \pm 3.9\%$  (Fig. 2.5B). Total emergence at 35 dap for the non-inoculated control was  $92.2 \pm 1.6\%$ . Total emergence at 35 dap was not significantly different for rates of inoculation of 10,000, 25,000, or 50,000 ppg, ranging from  $68.9 \pm 3.2$  to  $75.6 \pm 1.4\%$ , while total emergence at 75,000 ppg was significantly lower at  $40.6 \pm 3.0\%$  (Fig. 2.5B). In trial 2, post-emergence damping-off was first observed 7 dap. Emergence for the non-inoculated control flats was significantly higher compared to all other rates of inoculation at each weekly rating. Pre-emergence damping-off was significantly higher for the 75,000 ppg rate of inoculation at each weekly rating compared to all other rates of inoculation. Results of the statistical analyses for  $AUEPC$ ,  $AUDPC_{pre}$ ,  $AUDPC_{post}$ , and  $AUDPC_{total}$  were similar to the 35 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for

trial 2 (Fig. 2.7). Biomass of seedlings did decline with increasing rate of inoculation, but was not significantly different at inoculation rates of 10,000, 25,000, and 50,000 ppg, ranging from  $2.68 \pm 0.28$  to  $3.22 \pm 0.33$  g. Biomass at 35 dap was significantly highest for the non-inoculated control flats ( $4.08 \pm 0.29$  g), and significantly lowest for the 75,000 ppg rate of inoculation ( $1.62 \pm 0.10$  g).

The decision for which inoculation rate to use in subsequent greenhouse seed and drench treatment trials was made based on results from the first greenhouse inoculation rate trial, as the second inoculation rate trial was not completed when the first seed and drench treatment trial was set up. The 50,000 ppg inoculation rate was selected for greenhouse seed and drench treatment trials, because total damping-off at this rate in trial 1 was  $40.5 \pm 9.9\%$  at 32 dap. Similarly, for trial 2, total damping-off at the 50,000 ppg rate was  $43.0 \pm 4.5\%$ .

**2.3.5. *Fusarium oxysporum* f. sp. *spinaciae* inoculation rate trials.** Results of the inoculation rate trials indicated that the soil/oatmeal method used for *F. oxysporum* f. sp. *spinaciae* effectively produced post-emergence wilt of spinach seedlings compared to seedlings in non-inoculated flats. However, this pathogen did not have a significant effect on emergence or pre-emergence damping-off, unlike *P. ultimum* and *R. solani*. Based on the ANOVAs, the rate of inoculation did have a significant effect on post-emergence wilt and total wilt at the 21, 28, and 32 dap ratings in the first trial, and on the 28, 35, 42, 49, and 56 dap ratings in the second and third trials. Also based on the ANOVAs, the rate of inoculation did not have a significant effect on emergence or pre-emergence damping-off for any of the weekly ratings in any of the three trials. Additionally, the rate of inoculation did have a significant effect on the AUDPC<sub>post</sub> and AUDPC<sub>total</sub> in each trial at 32 or 56 dap, and on the biomass of seedlings in trials 2 and 3 at 56 dap, but not on AUEPC or AUDPC<sub>pre</sub>. All isolations conducted from symptomatic seedlings

in each trial demonstrated the presence of *Fusarium*, *Penicillium*, and *Trichoderma* spp. Only *Trichoderma* and *Penicillium* spp. were isolated from asymptomatic seedlings (*data not shown*).

The initial inoculation rates evaluated (0, 50, 100, 500, 1,000, and 5,000 ppg) proved too low for achieving approximately 50% total wilt for *F. oxysporum* f. sp. *spinaciae*, as the highest incidence of wilt was  $7.8 \pm 1.5\%$  at the 500 ppg rate of inoculation (*data not shown for this trial*). Therefore, the trial was repeated at higher rates of inoculation, i.e., 0, 10,000, 50,000, 100,000, and 500,000 ppg, which resulted in significantly higher percentage total wilt. Results from this first *F. oxysporum* f. sp. *spinaciae* inoculation rate trial are summarized in Fig. 2.8A and Fig. 2.9. Emergence at 32 dap in the first trial ranged from  $83.3 \pm 2.0$  to  $89.4 \pm 3.5\%$  across all treatments. Pre-emergence damping-off at 32 dap was not significantly different among the inoculation rates. Post-emergence wilt was first observed 14 dap, but the majority of the wilt did not occur until the 21, 28, and 32 dap ratings. Post-emergence wilt at 32 dap was not significantly different for the 50,000, 100,000, or 500,000 ppg rates of inoculation ( $91.8 \pm 1.4$ ,  $98.1 \pm 1.3$ , and 100%, respectively) but post-emergence and total wilt resulting from the 10,000 ppg rate of inoculation was  $52.1 \pm 8.0\%$ , which was significantly higher compared to that of the 0 ppg rate ( $3.9 \pm 1.5\%$ ). Results of the statistical analyses for the AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub>, and AUDPC<sub>total</sub> were similar to the 32 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off for trial 1 (Fig. 2.9). Biomass was not measured for trial 1.

Results from the second *F. oxysporum* f. sp. *spinaciae* inoculation rate trial are summarized in Fig. 2.8B and Fig. 2.10. Inoculation rates evaluated in trial 2 included 0, 5000, 10,000, 25,000, and 50,000 ppg, and the duration of trial 2 was 56 days. The same jar of soil/oatmeal inoculum was used for trial 2 as was used in trial 1, which had a mean inoculum

density of  $2.9 \times 10^6$  ppg. Emergence in the second trial at 56 dap was not significantly different for any rate of inoculation, ranging from  $91.7 \pm 2.5$  to  $96.1 \pm 1.4\%$ . Pre-emergence damping-off at 56 dap also was not significantly different among the inoculation rates. Post-emergence wilt and total wilt observed at 56 dap for the 10,000 ppg rate of inoculation was  $79.2 \pm 8.3$  and  $83.1 \pm 9.6\%$ , respectively, which was significantly higher than that of any other inoculation rate, including the two higher rates of inoculation of 25,000 and 50,000 ppg ( $51.3 \pm 10.0$  and  $58.4 \pm 8.9$  post-emergence wilt, respectively; and  $55.8 \pm 12.8$  and  $59.5 \pm 9.3\%$  total wilt, respectively). These results raised concerns that perhaps there was an error while setting up this trial, and, therefore, the trial was repeated. Results of the statistical analyses for the  $AUDPC_{post}$  and  $AUDPC_{total}$  were similar to the 56 dap ratings for post-emergence wilt and total wilt, respectively, for trial 2 (Fig. 2.10). Biomass of seedlings from the 25,000 and 50,000 ppg rates of inoculation were not significantly different ( $5.45 \pm 0.44$  and  $5.59 \pm 0.47$  g, respectively). However, biomass of seedlings from the 10,000 ppg rate of inoculation ( $3.16 \pm 0.33$  g) was significantly lower than that for all other treatments. Biomass of seedlings from the 0 ppg rate of inoculation was significantly higher than that of all other treatments ( $6.34 \pm 0.15$  g)

Results of the third *F. oxysporum* f. sp. *spinaciae* inoculation rate trial are summarized in Fig. 2.8C and Fig. 2.11. Inoculation rates evaluated in trial 3 included 0, 5,000, 10,000, 25,000, and 50,000 ppg, and the duration of trial 3 was 56 days. However, when the same jar of the soil/oatmeal inoculum of *F. oxysporum* f. sp. *spinaciae* was re-quantified to confirm that pathogenicity had not been lost while the inoculum was in storage for 6 months, the results indicated that the inoculum density for the same jar of inoculum used in trials 1 and 2 had increased from  $2.9 \times 10^6$  ppg to  $6.1 \times 10^6$  ppg. Therefore, a smaller volume of inoculum was used for each rate in trial 3, compared to trial 2. Emergence in the third trial at 56 dap was not

significantly different for any rate of inoculation, ranging from  $88.9 \pm 2.0$  to  $91.7 \pm 2.3\%$  (Fig. 2.8C). Pre-emergence damping-off at 56 dap also was not significantly different among the rates of inoculation and was negligible ( $1.4 \pm 0.6\%$  across all treatments). Post-emergence wilt and total wilt at 56 dap were only  $41.3 \pm 3.8$  and  $43.5 \pm 4.6\%$ , respectively, for the 50,000 ppg rate of inoculation. Post-emergence and total wilt were only  $11.8 \pm 3.2$  and  $15.2 \pm 2.9\%$ , respectively for the 10,000 ppg rate of inoculation, which was significantly lower than that of the same rate used in trial 2. Results of the statistical analyses for the  $AUDPC_{\text{post}}$  and  $AUDPC_{\text{total}}$  were similar to the 56 dap ratings for post-emergence wilt and total wilt, respectively, for trial 3 (Fig. 2.11). Biomass of seedlings from the 0, 5,000, and 10,000 ppg rates of inoculation was not significantly different, ranging from  $19.59 \pm 0.89$  to  $21.75 \pm 0.26$  g. Biomass resulting from seedlings from the 25,000 and 50,000 ppg rates of inoculation was not significantly different ( $16.95 \pm 0.68$  and  $14.92 \pm 1.08$  g, respectively), but were significantly lower compared to that of the seedlings from the 0, 5,000, and 10,000 ppg rates of inoculation.

**2.3.6. *Pythium ultimum* greenhouse seed and drench treatment trials.** Based on the ANOVAs, the seed and drench treatments did have a significant effect on emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off in each trial for all weekly ratings (7, 14, 21, 28, and 35 dap), compared to the non-treated seed planted into non-inoculated medium (non-treated, non-inoculated control treatment), with the exception of post-emergence damping-off 7 dap in each trial. Additionally, based on the ANOVAs, the seed and drench treatments did have a significant effect on AUEPC,  $AUDPC_{\text{pre}}$ ,  $AUDPC_{\text{post}}$ , and  $AUDPC_{\text{total}}$ , as well as biomass at the final rating (28 or 35 dap) in each trial. Both pre- and post-emergence damping-off contributed similarly to the negative impacts of *P. ultimum* on the spinach seedlings. All isolations conducted from symptomatic seedlings in this trial

demonstrated the presence of *Pythium* and *Trichoderma* spp. Only *Trichoderma* spp. were isolated from asymptomatic seedlings (*data not shown*).

Results from the first *P. ultimum* greenhouse seed and drench treatment trial are summarized in Table 2.3 and Fig. 2.12. For trial 1, percentage total damping-off observed for the non-treated seed planted into inoculated medium (non-treated, inoculated control treatment) was  $61.3 \pm 10.2\%$  at 35 dap (Table 2.3), which was slightly higher than the anticipated target of 50% damping-off expected at the 1,000 ppg rate of inoculation used. Emergence was first observed in flats that were treated with Experimental #1 and Experimental #2 at 4 dap, but not at least until 5 dap for any other treatments. Percentage emergence observed 35 dap for the non-treated, non-inoculated control was  $90.6 \pm 1.1\%$  vs.  $77.8 \pm 2.3\%$  for the non-treated, inoculated control (Table 2.3). Emergence at 35 dap for Experimental #1, Experimental #2, Natural II, and Natural X seed treatments was not significantly different than emergence for the Apron XL LS conventional fungicide seed treatment ( $94.4 \pm 2.5\%$ ), and was significantly higher than that of the non-treated, inoculated control. Prestop drench treatment resulted in significantly lower emergence than any other treatment ( $47.8 \pm 4.3\%$ ). In addition, treatment with Micro 108, Mycostop Mix, and SoilGard resulted in significantly lower emergence ( $67.2 \pm 3.0$ ,  $67.8 \pm 3.1$ , and  $64.4 \pm 2.7\%$ , respectively) compared to that of the inoculated, non-treated control. Treatment with compost tea, Kodiak, PGPR Galaxy, Subtilex, T-22 Planter Box, and Yield Shield did not have any significant effect on emergence compared the non-treated, inoculated control.

Pre-emergence damping-off at 35 dap in the first trial was significantly higher for the Micro 108, Mycostop Mix, Prestop, and SoilGard treatments ( $23.3 \pm 3.9$ ,  $22.8 \pm 3.6$ ,  $42.8 \pm 3.8$ , and  $26.1 \pm 3.1\%$ , respectively) compared to that of the non-treated, inoculated control ( $12.8 \pm$

2.6%). Pre-emergence damping-off observed for Experimental #1, Experimental #2, Natural II, and Natural X seed treatments ( $0$ ,  $0.6 \pm 0.6$ ,  $1.7 \pm 1.7$ , and  $2.8 \pm 1.5\%$ , respectively) was not significantly different than pre-emergence damping-off for the Apron XL LS conventional treatment ( $0\%$ ), which was significantly lower than that of the non-treated, inoculated control. Treatment with compost tea, Kodiak, PGPR Galaxy, Subtilex, T-22 Planter Box, and Yield Shield did not have any significant effect on pre-emergence damping-off compared to that of the non-treated, inoculated control.

Post-emergence damping-off in trial 1 was first observed 7 dap. Post-emergence damping-off 35 dap was highest for the Yield Shield treatment ( $61.3 \pm 5.9\%$ ), which was significantly higher than that of all treatments including the non-treated inoculated control, with the exceptions of Mycostop Mix, PGPR Galaxy, Prestop, Subtilex and T-22 Planter Box (Table 2.3). In contrast, treatment with compost tea resulted in significantly lower post-emergence damping-off ( $16.6 \pm 4.5\%$ ) compared to that of the non-treated, inoculated control ( $48.5 \pm 7.9\%$ ). Treatment with Experimental #1, Experimental #2, Natural II, and Natural X resulted in post-emergence damping-off ( $3.5 \pm 1.6$ ,  $6.4 \pm 1.8$ ,  $0.6 \pm 0.6$ , and  $7.4 \pm 1.9\%$ , respectively) that was both significantly lower than that of the non-treated, inoculated control, and not significantly different than that of the Apron XL LS conventional treatment ( $2.4 \pm 0.6\%$ ). Post-emergence damping-off observed for treatments with Kodiak, Micro 108, Mycostop Mix, PGPR Galaxy, Prestop, SoilGard, Subtilex, and T-22 Planter Box was not significantly different compared to that of the non-treated, inoculated control.

Total damping-off at 35 dap in trial 1 for treatments with compost tea, Experimental #1, Experimental #2, Natural II, and Natural X ( $27.7 \pm 4.5$ ,  $3.5 \pm 1.6$ ,  $7.0 \pm 2.0$ ,  $2.3 \pm 1.6$ , and  $10.2 \pm 2.1\%$ , respectively) was significantly lower compared to that of the non-treated, inoculated

control ( $61.3 \pm 10.2\%$ ) (Table 2.3). Total damping-off for the Experimental #1 and Natural II treatments was not significantly different from that of the Apron XL LS conventional treatment ( $2.4 \pm 0.6\%$ ). Prestop had the highest percentage total-damping-off ( $95.4 \pm 3.3\%$ ), and was the only treatment that resulted in significantly higher total damping-off compared to that of the non-treated, inoculated control. Percentage total damping-off for treatments with Kodiak, Micro 108, Mycostop Mix, PGPR Galaxy, SoilGard, Subtilex, T-22 Planter Box, and Yield Shield was not significantly different compared to that of the non-treated, inoculated control.

Biomass at 35 dap in trial 1 was highest for seedlings that developed from seed treated with Experimental #1 and Experimental #2 ( $5.39 \pm 0.39$  and  $6.06 \pm 0.57$  g, respectively), and only Experimental #2 treatment resulted in significantly higher biomass compared to that of both the non-treated, non-inoculated control and the Apron XL LS conventional control ( $4.55 \pm 0.29$ , and  $5.07 \pm 0.39$  g, respectively). Experimental #1 and Natural II seed treatments also resulted in total biomass that was not significantly different than that of the Apron XL LS treatment. Prestop was the only treatment that resulted in seedling biomass that was significantly lower ( $0.85 \pm 0.14$  g) than that of the non-treated, inoculated control. Biomass for seedlings that developed from treatments with Kodiak, Micro 108, Mycostop Mix, PGPR Galaxy, SoilGard, Subtilex, T-22 Planter Box, and Yield Shield was not significantly different compared to that of the non-treated, inoculated control. Results of the statistical analyses for the AUEPC,  $AUDPC_{pre}$ ,  $AUDPC_{post}$ , and  $AUDPC_{total}$  were similar to the 35 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 1 (Fig. 2.12).

Results from the second *P. ultimum* greenhouse seed and drench treatment trial are summarized in Table 2.4 and Fig. 2.13. For trial 2, total damping-off for the non-treated,

inoculated control was  $52.5 \pm 4.8\%$  at 28 dap, which was not significantly different from the anticipated target of 50% damping-off expected at the 1,000 ppg rate of inoculation used (Table 2.4). Emergence was first observed 4 dap in flats that were treated with Experimental #1 and Experimental #2, but not until at least 5 dap for any other treatments. Emergence at the final rating (28 dap) for the non-treated, non-inoculated control was  $91.7 \pm 2.3\%$  vs.  $72.2 \pm 3.8\%$  for the non-treated, inoculated control. Emergence for treatments with Experimental #1, Experimental #2, and Subtilex ( $92.2 \pm 2.0$ ,  $95.6 \pm 1.7$ , and  $85.6 \pm 3.1\%$ , respectively) was not significantly different than that of the Apron XL LS conventional control ( $91.1 \pm 2.0\%$ ) or the non-treated, non-inoculated control. Emergence for treatments with compost tea, Natural II, Prestop, and SoilGard ( $62.8 \pm 1.1$ ,  $54.5 \pm 4.8$ ,  $55.6 \pm 5.6$ , and  $48.3 \pm 6.0\%$ , respectively) was significantly lower compared to that of the non-treated, inoculated control. Treatment with Kodiak, Micro 108, Mycostop Mix, Natural X, PGPR Galaxy, T-22 Planter Box, and Yield Shield did not have any significant effect on emergence compared to the non-treated, inoculated control (Table 2.4).

Pre-emergence damping-off observed 28 dap for treatments with compost tea, Natural II, Prestop, and SoilGard ( $28.9 \pm 2.4$ ,  $37.2 \pm 3.8$ ,  $36.1 \pm 4.6$ , and  $43.3 \pm 6.6\%$ , respectively) was significantly higher compared to that of the non-treated, inoculated control ( $19.4 \pm 2.9\%$ ) (Table 2.4). In contrast, pre-emergence damping-off at 28 dap observed for treatments with Experimental #1, Experimental #2, and Subtilex ( $0.6 \pm 0.6$ ,  $0$ , and  $6.7 \pm 3.6\%$ , respectively) was not significantly different compared to that of the non-treated, non-inoculated control ( $0\%$ ) or the Apron XL LS conventional control ( $1.7 \pm 1.1\%$ ). Treatment with Kodiak, Micro 108, Mycostop Mix, Natural X, PGPR Galaxy, T-22 Planter Box, and Yield Shield did not have any significant effect on pre-emergence damping-off compared to the non-treated, inoculated control.

Post-emergence damping-off was first observed 7 dap in trial 2. Post-emergence damping-off at 28 dap observed for treatments with Natural II, Prestop, and T-22 Planter Box ( $64.1 \pm 8.8$ ,  $54.1 \pm 6.5$ , and  $57.8 \pm 6.7\%$ , respectively) was significantly higher compared to that of the non-treated, inoculated control ( $33.0 \pm 3.4\%$ ). In contrast, post-emergence damping off observed for treatment with Subtilex ( $11.2 \pm 2.1\%$ ) was not significantly different than that of the Apron XL LS conventional control ( $3.7 \pm 2.3\%$ ), and was the only treatment that resulted in significantly lower post-emergence damping-off compared to that of the non-treated, inoculated control. Treatment with compost tea, Experimental #1, Experimental #2, Kodiak, Micro 108, Mycostop Mix, Natural X, PGPR Galaxy, SoilGard, and Yield Shield did not have any significant effect on post-emergence damping-off compared to the non-treated, inoculated control.

Total damping-off at 28 dap in trial 2 was significantly higher for treatment with Natural II, Prestop, and SoilGard ( $91.0 \pm 5.6$ ,  $85.6 \pm 6.7$ , and  $89.4 \pm 6.3\%$ , respectively) compared to that of the non-treated, inoculated control ( $52.5 \pm 4.8\%$ ). Total damping-off was significantly lower for treatments with Experimental #1, Experimental #2, and Subtilex ( $16.3 \pm 1.8$ ,  $29.5 \pm 9.8$ , and  $17.8 \pm 4.6\%$ , respectively) compared to that of the non-treated, inoculated control. However, no treatments resulted in total damping-off as low as that observed for the Apron XL LS conventional control ( $5.4 \pm 2.1\%$ ). Treatment with compost tea, Kodiak, Micro 108, Mycostop Mix, Natural X, PGPR Galaxy, T-22 Planter Box, and Yield Shield did not have any significant effect on total damping-off compared to the non-treated, inoculated control.

Biomass at 28 dap in trial 2 for seedlings that developed from the Experimental #1 seed treatment ( $4.37 \pm 0.48$  g) was not significantly different compared to that of the non-treated, non-inoculated control ( $5.20 \pm 0.21$  g) or the Apron XL LS conventional control ( $4.61 \pm 0.24$  g)

treatments. In addition, biomass of seedlings from the Experimental #2 seed treatment ( $3.70 \pm 0.67$  g) was not significantly different than that of the seedlings from the Apron XL LS conventional treatment. Biomass for seedlings that developed from treatments with compost tea, Natural II, Prestop, SoilGard, and T-22 Planter Box ( $1.24 \pm 0.21$ ,  $1.24 \pm 0.32$ ,  $1.34 \pm 0.20$ ,  $1.40 \pm 0.24$ , and  $1.76 \pm 0.32$  g, respectively) was significantly lower compared to that of the non-treated, inoculated control ( $2.82 \pm 0.28$  g). Biomass for seedlings that developed from treatments with Experimental #2, Kodiak, Micro 108, Mycostop Mix, Natural X, PGPR Galaxy, Subtilex, and Yield Shield was not significantly different compared to that of the non-treated, inoculated control. Results of the statistical analyses for AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub>, and AUDPC<sub>total</sub> were similar to the 28 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 2 (Fig. 2.13).

In both trials, seed treatments with Experimental #1 and Experimental #2 resulted in emergence and pre-emergence damping-off that was not significantly different from that of the non-treated, non-inoculated control or the Apron XL LS conventional control (Tables 2.3 and 2.4). Experimental #1 and Experimental #2 treatments also resulted in significantly lower total damping-off from *P. ultimum* compared to that of the non-treated, inoculated control in each trial. In contrast, treatment with Prestop and SoilGard resulted in significantly lower emergence and significantly higher pre-emergence damping-off compared to that of the non-treated, inoculated control in each trial (Tables 2.3 and 2.4). Treatment with Prestop also resulted in significantly higher total damping-off compared to that of the non-treated, inoculated control in each trial. Treatment with Natural II, Natural X, and Subtilex resulted in significantly lower total damping-off compared to the non-treated, inoculated control in only one of the two trials.

**2.3.7. *Rhizoctonia solani* greenhouse seed and drench treatment trials.** Based on the ANOVAs, the seed and drench treatments did have a significant effect on emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off in each trial for all weekly ratings (7, 14, 21, and 28), with the exception of post-emergence damping-off at 7 dap in trial 1, and post-emergence damping-off and total damping-off at each weekly rating in trial 2, compared to the non-treated, non-inoculated control. Additionally, based on the ANOVAs, the seed and drench treatments did have a significant effect on AUEPC, AUDPC<sub>pre</sub>, and AUDPC<sub>total</sub>, as well as biomass at the final rating (28 dap) in each trial; and for AUDPC<sub>post</sub> in trial 1 but not in trial 2. Both pre-and post-emergence damping-off contributed to the negative impacts of *R. solani* on the spinach seedlings, but pre-emergence damping-off was more significant for this pathogen. All isolations conducted from symptomatic seedlings in this trial demonstrated the presence of *Rhizoctonia* and *Trichoderma* spp. Only *Trichoderma* spp. were isolated from asymptomatic seedlings (*data not shown*).

Results from the first *R. solani* seed and drench treatment trial are summarized in Table 2.5 and Fig. 2.14. For trial 1, percentage total damping-off observed at 28 dap for the non-treated, inoculated control treatment was  $74.6 \pm 3.9\%$  (Table 2.5), which was higher than the anticipated target of 50% damping-off expected at the 50,000 ppg rate of inoculation used. Emergence was first observed in flats planted with seed that was treated with Experimental #1 or Experimental #2 at 4 dap, but not until at least 5 dap for any other treatments. Percentage emergence observed at 35 dap for the non-treated, non-inoculated control was  $91.0 \pm 2.1\%$  vs.  $23.6 \pm 8.1\%$  for the non-treated, inoculated control (Table 2.5). Emergence at 28 dap for Experimental #1 and Natural II seed treatments ( $63.9 \pm 2.5$ , and  $65.6 \pm 10.7\%$ , respectively) was not significantly different than emergence for the Terraclor conventional fungicide drench

treatment ( $81.3 \pm 2.9\%$ ), but was significantly higher than that of the non-treated, inoculated control. Additionally, treatment with compost tea, Experimental #2, Natural X, Prestop, and Subtilex resulted in significantly higher emergence compared to that of the non-treated, inoculated control. Treatment with Yield Shield resulted in significantly lower emergence than any other treatment (0%). Treatment with Kodiak, Micro 108, Mycostop Mix, PGPR Galaxy, SoilGard, and T-22 Planter Box did not have any significant effect on emergence compared to the non-treated, inoculated control.

Pre-emergence damping-off at 28 dap in trial 1 was significantly higher for the Yield Shield treatment ( $91.0 \pm 2.1\%$ ) compared to that of the non-treated, inoculated control ( $67.4 \pm 7.7\%$ ) (Table 2.5). Pre-emergence damping-off for Experimental #1 and Natural II seed treatments ( $27.1 \pm 2.4$ , and  $27.2 \pm 11.0\%$ , respectively) was not significantly different than pre-emergence damping-off for the Terraclor conventional treatment ( $10.4 \pm 4.2\%$ ), which was significantly lower than that of the non-treated, inoculated control. In addition, pre-emergence damping-off observed for treatment with compost tea, Experimental #2, Natural X, Prestop, and Subtilex ( $46.5 \pm 7.5$ ,  $50.0 \pm 4.1$ ,  $48.2 \pm 4.9$ ,  $56.3 \pm 5.7$ , and  $52.1 \pm 2.9\%$ , respectively) was significantly lower compared to that of the non-treated, inoculated control. Treatment with Kodiak, Micro 108, Mycostop Mix, PGPR Galaxy, SoilGard, and T-22 Planter Box did not have any significant effect on pre-emergence damping-off compared to the non-treated, inoculated control.

Post-emergence damping-off in trial 1 was first observed 6 dap. Post-emergence damping-off at 28 dap was significantly higher for the Mycostop Mix, SoilGard, Subtilex, and T-22 Planter Box treatments ( $22.9 \pm 7.9$ ,  $22.2 \pm 5.1$ ,  $26.2 \pm 6.7$ , and  $28.9 \pm 9.1\%$ , respectively) compared to that of the non-treated inoculated control (Table 2.5). No post-emergence damping-

off was recorded for the Yield Shield treatment because no seedlings emerged in any flat with that treatment. No treatments resulted in post-emergence damping-off that was significantly lower than that of the non-treated, inoculated control or the Terraclor conventional control ( $1.7 \pm 1.7\%$ ). Post-emergence damping-off for treatments with compost tea, Experimental #1, Experimental #2, Kodiak, Micro 108, Natural II, Natural X, PGPR Galaxy, Prestop, Yield Shield, and Terraclor was not significantly different compared to the non-treated, inoculated control.

Total damping-off at 28 dap in trial 1 for treatments with Experimental #1, and Natural II ( $38.8 \pm 3.2$  and  $35.7 \pm 12.4\%$ , respectively) was significantly lower compared to that of the non-treated, inoculated control ( $74.6 \pm 3.9\%$ ), and was not significantly different from that of the Terraclor conventional control ( $12.1 \pm 4.1\%$ ) (Table 2.5). Yield Shield resulted in the highest total damping-off ( $91.0 \pm 2.1\%$ ), and was the only treatment that caused significantly higher total damping-off compared to that of the non-treated, inoculated control. Percentage total damping-off for treatments with compost tea, Experimental #2, Kodiak, Micro 108, Mycostop Mix, Natural X, PGPR Galaxy, Prestop, SoilGard, Subtilex, and T-22 Planter Box was not significantly different compared to that of the non-treated, inoculated control.

Biomass at 28 dap in trial 1 was highest for seedlings that developed from seed with Experimental #1 and Natural X treatments ( $3.89 \pm 0.5$  and  $2.03 \pm 0.5$  g, respectively), which did not differ significantly from that of the non-treated, non-inoculated control or Terraclor conventional control ( $2.57 \pm 0.05$ , and  $3.54 \pm 0.4$  g, respectively). Seedlings that developed from treatments with compost tea, Experimental #2, Natural II, and T-22 Planter Box resulted in significantly higher biomass ( $1.78 \pm 0.4$ ,  $1.75 \pm 0.2$ ,  $2.17 \pm 0.6$ , and  $1.47 \pm 0.4$  g, respectively) compared to that of the non-treated, inoculated control ( $0.76 \pm 0.3$  g). Biomass for seedlings that

developed from treatments with Kodiak, Micro 108, Mycostop Mix, PGPR Galaxy, Prestop, SoilGard, and Subtilex was not significantly different compared to that of the non-treated, inoculated control. Results of the statistical analyses for the AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub>, and AUDPC<sub>total</sub> were similar to the 35 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 1 (Fig. 2.14).

Results from the second *R. solani* greenhouse seed and drench treatment trial are summarized in Table 2.6 and Fig. 2.15. Due to the high percentage total damping-off for the non-treated, inoculated control in the first trial, the rate of inoculation used for the second trial was reduced from 50,000 to 25,000 ppg. For trial 2, total damping-off for the non-treated, inoculated control was  $6.4 \pm 2.8\%$  at 28 dap, which was significantly lower than the anticipated target of 50% damping-off expected at the 25,000 ppg rate of inoculation used. Emergence was first observed in flats that were treated with Experimental #1 and Experimental #2 at 4 dap, but not until at least 5 dap for any other treatments. Surprisingly, emergence at 28 dap for the non-treated, non-inoculated control was  $78.3 \pm 4.0\%$ , which was not significantly different than emergence for the non-treated, inoculated control at  $82.8 \pm 1.6\%$ . Emergence for treatments with each product except compost tea, Prestop, and SoilGard was not significantly different than that of the Terraclor conventional control ( $81.1 \pm 2.2\%$ ), the non-treated, inoculated control, and the non-treated, non-inoculated control. No treatment resulted in significantly higher emergence compared to any of the three control treatments. Emergence for treatments with compost tea, Prestop, SoilGard and T-22 Planter Box ( $65.0 \pm 4.4$ ,  $66.1 \pm 4.3$ ,  $68.3 \pm 2.4$ , and  $72.2 \pm 4.7\%$ , respectively) was significantly lower compared to that of the non-treated, inoculated control.

Pre-emergence damping-off observed at 28 dap in trial 2 for treatments with compost tea, PGPR Galaxy, Prestop, and SoilGard ( $15.0 \pm 5.4$ ,  $9.5 \pm 4.4$ ,  $12.8 \pm 3.6$ , and  $10.0 \pm 4.2\%$ , respectively) was significantly higher compared to the non-treated, inoculated control ( $1.7 \pm 1.1\%$ ) (Table 2.6). Pre-emergence damping-off observed for treatments with Experimental #1, Experimental #2, Kodiak, Micro 108, Mycostop Mix, Natural II, Natural X, Subtilex, and Yield Shield was not significantly different compared to the non-treated, non-inoculated control (0%) or the Terraclor conventional control ( $2.8 \pm 1.2\%$ ). Post-emergence damping-off was first observed 7 dap in trial 2. No significant difference in post-emergence damping-off was detected among any of the treatments, including the three control treatments in this trial in which disease incidence was relatively low compared to trial 1. Additionally, no significant difference in total damping-off was detected among any of the treatments, including the three control treatments.

Biomass at 28 dap in trial 2 for seedlings that developed from the Experimental #1, Experimental #2, Mycostop Mix, Natural II, PGPR Galaxy, and T-22 Planter box, ranging from  $4.18 \pm 0.21$  to  $4.78 \pm 0.32$ , was not significantly different compared to that of the non-treated, inoculated control ( $4.93 \pm 0.19$  g) (Table 2.6). Biomass for seedlings that developed from treatments with compost tea, Kodiak, Micro 108, Natural X, Prestop, SoilGard, Subtilex, Yield Shield, and Terraclor, ranging from  $1.92 \pm 0.30$  to  $4.08 \pm 0.24$  g, was significantly lower compared to that of the non-treated, inoculated control. Results of the statistical analyses for AUEPC,  $AUDPC_{pre}$ ,  $AUDPC_{post}$ , and  $AUDPC_{total}$  were similar to the 28 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 2. However, the results from the  $AUDPC_{post}$  for this trial were not significant based on the ANOVAs (Fig. 2.15C). Because of the low incidence of

damping-off in the second trial, few similarities were evident between the two trials with *R. solani*.

**2.3.8. *Fusarium oxysporum* f. sp. *spinaciae* greenhouse seed and drench treatment trials.** Based on the ANOVAs, the seed and drench treatments did have a significant effect on emergence, pre-emergence damping-off, post-emergence wilt, and total disease in trial 1 for all weekly ratings (7, 14, 21, 28, 35, 42, and 49 dap) compared to the non-treated, non-inoculated control. The seed and drench treatments also had a significant effect on emergence, pre-emergence damping-off, post-emergence wilt, and total disease in trial 2 for all weekly ratings (7, 14, 21, 28, 35, and 42 dap) with the exception of post-emergence wilt 7, 14, and 21 dap, compared to the non-treated, non-inoculated control. Additionally, based on the ANOVAs, the seed and drench treatments did have a significant effect on AUEPC, AUDPC<sub>pre</sub>, AUDPC<sub>post</sub>, and AUDPC<sub>total</sub>, as well as biomass at the final rating (42 or 49 dap) in each trial. Post-emergence wilt primarily contributed to the negative impacts of *F. oxysporum* f. sp. *spinaciae* on the spinach seedlings, unlike *P. ultimum* and *R. solani*. All isolations conducted from symptomatic seedlings in this trial demonstrated the presence of *Fusarium*, *Penicillium* and *Trichoderma* spp. Only *Trichoderma* and *Penicillium* spp. were isolated from asymptomatic seedlings (*data not shown*).

Results from the first *F. oxysporum* f. sp. *spinaciae* greenhouse seed and drench treatment trial are summarized in Table 2.7 and Fig. 2.16. For trial 1, the percentage total damping-off observed for the non-treated, inoculated control was  $42.9 \pm 7.3\%$  at 42 dap, which was not significantly different than the anticipated target of 50% damping-off expected at the 10,000 ppg rate of inoculation used. Emergence was first observed in flats that were treated with Experimental #1 and Experimental #2 at 4 dap, but not until at least 5 dap for any other treatment. Percentage emergence observed at 42 dap for the non-treated, non-inoculated control

was  $93.9 \pm 2.8\%$ , which was not significantly different than a mean emergence of  $95.6 \pm 1.4\%$  for the non-treated, inoculated control, because emergence counts included healthy and wilting plants. Emergence at 42 dap for compost tea, Experimental #1, Experimental #2, Kodiak, Mycostop Mix, Natural II, Natural X, PGPR Galaxy, and Subtilex treatments, ranging from  $88.3 \pm 3.0$  to  $96.7 \pm 1.0\%$ , was not significantly different than emergence for the Mertect 340F conventional fungicide seed treatment control ( $92.8 \pm 2.4\%$ ), the non-treated, inoculated control, and the non-treated, non-inoculated control. Treatment with T-22 Planter Box resulted in the lowest emergence of all other treatments ( $80.0 \pm 2.0\%$ ), but this was not significantly different from that of the Micro 108, Prestop, SoilGard, and Yield Shield treatments ( $83.3 \pm 5.1$ ,  $85.6 \pm 4.6$ ,  $84.4 \pm 4.2$ , and  $86.7 \pm 1.8\%$ , respectively), all of which resulted in significantly lower emergence compared to that of the non-treated, inoculated control.

Pre-emergence damping-off at 42 dap in the first trial was low overall, but was significantly higher for the Micro 108, SoilGard, T-22 Planter Box, and Yield Shield treatments ( $10.6 \pm 4.8$ ,  $12.2 \pm 3.6$ ,  $13.9 \pm 4.2$ , and  $8.9 \pm 2.7\%$ , respectively), compared to that of the non-treated, inoculated control ( $2.2 \pm 1.4\%$ ) (Table 2.7). Pre-emergence damping-off for all other treatments ranged from  $2.2 \pm 1.0$  to  $6.7 \pm 12.6\%$ , and was not significantly different than pre-emergence damping-off resulting from the Mertect 340F conventional treatment ( $3.3 \pm 2.2\%$ ), or the non-treated, inoculated control. Post-emergence wilt in trial 1 was first observed 28 dap. Post-emergence wilt at 42 dap was significantly higher for treatments with Experimental #1, Experimental #2, Kodiak, PGPR Galaxy, SoilGard, and T-22 Planter Box, ranging from  $55.8 \pm 2.9$  to  $66.5 \pm 9.6\%$ , compared to the non-treated, inoculated control ( $40.7 \pm 6.9\%$ ) (Table 2.7). In comparison, treatment with Prestop and Yield Shield resulted in significantly lower post-emergence wilt ( $25.5 \pm 5.1\%$  and  $22.3 \pm 4.8$ , respectively) compared to the non-treated,

inoculated control. Post-emergence wilt for treatments with Experimental #2, Micro 108, Mycostop Mix, Natural II, Natrual X, and Subtilex was not significantly different compared to that of the non-treated, inoculated control.

None of the treatments in trial 1 resulted in total disease significantly lower than that of the non-treated, inoculated control at 42 dap ( $42.9 \pm 7.3\%$ ) (Table 2.7). Total disease for the compost tea, Prestop, and Yield Shield treatments ( $33.4 \pm 5.8$ ,  $33.8 \pm 6.9$ , and  $31.2 \pm 5.5\%$ , respectively) was significantly lower than that of the Mertect 340F conventional control ( $51.6 \pm 6.1\%$ ). Total disease for the Kodiak, PGPR Galaxy, SoilGard, and T-22 Planter Box treatments ( $64.0 \pm 8.0$ ,  $62.5 \pm 3.8$ ,  $75.0 \pm 8.5$ , and  $77.7 \pm 8.0\%$ , respectively) was significantly higher than that of the non-treated, inoculated control. Percentage total disease for treatments with Experimental #1, Experimental #2, Micro 108, Mycostop Mix, Natural II, Natural X, Prestop, Subtilex, and Yield Shield was not significantly different compared to that of the non-treated, inoculated control.

Biomass at 42 dap in trial 1 was significantly higher for seedlings that developed from compost tea, Prestop, Subtilex, and Yield Shield treatments ( $6.64 \pm 0.55$ ,  $4.65 \pm 0.57$ ,  $5.06 \pm 0.38$ , and  $5.81 \pm 0.44$  g, respectively) compared to that of the non-treated, inoculated control ( $3.48 \pm 0.33$  g) and Mertect 340F conventional control ( $3.54 \pm 0.38$  g) (Table 2.7). Biomass was significantly lower for seedlings that developed from SoilGard and T-22 Planter Box treatments ( $2.40 \pm 0.38$  and  $2.14 \pm 0.41$  g, respectively) compared to that of the non-treated, inoculated control. Biomass for seedlings that developed from treatments with Experimental #1, Experimental #2, Kodiak, Micro 108, Mycostop Mix, Natural II, Natural X, and PGPR Galaxy was not significantly different compared to that of the non-treated, inoculated control. Results of the statistical analyses for AUEPC,  $AUDPC_{pre}$ ,  $AUDPC_{post}$ , and  $AUDPC_{total}$  were similar to the

42 dap ratings for percentage emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 1 (Fig. 2.16).

Results from the second *F. oxysporum* f. sp. *spinaciae* greenhouse seed and drench treatment trial are summarized in Table 2.8 and Fig. 2.17. For trial 2, total damping-off for the non-treated, inoculated control was only  $29.9 \pm 6.9\%$  at 49 dap, which was significantly lower than the anticipated target of 50% damping-off expected at the 10,000 ppg rate of inoculation used. As for the other pathogens, emergence was first observed in flats that were treated with Experimental #1 and Experimental #2 at 4 dap, but not until at least 5 dap for any other treatments. Emergence at 49 dap for the non-treated, non-inoculated control was  $77.8 \pm 3.4\%$ , which was not significantly different than  $79.4 \pm 4.3\%$  emergence for the non-treated, inoculated control. Emergence for treatments with Micro 108 and Prestop ( $57.8 \pm 8.6$  and  $60.0 \pm 7.2\%$ , respectively) was significantly lower compared to that of the non-treated, inoculated control. Emergence for treatments with compost tea, Experimental #1, Experimental #2, Kodiak, Mycostop Mix, Natrua II, Natrua X, PGPR Galaxy, SoilGard, Subtilex, T-22 Planter Box, and Yield Shield ranged from  $72.2 \pm 7.8$  to  $87.2 \pm 2.6\%$ , and was not significantly different than that of the three control treatments.

Pre-emergence damping-off observed at 49 dap in trial 2 for treatments with Micro 108 and Prestop ( $20.6 \pm 11.5$  and  $20.0 \pm 8.7\%$ , respectively) was significantly higher compared to that of the non-treated, inoculated control ( $3.9 \pm 3.9\%$ ) (Table 2.8). Pre-emergence damping-off resulting from all other treatments was not significantly different than that of the non-treated, inoculated control or Mertect 340 conventional control ( $3.3 \pm 2.2\%$ ). Post-emergence wilt was first observed at 14 dap in trial 2. Post-emergence wilt at 49 dap observed for treatments with compost tea and Prestop ( $11.7 \pm 3.9$  and  $11.6 \pm 5.8\%$ , respectively) was significantly lower

compared to that of the non-treated, inoculated control ( $26.1 \pm 4.3\%$ ). Post-emergence wilt resulting from all other treatments was not significantly different than that of the non-treated, inoculated control. However, treatment with PGPR Galaxy resulted in the highest post-emergence wilt ( $34.4 \pm 7.1\%$ ), which was significantly higher compared to that of the Mertect 340F conventional control ( $21.9 \pm 6.1\%$ ).

Total disease at 49 dap in trial 2 was significantly lower for treatment with compost tea ( $19.5 \pm 8.6\%$ ) compared to that of the non-treated, inoculated control ( $29.9 \pm 6.9\%$ ) (Table 2.8). Total disease observed for all other treatments was not significantly different than that of the non-treated, inoculated control. However, treatment with Micro 108 resulted in the highest total disease ( $47.2 \pm 15.3\%$ ), which was significantly higher compared to that of the Mertect 340F conventional control ( $25.3 \pm 8.2\%$ ).

Biomass at 49 dap in trial 2 for seedlings that developed from the Experimental #1 seed treatment ( $13.71 \pm 0.46$  g) was not significantly different compared to that of the non-treated, non-inoculated control ( $14.90 \pm 0.39$  g) or the Mertect 340F conventional control ( $13.44 \pm 0.45$  g), and was significantly higher than that of the non-treated, inoculated control ( $12.85 \pm 0.68$  g). Biomass for seedlings that developed from the treatment with Micro 108 ( $10.51 \pm 0.94$  g) was significantly lower compared to that of the non-treated, inoculated control. Biomass for seedlings that developed from treatments with compost tea, Experimental #2, Kodiak, Mycostop Mix, Natural II, Natural X, PGPR Galaxy, Prestop, SoilGard, Subtilex, T-22 Planter Box, and Yield Shield was not significantly different compared to that of the non-treated, inoculated control. Results of the statistical analyses for the AUEPC,  $AUDPC_{pre}$ ,  $AUDPC_{post}$ , and  $AUDPC_{total}$  were similar to the 49 dap ratings for percentage emergence, pre-emergence

damping-off, post-emergence damping-off, and total damping-off, respectively, for trial 2 (Fig. 2.17).

In both trials, treatments with Micro 108 and Prestop resulted in emergence that was significantly lower than that of the non-treated, inoculated control (Tables 2.7 and 2.8). Also in both trials, treatment with Micro 108 resulted in pre-emergence damping-off that was significantly higher compared to that of the non-treated, inoculated control. Treatment with Prestop resulted in significantly lower post-emergence wilt compared to that of the non-treated, inoculated control in each trial.

**2.3.9. Seed health assays of treated seed.** Results from the seed health assays of treated seed are summarized in Table 2.9. For the first assay, the incidence of *Verticillium* spp. detected on the seed was reduced significantly from that of non-treated seed ( $49.75 \pm 2.06\%$ ) by treatment with Experimental #1 (10.5%), Experimental #2 (0.75%), Mycostop Mix (13.00%), Natural II (4.75%), Natural X (20.25%), and Mertect 340F (0.75%), but not by any other treatment. The greatest reduction in incidence of seedborne *Verticillium* spp. was observed on seed treated with Experimental #2 ( $0.75 \pm 0.48\%$ ) compared to the non-treated seed. In contrast, the incidence of *Verticillium* spp. detected for all seed treatments in the second assay was not significantly different from that of the non-treated seed ( $1.25 \pm 0.25\%$ ) because of the low level of this genus observed on that seed lot, with the exception of Experimental #1 which resulted in a significantly higher incidence of *Verticillium* spp. detected ( $10.25 \pm 1.03\%$ ). In contrast, the T-22 Planter Box and Mertect 340F treatments resulted in significantly lower incidences of *Verticillium* spp. (0%) compared to the non-treated seed. Most isolates of *Verticillium* observed resembled *V. dahliae*, a known pathogen of spinach (du Toit et al., 2005).

The prevalence of seedborne *S. botryosum* was significantly higher for the seed treated with Experimental #1 in the first assay ( $17.50 \pm 3.10\%$ ) compared to the non-treated seed ( $4.00 \pm 1.35\%$ ). Similarly, seed treatments with both Experimental #1 and Experimental #2 in the second assay resulted in significantly higher incidences of *S. botryosum* ( $11.25 \pm 1.75\%$  and  $9.50 \pm 1.19\%$ , respectively) compared to the non-treated seed ( $5.50 \pm 0.87\%$ ). In comparison, the prevalence of *S. botryosum* was significantly reduced in the first assay by Mycostop Mix, Natural II, and Apron XL LS ( $0.50 \pm 0.29$ ,  $0.50 \pm 0.50$ , and  $1.50 \pm 0.96\%$ , respectively) compared to that of the non-treated seed. The incidence of seedborne *S. botryosum* was significantly reduced in the second assay only by Mycostop Mix ( $0.50 \pm 0.29\%$ ) compared to the non-treated seed. No significant differences were detected in either trial in the prevalence of *Fusarium* spp. observed among seed treatments compared to the non-treated seed ( $2.25 \pm 1.31\%$  and  $0\%$  in trials 1 and 2, respectively). The seed lot used in the second assay was highly infested with *Alternaria* spp., with  $96.25 \pm 1.65\%$  incidence on the non-treated seed compared to  $13.25 \pm 1.70\%$  incidence of *Alternaria* spp. in the first assay. Seed treatment with Experimental #1, Experimental #2, and Mycostop Mix significantly reduced the incidence of *Alternaria* spp. in the second assay ( $3.00 \pm 2.04$ ,  $2.25 \pm 0.25$ , and  $88.00 \pm 3.72\%$ , respectively).

The prevalence of *Trichoderma* spp. and actinomycetes observed on the seed were also recorded in these assays. For the first assay, the incidence of *Trichoderma* spp. was significantly higher for seed treated with Experimental #2 ( $99.25 \pm 0.75\%$ ) and T-22 Planter box ( $48.50 \pm 7.14\%$ ) compared with the non-treated seed ( $0\%$ ). For trial 2, the incidence of *Trichoderma* spp. was again significantly higher for seed treated with Experimental #2 ( $54.00 \pm 16.25\%$ ) and T-22 Planter box ( $72.75 \pm 6.54\%$ ) compared with the non-treated seed ( $0\%$ ). These results reflected the fact that Experimental #2 and T-22 Planter Box both contained *T. harzianum* as the active

ingredient (Table 2.1). For the first assay, the incidence of seedborne actinomycetes was not significantly different for any of the treatments compared to the non-treated seed (*data not shown*). However, in the second assay, the incidence of actinomycetes was significantly higher for seed treated with Mycostop Mix ( $91.0 \pm 1.6\%$ ) and Natural II ( $4.0 \pm 1.6\%$ ) compared with the non-treated seed ( $1.8 \pm 1.5\%$ ). The active ingredient in each of Mycostop Mix and Natural II is an actinomycete (Table 2.1).

**2.3.10. Germination assays of treated seed.** Results of the seed germination assays are summarized in Table 2.10. For the 7 day reading of the first assay, seed treatment with Experimental #1 and Experimental #2 were the only treatments that resulted in significantly higher germination ( $70.0 \pm 3.2$  and  $64.5 \pm 3.4\%$ , respectively) compared with the non-treated seed ( $50.3 \pm 4.3\%$ ). This was not the situation in the second assay at the 7 day reading, at which time the germination for treatments with Experimental #1 and Experimental #2 was not significantly different compared to that of the non-treated seed. However, in the second assay, the 5 day reading revealed that seed treated with Experimental #1 and Experimental #2 resulted in significantly higher germination ( $38.00 \pm 5.99\%$  and  $29.25 \pm 7.50\%$ , respectively) compared to the non-treated seed ( $16.00 \pm 4.10\%$ ). No other treatments in the second assay had significantly higher or lower germination for the 5 day reading compared to the non-treated seed. In the first assay at the 7 day reading, seed treated with Natural II or Natural X resulted in significantly lower germination ( $34.75 \pm 2.32\%$  and  $40.50 \pm 2.02\%$ , respectively) compared to the non-treated seed ( $50.25 \pm 4.31\%$ ). However, no significant differences in germination were detected among any of the seed treatments at the 14 and 21 day readings of the first assay. For the 14 day reading in the second assay, Experimental #1 was the only seed treatment that resulted in significantly higher germination ( $92.50 \pm 0.87\%$ ) than the non-treated seed ( $88.50 \pm$

0.87%). For the 21 day reading in the second assay, seed treated with Experimental #1, Experimental #2, and Mycostop Mix resulted in significantly higher germination ( $93.25 \pm 0.95$ ,  $92.75 \pm 1.65$ , and  $92.50 \pm 0.65\%$ , respectively) compared to that of the non-treated seed ( $88.50 \pm 0.87\%$ ). Total percentage abnormal germination, rotten seed, and non-germinated seed were not significantly different among the seed treatments evaluated (*data not shown*).

**2.3.11. Potting mix pH from treatment trials.** The pH of the potting mix measured prior to planting the *P. ultimum* experiment averaged 6.46, and averaged 6.40 prior to planting the *F. oxysporum* f. sp. *spinaciae* experiment. The pH of the potting mix at the end of the *F. oxysporum* f. sp. *spinaciae* experiment was still 6.40.

## 2.4. DISCUSSION

The efficacy of a range of seed and drench treatments for control of soilborne seedling blight and damping-off pathogens was investigated in this study to provide an objective evaluation of EPA registered, OMRI-listed products approved for organic production, or products currently being developed for approval for use in organic production, using spinach as a model small-seeded vegetable. Greenhouse trials evaluating inoculation rates of *P. ultimum*, *R. solani*, and *F. oxysporum* f. sp. *spinaciae* were conducted to determine a rate of inoculation for each pathogen that resulted in approximately 50% damping-off or post-emergence wilt, in order to effectively differentiate among seed and drench treatments evaluated under the conditions of this study. For *P. ultimum*, a rate of 1,000 ppg of the soil/oatmeal inoculum consistently achieved this goal. For *R. solani*, a rate of 50,000 ppg of the soil/oatmeal inoculum was effective in the first trial. For *F. oxysporum* f. sp. *spinaciae*, a rate of 10,000 ppg was selected. However,

subsequent trials revealed that these rates for *R. solani* and *F. oxysporum* f. sp. *spinaciae* did not always give consistent levels of damping-off or wilt.

Fourteen seed and/or drench treatments were selected and evaluated in a greenhouse against each of the three soilborne pathogens. However, each treatment evaluated was not necessarily labeled for management of all three pathogens. Although the high bacterial diversity compost tea was not registered as a disease control product, it has been shown to suppress damping-off caused by *P. ultimum* (Scheuerell and Mahaffee, 2004). The two proprietary seed treatments, Experimental #1 (an organic disinfectant) and Experimental #2 (an organic disinfectant plus *T. harzianum* T-22), were not EPA registered at the time of this study. Although there was no label to claim which pathogens the two treatments might be effective at suppressing, the manufacturer of these products was interested in evaluating the products against all three pathogens. Kodiak (*B. subtilis*) seed treatment was labeled for control of *Fusarium* spp. and *Rhizoctonia* spp. by means of colonizing the root system and competing with pathogens. The Micro 108 (*S. griseoviridis*) seed and drench treatment labels did not list specific pathogens for control. However, the label did state that the products enhance growth and vigor of seedlings by enriching soil nutrients around the root system. The Mycostop Mix (*S. griseoviridis*) seed treatment label claimed the treatment would suppress seed and root rots caused by *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. The Natural II (actinomycetes) seed treatment label did not list control of any specific pathogens, and the Natural X treatment did not have a label at the time of this study. PGPR Galaxy (*B. azotofixans*, *A. chroococum*, *P. putida*, and *P. fluorescens*) was applied as a seed treatment for this study, as suggested by the manufacturer. However, PGPR galaxy is labeled as a drench treatment for improving crop establishment by stimulating root systems and plant defense mechanisms. Prestop (*G. catenulatum*) is labeled as a drench for

control of *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. SoilGard (*G. virens*) is labeled as a drench treatment for management of damping-off caused by *Pythium* spp. and *Rhizoctonia* spp. through mechanisms of antagonism. Subtilex (*B. subtilis*) seed treatment is labeled for control of *Rhizoctonia* spp. and *Fusarium* spp. by colonizing root systems and competing with the pathogens. The T-22 Planter Box (*T. harzianum* T-22) seed and drench treatment label claims efficacy at suppressing diseases caused by *Pythium* spp., *Rhizoctonia* spp., and *Fusarium* spp. through colonization of root systems and competition with the pathogens. The Yield Shield (*B. pumilus*) seed treatment label claims efficacy in management of *Fusarium* spp. and *Rhizoctonia* spp. by activating host resistance to the pathogens. Each seed and/or drench treatment was evaluated against each pathogen to determine whether or not the claims on the labels were accurate, as well as to determine if each product may have had a wider range of efficacy against more pathogens than were listed on each label.

*P. ultimum* invades the food-rich environment of seeds, which exude nutrients into the surrounding wet soil during germination, resulting in seed rot and pre- or post-emergence damping-off of seedlings (Bruehl, 1987; Hendrix and Campbell, 1973; Mitchell, 1979). Low temperatures unsuitable for plant growth result in a longer period of time for the pathogen to attack seeds and seedlings, and are associated with an increase in inoculum potential of the pathogen (Mitchell, 1979). Conducting the additional *P. ultimum* inoculation rate trial in growth chambers set at  $15 \pm 2^\circ\text{C}$  vs. in the greenhouse at  $25 \pm 5^\circ\text{C}$  had no significant impact on disease incidence, even though it was anticipated that this pathogen might require the lower temperatures for maximum disease expression (K. Schroeder, *personal communication*).

The method and rate of inoculation used in the greenhouse seed and drench treatment trials for *P. ultimum* offered good differentiation of efficacy of the treatments evaluated. *P.*

*ultimum* thrives under wetter conditions than *R. solani* or *F. oxysporum* f. sp. *spinaciae* (Hendrix and Campbell, 1973). *P. ultimum* spores germinate and grow rapidly in wet soils in the presence of host exudates (Bruehl, 1987; Hendrix and Campbell, 1973). The zoospores produced by *Pythium* spp. require free moisture to move to a host plant. In each of these trials, the drench treatments with Micro 108, Prestop, SoilGard, and T-22 Planter Box, and the compost tea drench treatment in the second trial, caused significantly higher total damping-off than the other treatments. This may be attributed to the fact that these drench treatments added a significant amount of moisture to the potting medium into which the seed was planted. This additional moisture may have enhanced the inoculum potential of the pathogen in the potting mix compared to the seed treatments. To test this hypothesis in the second trial, a water drench of the same volume of liquid used for the drench treatments (with the exception of the compost tea) was included. However, emergence and disease incidence observed in the flats drenched with water were not significantly different than those of the non-treated seed planted in medium without the water drench (*data not shown*).

For *P. ultimum*, the two experimental seed treatments, Experimental #1 and Experimental #2, provided equivalent control of damping-off to that provided by the conventional fungicide, Apron XL LS (mefenoxam) in each of two trials; while Natural II, Natural X, and Subtilex seed treatments each suppressed damping-off significantly in one of the two trials. Damping-off results for the Natural II seed treatment were inconsistent in the *P. ultimum* trials, as for emergence ratings. The treatment significantly suppressed disease in the first trial, but resulted in significantly higher disease in the second trial. Additionally, results for the compost tea drench were not consistent with results for the same tea evaluated by Scheuerell and Mahaffee (2004).

These results indicated that seed treatment with Experimental #1 and Experimental #2 consistently provided the best protection from damping-off compared to all other treatments evaluated. *T. harzianum* is one of the active ingredients in Experimental #2. These results are reflective of a study by Howell (2007), in which seed treatment with a variety of strains of *Trichoderma* spp. effectively reduced pre-emergence damping-off of cotton caused by *P. ultimum*. Other BCAs, including *E. cloaceae*, *E. herbicola*, *P. fluorescens*, *R. leguminosarum* bv. *viceae*, have also been shown to effectively reduce damping-off caused by *Pythium* spp. on a variety of crops (Huang and Erickson, 2007; Nelson, 1988; Trapero-Casas and Ingram, 1990). However, in this study, treatments evaluated with the same active ingredients listed in those studies (PGPR Galaxy and T-22 Planter Box) did not result in a significant reduction in damping-off caused by *P. ultimum*. Labels for Mycostop Mix, Prestop, SoilGard, and T-22 Planter Box claim efficacy in suppressing damping-off caused by *Pythium* spp.

For *R. solani*, pre-emergence damping-off was more prevalent than post-emergence damping-off in the inoculation rate trials. *R. solani* is known for the ability to respond quickly to energy sources and to grow rapidly to increase the inoculum potential (Mitchell, 1979). The pathogen attacks seeds before and during germination, causing low emergence of seedlings (Baker, 1970). This was evident in the greenhouse inoculation rate trials as emergence of seedlings declined as the rate of inoculation of *R. solani* increased.

Quantifying *Rhizoctonia* spp. using soil-dilution methods can be difficult (Paulitz and Schroeder, 2005). Quantifying inoculum density of the jars of *R. solani* soil/oatmeal inoculum for the inoculation rate trials in this study proved difficult using the soil-dilution plating method, as there was often high variation in inoculum density among jars of inoculum, ranging from  $3.1 \times 10^4$  to  $3.26 \times 10^5$  ppg. The soil/oatmeal medium contained large masses of *R. solani* mycelia

which made it difficult to quantify propagules of the pathogen. For comparison, soil-dilution plating was also conducted using *R. solani* soil/oatmeal inoculum that was ground using a coffee grinder (Braun, Kronberg, Germany) to compare with non-ground inoculum. No significant difference was detected in dilution plate counts from the same jars of inoculum between the ground and non-ground inocula, but there was still high variation among jars of inoculum, even when the *R. solani* soil/oatmeal inoculum was ground (*data not shown*).

Results from the first *R. solani* seed and drench treatment trial indicated that an inoculum density of 50,000 ppg was higher than anticipated to achieve 50% damping-off in the non-treated, inoculated flats for effective separation of efficacy among the treatments evaluated. This may have been related to the high variation in inoculum density among jars of the soil/oatmeal inoculum. It is also possible that the dilution plating method of quantifying the inoculum was not as accurate as desired. To attempt to compensate for the high level of disease, the rate of inoculation was reduced to 25,000 ppg in the second trial. However, this proved too low to effectively differentiate efficacy among the seed and drench treatments evaluated, as emergence for the inoculated, non-treated control flats was high ( $82.8 \pm 1.6\%$ ), while total damping-off was very low ( $6.4 \pm 2.8\%$ ). Although significant differences among treatments were detected for all variables measured in trial 1 (emergence, pre-emergence, post-emergence, and total damping-off), and only for emergence and pre-emergence damping-off ratings in trial 2, neither trial gave as accurate an assessment of the treatments evaluated at a rate of inoculation anticipated to accomplish approximately 50% total damping-off of the non-treated, inoculated control as for the *P. ultimum* trials.

Based on the results from both *R. solani* seed and drench treatment trials, treatment with Experimental #1 and Natural II resulted in emergence that was not significantly different from

that of the non-treated, non-inoculated control, or the Terraclor conventional fungicide drench. The active ingredients of Experimental #1 and Natural II are an organic disinfectant and actinomycetes, respectively. Other BCAs, including *P. aeruginosa* and *P. fluorescens*, *P. putida*, *T. harzianum*, *Gliocladium* spp., and *B. amyloliquifaciens*, have been shown to effectively reduce damping-off caused by *Rhizoctonia* spp. on a variety of crops (Baker and Paulitz, 1996; Chung et al., 2005; Grosch et al., 2005; Harris and Adkins, 1999; Lewis and Papavizas, 1985; Marshall, 1982; and Siddiqui and Shaukat, 2002). However, in this study, treatments evaluated that had the same active ingredients as in those studies (Experimental #2, PGPR Galaxy, PreStop, SoilGard, and T-22 Planter Box) did not result in a significant reduction in damping-off caused by *Rhizoctonia* spp. Labels for Kodiak, Mycostop Mix, Prestop, SoilGard, Subtilex, T-22 Planter Box, and Yield Shield claim efficacy in suppressing *R. solani*. A study by Marshall (1982) revealed that seed treatment with *T. harzianum* resulted in the most significant reduction in disease caused by *R. solani* at a pH of 3.5 vs. pH 5.6. This may indicate that the products evaluated in this study that contained *Trichoderma* spp. as the active ingredient may have been limited in efficacy by the pH of the potting medium, which ranged from 6.40 to 6.46.

Fusarium wilts are generally more severe in warm soil, and are favored at temperatures near 28°C (Bruehl, 1987). Members of the genus *Fusarium* have the ability to undergo extensive mycelial growth, with quick response to nutrients and efficient use of ephemeral substrates that become available to maintain the pathogen inoculum potential (Mitchell, 1979). For the *F. oxysporum* f. sp. *spinaciae* inoculation rate trials in this study, post-emergence wilt was significantly more prevalent than pre-emergence damping-off. Therefore, the total wilt and post-emergence wilt ratings were nearly identical, whereas pre-emergence damping-off was negligible and total emergence remained relatively constant among the rates of inoculation

assessed. The duration of the second inoculation rate trial for *F. oxysporum* f. sp. *spinaciae* was longer than that of the *P. ultimum* and *R. solani* trials because the former took longer to cause disease than the latter two pathogens. Additionally, trials with *F. oxysporum* f. sp. *spinaciae* were maintained at higher temperatures than other two pathogens to induce a higher transpirational demand and promote expression of vascular wilt. Although pathogenicity of the *F. oxysporum* f. sp. *spinaciae* 001 isolate was confirmed on the spinach inbred '9420.533', post-emergence wilt was less severe and slower to develop on the hybrid 'Lazio' in the greenhouse inoculation rate trials and the seed and drench treatment trials. It was later determined that one of the parent lines in this hybrid has partial resistance to Fusarium wilt, which may be expressed in the hybrid (J. de Visser, spinach breeder, Pop Vriend Seeds, *personal communication*).

Results from trial 1 of the *F. oxysporum* f. sp. *spinaciae* seed and drench treatment evaluations indicated that 10,000 ppg was an appropriate rate of inoculation for achieving a total wilt incidence of approximately 50% for the inoculated, non-treated control, and provided a clear differentiation among treatments for control of this pathogen. However, for trial 2, this rate of inoculation resulted in a lower incidence of total wilt of the inoculated, non-treated control than the targeted 50%, which limited the ability to differentiate among the seed and drench treatments at the anticipated level of disease. In addition, the fact that these trials were carried out for 42 and 49 days (trial 1 and 2, respectively) may have impacted the efficacy of the treatments, as many seed treatments have efficacy against specific pathogens for a limited duration after planting (Harman, 1991). Confounding this further is the evidence that the spinach hybrid 'Lazio' has partial resistance to Fusarium wilt. Therefore, some of the treatments evaluated in these trials may no longer have been efficacious against *F. oxysporum* f. sp. *spinaciae* by the time the disease was induced in the plants.

According to results from the *F. oxysporum* f. sp. *spinaciae* seed and drench treatment trials, none of the treatments evaluated consistently provided lower post-emergence wilt compared to that of the non-treated, inoculated control in both trials. However, compost tea drench, Prestop drench, and Yield Shield seed treatment each suppressed post-emergence wilt significantly in one of the two trials. Other BCAs, including *P. fluorescens*, *P. putida*, *Glomus intraradices*, *G. mosseae*, *E. cloaceae*, *R. leguminosarum*, *Trichoderma* spp., *G. virens*, *B. cepacia*, and non-pathogenic species of *Fusarium* have been shown to effectively reduce wilt caused by *Fusarium* spp. on a variety of crops (Akkopru and Demir, 2005; Fravel et al., 2003; Hassan Dar et al., 1997; Larkin and Fravel, 1998; Leeman et al., 1995; and Raaijmakers et al., 1995). However, in this study, treatments evaluated with the same active ingredients listed in those studies (Experimental #2, PGPR Galaxy, SoilGard, and T-22 Planter Box) did not result in a significant reduction in post-emergence wilt caused by *F. oxysporum* f. sp. *spinaciae*. Labels for Kodiak, Mycostop Mix, Prestop, Subtilex, T-22 Planter Box, and Yield Shield claim efficacy in suppressing *Fusarium* spp. In each trial, total wilt for the Mertect 340F conventional fungicide was not significantly different than that of the inoculated, non-treated control. This indicates that Mertect 340F was not an effective seed treatment for management of *Fusarium* wilt for this spinach hybrid under the conditions of these trials.

A very low incidence (<4%) of post-emergence wilt was sometimes evident for the non-inoculated control treatments in the greenhouse inoculation rate trials. *Fusarium* spp. can be seed transmitted (Fravel et al., 2003; Neergaard, 1977). Although these fungi are predominantly soilborne pathogens, seed transmission of *Fusarium* spp. can be a significant source of inoculum, even at low incidences, when the pathogens are introduced to non-infested soils (Neergaard, 1977). The *Fusarium* observed in the seed health assay on the seed lot used for the inoculation

rate trials (at an incidence of  $4.8 \pm 1.7\%$ ) may have been the cause of this wilt although these seedborne isolates were not identified to species. An isolate of a *Fusarium* species found on one of the spinach seed was tested for pathogenicity on spinach, but did not prove pathogenic (*data not shown*). However, this was not a representative sample of the seedborne *Fusarium* isolates on the two seed lots used in this study.

Environmental conditions greatly influence the type and amount of inoculum produced by seedborne pathogens, as well as the significance of seedborne inoculum relative to other sources of infection (Neergaard, 1977). Many plant pathogens survive in seeds, and seeds can be an important factor in the perpetuation of some plant pathogens (Agarwal and Sinclair, 1997). Seed treatments can provide curative and/or protective control of seedborne as well as soilborne pathogens (Neergaard, 1977; Taylor and Harman, 1990). The two seed lots used for the greenhouse seed and drench treatment trials in this study were commercially available seed lots. Results from the seed health assays revealed that the two seed lots showed significant differences in the incidences of seedborne *Verticillium* spp. and *Alternaria* spp. The second seed lot, which was used in all of the repeat greenhouse seed and drench treatment trials, was highly infested with *Alternaria* spp. This high incidence of *Alternaria* spp. likely affected results for seed treatments in that assay. This amount of *Alternaria* spp. on the seed may have suppressed development of other fungi on the seed, e.g., *Verticillium* spp. and *S. botryosum*. This was apparent for seed treated with Experimental #1 and Experimental #2, which significantly reduced the incidence of *Alternaria* spp. in the second assay compared to the non-treated seed. For those two treatments, the incidences of *Verticillium* spp., and *S. botryosum* were significantly higher than for the non-treated seed, whereas none of the other treatments resulted in significant differences in incidences of those fungi compared to the non-treated seed. *S. botryosum*

infections of spinach seed have been shown to be deep in the pericarp and even in the embryo (Hernandez-Perez and du Toit, 2006) compared with many other seedborne pathogens of spinach, which might explain why the organic disinfectant in Experimental #1 and Experimental #2 did not eliminate *S. botryosum* from the seed.

The consistency in performance of seed treatments varies among crop species, seed treatment products, seedborne or soilborne diseases, soil types and conditions, etc. (Taylor and Harman, 1990). The prevalence of both *Trichoderma* spp. and actinomycetes on the treated spinach seed was recorded in this study because the active ingredients of some of the products evaluated included *Trichoderma* spp. or actinomycetes. By recording the prevalence of these organisms during each assay, the degree of coverage or colonization of the seed with each BCA could be assessed. For the first seed lot, the fact that there was no significant difference in the low prevalence of actinomycetes among the treatments assessed suggests that the entire seed lot was naturally infected with a low level of actinomycetes. Very few actinomycetes were recorded for the Micro 108, Natural II, and Natural X seed treatments in each assay, even though the active ingredients for these products were a strain of *S. lydicus* for Micro 108 and a proprietary actinomycete for Natural II and Natural X. These results suggest that the products may not have adhered well to the seed during treatment, that the products were not viable, or that viability of the actinomycetes degraded after seed treatment, although the products were stored and seed were treated strictly according to the manufacturer's label or even by the registrant (for Natural II and Natural X).

The earlier germination of seed treated with Experimental #1 and Experimental #2 in the germination assays demonstrated the consistency of these two treatments at promoting early germination of spinach seed. This early germination and emergence could be beneficial at

reducing the effects of pre-emergence damping-off caused by *P. ultimum* and *R. solani*.

Although not significantly different from that of the non-treated seed by the final reading, Natural II treated seed had the lowest germination at the first reading in each assay. This is consistent with results from previous spinach seed treatment trials with this product (du Toit et al., 2007). *Alternaria* spp. are known to cause seed rot of a variety of crops (Agarwal and Sinclair, 1997). Experimental #1, Experimental #2, and Mycostop Mix, significantly reduced the incidence of seedborne *Alternaria* spp. observed on the second seed lot, and also resulted in significantly higher final germination for this seed lot compared to the non-treated seed.

The soil/oatmeal form of inoculum used for the greenhouse trials in this study was effective for achieving approximately 50% damping-off in all of the *P. ultimum* inoculation rate and treatment trials. However, this form of inoculum was less reliable or consistent for the other two pathogens, *R. solani* and *F. oxysporum* f. sp. *spinaciae*. Because of this variability, perhaps a different form of inoculum for *R. solani* should be used in future studies, such as the ground, inoculated rye seed used for storage of the isolate, which might be more effective and consistent in performance than the soil/oatmeal inoculum, since the rye inoculum contained dormant resting structures of the pathogen rather than masses of mycelia can be more difficult to quantify. Other studies have used forms of inoculum similar to the ground, inoculated rye seed, including inoculated oat seed, hulls, or kernels (Marshall, 1982; Grosch et al., 2005), or quartz sand and corn meal inoculum (Lewis and Papavizas, 1985), in which no difficulties with these forms of inoculum were reported.

Because of variability in results for the *F. oxysporum* f. sp. *spinaciae* trials, perhaps a different form of inoculum would also be more appropriate to use for *F. oxysporum* f. sp. *spinaciae*, such as chlamydospore inoculum (Kraft and Roberts, 1969; Marois and Mitchell,

1981; Rush and Kraft, 1986), a malt extract medium inoculum (Raaijmakers et al., 1995), or a conidial suspension (Larkin and Fravel, 1998). The fact that the pathogen produced wilt within only 32 days in the first inoculation rate trial, not until 56 dap for the second and third inoculation rate trials, and 42 and 49 dap in the greenhouse seed and drench treatment trials may be evidence that the isolate in this form of inoculum was less effective than anticipated. This isolate was stored in a sterile soil/sand mixture (1:1 ratio) at 4°C for six years. This soil storage method for *Fusarium* spp. is commonly used, but is not recommended by some researchers (Leslie and Summerell, 2006). Studies by both Booth (1971) and Windels et al. (1988 and 1993) discovered that the fungus can colonize the soil medium and grow during storage, which increases the possibility of mutation, and of recovering strains in which the morphology/phenotype has been altered. However, this was not evident in this study. Additionally, a study by Gaylarde and Kelley (1995) revealed that *F. merismoides* stored in a similar soil medium appeared to senesce; evidence for this senescence was the appearance of additional DNA restriction fragments associated with a plasmid in this isolate. However, this was not assessed in this study. Using a different isolate of the pathogen or method of inoculation that would result in earlier disease expression than in these trials, or a different spinach cultivar than Lazio that does not have resistance to *F. oxysporum* f. sp. *spinaciae* may have led to different results.

Variation in efficacy from trial to trial of some of the seed and drench treatments evaluated in this study was observed. This could be attributable to a number of things, e.g., the higher incidence of *Alternaria* spp. on the seed lot used in the second trial vs. the first trial may have inhibited efficacy of some seed treatments. However, as previously stated, the two seed lots used in these trials were commercially available. It is important to know if the seed or

drench treatments evaluated do not show consistency in efficacy under different conditions or with seed lots that have different incidences of seedborne fungi.

Further research involving combinations of organic seed or drench treatments against each pathogen separately, or against combinations of the pathogens is needed. None of the selected treatments will serve as a 'silver bullet' to provide protection of all crop species against all pathogens. Furthermore, a specific treatment may be effective under specific conditions, but not under other conditions, as BCAs are readily affected by soil type, pH, temperature, etc. It is important to assess the strengths of each treatment, and to determine how the treatments could work synergistically to reduce damping-off and seedling blights in organic production for a variety of crop species under a variety of conditions.

## **2.5. LITERATURE CITED**

Agarwal, V. K., and Sinclair, J. B. 1997. Principles of Seed Pathology, Second Edition. CRC Press, Inc., Boca Raton, FL.

Akkopru, A., and Demir, S. 2005. Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* by AMF *Glomus intraradices* and some Rhizobacteria. J. Phytopathology 153:544-550.

Baker, K. F. 1970. Types of *Rhizoctonia* diseases and their occurrence. Pages 125-148, in: *Rhizoctonia solani*, Biology and Pathology. University of California Press, Berkeley and Los Angeles, CA.

Baker, R., and Paulitz, T. C. 1996. Theoretical basis for microbial interaction leading to biological control of soilborne plant pathogens. Pages 50-79, in: Principles and Practice of Managing Soilborne Plant Pathogens. R. Hall, Editor. APS Press, St. Paul, MN.

- Bassi, A., Jr., and Goode, M. J. 1978. *Fusarium oxysporum* f. sp. *spinaciae* seedborne in spinach. Plant Dis. Rep. 62:203-205.
- Booth, C. 1971. The genus *Fusarium*. Commonwealth Mycological Institute, Kew, Surrey, United Kingdom.
- Bruehl, G. W. 1987. Soilborne Plant Pathogens. Macmillan Publishing Co., New York, NY.
- Campbell, C. L., and Neher, D. A. 1996. Challenges, opportunities, and obligations in root disease epidemiology and management. Pages 20-49, in: Principles and Practice of Managing Soilborne Plant Pathogens. R. Hall, Editor. APS Press, St. Paul, MN.
- Chung, W. C., Huang, J. W., and Huang, H. C. 2005. Formulation of a soil biofungicide for control of damping-off of Chinese cabbage (*Brassica chinensis*) caused by *Rhizoctonia solani*. Biological Control 32:287-294.
- du Toit, L. J., Derie, M. L., Brissey, L. M., Brissey, L. M. 2007. Evaluation of fungicides for control of seedborne *Stemphylium botryosum* on spinach. Plant Dis. Mngmt Rep. 1:ST003.
- du Toit, L. J., Derie, M. L., Brissey, L. M., Cummings, J.A. 2007. Evaluation of limestone amendments for control of Fusarium wilt in a spinach seed crop. Plant Dis. Mngmt Rep. 1:V091.
- du Toit, L. J., Derie, M. L., and Hernandez-Perez, P. 2005a. Verticillium wilt in spinach seed production. Plant Dis. 89:4-11.
- du Toit, L. J., Inglis, D. A., and Miles, C. M. 2005b. USDA Organic Cropping Research for the Northwest: Organic Seed Treatments: 2005 Progress Report. Washington State University-Mount Vernon NWREC, Mount Vernon, WA.

- Farr, D. F., Rossman, A. Y., Palm, M. E., and McCray, E. B. (n.d.) Fungal Databases, Systematic Botany & Mycology Laboratory, USDA ARS. Retrieved 3 February 2007 from <http://nt.ars-grin.gov/fungaldatabases>.
- Fravel, D., Olivain, C., and Alabouvette, C. 2003. *Fusarium oxysporum* and its biocontrol. *New Phytologist* 157:493-502.
- Gaylarde, C., and Kelley, J. 1995. Genetic variation in *Fusarium merismoides* preserved by two different methods. *World J. Microbiol. Biotechnol.* 11:319-321.
- Gindrat, D. 1979. Side effects of pesticides: Concluding remarks. Pages 531-534, in: *Soil-borne Plant Pathogens*. B. Schippers and W. Gams, Editors. Academic Press, New York, NY.
- Groot, S., van der Wolf, J. M., Jalink, H., Langerak, C. J., and van den Bulk, R. W. 2004. Challenges for the production of high quality organic seeds. *Seed Testing International ISTA News Bulletin* No. 127:12-15.
- Grosch, R., Franziska, F., Lottmann, J., Kofoet, A., and Berg, G. 2005. Effectiveness of three antagonistic bacterial isolates to control *Rhizoctonia solani* Kuhn on lettuce and potato. *Can. J. Microbiol.* 51:345-353.
- Harman, G. E. 1991. Seed treatments for biological control of plant disease. *Crop Protection* 10:166-171.
- Harris, A. R., and Adkins, P. G. 1999. Versatility of fungal and bacterial isolates for biological control of damping-off disease caused by *Rhizoctonia solani* and *Pythium* species. *Biological Control* 15:10-18.

- Hassan Dar, G., Zargar, M. Y., and Beigh, G. M. 1997. Biocontrol of Fusarium root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mossea* and *Rhizobium leguminosarum*. *Microbial Ecology* 34:74-80.
- Hendrix, F. F., and Campbell, W. A. 1973. Pythiums as plant pathogens. *Annu. Rev. Phytopath.* 11:77-98.
- Hernandez-Perez, P., du Toit, L. J. 2006. Seedborne *Cladosporium variabile* and *Stemphylium botryosum* in spinach. *Plant Dis.* 90:137-145.
- Howell, C. R. 2007. Effect of seed quality and combination fungicide-*Trichoderma* spp. seed treatments on pre- and post-emergence damping-off in cotton. *Phytopathology* 97:66-71.
- Huang, H. C., and Erickson, R. S. 2007. Effect of seed treatment with *Rhizobium leguminosarum* on Pythium damping-off, seedling height, root nodulation, root biomass, shoot biomass, and seed yield of pea and lentil. *J. Phytopathology* 155:31-37.
- Irish, B. M., Correll, J. C., Koike, S. T., and Morelock, T. E. 2007. Three new races of the spinach downy mildew pathogen identified by a modified set of spinach differentials. *Plant Dis.* 91:1392-1396.
- Kerr, A. 1963. The root rot Fusarium wilt complex of pea. *Aus. J. Biol. Sci.* 16:55-69.
- Klungland, M. W., and McArthur, M. 1989. Soil Survey of Skagit County Area, Washington. USDA Soil Conservation Service.
- Koenig, R. L., and Baker, B. 2002. U.S. National Organic Program Standards: Implications for researchers. APSnet <http://www.apsnet.org/online/feature/organic/>
- Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8:114-125.

- Kraft, J. M., and Roberts, D. D. 1969. Influence of soil water and temperature on the pea root rot complex caused by *Pythium ultimum* and *Fusarium solani* f. sp. *lisi*. *Phytopathology* 59:149-152.
- Larkin, R. P., and Fravel, D. R. 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of *Fusarium* wilt of tomato. *Plant Dis.* 82:1022-1028.
- Leeman, M., van Pelt, J. A., Hendrickx, M. J., Scheffer, R. J., Bakker, P. A. H. M., and Schippers, B. 1995. Biocontrol of *Fusarium* wilt of radish in commercial greenhouse trials by seed treatment with *Pseudomonas fluorescens* WCS374. *Phytopathology* 85:1301-1305.
- Leslie, J. F., and Summerell, B. A. 2006. Techniques for growing and maintaining *Fusarium*. Pages 21-30, in: *The Fusarium Laboratory Manual*. Blackwell Publishing, Ames, IA.
- Lewis, J. A., and Papavizas, G. C. 1985. Effect of mycelial preparations of *Trichoderma* and *Gliocladium* on populations of *Rhizoctonia solani* and the incidence of damping-off. *Phytopathology* 75:812-817.
- Marois, J. J., and Mitchell, D. J. 1981. Effects of fungal communities on the pathogenic and saprophytic activities of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology* 71:1251-1256.
- Marshall, D. S. 1982. Effect of *Trichoderma harzianum* seed treatment and *Rhizoctonia solani* inoculum concentration on damping-off of snap bean in acidic soils. *Plant Dis.* 66:788-789.
- Mircetich, S. M., and Kraft, J. M. 1973. Efficiency of various selective media in determining *Pythium* populations in soil. *Mycopathol. Mycol. Appl.* 50:151-161.
- Mitchell, J. E. 1979. The dynamics of the inoculum potential of populations of soil-borne plant pathogens in the soil ecosystem. Pages 3-20, in: *Soil-borne Plant Pathogens*. B. Schippers and W. Gams, editors. Academic Press, New York, NY.

- National Organic Program. 2005. Organic Foods Production Act of 1990. Section 2109:  
Prohibited Crop Production Practices and Materials.  
<http://www.ams.usda.gov/nop/NOP/NOPhome.html>.
- Neergaard, P. 1977. Seed Pathology. John Wiley & Sons, Inc., New York, NY.
- Nelson, E. B. 1988. Biological control of Pythium seed rot and preemergence damping-off of cotton with *Enterobacter cloacae* and *Erwinia herbicola* applied as seed treatments. Plant Dis. 72:140-142.
- Oparaocha, E. T., and Okigbo, R. N. 2003. Thrips (*Thysanoptera*) of vegetable crops (okra, spinach, garden egg and pumpkin) grown in Southeastern Nigeria. Plant Protect. Sci. 39:132-138.
- Organic Materials Review institute. <http://www.omri.org/>.
- Paulitz, T. C., and Schroeder, K. L. 2005. A new method for the quantification of *Rhizoctonia solani* and *R. oryzae* from soil. Plant Dis. 89:767-772.
- Peever, T. L., Canihos, Y., Olsen, L., Ibanez, A., Liu, Y. C., and Timmer, L. W. 1999. Population genetic structure and host specificity of *Alternaria* spp. causing brown spot of *Minneola tangelo* and rough lemon in Florida. Phytopathology 89:851-860.
- Raaijmakers, J. M., Leeman, M., van Oorschot, M. M. P., van der Sluis, I., Schippers, B., and Bakker, P. A. H. M. 1995. Dose-response relationships in biological control of Fusarium wilt of radish by *Pseudomonas* spp. Phytopathology 85:1075-1081.
- Rush, C. M., and Kraft, J. M. 1986. Effects of inoculum density and placement on Fusarium root rot of peas. Phytopathology 76:1325-1329.

- Scheuerell, S., and Mahaffee, W. 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. *Phytopathology* 94:1156-1163.
- Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67:1051-1056.
- Siddiqui, I. A., and Shaukat, S. S. 2002. Resistance against the damping-off fungus *Rhizoctonia solani* systemically induced by the plant-growth-promoting rhizobacteria *Pseudomonas aeruginosa* (IE-6S+) and *P. fluorescens* (CHA0). *J. Phytopathology* 150:500-506.
- Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* Species. APS Press, St. Paul, MN.
- Steele, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2<sup>nd</sup> ed. McGraw Hill, New York, NY.
- Taylor, A. G., and Harman G. E. 1990. Concepts and technologies of selected seed treatments. *Annu. Rev. Phytopathology* 28:321-339.
- Trapero-Casas, A., and Ingram, D. M. 1990. Control of Pythium seed rot and preemergence damping-off of chickpea in the U.S., Pacific Northwest and Spain. *Plant Dis.* 74:563-569.
- Windels, C. E., Barnes, P. M., and Kommendahl, T. 1988. Five-year preservation of *Fusarium* species on silica gel and soil. *Phytopathology* 78:107-109.
- Windels, C. E., Barnes, P. M., and Kommendahl, T. 1993. *Fusarium* species stored in silica gel and soil for ten years. *Mycologia* 85:21-23.
- Yaklich, R. W. 1985. Germination tests. Pages 30-36 in: Rules for Testing Seeds. Association of Official Seed Analysts. *J. Seed Technol.* 8:2.

**Table 2.1.** Seed and drench treatments evaluated in greenhouse trials for efficacy against damping-off and vascular wilt of spinach caused by *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum* f. sp. *spinaciae*<sup>a</sup>

Treatment <sup>b</sup>	Active ingredient (rate in product)	Registrant or manufacturer	Rate of application of product <sup>c</sup>	Method of application	OMRI-listed <sup>d</sup>	Registered for spinach in WA State in 2007 <sup>e</sup>
Compost tea	High bacterial diversity compost tea <sup>f</sup>	Washington State University, Pullman, WA	646 liters/100 m <sup>3</sup> potting mix	Drench immediately after planting	Yes	No
Experimental #1	Proprietary organic disinfectant	Proprietary	Proprietary	Seed treatment	Not yet applied	No
Experimental #2	Proprietary organic disinfectant + <i>Trichoderma harzianum</i> T-22	Proprietary	Proprietary	Seed treatment	Not yet applied	No
Kodiak Concentrate Biological Fungicide	<i>Bacillus subtilis</i> (1.37%)	Bayer CropScience, Research Park Triangle, NC	31.2 g/100 kg seed	Slurry seed treatment	Yes	No
Micro 108 Seed Inoculant + Actinovate AG	<i>Streptomyces lydicus</i> (10 <sup>8</sup> cfu/g) + <i>S. lydicus</i> (10 <sup>7</sup> cfu/g)	Natural Industries, Houston, TX	1.76 kg/100 kg seed + 1.29 kg/100 liters water	Dry seed coating + drench immediately after planting	Yes	Yes
Mycostop Mix	<i>Streptomyces griseoviridis</i> (4%)	Verdera Oy, Luoteisrinne, Finland	625.7 g/100 kg seed	Dry seed coating	Yes	Yes
Natural II	Actinomycete (0.6%)	Agricoat LLC, Soledad, CA	750.7 g/100 kg seed	Seed treatment	No	No
Natural X	Actinomycete (0.6%)	Agricoat LLC	750.7 g/100 kg seed	Seed treatment	No	No
PGPR Galaxy	Bacterial mixture <sup>g</sup>	Holmes ENVIRO, LLC, Philomath, OR	7 liters/100 kg seed	Slurry seed treatment	Yes	No
Prestop	<i>Gliocladium catenulatum</i> (32%)	Verdera Oy	180 g/100 liters water	Drench immediately after planting	Not yet applied in US, but approved in EU	No

**Table 2.1.** Continued

Treatment <sup>b</sup>	Active ingredient (rate in product)	Registrant or manufacturer	Rate of application of product <sup>c</sup>	Method of application	OMRI-listed <sup>d</sup>	Registered for spinach in WA State in 2007 <sup>e</sup>
SoilGard 12G	<i>Gliocladium virens</i> (12%)	Certis USA, Columbia, MD	239.7 g/100 liters water	Drench >24 h before planting	Yes	No
Subtilex	<i>Bacillus subtilis</i> (2.75%)	Becker Underwood, Ames, IA	15.6 g/100 kg seed	Slurry seed treatment	No	No
T-22 Planter Box	<i>Trichoderma harzianum</i> T-22 (1.15%)	BioWorks, Inc., Victor, NY	250 g/100 kg seed	Dry seed coating + drench 4 days after planting	Yes	Yes
Yield Shield	<i>Bacillus pumilus</i> (0.28%)	Bayer CropScience	6.26 g/100 kg seed	Slurry seed treatment	Yes	No
Apron XL LS	Mefenoxam (33%)	Syngenta Crop Protection, Greensboro, NC	20.8 ml/100 kg seed	Slurry seed treatment	No	Yes
Mertect 340F	Thiabendazole (42.3%)	Syngenta Crop Protection	122.4 ml/100 kg seed	Slurry seed treatment	No	No
Terraclor 75% WP	Pentachloro-nitrobenzene (75%)	Crompton Uniroyal Chemical, Middlebury, CT	59.9 g/100 liters water	Drench immediately after planting	No	No
Non-treated seed	-	-	-	-	-	-

<sup>a</sup> Each trial was set up as a randomized complete block design with 4 or 5 replications. Each experimental unit consisted of one 30.5 cm x 30.5 cm x 6.4 cm tall flat containing 1200 g wetted potting mix (Sunshine Organic Growers Mix, Sun Gro Horticulture, Bellevue, WA) inoculated with the selected pathogen at a rate determined by inoculation rate trials, and planted with 6 rows of 6 seed of the spinach hybrid ‘Lazio’, as described in the text.

<sup>b</sup> Products were selected for evaluation against each of the three pathogens. Not all products were approved by the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) for use in organic systems in 2007. Apron XL LS, Mertect 340F, and Terraclor 75% WP were included as conventional fungicide seed or drench treatments for control of *Pythium* spp., *Fusarium* spp., and *Rhizoctonia* spp., respectively. Non-treated seed was included as a control treatment.

- <sup>c</sup> Each product was evaluated at the highest recommended label rate for spinach and/or a crop with similar size seed to spinach, or according to registrant recommendations. Drench treatments were applied according to the labels based on surface area or volume of potting medium treated.
- <sup>d</sup> The Organic Materials Review Institute (OMRI) provides organic certifiers, growers, manufacturers, and suppliers an independent review of products intended for use in certified organic production, handling, and processing in the U.S. (<http://www.omri.org/>). “Not yet applied” means registrant had not yet applied for OMRI approval.
- <sup>e</sup> Approved by the Washington State Department of Agriculture for use on certified organic spinach crops in Washington State in 2007.
- <sup>f</sup> Ingredients of the compost tea included vermicompost (50 ml), seaweed powder (1 ml), liquid humic acids (2 ml), and Azomite rock dust (Scheuerell and Mahaffee, 2004). The compost tea was brewed by C. Crosby at Washington State University, Pullman, WA.
- <sup>g</sup> PGPR Galaxy contains *Bacillus azotofixans* (304 billion cells/liter), *Azotobacter chroococcum* (304 billion cells/liter), *Pseudomonas putida* (304 billion cells/liter), and *Pseudomonas fluorescens* (304 billion cells/liter).

**Table 2.2.** Incidence of necrotrophic fungi on a seed lot of the spinach hybrid ‘Lazio’ used for greenhouse inoculation rate trials for damping-off and wilt pathogens

Seed treatment <sup>a</sup>	Mean ± standard error (%) of 400 spinach seed infested or infected					
	<i>Fusarium</i> spp.	<i>Verticillium</i> spp.	<i>Stemphylium botryosum</i>	<i>Cladosporium variabile</i>	Other <i>Cladosporium</i> spp.	<i>Alternaria</i> spp.
Non-surface-sterilized	4.8 ± 1.7	42.0 ± 4.3	35.0 ± 2.4	2.5 ± 1.0	33.5 ± 6.0	36.5 ± 4.8
Surface-sterilized	0	31.0 ± 6.2	37.0 ± 6.4	0.3 ± 0.5	7.0 ± 2.4	24.8 ± 9.6

<sup>a</sup> Four samples of 100 seed were surface-sterilized in 1.2% NaOCl for 60 s, triple-rinsed, dried, and subjected to a freeze-blotter seed health assay as described by du Toit et al. (2005). For the non-surface-sterilized assay, seed was not soaked or rinsed prior to plating.

**Table 2.3.** Evaluation of seed and drench treatments for control of *Pythium ultimum* as a damping-off pathogen of spinach: First greenhouse trial<sup>a</sup>

Treatment <sup>b</sup>	% Emergence		% Damping-off <sup>c</sup>				Total dry weight <sup>d</sup>
	Pre-emergence	Post-emergence	Total	Pre-emergence	Post-emergence	Total	
Compost tea	80.0 b	11.1 e	16.6 e	27.7 d	4.09 c		
Experimental #1	95.6 a	0.0 f	3.5 fg	3.5 f	5.39 ab		
Experimental #2	95.6 a	0.6 f	6.4 f	7.0 e	6.06 a		
Kodiak	79.5 b	11.1 e	43.5 cd	54.6 c	2.61 d		
Micro 108	67.2 de	23.3 bc	41.3 d	64.6 bc	2.30 def		
Mycostop Mix	67.8 de	22.8 bc	59.4 ab	81.9 ab	1.87 f		
Natural II	93.9 a	1.7 f	0.6 g	2.3 f	4.49 bc		
Natural X	90.6 a	2.8 f	7.4 f	10.2 e	4.03 c		
PGPR Galaxy	71.1 bcde	19.5 bcd	51.8 abcd	71.2 abc	2.30 def		
Prestop	47.8 f	42.8 a	57.3 ab	95.4 a	0.85 g		
Soilgard	64.4 e	26.1 ab	41.4 d	67.5 bc	2.41 def		
Subtilex	75.0 bcd	15.6 cde	50.6 abcd	66.1 abc	2.56 de		
T-22 Planter Box	69.4 cde	21.1 bcd	54.7 abc	75.8 abc	1.99 def		
Yield Shield	78.3 bc	12.2 e	61.3 a	73.5 abc	1.95 ef		
Apron XL LS	94.4 a	0.0 f	2.4 fg	2.4 f	5.07 b		
Non-treated seed in inoculated medium	77.8 bc	12.8 de	48.5 bcd	61.3 bc	2.28 def		
Non-treated seed in non-inoculated medium	90.6 a	0.0 f	3.7 fg	3.7 f	4.55 bc		
LSD (Pr < 0.05) <sup>c</sup>	9.09	Rank	Square root	Log	Log		

<sup>a</sup> This table presents results of the first of two *P. ultimum* greenhouse trials evaluating seed and drench treatments on spinach for use in organic production. A randomized complete block design with 5 replications was used. Each experimental unit consisted of one 30.5 cm x 30.5 cm x 6.4 cm tall flat containing 1,200 g moistened organic potting mix (Sunshine Organic Growers Mix, Sun Gro Horticulture, Bellevue, WA) inoculated with the pathogen at a rate determined by inoculation rate trials (see main text). Six rows of six seed of the spinach hybrid ‘Lazio’ were planted in each flat. The number of emerged seedlings and the number of wilted seedlings was recorded at weekly intervals for 5 weeks. Results are shown for the final rating (35 days after planting).

<sup>b</sup> Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 2.1 for details of the treatments. Apron XL LS (mefenoxam) was included as a conventional fungicide seed treatment for control of *P. ultimum*. For both control treatments, the seed was not treated. For all treatments except the non-treated seed planted into

non-inoculated medium, seed was planted into potting mix that was inoculated with *P. ultimum* at a rate of 1,000 ppg (w/w) based on results from inoculation rate trials described in Chapter 2.

- <sup>c</sup> Pre-emergence damping-off was determined as a percentage of non-emerged seedlings in each flat compared to the non-inoculated control flats in each replication. Post-emergence damping-off was determined as the percentage of emerged seedlings in each flat that died or exhibited damping-off symptoms. Total damping-off was determined as pre- plus post- emergence damping-off.
- <sup>d</sup> Biomass was determined as above-ground dry weight of all the emerged seedlings present in each flat at the final rating. The seedlings were cut at the soil line, and the tissue was dried and weighed.
- <sup>e</sup> LSD = Fisher's protected least significant difference (Steele and Torrie, 1980). Means followed by the same letter within a column are not significantly different. 'Log', 'square root', or 'rank' indicate the original mean values are presented, but means separation by LSD was based on transformation (logarithmic or square root transformation) or Friedman's non-parametric rank test of the data because of heterogeneous variances and/or non-normal distribution of residuals (Steele and Torrie, 1980).

**Table 2.4.** Evaluation of seed and drench treatments for control of *Pythium ultimum* as a damping-off pathogen of spinach: Second greenhouse trial<sup>a</sup>

Treatment <sup>b</sup>	% Emergence		% Damping-off <sup>c</sup>			Total dry weight <sup>d</sup>	
	Pre-emergence	Post-emergence	Total				
Compost tea	62.8 de	28.9 ab	47.2 abcde	71.7 abc	1.24	g	
Experimental #1	92.2 a	0.6 e	15.8 gh	16.3 d	4.37	ab	
Experimental #2	95.6 a	0.0 e	29.5 fgh	29.5 d	3.70	bc	
Kodiak	7222 bc	19.4 cd	31.1 defg	50.6 bc	3.09	cd	
Micro 108	73.9 b	17.8 d	34.4 bcdefg	52.2 bc	2.65	cde	
Mycostop Mix	73.3 b	18.3 d	36.7 bcdef	52.2 c	2.80	cd	
Natural II	54.5 e	37.2 a	64.1 a	91.0 a	1.24	g	
Natural X	68.3 bc	23.3 cd	32.2 efg	55.5 bc	2.70	cde	
PGPR Galaxy	71.1 bc	20.6 cd	30.1 efg	50.6 bc	2.78	cd	
Prestop	55.6 de	36.1 ab	54.1 ab	85.6 a	1.34	fg	
Soilgard	48.3 e	43.3 a	51.6 abcd	89.4 a	1.40	fg	
Subtilex	85.6 a	6.7 e	11.2 hi	17.8 d	3.06	cd	
T-22 Planter Box	72.8 b	18.9 d	57.8 a	76.7 abc	1.76	efg	
Yield Shield	66.1 cd	25.6 bc	53.2 abc	78.7 ab	2.16	def	
Apron XL LS	91.1 a	1.7 e	3.7 ij	5.4 e	4.61	ab	
Non-treated seed in inoculated medium	72.2 bc	19.4 cd	33.0 cdefg	52.5 bc	2.82	cd	
Non-treated seed in non-inoculated medium	91.7 a	0.0 e	1.8 j	1.8 e	5.20	a	
LSD (Pr < 0.05) <sup>c</sup>	Rank	Rank	Arcsin	Log	Arcsin		

<sup>a</sup> This table presents results of the second of two *P. ultimum* greenhouse trials evaluating seed and drench treatments on spinach for use in organic production. A randomized complete block design with 5 replications was used. Each experimental unit consisted of one 30.5 cm x 30.5 cm x 6.4 cm tall flat containing 1,200 g moistened organic potting mix (Sunshine Organic Growers Mix, Sun Gro Horticulture, Bellevue, WA) inoculated with the pathogen at a rate determined by inoculation rate trials (see main text). Six rows of six seed of the spinach hybrid ‘Lazio’ were planted in each flat. The number of emerged seedlings and the number of wilted seedlings was recorded at weekly intervals for 4 weeks. Results are shown for the final rating (28 days after planting).

<sup>b</sup> Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 2.1 for details of the treatments. Apron XL LS (mefenoxam) was included as a conventional fungicide seed treatment for control of *P. ultimum*. For both control treatments, the seed was not treated. For all treatments except the non-treated seed planted into

non-inoculated medium, seed was planted into potting mix that was inoculated with *P. ultimum* at a rate of 1,000 ppg (w/w) based on results from inoculation rate trials described in Chapter 2.

- <sup>c</sup> Pre-emergence damping-off was determined as a percentage of non-emerged seedlings in each flat compared to the non-inoculated control flats in each replication. Post-emergence damping-off was determined as the percentage of emerged seedlings in each flat that died or exhibited damping-off symptoms. Total damping-off was determined as pre- plus post- emergence damping-off.
- <sup>d</sup> Biomass was determined as above-ground dry weight of all the emerged seedlings present in each flat at the final rating. The seedlings were cut at the soil line, and the tissue was dried and weighed.
- <sup>e</sup> LSD = Fisher's protected least significant difference (Steele and Torrie, 1980). Means followed by the same letter within a column are not significantly different. 'Log', 'arcsin', or 'rank' indicate the original mean values are presented, but means separation by LSD was based on transformation (logarithmic or square root transformation) or Friedman's non-parametric rank test of the data because of heterogeneous variances and/or non-normal distribution of residuals (Steele and Torrie, 1980).

**Table 2.5.** Evaluation of seed and drench treatments for control of *Rhizoctonia solani* as a damping-off pathogen of spinach: First greenhouse trial<sup>a</sup>

Treatment <sup>b</sup>	% Emergence		% Damping-off <sup>c</sup>				Total dry weight <sup>d</sup>			
			Pre-emergence	Post-emergence		Total				
Compost tea	44.4	cd	46.5	fg	15.7	abcde	62.3	cd	1.78	bcdef
Experimental #1	63.9	ab	27.1	hi	11.7	bcdef	38.8	de	3.89	a
Experimental #2	41.0	cd	50.0	fg	16.5	abcd	66.5	cd	1.75	bcd
Kodiak	22.9	fgh	68.1	bcd	17.1	abcd	84.3	ab	0.97	fghij
Micro 108	27.1	fgh	63.9	bcd	11.8	bcdef	75.6	bc	0.81	ghij
Mycostop Mix	20.1	ghi	70.8	abc	22.9	abc	90.3	ab	0.60	ijk
Natural II	65.6	abc	27.2	ghi	8.5	cdef	35.7	de	2.17	bcde
Natural X	41.7	bcd	48.2	fgh	13.4	bcdef	61.6	cd	2.03	abc
PGPR Galaxy	16.0	hi	75.0	ab	11.3	cdef	86.1	ab	0.53	jk
Prestop	34.7	de	56.3	ef	7.1	def	63.4	cd	1.31	efgh
Soilgard	32.6	def	58.3	def	22.2	abc	80.4	ab	1.04	fghi
Subtilex	38.9	de	52.1	ef	26.2	ab	77.3	abc	1.19	defgh
T-22 Planter Box	27.8	efg	63.2	cde	28.9	a	87.9	ab	1.47	cdefg
Yield Shield	0.0	i	91.0	a	0.0	f	91.0	a	0.00	k
Terraclor	81.3	a	10.4	i	1.7	ef	12.1	e	3.54	a
Non-treated seed in inoculated medium	23.6	fgh	67.4	bcd	7.2	def	74.6	bc	0.76	hijk
Non-treated seed in non-inoculated medium	91.0	a	0.0	i	1.5	ef	1.5	e	2.57	ab
LSD (Pr < 0.05) <sup>e</sup>	Rank		Rank		14.54		Rank		Rank	

<sup>a</sup> This table presents results of the first of two *R. solani* greenhouse trials evaluating seed and drench treatments on spinach for use in organic production. A randomized complete block design with 4 replications was used. Each experimental unit consisted of one 30.5 cm x 30.5 cm x 6.4 cm tall flat containing 1,200 g moistened organic potting mix (Sunshine Organic Growers Mix, Sun Gro Horticulture, Bellevue, WA) inoculated with the pathogen at a rate determined by inoculation rate trials (see main text). Six rows of six seed of the spinach hybrid ‘Lazio’ were planted in each flat. The number of emerged seedlings and the number of wilted seedlings was recorded at weekly intervals for 4 weeks. Results are shown for the final rating (28 days after planting).

<sup>b</sup> Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 2.1 for details of the treatments. Terraclor 75% WP (PCNB) was included as a conventional fungicide drench treatment for control of *R. solani*. For both control treatments, the seed was not treated. For all treatments except the non-treated seed planted into non-inoculated medium, seed was planted into potting mix that was inoculated with *R. solani* at a rate of 50,000 ppg (w/w) based on results from inoculation rate trials described in Chapter 2.

- <sup>c</sup> Pre-emergence damping-off was determined as a percentage of non-emerged seedlings in each flat compared to the non-inoculated control flats in each replication. Post-emergence damping-off was determined as the percentage of emerged seedlings in each flat that died or exhibited damping-off symptoms. Total damping-off was determined as pre- plus post- emergence damping-off.
- <sup>d</sup> Biomass was determined as above-ground dry weight of all the emerged seedlings present in each flat at the final rating. The seedlings were cut at the soil line, and the tissue was dried and weighed.
- <sup>e</sup> LSD = Fisher's protected least significant difference (Steele and Torrie, 1980). Means followed by the same letter within a column are not significantly different. 'Rank' indicates the original mean values are presented, but means separation by LSD was based on Friedman's non-parametric rank test of the data because of heterogeneous variances and/or non-normal distribution of residuals (Steele and Torrie, 1980).

**Table 2.6.** Evaluation of seed and drench treatments for control of *Rhizoctonia solani* as a damping-off pathogen of spinach: Second greenhouse trial<sup>a</sup>

Treatment <sup>b</sup>	% Emergence	% Damping-off <sup>c</sup>			Total	Total dry weight <sup>d</sup>
		Pre-emergence	Post-emergence	Total		
Compost tea	65.0 f	15.0 a	4.7 a	19.7 a	1.92 i	
Experimental #1	79.4 abc	5.0 bcde	3.4 a	8.4 a	4.74 ab	
Experimental #2	78.3 abcd	3.9 cde	7.6 a	11.5 a	4.78 ab	
Kodiak	80.0 abc	5.0 cde	4.9 a	9.9 a	4.08 bcde	
Micro 108	78.9 abc	5.6 abcde	7.4 a	12.9 a	3.92 cde	
Mycostop Mix	85.6 a	0.6 e	4.0 a	4.5 a	4.18 abcd	
Natural II	81.1 abc	3.3 cde	3.4 a	6.7 a	4.42 abc	
Natural X	73.9 bcdef	5.0 abcde	6.5 a	11.5 a	3.52 def	
PGPR Galaxy	75.6 abcde	9.5 abc	7.0 a	16.5 a	4.24 abcd	
Prestop	66.1 ef	12.8 ab	6.2 a	19.0 a	2.47 hi	
Soilgard	68.3 def	10.0 abc	7.4 a	17.4 a	3.10 fgh	
Subtilex	75.0 bcdef	6.1 bcde	8.4 a	14.5 a	3.77 cdef	
T-22 Planter Box	72.2 cdef	8.9 abcd	3.4 a	12.3 a	4.27 abcd	
Yield Shield	80.6 abc	2.8 bcde	11.5 a	14.3 a	3.85 cdef	
Terraclor	81.1 abc	2.3 bcde	0.7 a	3.5 a	2.70 ghi	
Non-treated in inoculated medium	82.8 ab	1.7 de	4.7 a	6.4 a	4.93 a	
Non-treated in non-inoculated medium	78.3 abcd	0.0 e	1.6 a	1.6 a	3.28 efg	
LSD (Pr < 0.05) <sup>c</sup>	10.36	Rank	NS	NS	0.806	

<sup>a</sup> This table presents results of the second of two *R. solani* greenhouse trials evaluating seed and drench treatments on spinach for use in organic production. A randomized complete block design with 5 replications was used. Each experimental unit consisted of one 30.5 cm x 30.5 cm x 6.4 cm tall flat containing 1,200 g moistened organic potting mix (Sunshine Organic Growers Mix, Sun Gro Horticulture, Bellevue, WA) inoculated with the pathogen at a rate determined by inoculation rate trials (see main text). Six rows of six seed of the spinach hybrid ‘Lazio’ were planted in each flat. The number of emerged seedlings and the number of wilted seedlings was recorded at weekly intervals for 4 weeks. Results are shown for the final rating (28 days after planting).

<sup>b</sup> Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 2.1 for details of the treatments. Terraclor 75% WP (PCNB) was included as a conventional fungicide drench treatment for control of *R. solani*. For both control treatments, the seed was not treated. For all treatments except the non-treated seed planted into non-inoculated medium, seed was planted into potting mix that was inoculated with *R. solani* at a rate of 25,000 ppg (w/w) based on results from inoculation rate trials described in Chapter 2.

- <sup>c</sup> Pre-emergence damping-off was determined as a percentage of non-emerged seedlings in each flat compared to the non-inoculated control flats in each replication. Post-emergence damping-off was determined as the percentage of emerged seedlings in each flat that died or exhibited damping-off symptoms. Total damping-off was determined as pre- plus post- emergence damping-off.
- <sup>d</sup> Biomass was determined as above-ground dry weight of all the emerged seedlings present in each flat at the final rating. The seedlings were cut at the soil line, and the tissue was dried and weighed.
- <sup>e</sup> LSD = Fisher's protected least significant difference (Steele and Torrie, 1980). Means followed by the same letter within a column are not significantly different. 'Rank' indicates the original mean values are presented, but means separation by LSD was based on Friedman's non-parametric rank test of the data because of heterogeneous variances (Steele and Torrie, 1980).

**Table 2.7.** Evaluation of seed and drench treatments for control of *Fusarium oxysporum* f. sp. *spinaciae* as a wilt pathogen of spinach: First greenhouse trial<sup>a</sup>

Treatment <sup>b</sup>	% Emergence		% Damping-off or wilt <sup>c</sup>			Total dry weight <sup>d</sup>	
	Pre-emergence	Post-emergence	Total				
Compost tea	90.6 abcde	5.0 bcdefg	28.4 gh	33.4 g	6.64 a		
Experimental #1	93.3 abcd	2.2 defg	56.5 abc	58.7 bcd	4.03	efg	
Experimental #2	93.8 abc	1.1 fg	54.0 abcd	55.4 cde	3.50	fgh	
Kodiak	90.6 abcde	3.3 cdefg	60.7 ab	64.0 abc	3.21	ghi	
Micro 108	83.3 ef	10.6 abcd	44.5 cdef	55.1 cde	3.00	hij	
Mycostop Mix	92.8 abcd	2.8 cdefg	53.9 abcd	56.7 cd	3.32	gh	
Natural II	88.3 bcde	5.6 abcdef	33.7 efgh	39.3 efg	4.23	def	
Natural X	96.7 a	1.7 fg	43.3 cdefg	44.9 defg	4.03	efg	
PGPR Galaxy	90.6 abcde	6.7 bcdef	55.8 abc	62.5 abc	3.23	ghi	
Prestop	85.6 def	8.3 abcde	25.5 h	33.8 g	4.65	cde	
Soilgard	84.4 ef	12.2 ab	66.5 a	75.0 ab	2.40	ij	
Subtilex	91.1 abcde	3.9 cdefg	33.2 fgh	37.1 fg	5.06	bcd	
T-22 Planter Box	80.0 f	13.9 a	66.1 a	77.7 a	2.14	j	
Yield Shield	86.7 cdef	8.9 abc	22.3 hi	31.2 g	5.81	ab	
Mertect 340F	92.8 abcd	3.3 cdefg	48.3 bcde	51.6 cdef	3.54	fgh	
Non-treated seed in inoculated medium	95.6 ab	2.2 efg	40.7 defg	42.9 defg	3.48	fgh	
Non-treated seed in non-inoculated medium	93.9 abc	0.0 g	10.2 i	10.2 h	5.17	bc	
LSD (Pr < 0.05) <sup>e</sup>	7.95	Log	14.97	16.36	0.908		

<sup>a</sup> This table presents results of the first of two *F. oxysporum* f. sp. *spinaciae* greenhouse trials evaluating seed and drench treatments on spinach for use in organic production. A randomized complete block design with 5 replications was used. Each experimental unit consisted of one 30.5 cm x 30.5 cm x 6.4 cm tall flat containing 1,200 g moistened organic potting mix (Sunshine Organic Growers Mix, Sun Gro Horticulture, Bellevue, WA) inoculated with the pathogen at a rate determined by inoculation rate trials (see main text). Six rows of six seed of the spinach hybrid ‘Lazio’ were planted in each flat. The number of emerged seedlings and the number of wilted seedlings was recorded at weekly intervals for 6 weeks. Results are shown for the final rating (42 days after planting).

<sup>b</sup> Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 2.1 for details of the treatments. Mertect 340F (thiabendazole) was included as a conventional fungicide seed treatment for control of *F. oxysporum* f. sp. *spinaciae*. For both control treatments, the seed was not treated. For all treatments except the non-treated seed planted into non-inoculated medium, seed was planted into potting mix that was inoculated with

*F. oxysporum* f. sp. *spinaciae* at a rate of 10,000 ppg (w/w) based on results from inoculation rate trials described in Chapter 2.

- <sup>c</sup> Pre-emergence damping-off was determined as a percentage of non-emerged seedlings in each flat compared to the non-inoculated control flats in each replication. Post-emergence damping-off was determined as the percentage of emerged seedlings in each flat that died or exhibited damping-off symptoms. Total damping-off was determined as pre- plus post- emergence damping-off.
- <sup>d</sup> Biomass was determined as above-ground dry weight of all the emerged seedlings present in each flat at the final rating. The seedlings were cut at the soil line, and the tissue was dried and weighed.
- <sup>e</sup> LSD = Fisher's protected least significant difference (Steele and Torrie, 1980). Means followed by the same letter within a column are not significantly different. 'Log' indicates the original mean values are presented, but means separation by LSD was based on transformation (logarithmic) because of heterogeneous variances (Steele and Torrie, 1980).

**Table 2.8.** Evaluation of seed and drench treatments for control of *Fusarium oxysporum* f. sp. *spinaciae* as a wilt pathogen of spinach: Second greenhouse trial<sup>a</sup>

Treatment <sup>b</sup>	% Damping-off or wilt <sup>c</sup>				Total dry weight <sup>d</sup>
	% Emergence	Pre-emergence	Post-emergence	Total	
Compost tea	74.4 abc	7.8 cde	11.7 de	19.5 d	12.95 bcd
Experimental #1	73.9 abc	8.3 abc	19.1 cd	27.5 abcd	13.71 ab
Experimental #2	67.8 cde	13.3 bc	20.3 bcd	33.7 abc	12.37 cde
Kodiak	76.1 abc	8.9 abc	26.5 abc	35.4 abc	11.60 ef
Micro 108	57.8 e	20.6 ab	26.6 abc	47.2 a	10.51 f
Mycostop Mix	73.3 bcd	6.7 cd	24.3 abc	31.0 abc	12.63 de
Natural II	83.9 ab	4.4 cde	25.6 abc	30.1 abc	12.40 de
Natural X	87.2 a	1.7 de	21.3 bcd	23.0 cd	12.85 cde
PGPR Galaxy	80.6 abc	5.6 cde	34.4 a	39.9 abc	12.44 de
Prestop	60.0 de	20.0 a	11.6 de	31.6 abc	11.74 ef
Soilgard	73.9 abc	9.4 cd	31.8 ab	41.3 abc	11.82 de
Subtilex	72.2 bcd	11.1 cde	29.5 abc	40.6 ab	12.71 cde
T-22 Planter Box	83.9 ab	1.7 cde	26.0 abc	27.7 abc	13.27 bcd
Yield Shield	77.2 abc	6.7 cde	23.0 abcd	29.7 abcd	11.78 ef
Mertect 340F	85.0 ab	3.3 cde	21.9 bcd	25.3 bcd	13.44 abc
Non-treated seed in inoculated medium	79.4 abc	3.9 cde	26.1 abc	29.9 abc	12.85 cde
Non-treated seed in non-inoculated medium	77.8 abc	0.0 e	0.7 e	0.7 e	14.90 a
LSD (Pr < 0.05) <sup>c</sup>	13.44	Rank	12.19	Log	Rank

<sup>a</sup> This table presents results of the second of two *F. oxysporum* f. sp. *spinaciae* greenhouse trials evaluating seed and drench treatments on spinach for use in organic production. A randomized complete block design with 5 replications was used. Each experimental unit consisted of one 30.5 cm x 30.5 cm x 6.4 cm tall flat containing 1,200 g moistened organic potting mix (Sunshine Organic Growers Mix, Sun Gro Horticulture, Bellevue, WA) inoculated with the pathogen at a rate determined by inoculation rate trials (see main text). Six rows of six seed of the spinach hybrid ‘Lazio’ were planted in each flat. The number of emerged seedlings and the number of wilted seedlings was recorded at weekly intervals for 7 weeks. Results are shown for the final rating (49 days after planting).

<sup>b</sup> Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Refer to Table 2.1 for details of the treatments. Mertect 340F (thiabendazole) was included as a conventional fungicide seed treatment for control of *F. oxysporum* f. sp. *spinaciae*. For both control treatments, the seed was not treated. For all treatments except the

non-treated, non-inoculated control, seed was planted into potting mix that was inoculated with *F. oxysporum* f. sp. *spinaciae* at a rate of 10,000 ppg (w/w) based on results from inoculation rate trials described in Chapter 2.

<sup>c</sup> Pre-emergence damping-off was determined as a percentage of non-emerged seedlings in each flat compared to the non-inoculated control flats in each replication. Post-emergence damping-off was determined as the percentage of seedlings emerged in each flat that died or exhibited damping-off symptoms. Total damping-off was determined as pre- plus post- emergence damping-off.

<sup>d</sup> Biomass was determined as above-ground dry weight of all the emerged seedlings present in each flat at the final rating. The seedlings were cut at the soil line, and the tissue was dried and weighed.

<sup>e</sup> LSD = Fisher's protected least significant difference (Steele and Torrie, 1980). Means followed by the same letter within a column are not significantly different. 'Rank' and 'Log' indicates the original mean values are presented, but means separation by LSD was based on transformation (logarithmic) or Friedman's non-parametric rank test of the data because of heterogeneous variances and/or non-normal distribution of residuals (Steele and Torrie, 1980).

**Table 2.9.** Results of a freeze-blotter seed health assay of spinach seed with seed treatments evaluated in greenhouse and field trials for control of damping-off or seedling blight of spinach<sup>a</sup>

Seed treatment <sup>b</sup>	% Seed infected or infested (14 days after plating)							
	Trial 1			Trial 2				
	<i>Stemphylium botryosum</i> spp.	<i>Verticillium</i> spp.	<i>Fusarium</i> spp.	<i>Alternaria</i> spp.	<i>Stemphylium botryosum</i> spp.	<i>Verticillium</i> spp.	<i>Fusarium</i> spp.	<i>Alternaria</i> spp.
Non-Treated	4.00 bcd	49.75 a	2.25 abcd	13.25 ab	5.50 c	1.25 bc	0.00 a	96.25 ab
Experimental #1	17.50 a	10.50 c	1.75 abcd	5.00 de	11.25 a	10.25 a	0.00 a	3.00 d
Experimental #2	3.50 bcde	0.75 e	0.25 cd	0.50 f	9.50 ab	8.50 ab	0.75 a	2.25 d
Kodiak	3.50 bcde	48.50 a	1.75 abc	10.25 ab	5.00 c	0.75 cd	0.00 a	98.00 ab
Micro 108	4.50 bc	48.75 a	4.75 a	14.00 a	4.25 c	1.00 cd	0.00 a	98.50 a
Mycostop Mix	0.50 f	13.00 c	3.50 ab	2.00 ef	0.50 d	0.75 cd	0.00 a	88.00 c
Natural II	0.50 f	4.75 d	1.25 abcd	0.25 f	6.25 bc	1.00 cd	0.00 a	97.75 ab
Natural X	1.75 def	20.25 b	0.75 bcd	1.00 f	3.75 cd	0.50 cd	0.00 a	97.25 ab
PGPR Galaxy	4.50 bc	45.75 a	2.25 ab	9.25 bc	4.25 c	0.75 cd	0.00 a	98.00 ab
Subtlex	2.00 cdef	45.25 a	2.75 ab	7.25 cd	3.25 cd	0.75 cd	0.25 a	97.50 ab
T-22 Planter Box	1.75 def	41.50 a	3.00 a	10.50 ab	5.25 c	0.00 d	0.50 a	96.00 b
Yield Shield	3.00 bcde	49.25 a	1.25 abcd	11.75 ab	3.00 cd	1.00 cd	0.00 a	98.50 ab
Apron XL LS	1.50 ef	40.75 a	2.00 ab	3.75 def	3.25 cd	0.50 cd	0.00 a	98.25 ab
Mertect 340F	6.00 b	0.75 e	0.00 d	11.5 ab	4.50 c	0.00 d	0.00 a	97.25 ab
LSD (Pr < 0.05) <sup>c</sup>	Log	Log	Rank	Rank	3.567	Rank	NS	Arcsin

<sup>a</sup> The freeze-blotter seed health assay was completed for 4 replications of 100 seed for each seed treatment, as described by du Toit et al. (2005). A different commercial seed lot of the spinach hybrid 'Lazio' was evaluated in each trial.

<sup>b</sup> Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Apron XL LS and Mertect 340F were included as conventional fungicide seed treatments for control of *Pythium* spp. and *Fusarium* spp., respectively. Each product was evaluated at the highest recommended label rate for spinach and/or a crop with similar sized seed, or according to registrant recommendations. Refer to Table 2.1 for further details on the seed treatments.

<sup>c</sup> LSD = Fisher's protected least significant difference. Means followed by the same letter within a column are not significantly different. 'Log', 'square root', 'Arcsin', and 'rank' indicate original mean % values are presented, but means separation by LSD is based on transformation of the data (logarithmic, square root, or arcsin square root transformation) or Friedman's non-parametric rank test because of heterogeneous variances and/or non-normal distribution of residuals. NS = not significantly different at  $P = 0.05$  (Steele and Torrie, 1980).

**Table 2.10.** Results from germination assays of spinach seed with seed treatments evaluated in greenhouse and field trials for control of damping-off and seedling blight of spinach<sup>a</sup>

Seed treatment <sup>b</sup>	% Germination (days)									
	Trial 1					Trial 2				
	7	14	21	5	7	14	21	5	7	21
Non-Treated	50.25 cd	88.75 a	90.25 a	16.00 cde	80.50 abc	88.50 bcde	88.50 cdef			
Experimental #1	70.00 a	92.00 a	92.50 a	38.00 a	78.00 cd	92.50 a	93.25 a			
Experimental #2	64.50 ab	89.50 a	91.00 a	29.25 b	76.25 cd	90.75 abc	92.75 a			
Kodiak	52.50 cd	89.50 a	90.50 a	19.00 cd	78.00 cd	85.25 ef	85.50 fg			
Micro 108	51.75 cd	88.25 a	88.75 a	18.50 cd	82.75 abc	88.75 abcde	88.75 bcdef			
Mycostop Mix	47.00 de	88.00 a	89.00 a	21.75 bc	85.25 ab	92.25 ab	92.50 ab			
Natural II	34.75 f	88.75 a	90.75 a	10.50 e	80.50 abc	91.25 abc	91.50 abcd			
Natural X	40.50 ef	89.00 a	90.25 a	16.75 cde	79.25 bcd	90.50 abc	90.50 abcde			
PGPR Galaxy	53.75 cd	85.75 a	87.25 a	17.50 cde	78.50 cd	86.50 def	87.00 efg			
Subtilex	57.00 bc	90.00 a	91.00 a	17.25 cde	81.00 abc	88.00 cdef	88.50 cdef			
T-22 Planter Box	55.00 cd	89.25 a	90.25 a	12.25 de	86.50 a	92.25 ab	92.25 abc			
Yield Shield	49.50 cd	89.50 a	90.75 a	17.25 cde	81.50 abc	90.00 abcd	90.00 abcde			
Apron XL LS	55.75 c	90.25 a	91.25 a	15.25 cde	73.75 d	84.25 f	84.25 g			
Mertect 340F	42.50 cd	89.50 a	91.50 a	16.25 cde	82.25 abc	87.75 cdef	87.75 defg			
LSD (Pr < 0.05) <sup>c</sup>	8.311	NS	NS	7.521	6.737	3.940	3.771			

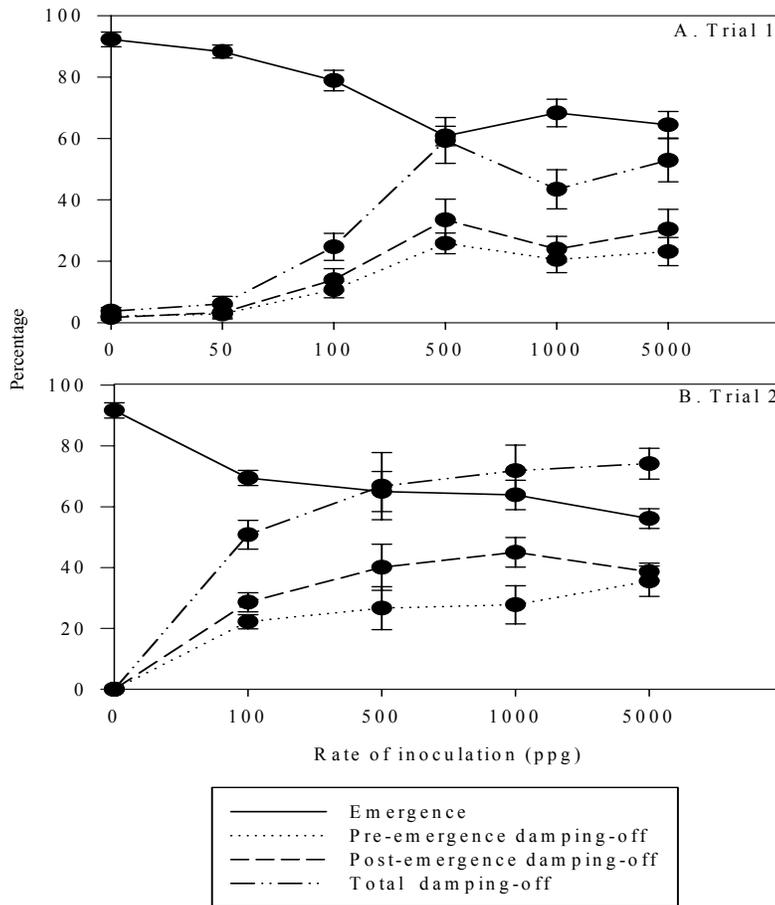
<sup>a</sup> The Association of Official Seed Analysts (AOSA) blotter germination assay was completed for 4 replications of 100 seed for each seed treatment. A different commercial seed lot of the spinach hybrid 'Lazio' was evaluated in each trial. The percentage abnormally-germinated seed, rotten seed, and non-germinated seed were also recorded at 21 days, but there were no significant differences among treatments based on analyses of variance for each of these variables (*data not shown*)

<sup>b</sup> Not all products were EPA registered or reviewed for compliance with the USDA National Organic Program (NOP) or the Organic Materials Review Institute (OMRI) in 2007. Apron XL LS and Mertect 340F were included as conventional fungicide seed treatments for control of *Pythium* spp. and *Fusarium* spp., respectively. Each product was evaluated at the highest recommended label rate for spinach and/or a crop with similar sized seed, or according to registrant recommendations. Refer to Table 2.1 for details of each treatment.

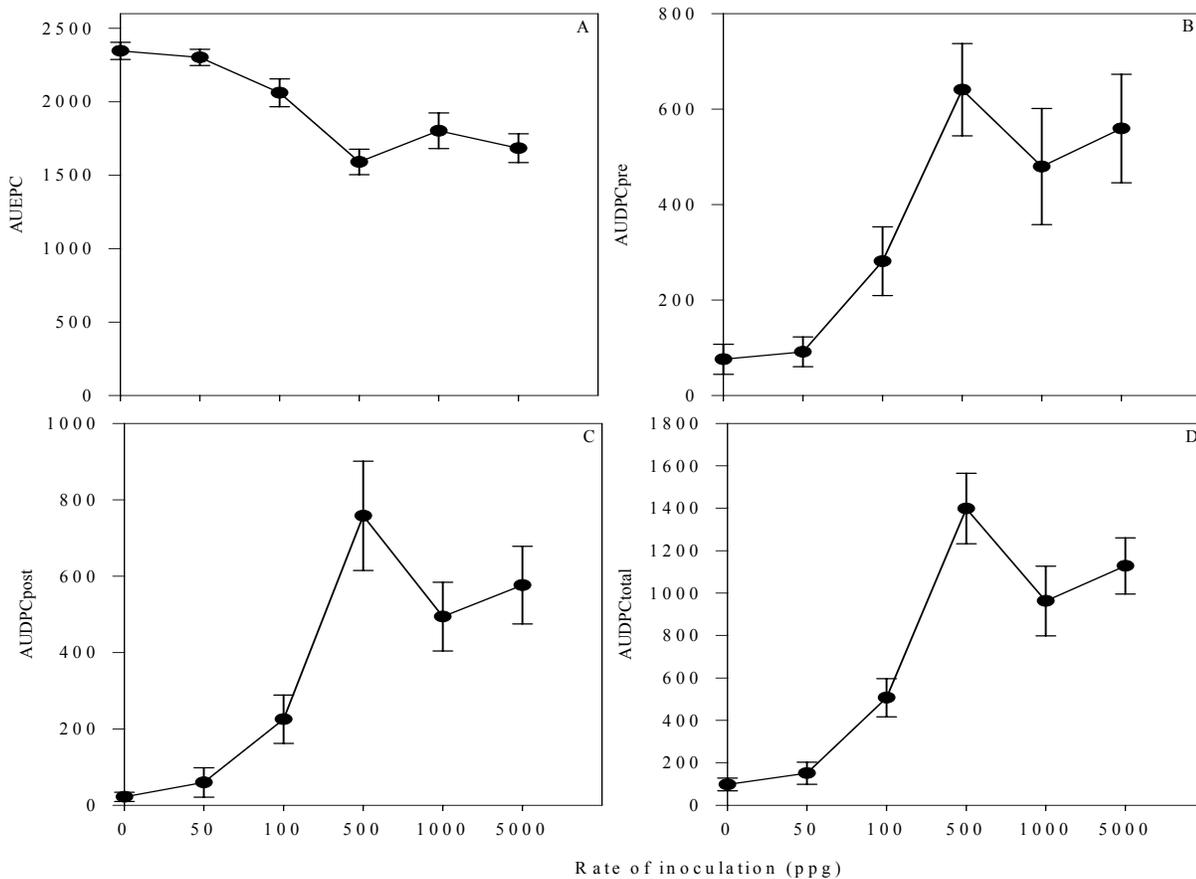
<sup>c</sup> LSD = Fisher's protected least significant difference. Means followed by the same letter within a column are not significantly different. 'Log', 'square root', 'Arcsin', and 'rank' indicate original mean % values are presented, but means separation by LSD is based on transformation of the data (logarithmic, square root, or arcsin square root transformation) or Friedman's non-parametric rank test because of heterogeneous variances and/or non-normal distribution of residuals. NS = not significantly different at  $P = 0.05$  (Steele and Torrie, 1980).



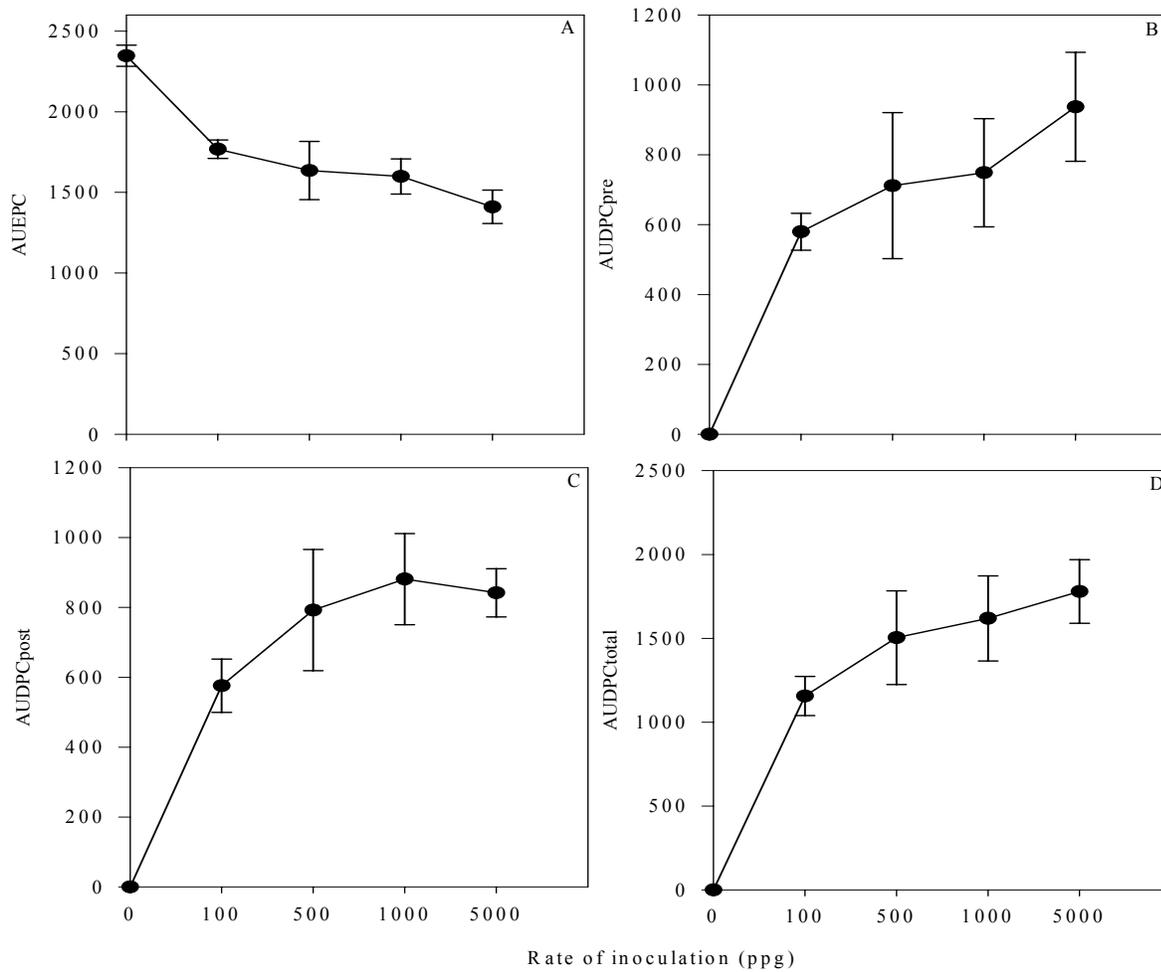
**Fig. 2.1.** Greenhouse pathogenicity test of *Rhizoctonia solani* AG4 HGII isolates ‘VSP 05-01A’ and ‘VSP 05-01B’ on spinach seedlings. The non-inoculated control seedling (left), and seedlings inoculated with rye seed colonized by isolate ‘VSP 05-01A’ (top right), and isolate ‘VSP 05-01B’ (bottom right) were three weeks old at the time of inoculation. The photo was taken 4 weeks after inoculation. Refer to the text for details on the method of inoculation.



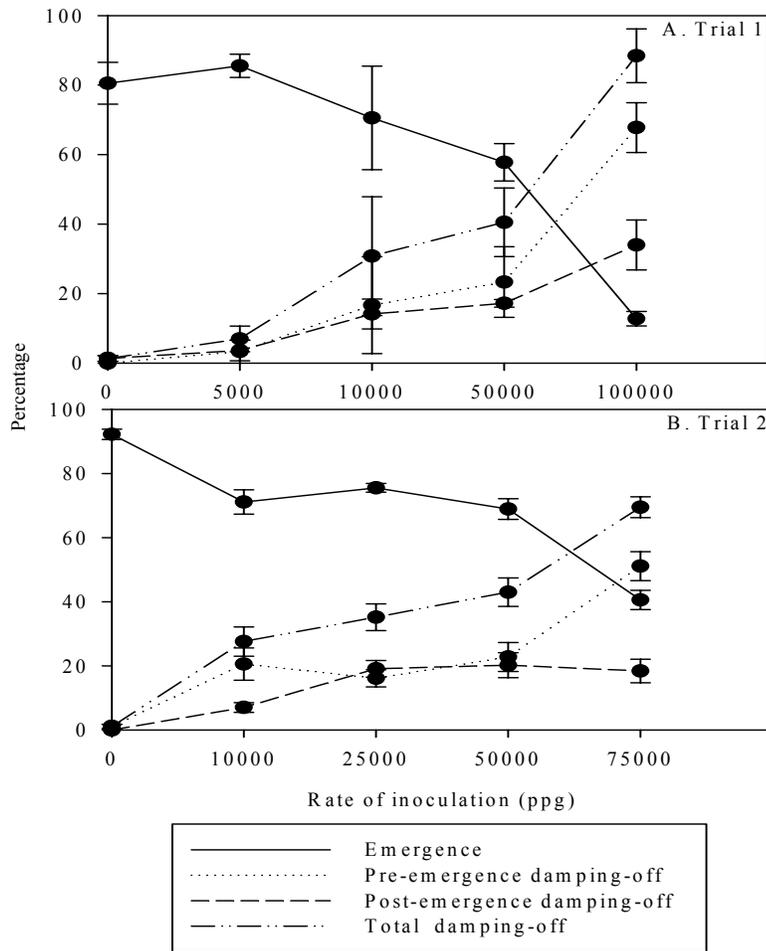
**Fig. 2.2.** Emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off (pre- + post-emergence) caused by *Pythium ultimum* in greenhouse trials evaluating the effect of inoculation rate on spinach seedlings. Each trial was set up as a randomized complete block design with five replications of four or five rates of inoculation (measured in propagules/g, ppg). Trial 1 (A) was conducted in June to July 2006, and trial 2 (B) was conducted in January to February 2007, each at  $25 \pm 5^\circ\text{C}$ . The durations of trials 1 and 2 were 32 and 35 days, respectively, with emergence and damping-off measured weekly. The 50 ppg rate was not included in trial 2 based on results of trial 1. Each data point shows the mean and standard error of five replications.



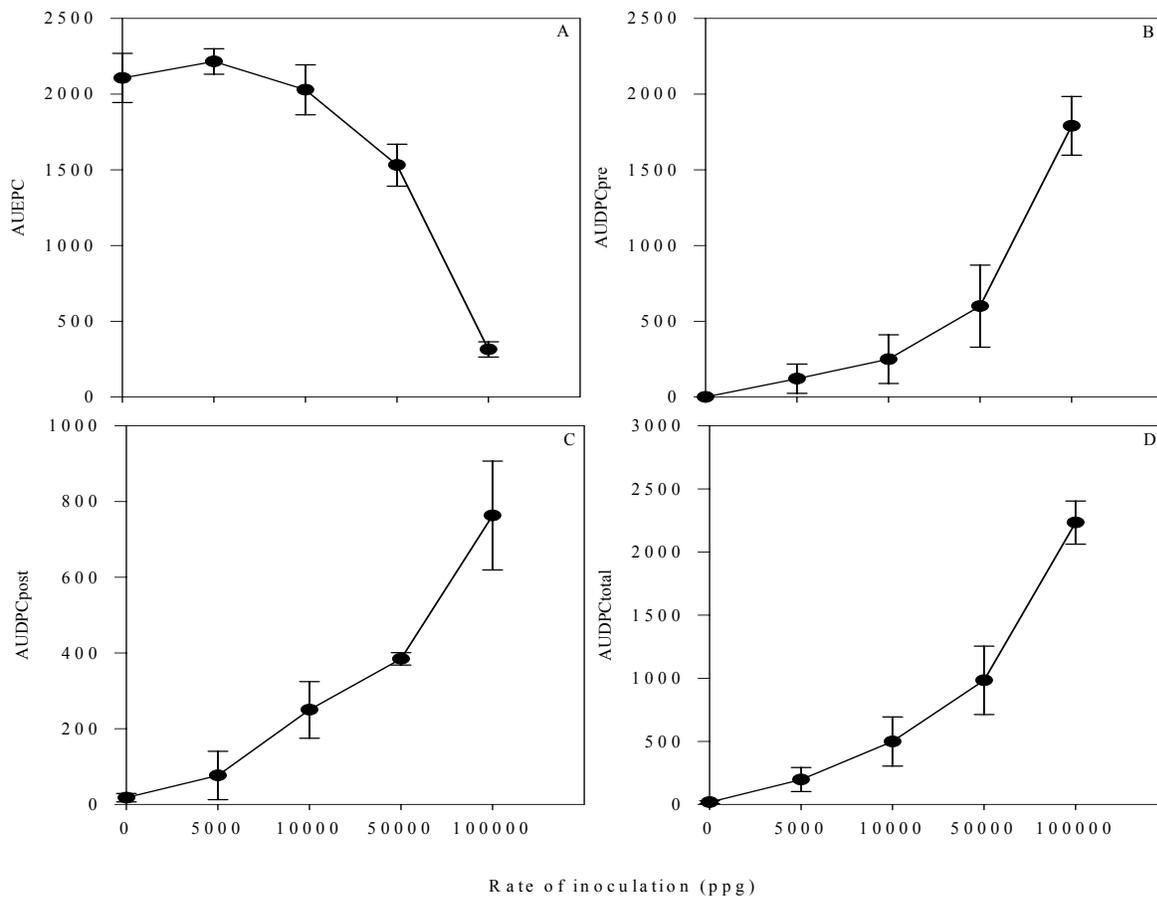
**Fig. 2.3.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence damping-off (AUDPC<sub>post</sub>), and D) total damping-off (AUDPC<sub>total</sub>) for trial 1 of *Pythium ultimum* inoculation rate trials on spinach carried out in a greenhouse at  $25 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of six rates of inoculation, and was conducted in June to July 2006. The duration of the trial was 32 days, with emergence and damping-off measured weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.



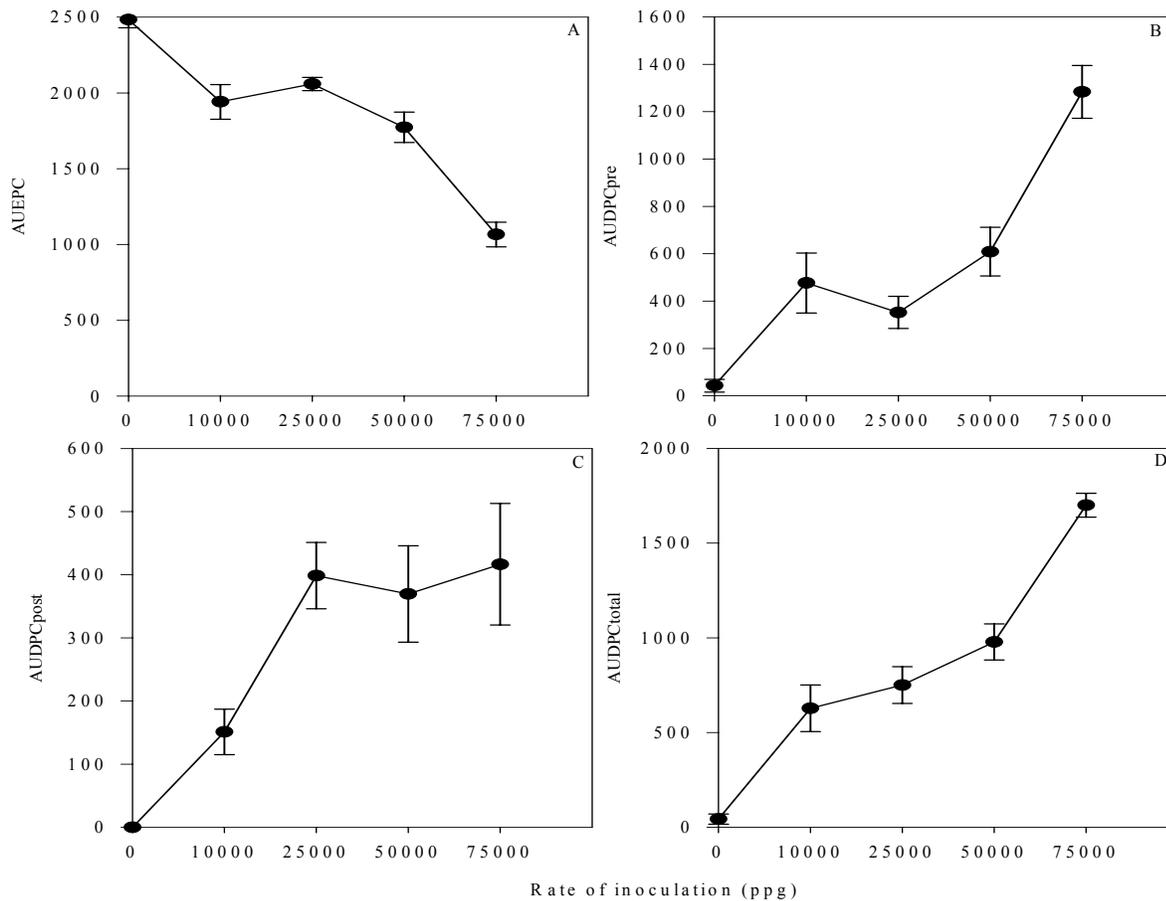
**Fig. 2.4.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence damping-off (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of *Pythium ultimum* inoculation rate trials carried out in a greenhouse at  $25 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation and was conducted in January to February 2007. The duration of the trial was 35 days, with emergence and damping-off measured weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.



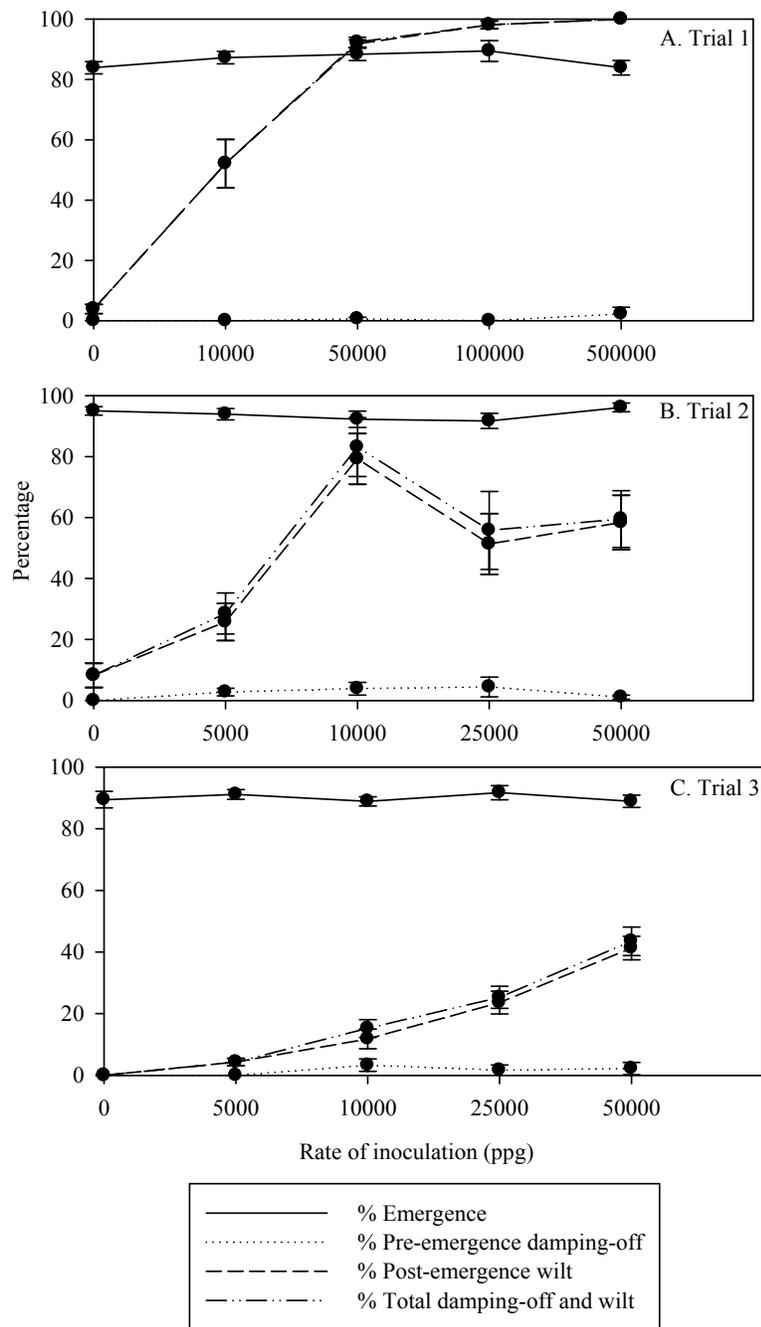
**Fig. 2.5.** Emergence, pre-emergence damping-off, post-emergence damping-off, and total damping-off (pre- + post-emergence) caused by *Rhizoctonia solani* in greenhouse trials evaluating the effect of inoculation rate on spinach seedlings. Each trial was set up as a randomized complete block design with five replications of five rates of inoculation (measured in propagules/g, ppg). Trial 1 was conducted July to August 2006, and trial 2 was conducted in February to March 2007, each at  $25 \pm 5^\circ\text{C}$ . The duration of trial 1 was 32 days, and trial 2 was 35 days, with emergence and damping-off measured weekly. The 5,000 and 100,000 ppg rates were not included in trial 2 based on results of trial 1. Each data point shows the mean and standard error of five replications.



**Fig. 2.6.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence damping-off (AUDPC<sub>post</sub>), and D) total damping-off (AUDPC<sub>total</sub>) for trial 1 of *Rhizoctonia solani* inoculation rate trials carried out in a greenhouse at  $25 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation and was conducted in July to August 2006. The duration of the trial was 32 days, with emergence and damping-off measured weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.

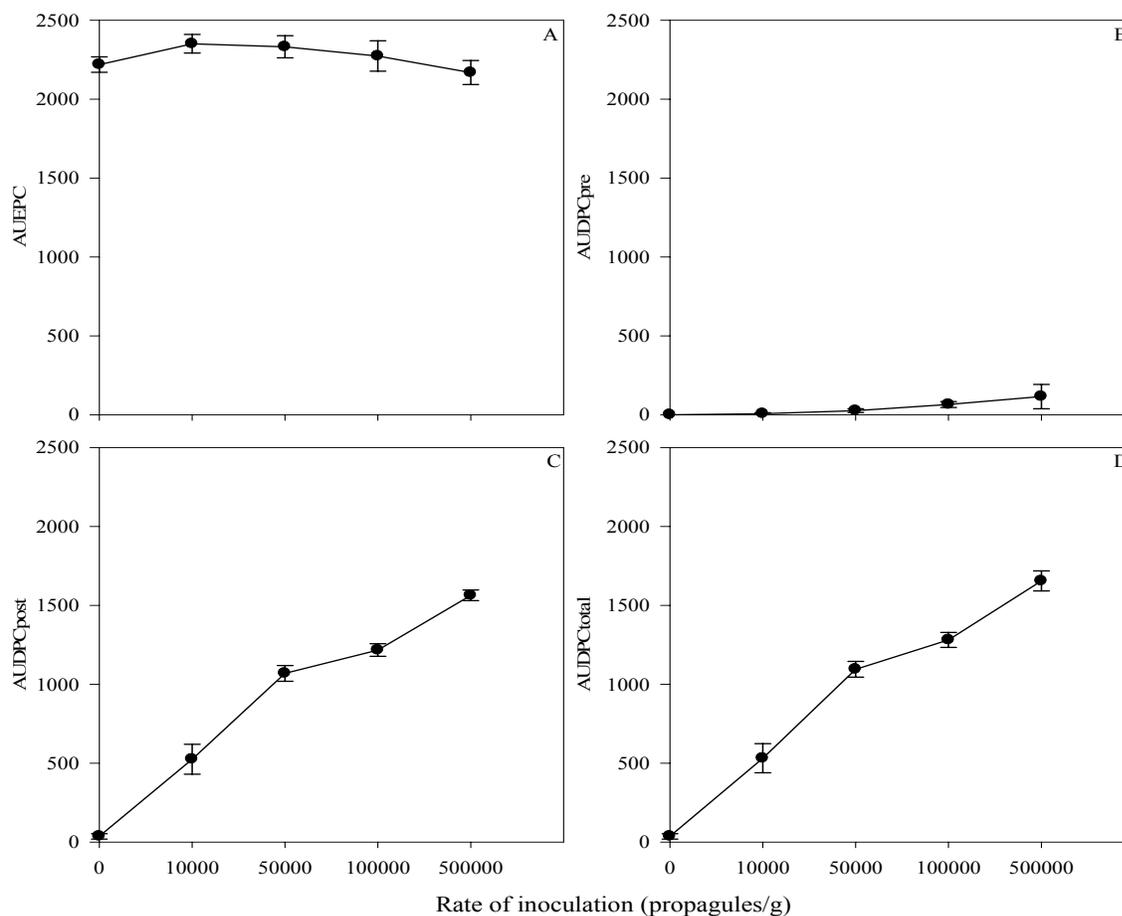


**Fig. 2.7.** Area under progress curves (AUEPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence damping-off (AUDPC<sub>post</sub>), and D) total damping-off (AUDPC<sub>total</sub>) for trial 2 of *Rhizoctonia solani* inoculation rate trials carried out in a greenhouse at  $25 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation and was conducted in February to March 2007. The duration of the trial was 35 days, with emergence and damping-off measured weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.

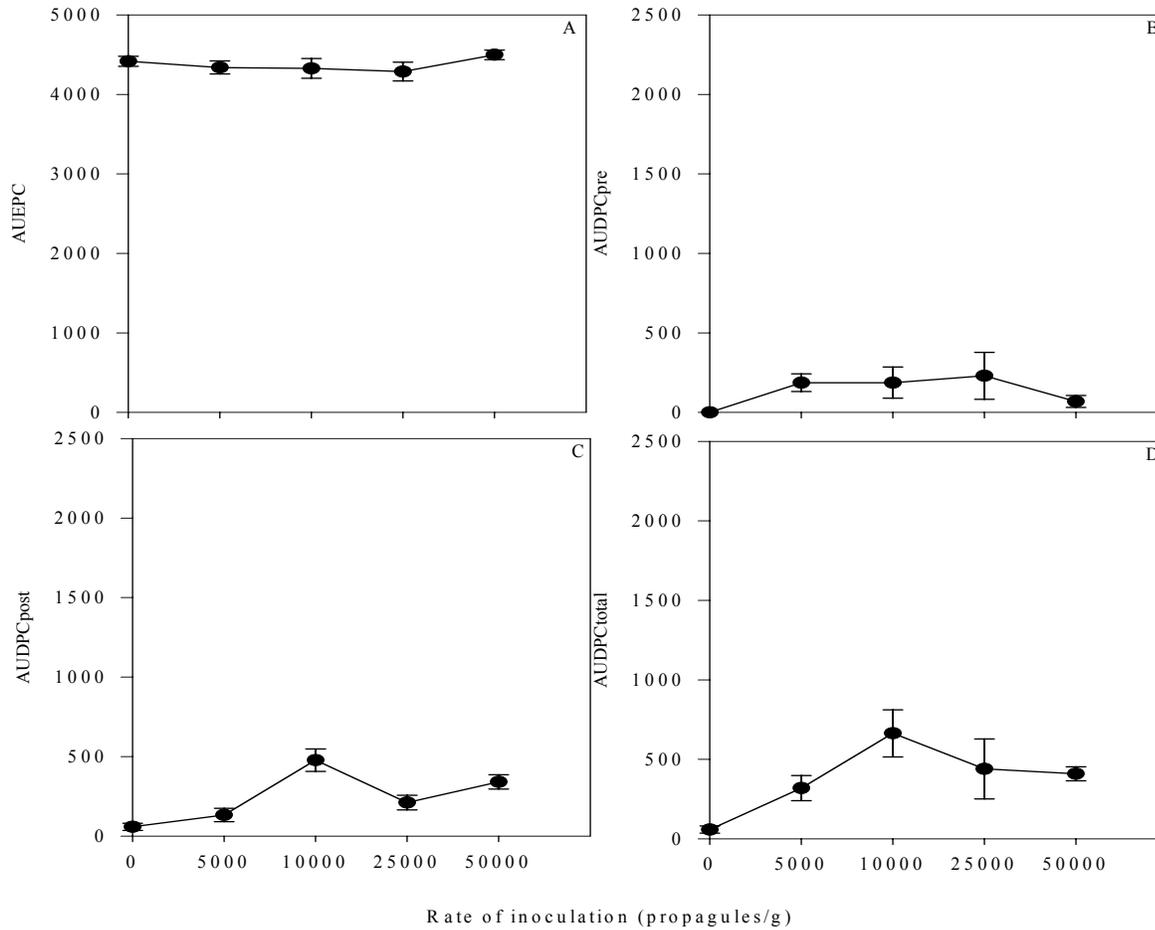


**Fig. 2.8.** Emergence, pre-emergence damping-off, post-emergence wilt, and total damping-off or wilt (pre- + post-emergence) caused by *Fusarium oxysporum* f. sp. *spinaciae* in greenhouse trials evaluating the effect of inoculation rate on spinach seedlings. Each trial was set up as a randomized complete block design with five replications of five rates of inoculation (measured

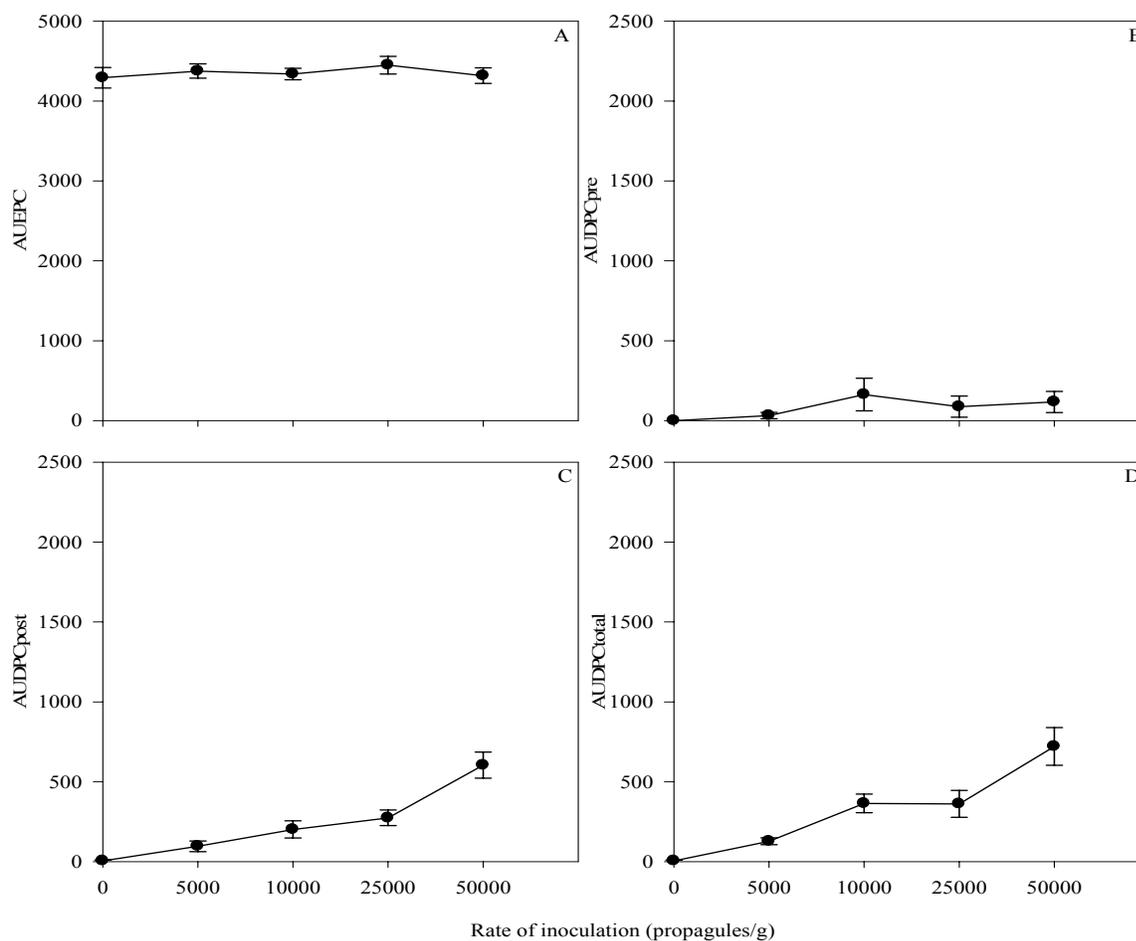
in propagules/g, ppg). Trial 1 (A) was conducted in July to August 2006, trial 2 (B) was conducted in February to March 2007, and trial 3 (C) was conducted July to September 2007, each at 25 or 28 ± 5°C. The duration of trials 1, 2 and 3 was 32, 56 and 56 days, respectively, with emergence and damping-off measured weekly. The 5,000 ppg and 100,000 ppg rates were not included in trials 2 or 3 based on results of trial 1. Each data point shows the mean and standard error of five replications.



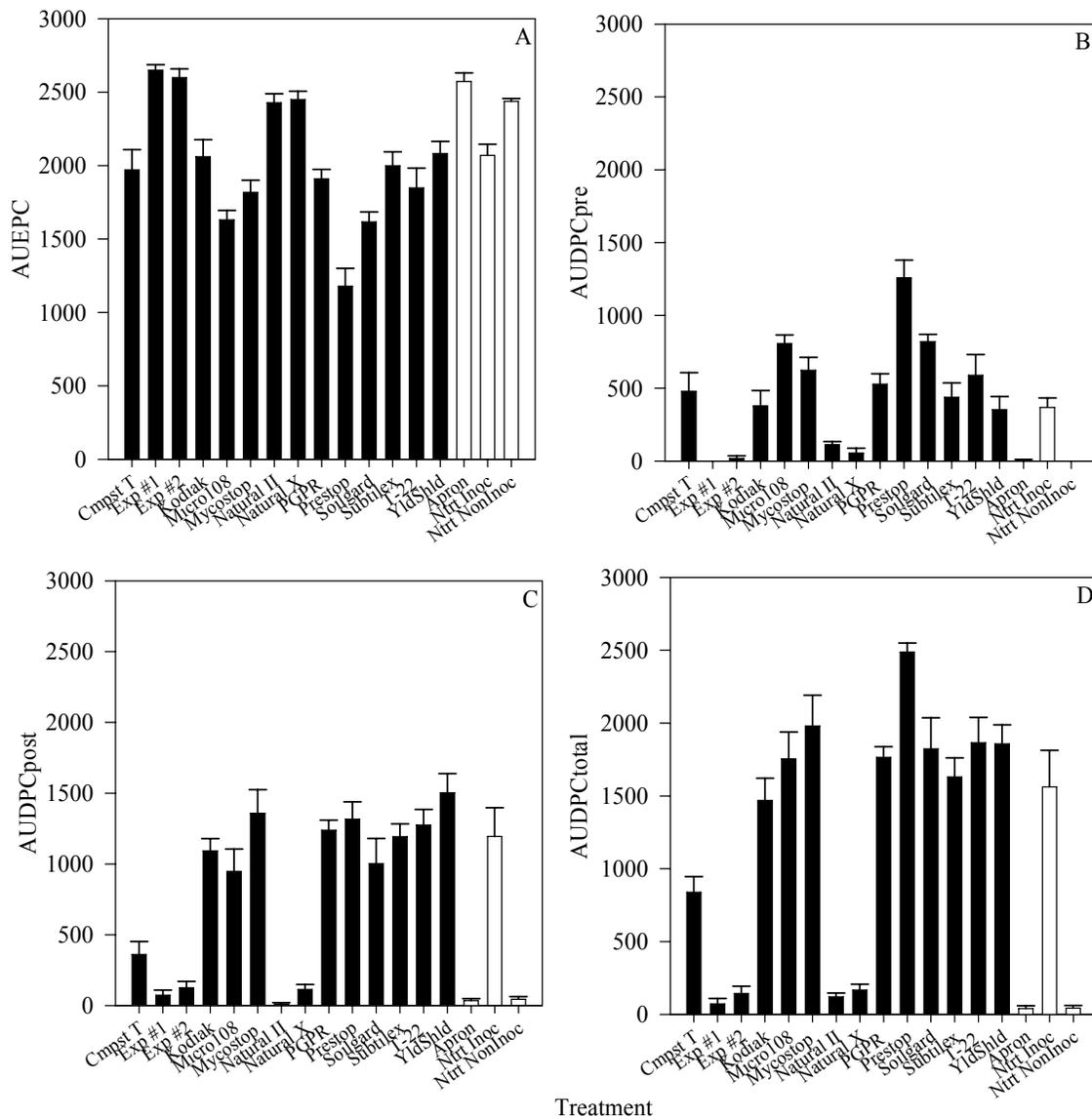
**Fig. 2.9.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 1 of *Fusarium oxysporum* f. sp. *spinaciae* inoculation rate trials carried out in a greenhouse at  $25 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation, and was conducted in July to August 2006. The duration of the trial was 32 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.



**Figure 2.10.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of *Fusarium oxysporum* f. sp. *spinaciae* inoculation rate trials carried out in a greenhouse at  $28 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation, and was conducted in February to March 2007. The duration of the trial was 56 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.

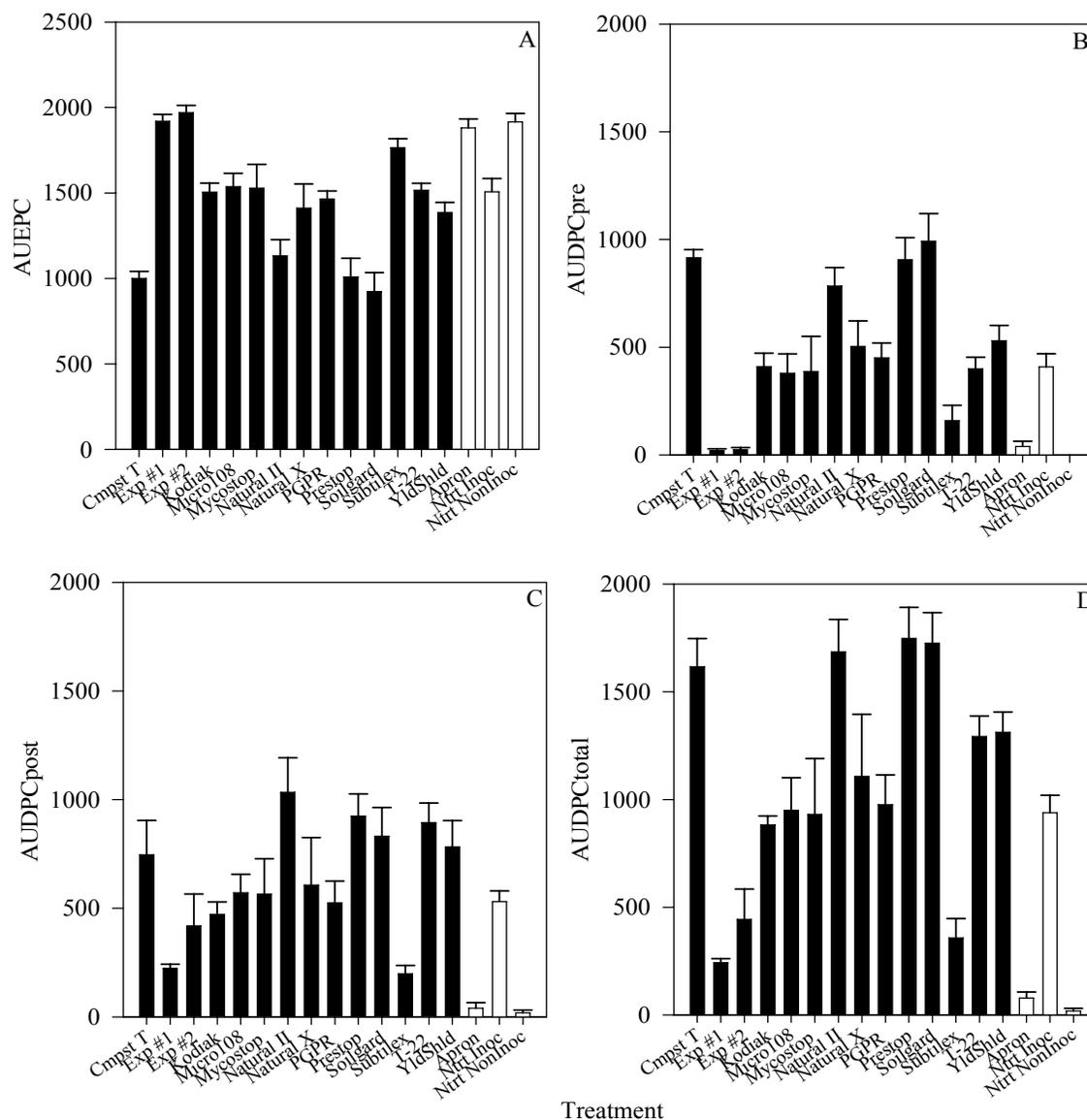


**Fig. 2.11.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 3 of *Fusarium oxysporum* f. sp. *spinaciae* inoculation rate trials carried out in a greenhouse at  $28 \pm 5^\circ\text{C}$ . Inoculation rate was measured in propagules/g (ppg). The trial was set up as a randomized complete block design with five replications of five rates of inoculation, and was conducted in July to September 2007. The duration of the trial was 56 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each data point shows the mean and standard error of five replications.



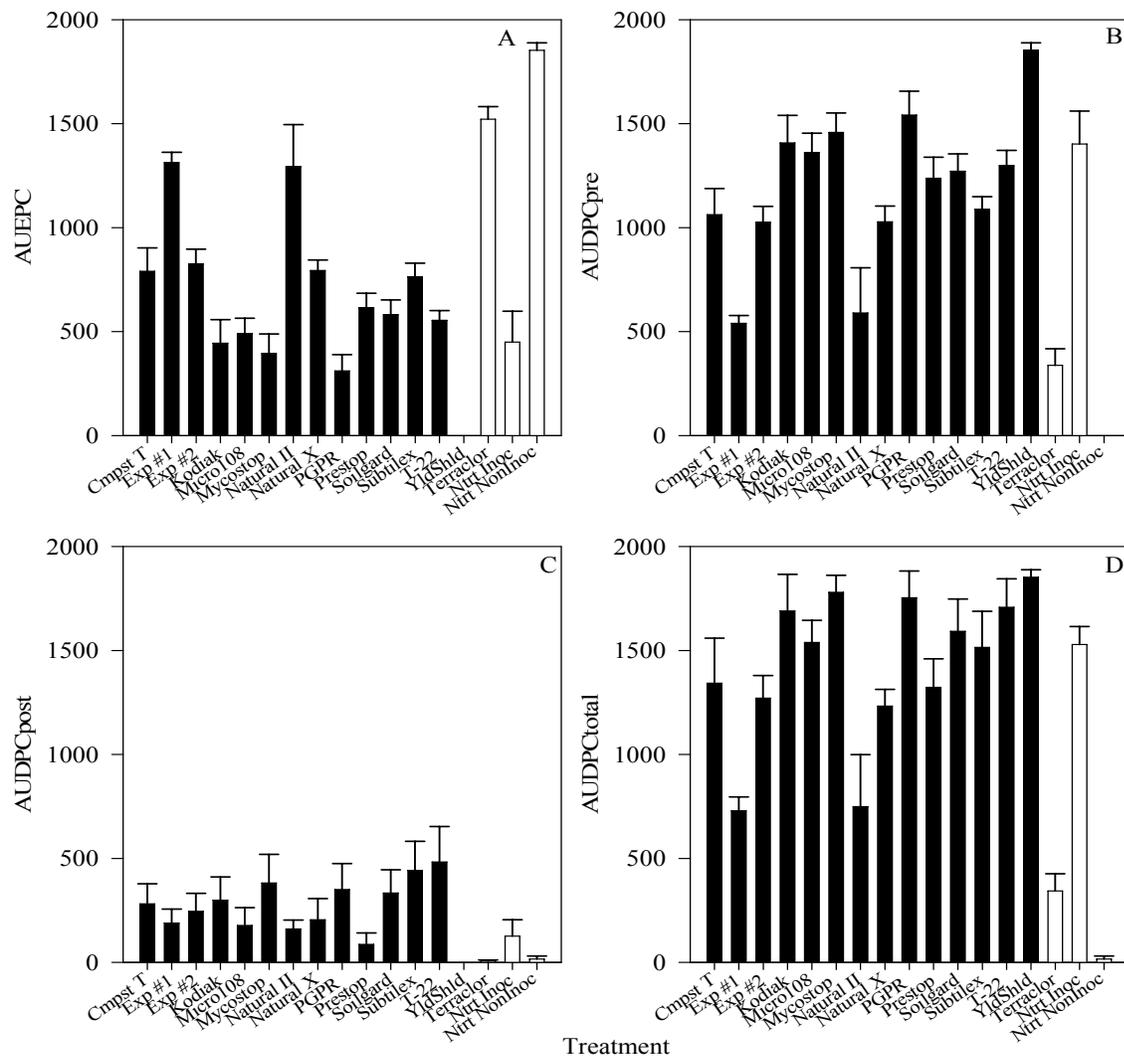
**Fig. 2.12.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 1 of seed and drench treatment trials carried out in a greenhouse at 25 ± 5°C for control of *Pythium ultimum* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in February to April 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names, which appear in the same order as in this figure. The duration of the trial was

35 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.



**Fig. 2.13.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of seed and drench treatment trials carried out in a greenhouse at 25 ± 5°C for control of *Pythium ultimum* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in April to May 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names, which appear in the same order as in this figure. The duration of the trial was

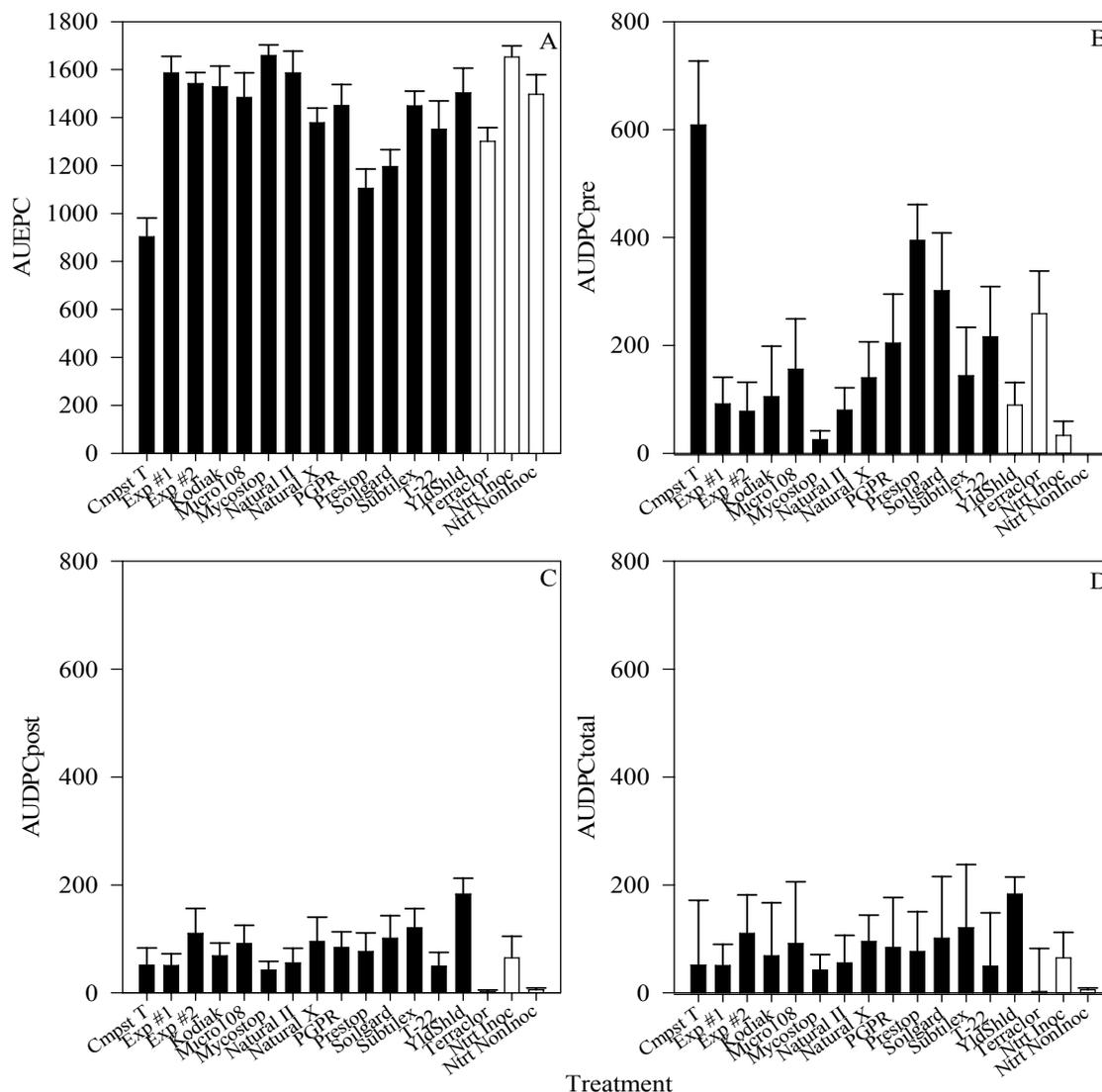
28 days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.



**Fig. 2.14.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 1 of seed and drench treatment trials carried out in a greenhouse at 25 ± 5°C for control of *Rhizoctonia solani* on spinach. The trial was set up as a randomized complete block design with four replications of 17 treatments, and was conducted in April to May 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names, which appear in the same order as in this figure. The duration of the trial was 28 days, with emergence and disease rated weekly. Refer to the text for the formula used to

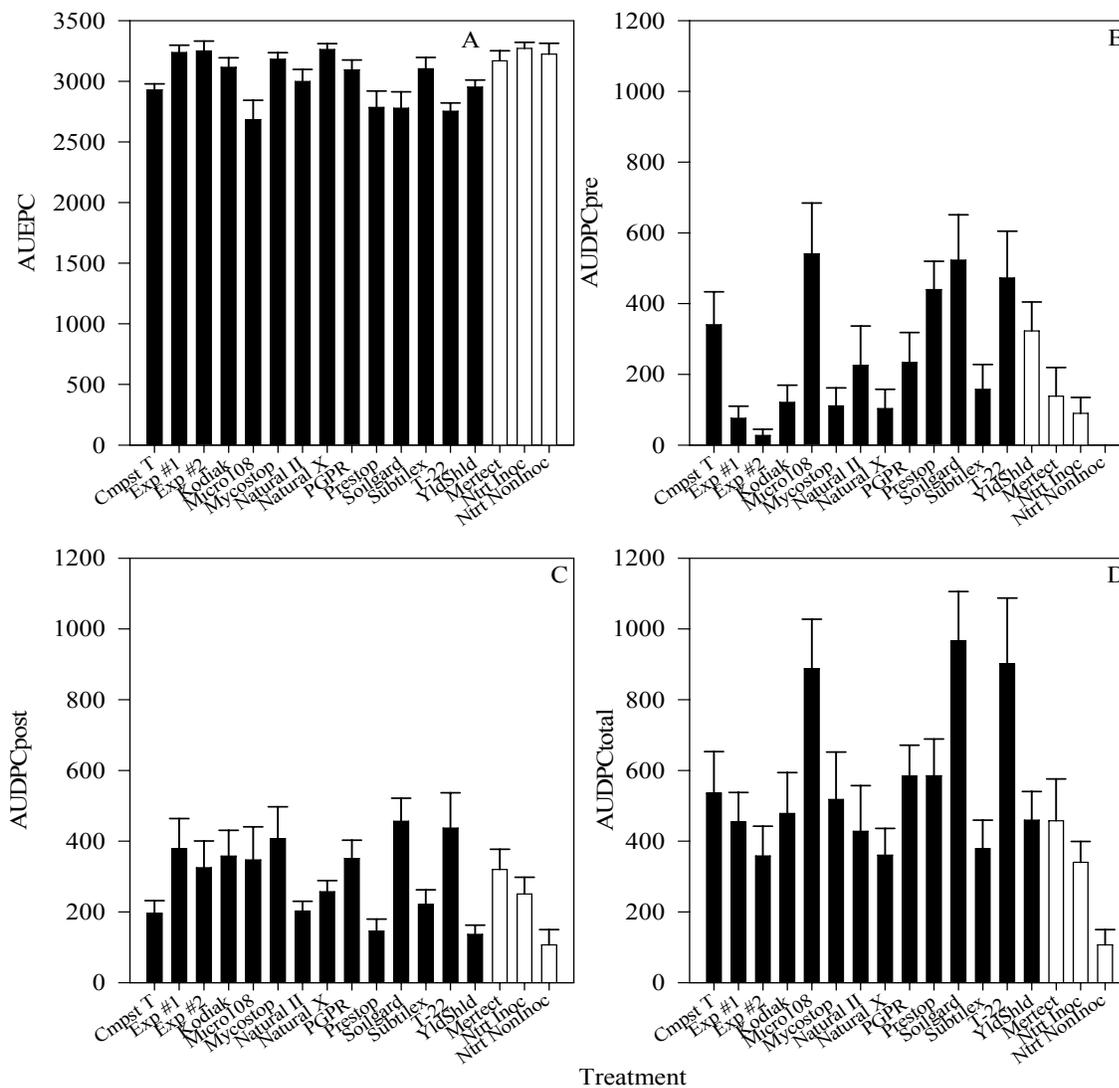
calculate the AUPC values. Each bar shows the mean and standard error of five replications.

The white bars represent the three control treatments.



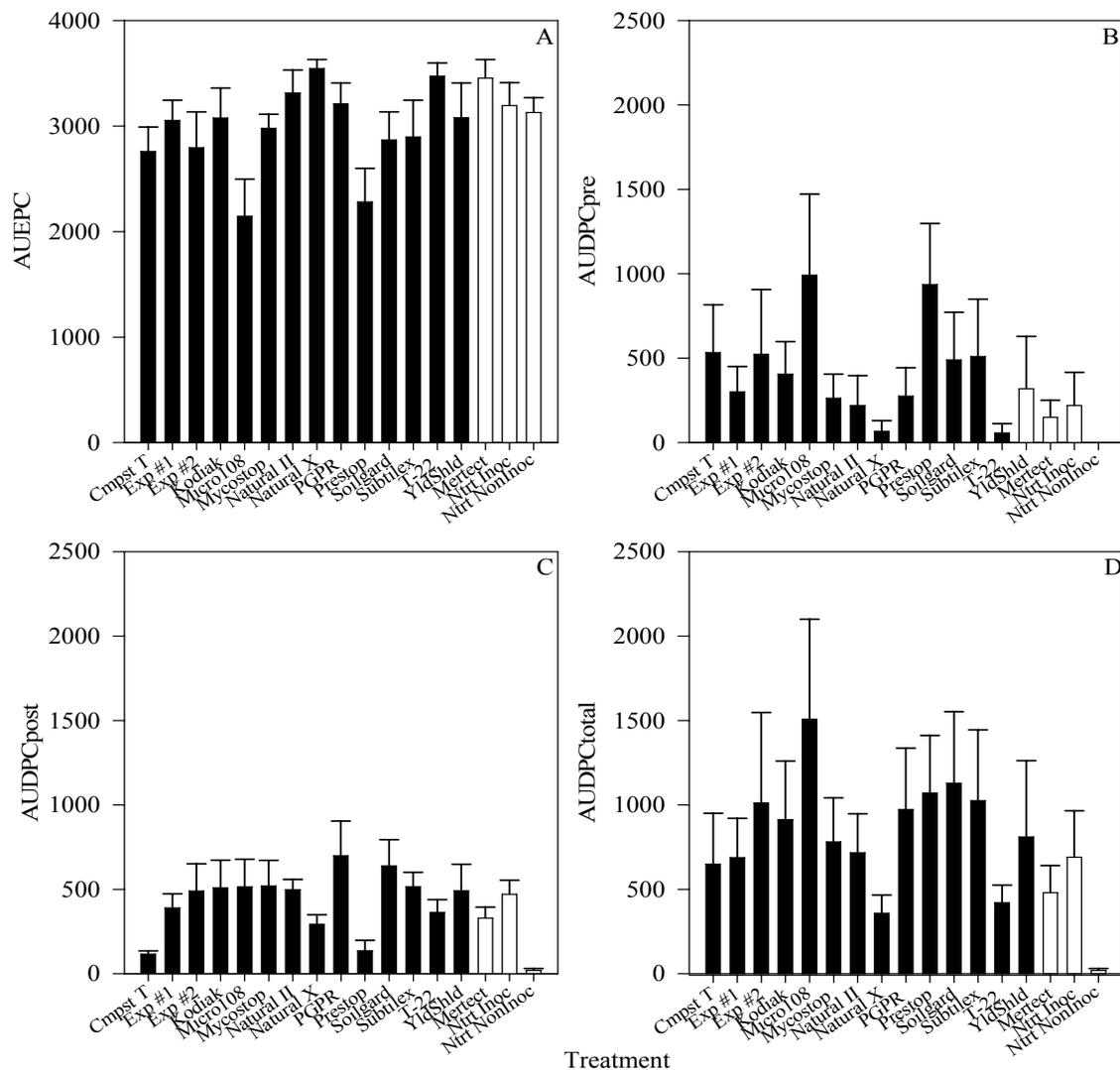
**Fig. 2.15.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of seed and drench treatment trials carried out in a greenhouse at 25 ± 5°C for control of *Rhizoctonia solani* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in May to June 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names which appear in the same order as in this figure. The duration of the trial was 28

days, with emergence and disease rated weekly. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.



**Fig. 2.16.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 1 of seed and drench treatment trials carried out in a greenhouse at 28 ± 5°C for control of *Fusarium oxysporum* f. sp. *spinaciae* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in March to May 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names which appear in the table in the same order as in this figure. The duration of the trial was 42 days, with emergence and disease rated weekly. Refer to the text

for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.



**Fig. 2.17.** Area under progress curves (AUPC) for A) emergence (AUEPC), B) pre-emergence damping-off (AUDPC<sub>pre</sub>), C) post-emergence wilt (AUDPC<sub>post</sub>), and D) total disease (AUDPC<sub>total</sub>) for trial 2 of seed and drench treatment trials carried out in a greenhouse at 28 ± 5°C for control of *Fusarium oxysporum* f. sp. *spinaciae* on spinach. The trial was set up as a randomized complete block design with five replications of 17 treatments, and was conducted in April to June 2007. Refer to Table 2.1 for details of the seed and drench treatments, and to Table 2.3 for full treatment names which appear in the table in the same order as in this figure. The duration of the trial was 49 days, with emergence and disease rated weekly. Refer to the text for

the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the three control treatments.

## Chapter 3

### Field evaluations of seed and drench treatments for control of damping-off and seedling blight pathogens of spinach for organic production

#### 3.1. INTRODUCTION

Soilborne plant pathogens, including *Pythium* spp., *Fusarium* spp., and *Rhizoctonia* spp., cause extensive damage to numerous crops worldwide (Campbell and Neher, 1996). Both the ecology of soilborne pathogens and the epidemiology of root diseases present challenges to researchers, because each pathosystem is unique. Three specific challenges applicable to many root disease systems include difficulties in quantifying inoculum, assessing disease, and designing effective studies (Campbell and Neher, 1996). Most soilborne pathogens survive in the soil as dormant propagules or resting structures, such as sclerotia, which can be extremely long-lived in the soil, with virtually no effective long-term cultural control options, making management of such pathogens difficult (Baker and Paulitz, 1996).

Disease management in organic production systems is especially challenging because organic producers do not have the option to utilize all of the conventional disease control methods such as synthetic chemical treatments. Thus, organic growers must rely on chemical formulations of biological control agents (BCAs) that are approved for use in organic production, as well as other disease management tools such as crop rotations, cover cropping, and use of resistant cultivars when available (Koenig and Baker, 2002). In 1990, Congress passed the Organic Foods Production Act (OFPA) in the Farm Bill, establishing consistent organic production standards nationwide by implementing federally mandated organic standards. As a result, the USDA created the National Organic Program (NOP, 2005) as a part of the Agriculture Marketing Service (AMS). According to the Organic Foods Production Act of 1990, Section

2109 (a), Seed, Seedlings and Planting Practices: “For a farm to be certified under this title, producers on such farm shall not apply materials to, or engage in practices on, seeds or seedlings that are contrary to, or inconsistent with the applicable organic certification program” ([www.ams.usda.gov/nop/NOP/NOPhome.html](http://www.ams.usda.gov/nop/NOP/NOPhome.html)).

The demand for organically produced seed has increased since the rules of the USDA NOP have required the use of organic seed in organic production (Groot et al., 2004). However, concern over losses due to seedborne and soilborne pathogens has also increased because of the limited effective options available for seed treatments that satisfy organic standards (du Toit et al., 2005). There is an obvious need for seed treatments that can be EPA registered for use in organic production systems that are more effective against the diversity of soilborne pathogens than those currently available commercially. A variety of products have been developed for which the registrants or labels state efficacy against such pathogens, but results of various independent studies have often been highly variable (Harman, 1991). Therefore, research is necessary to aid seed companies at improving organic seed production and to provide more effective treatments that protect organic seeds in the soils into which they are planted, e.g., through development and refinement of disease management tools approved for use in organic production systems (Groot et al., 2004).

Seed treatments can be inexpensive and very effective forms of plant disease control (Taylor and Harman, 1990). The main objectives of seed treatments are to prevent rotting of planted seeds and/or infection of the developing seedlings, either by killing the pathogens directly in or on the seed, by protecting the developing seedling from infection by soilborne pathogens, or directly improving plant growth through application of nutrients or microorganisms that improve nutrient uptake by the seedlings (Taylor and Harman, 1990). The

reliability of seed treatments at meeting these objectives varies among crop species, seed treatment products, seedborne or soilborne diseases, soil types and conditions, etc. (Taylor and Harman, 1990). Many biological seed and drench treatments have been developed to protect against soilborne plant pathogens. Baker and Paulitz (1996) outlined three strategies for obtaining biological control of soilborne plant pathogens: 1) protection of infection courts, 2) reduction of inoculum potential in sites not necessarily associated with the infection court, and 3) induction of host resistance. They concluded that perhaps the most efficient of these strategies is the protection of a fixed infection court, such as seed, since the infection court remains stationary and does not encounter new inoculum over time. Therefore, a single application of a BCA may provide ample protection of a fixed infection site. This is why a variety of BCAs have shown potential as seed treatments for protection against seed decay and seedling damping-off diseases (Baker and Paulitz, 1996). The activity expected from an antagonistic BCA applied to seed is short-term protection against damping-off pathogens, or longer-term protection of the root system through colonization of the roots and the rhizosphere of the host (Gindrat, 1979).

The efficacy of seed and drench treatment products for control of soilborne diseases may differ among greenhouse and field settings (Baker and Paulitz, 1996; Taylor and Harman, 1990; Alabouvette et al., 1979; Gerhardson and Larsson, 1991). Soil type may also have an impact on the efficacy of treatments. Differences in pH, soil moisture content, or soil organic matter can have dramatic effects on the activities of biological organisms in soil (Baker and Paulitz, 1996; du Toit et al., 2007; Harman, 1991; Gerhardson and Larsson, 1991; Pierson and Pierson, 2007). Few biological methods of control have proven successful enough to be used on a large scale, due to difficulties in production, storage, and application (Alabouvette et al., 1979). It is important that products intended for management of soilborne diseases be evaluated under a

variety of conditions to determine true efficacy of the product in the diversity of environments in which the products might be used.

The purpose of this research was to provide an objective evaluation of selected seed and drench treatment products EPA registered and approved for use in organic production, and products that have the potential for registration and approval. Products were selected based on results from greenhouse trials described in Chapter 2, for control of soilborne seedling blight or damping-off diseases of spinach under field conditions. The research was done at three locations in western Washington with three soil types to evaluate the treatment products under a variety of conditions. Pathogens from each of three phyla were selected for evaluating seed and drench treatments based on the individual and collective impacts these pathogens have on the seedling blight and damping-off complex for many small-seeded vegetables: *Rhizoctonia solani* Kühn, a basidiomycete anamorph of *Thanatephorus cucumeris* (Frank) Donk well-known for causing seed rot and damping-off (Sneh et al., 1991); *Fusarium oxysporum* Schlecht. f. sp. *spinaciae* (Sherb.) Snyder and Hans., an ascomycete anamorph that causes seedling blight and a vascular wilt of spinach (Bassi and Goode, 1978); and *Pythium ultimum* Trow, an oomycete responsible for causing severe losses to both pre- and post-emergence damping-off of seedlings (Hendrix and Campbell, 1973). The specific objectives of this study were to:

1. Evaluate selected seed and drench treatments under field conditions at two different certified organic sites in western Washington, using plots inoculated with *P. ultimum*, *R. solani*, and *F. oxysporum* f. sp. *spinaciae*.
2. Evaluate selected USDA NOP-approved seed and drench treatments under field conditions at a certified organic farm in western Washington that had known problems with damping-off of spinach.

## 3.2. MATERIALS AND METHODS

**3.2.1. Field trial locations and experimental designs.** Field trials were conducted to evaluate selected seed and drench treatments under organic field conditions at each of three locations. One trial was planted at the Washington State University Mount Vernon Northwestern Washington Research and Extension Center (WSU Mount Vernon NWREC) in Mount Vernon, WA on 18 May 2007. A second trial was planted at the WSU Vancouver Research and Extension Unit (WSU Vancouver REU) in Vancouver, WA on 5 June 2007. The third trial was planted on a grower-cooperator's certified organic farm in Sequim, WA on 7 August 2007. Each field site was certified for organic production by the Washington State Department of Agriculture. The soil type at the Mount Vernon site was a Puget silt loam, at the Vancouver site was a Hillsboro silt loam, and at the Sequim site was a Lummi silt loam (<http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>). The Mount Vernon field site had been planted to a mustard (*Brassica juncea*) winter cover crop (Caliente 119 Blend, High Performance Seeds Inc., Moses Lake, WA) in 2006-07, which was incorporated into the soil in April 2007. The Vancouver site had been used for a bean (*Phaseolus vulgaris*) cultivar trial in 2006 with replicated plots of 34 cultivars. The Sequim field site had been planted to Brussels sprouts (*Brassica oleracea*, var. *gemnifera*) in 2006 which was incorporated in the spring of 2007, and the field was left fallow until the trial was planted.

The Mount Vernon and Vancouver trials were each set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. The main plots received four inoculation treatments: inoculation of the soil with *P. ultimum*, *F. oxysporum* f. sp. *spinaciae*, or *R. solani*, and a non-inoculated control treatment. The split plot treatments included 12 seed or drench treatments (Table 3.1). Each split plot was 3.00 m long x 0.76 m

wide, with five rows of spinach planted at a 5 cm spacing between rows and with 250 seed in each 3 m of row. The Sequim trial was set up as a randomized complete block design with five replications of nine USDA- and OMRI-approved organic seed or drench treatments. This trial was not inoculated with the three pathogens because it was on a grower-cooperator's farm. The plots in that trial were 3.0 m long x 1.5 m wide, with four rows of spinach planted at a 38 cm spacing between rows with 100 seed in each 3 m of row.

**3.2.2. Quantification of pathogen populations in the soil at each location.** Natural soilborne populations of the three genera of interest (*Fusarium*, *Pythium*, and *Rhizoctonia*) were determined for each field site prior to inoculation of the plots and planting spinach seed. A random sample of 30 to 50 soil cores (20 mm diameter core) was collected at each field site to a 15 cm depth. Three 10 g subsamples of each soil sample were each suspended in 90 ml sloppy agar (0.1% water agar) and placed on a reciprocal shaker set at 250 rpm for 12 min. An aliquot of 5 ml of the soil solution (designated as  $10^0$  dilution) was added to 45 ml sloppy agar and shaken to prepare the  $10^{-1}$  dilution. The process was repeated to prepare a dilution series to  $10^{-4}$ . Three replicate 0.5 ml aliquots of the soil suspension from each dilution were transferred onto three Petri plates of the appropriate selective agar medium and spread evenly over the plates with a sterile glass rod. Depending on the agar medium and the pathogen, the plates were incubated for 2 to 7 days before the numbers of colonies of the appropriate morphology for the genus of interest were counted.

Komada's agar medium (Komada, 1975) was used to quantify populations of *F. oxysporum*, and a *Pythium* selective agar medium (Mircetich and Kraft, 1973) was used to quantify *Pythium* populations in each soil. A medium containing tannic acid and benomyl (Du Pont, Wilmington, DE) that is semi-selective for *R. solani*, called TAB (Sumner and Bell, 1982),

was initially used to attempt to quantify *R. solani* populations in each soil. However, this medium was not effective for quantifying *R. solani* from the soil dilutions as most colonies observed on the TAB plates were *Fusarium* spp. Another *Rhizoctonia* semi-selective medium containing chloramphenicol was used to attempt to quantify *R. solani* populations in each soil, as described by Paulitz and Schroeder (2005). This medium also was not effective at quantifying *R. solani* (*data not shown*). A method developed by Paulitz and Schroeder (2005) that involves baiting *R. solani* from the soil with wooden toothpicks, and placing colonized toothpicks onto the same *Rhizoctonia* selective medium containing chloramphenicol, will be carried out in the future to quantify *R. solani* in these field soil samples, which have been stored in plastic bags at 4°C.

Soil samples were collected again from both the Mount Vernon and Vancouver sites two weeks after inoculation and planting, and assayed by soil dilutions (or will be assayed using the toothpick method, as described above, for quantifying *Rhizoctonia* populations). Two soil cores were collected to a depth of 15 cm from each split plot, and all the cores within each main plot were combined into one main sample for each replication. The soil samples were shaken by hand in a bag to ensure thorough mixing, and three 10 g subsamples were removed from each main plot sample for dilution plating onto semi-selective media or for the toothpick assay, as described above. The Sequim site was not inoculated with any of the three pathogens; therefore, populations of these pathogens were not quantified after planting the spinach seed.

**3.2.3. Soil pH.** The pH of the soil samples collected from each site prior to planting was also measured. Three 10 g subsamples of soil from each site were each added to 10 ml deionized water. Each sample was stirred for 30 s, left for 10 min, and the process repeated two more times before the pH was measured using a VWR Symphony pH meter (VWR, West Chester, PA).

**3.2.4. Inoculum production.** The Mount Vernon and Vancouver field plots were inoculated with each of the three pathogens in the appropriate main plots to ensure disease pressure from each pathogen at these sites. The inocula for *F. oxysporum* f. sp. *spinaciae* and *R. solani* were produced on organic rye seed (Mountain High Organics, New Milford, CT). The rye seed was soaked overnight in batches of 1,200 g in approximately 1.9 liters deionized water in 3.8 liter plastic milk jugs. Each jug was then drained of excess water, capped with a foam plug, and the plug covered with aluminum foil. The jugs of seed were then autoclaved at 120°C and 15 psi for 90 min, twice at a 24 h interval. Each jug was then inoculated with 15 to 20 colonized agar plugs (1 cm in diameter) of three- to five-day old actively growing cultures of the appropriate pathogen taken from Petri plates of potato dextrose agar (PDA, Beckton, Dickinson & Co., Sparks, MD). The jugs of inoculated rye were left at room temperature (22 to 25°C) for 4 to 5 weeks until the rye seed was fully colonized, and were shaken by hand once a week to promote uniform colonization of the pathogen on the rye seed. Once fully colonized, the rye seed was dispensed onto butcher paper in a fume hood, and turned daily by hand for six days until completely dry. The colonized, dried rye seed was then ground using a grain mill (Kitchen Aid, Shelton, CT) and sieved to achieve a ground particle size ranging from 1.0 to 1.8 mm. The ground, sieved rye inocula were stored at  $6.2 \pm 2.0^\circ\text{C}$  and 45% relative humidity until used to inoculate field plots. The *P. ultimum* inoculum was prepared using the soil/oatmeal method described in Chapter 2 for the greenhouse trials. The inocula were quantified with the soil dilution plating method described above, using three samples per jug of inoculum. The *R. solani* inoculum contained  $7.3 \times 10^3 \pm 0.6 \times 10^3$  propagules/g (ppg), the *F. oxysporum* f. sp. *spinaciae* inoculum contained  $6.3 \times 10^6 \pm 1.6 \times 10^6$  ppg, and the *P. ultimum* inoculum contained  $4.5 \times 10^5 \pm 1.5 \times 10^5$  ppg.

Based on rates of inoculum used in the greenhouse trials, quantification of the inocula by soil dilutions, and the amount of inoculum available, 200 g *R. solani* inoculum, 150 g *F. oxysporum* f. sp. *spinaciae* inoculum, and 75 g *P. ultimum* inoculum were aliquoted into 0.95 liter Ziplock bags (S.C. Johnson Company, Racine, WI) to inoculate each split plot at the Mount Vernon field site. However, based on very low emergence of seedlings observed for the *R. solani*-inoculated plots at this site, the rate of *R. solani* inoculum applied to the Vancouver split plots was reduced to 100 g/split plot. The inoculum was applied over the appropriate split plot by hand using a soil sieve to ensure even distribution. A 1.8 mm sieve was used to apply the *R. solani* and *F. oxysporum* f. sp. *spinaciae* inocula, and a 1.0 mm sieve was used to apply the *P. ultimum* inoculum because of the smaller particle size for the soil-oatmeal inoculum compared to the rye seed inocula. After the inoculum was applied to the surface of each split plot, the inoculum was incorporated into the soil to a 7 to 10 cm depth using a Honda FR800 rototiller (Honda, Alpharetta, GA). The rototiller was operated in 3 m of non-inoculated soil between incorporating inoculum of each pathogen to avoid cross-contamination.

**3.2.5. Seed and drench treatments.** The 12 seed and drench treatments evaluated at the Mount Vernon and Vancouver sites were selected based on results of the greenhouse trials described in Chapter 2, in which 14 products were evaluated for control of each of *P. ultimum*, *F. oxysporum* f. sp. *spinaciae*, and *R. solani*. The 12 seed and drench treatments evaluated in the field trials are listed in Table 3.1. Treatments included 10 biological seed or drench treatments, non-treated seed, and a conventional fungicide combination treatment that included seed treatment with Apron XL LS (mefenoxam, for *P. ultimum*) and Mertect 340-F (thiabendazole, for *F. oxysporum* f. sp. *spinaciae*) followed by a drench with Terraclor 75% (pentachloronitrobenzene, for *R. solani*). The treatments evaluated at the Sequim trial included

only the eight EPA registered and WSDA- and OMRI-listed treatments that had been evaluated in the greenhouse trials described in Chapter 2, and non-treated seed for the control treatment, because this site was approved by the WSDA for commercial organic production.

The label recommendations for the SoilGard drench treatment required 24 h between application of the drench treatment and planting spinach. Therefore, this product was applied to the appropriate split plots immediately after rototilling the inocula into the soil. Spinach seed of the hybrid ‘Lazio’ (Pop Vriend Seeds BV, Andijk, The Netherlands) was planted to a depth of 1.3 cm by Dan Martin and Chris Becker of Alf Christianson Seed Company (Mount Vernon, WA) the day after inoculation, using a tractor-mounted Hege 100 cone planter (Wintersteiger AG, Niederlassungm, Austria). Lazio was selected for these trials because of the susceptibility of this hybrid to damping-off, and the popularity of this hybrid for organic ‘baby leaf’ spinach because of resistance of the hybrid to all 10 known races of the spinach downy mildew pathogen, *Peronospora farinosa* f. sp. *spinaciae* (Irish et al., 2007). All plots at the Mount Vernon trial were planted on 18 May 2007 with the exception of the Experimental #1 and Experimental #2 plots, because the seed treated with these products was only received on 21 May 2007 from the company that applied the treatments. The split plots for these two treatments were planted using a Plotmatic 1R cone planter (Wintersteiger AG, Niederlassungm, Austria) on 21 May 2007. All 12 treatments at the Vancouver field site were planted on 5 June 2007 using the same Hege 100 cone planter as used at the Mount Vernon site. The compost tea, Micro 108, and Terraclor drench treatments were applied to the appropriate split plots on the day of planting at both the Mount Vernon and Vancouver trial sites.

The seed and drench treatments were applied at the highest appropriate rate recommended by the label or the manufacturer (Tables 3.1 and 3.2). The Experimental #1,

Experimental #2, Natural II, and Natural X seed treatments were each carried out by the respective companies, and the treated seed was returned to the WSU Mount Vernon NWREC. The compost tea was brewed on-site 18 to 24 h prior to planting each trial. The compost tea ingredients were measured and shipped to the NWREC by Catherine Crosby at the WSU Crop and Soil Sciences Department, Pullman, WA. Crosby's M.S. thesis project was in progress at the time this study was carried out, and involved characterizing and evaluating compost tea communities for suppression of *Xanthomonas campestris* in cabbage seed production. This compost tea was developed specifically for high bacterial diversity, with the ingredients including vermicompost (5 L/100 liters), seaweed powder (100 ml/100 liters), liquid humic acids (200 ml/100 liters), and azomite rock dust (300g/100 liters) (Scheuerell and Mahaffee, 2004). The compost tea was brewed (94.6 liters) the day before planting each trial, using a plastic Rubbermaid can (Rubbermaid, Fairlawn, OH) that was disinfected with 10% NaOCl and rinsed thoroughly prior to the brewing process. The can was filled with approximately 75 liters tap water and aerated using a Soil Soup Bio-blender (Soil Soup, Seattle, WA) for 24 h prior to adding the compost tea ingredients, to ensure volatilization of chlorine in the municipal water source that may have been detrimental to living microorganisms in the compost. The suspension was aerated for an additional 18 to 24 h after the ingredients were added. Each appropriate split plot was treated with the compost tea by diluting 3.8 liters concentrated tea in 7.6 liters water (also aerated for 24 h) per plot.

Terraclor was applied at a rate of 59.9 g/100 liters water as 4.7 g product dissolved in 1.89 liters of water/split plot at the Mount Vernon site. However, the amount of water used for the Terraclor drench at the Vancouver trial was increased to 3.8 liters to improve distribution of the product over the surface of each split plot. The SoilGard drench treatment was applied at a

rate of 239.7 g/100 liters water. Following the label instructions, 283.75 g SoilGard was dissolved in 8 liters water for each replication for each trial, and stirred for 30 min. The liquid suspension was decanted to remove the solid precipitate. The decanted suspension was then divided into batches of 600 ml stock solution, and each 600 ml aliquot was diluted with 7.4 liters water/split plot. All drenches were applied by hand using 7.6 and 11.4 liter plastic watering cans.

A light rain occurred at both the Mount Vernon and Vancouver sites after planting and all drenches had been applied, and rain showers continued through the next two days at both sites. Average daily temperature at the Mount Vernon site was  $12.7 \pm 1.9^{\circ}\text{C}$ , and ranged from 4.2 to  $26.1^{\circ}\text{C}$  for the duration of the trial, with 47.0 mm total precipitation recorded. Average daily temperature at the Vancouver site was  $19.2 \pm 8.1^{\circ}\text{C}$ , and ranged from 6.2 to  $40.6^{\circ}\text{C}$  for the duration of the trial, with a total of 65.5 mm rainfall and irrigation recorded at this site. Average daily temperature at the Sequim site was  $14.8 \pm 4.0^{\circ}\text{C}$ , and ranged from 6.1 to  $30.4^{\circ}\text{C}$  for the duration of the trial, with 20.8 mm total precipitation recorded.

**3.2.6. Disease rating and plot maintenance.** Seedling emergence and wilt ratings were conducted at 7 day intervals for 5 or 6 weeks after planting at each field trial. The Experimental #1 and Experimental #2 plots at the Mount Vernon site were rated three days after the other plots for the first three weeks to compensate for the delay in planting these treatments. A 1.22 m x 0.5 m PVC pipe frame was laid down on each split plot to determine the length of the center three rows of each split plot to be rated (1.22 m of each of the middle three rows/split-plot). For the Sequim site, the full 3 m length of all four rows was rated for each plot. Plots were hand-weeded and alleys were rototilled to manage weeds. Plots at the Mount Vernon site were fertilized two weeks after planting with Alaska fish fertilizer (5-1-1) (Lilly Miller Brands, Clackamas, OR)

applied at 7.68 ml/liter water and Acadian Seaplants Seaweed Extract fertilizer (Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada) applied at 2.23 g/liter water to provide micronutrients. The fertilizers were applied in 7.6 liters/split plot using plastic watering cans. PAR4 (9-3-7) (Boyer Valley Organic Proteins, Arion, IA), a granular fertilizer, was applied at a rate of 4347 kg/ha and incorporated the day prior to planting at the Vancouver field site.

**3.2.7. Statistical analyses.** Analyses of variance (ANOVAs), means comparisons using Fisher's protected least significant difference (LSD at  $P < 0.05$ ), or Friedman's nonparametric rank test were carried out using PROC GLM of SAS (Version 9.1, SAS Institute, Cary, NC) on each of the dependent variables measured in each trial. Friedman's non-parametric rank test was used when the original data and transformations of the data (logarithmic, square root, or arcsin square root) did not meet assumptions for parametric analyses, i.e., normally distributed data with homogeneous variances (Steele and Torrie, 1980). Post-emergence damping-off or wilt was calculated as the number of emerged seedlings that damped-off or developed vascular wilt symptoms. Additionally, total above-ground, dry weight of the plants in each split plot for the Mount Vernon and Vancouver sites, and in each plot at the Sequim site was determined by sampling a 1.2 m section of the center three rows in each split-plot, placing the plants in a drier for two weeks at 32°C, then weighing the dried plant material. Area under emergence progress curve (AUEPC), and area under post-emergence damping-off (or wilt) progress curve (AUDPCpost) were calculated. The area under progress curve (AUPC) is a cumulative measurement over time of the dependent variable (emergence or disease), calculated as an average of emergence or disease ratings over time:  $[(\sum(y_i + y_{i+1}))(t_i - t_{i+1})]$ , where  $y_i$  = the number of emerged or diseased seedlings at the  $i^{\text{th}}$  rating,  $y_{i+1}$  = the number of emerged or

diseased seedlings at the (i+1) rating,  $t_i$  = the number of days at the  $i^{\text{th}}$  rating, and  $t_{i+1}$  = the number of days at (i+1)<sup>th</sup> rating (Shaner and Finney, 1977).

### 3.3. RESULTS

**3.3.1. Pathogen populations in the soil and soil pH.** Soil dilution-plating revealed a *F. oxysporum* population of  $4.5 \times 10^4 \pm 0.4 \times 10^4$  ppg, and a *Pythium* population of  $3.0 \times 10^2 \pm 1.5 \times 10^2$  ppg prior to inoculation at the Mount Vernon field site. The populations at the Vancouver field site were  $6.1 \times 10^3 \pm 1.9 \times 10^3$  ppg for *F. oxysporum*, and  $1.5 \times 10^2 \pm 7.1 \times 10^1$  ppg for *Pythium* spp. The Komada's agar medium used for detecting *Fusarium* spp. is only semi-selective for *F. oxysporum* (Komada, 1975). The percentage of *F. oxysporum* colonies detected that were the spinach pathogen, *F. oxysporum* f. sp. *spinaciae*, was not determined. At the Sequim site, natural soil populations of the pathogens were  $8.5 \times 10^3 \pm 1.5 \times 10^3$  ppg for *F. oxysporum*, and  $2.23 \times 10^3 \pm 1.0 \times 10^3$  ppg for *Pythium* spp. Soil pH at the Mount Vernon site prior to planting was  $6.42 \pm 0.10$ , at the Vancouver site was  $6.29 \pm 0.17$ , and at the Sequim site was  $7.61 \pm 0.08$ .

**3.3.2. Mount Vernon field site.** Results from the Mount Vernon seed and drench treatment field trial are summarized in Fig. 3.1 and Fig. 3.2. Emergence of spinach seedlings was negligible in many of the *R. solani*-inoculated plots ( $8.93 \pm 1.46$  plants/3.7 m row at 42 dap over all plots inoculated with this fungus), compared to  $92.25 \pm 2.55$  plants/3.7 m row at 42 dap in the non-inoculated plots, apparently due to a rate of inoculation that was too high for the purpose of differentiating efficacy of the seed and drench treatments against this pathogen. Therefore, only results from the *P. ultimum*-, *F. oxysporum* f. sp. *spinaciae*-, and non-inoculated plots are presented. Based on the ANOVAs without the *R. solani*-inoculated plots, there was no

significant interaction between the main plot factor (pathogen inoculation) and the split-plot factor (seed and drench treatments), for any dependent variable at any of the weekly ratings. However, both the inoculations and the seed and drench treatments had a significant effect on emergence for all weekly ratings (7, 14, 21, 28, 35, and 42 dap). The main plot inoculations only had a significant effect on post-emergence disease at 14 and 21 dap, and the seed and drench treatments only had a significant effect on post-emergence disease at 28 dap. Additionally, the seed and drench treatments had a significant effect on the AUEPC and AUDPC<sub>post</sub> values, as well as spinach biomass at the final rating (42 dap).

Results of the statistical analyses for the AUEPC values for pathogen inoculations (main plots) (Fig. 3.1A) were similar to those of the 42 dap ratings for emergence. Similarly, statistical analysis results for the AUEPC values for seed and drench treatments (Fig. 3.2A) were similar to those of the 42 dap ratings for emergence. Results for the AUDPC<sub>post</sub> values for pathogen inoculations (Fig. 3.1B) were similar to those of the 14 and 21 dap ratings for post-emergence disease. Inoculation of the soil with *F. oxysporum* f. sp. *spinaciae* resulted in significantly lower emergence ( $70.9 \pm 2.7$  plants/3.7 m row) and biomass ( $55.5 \pm 2.6$  g) at 42 dap compared to those of the *P. ultimum*-inoculated plots ( $87.6 \pm 2.4$  plants/3.7 m row and  $71.5 \pm 3.0$  g, respectively) and the non-inoculated plots ( $92.3 \pm 2.6$  plants/3.7 m row and  $79.5 \pm 3.3$  g, respectively) (Fig. 3.1). Emergence was significantly higher in the non-inoculated plots at 14, 21, 28, and 35 dap compared to that of the plots inoculated with either pathogen (*data not shown*). At 14 and 21 dap, there was significantly higher post-emergence disease in *F. oxysporum* f. sp. *spinaciae*-inoculated plots than all other plots, but no significant difference between the *P. ultimum*-inoculated plots and the non-inoculated plots.

Emergence at 7 dap across all inoculated plots was significantly higher for seed treated with Experimental #1 and Experimental #2 ( $29.0 \pm 2.9$  and  $28.4 \pm 2.8$  plants/3.7 m row, respectively) compared to that of all other treatments, for which emergence ranged from  $1.3 \pm 0.3$  to  $6.1 \pm 1.9$  plants/3.7 m row (*data not shown*), but was not significantly higher at any subsequent ratings. Emergence observed 42 dap for the non-treated seed planted into non-inoculated control plots was  $108.8 \pm 6.8$  plants/3.7 m row, compared to  $83.4 \pm 3.8$  plants/3.7 m row for the non-treated seed planted into *F. oxysporum* f. sp. *spinaciae*-inoculated plots, and  $101.0 \pm 6.1$  plants/3.7 m row for the non-treated seed planted into *P. ultimum*-inoculated plots. Averaged across all inoculated plots, emergence at 42 dap was highest for the combination conventional fungicide treatment ( $103.7 \pm 5.0$  plants/3.7 m row) compared to that of all other treatments with the exception of non-treated seed ( $97.3 \pm 4.2$  plants/3.7 m row). Emergence 42 dap for Natural II, Natural X, and Subtilex treatments ranged from  $88.8 \pm 4.5$  to  $90.9 \pm 5.8$  plants/3.7 m row, and was not significantly different than emergence for the non-treated seed. However, there was no significant difference in emergence of seedlings from split-plots with Natural X, Natural II, Subtilex, SoilGard, Kodiak, Yield Shield, or Micro 108 treatments. Emergence of seedlings was not significantly different at 42 dap for treatments with Experimental #1, Experimental #2, and compost tea, which ranged from  $59.4 \pm 3.0$  to  $65.5 \pm 3.1$  plants/3.7 m row, but was significantly lower than that of all other treatments.

Post-emergence disease was first observed 14 dap for all seed and/or drench treatments (*data not shown*). The number of seedlings with post-emergence disease observed 42 dap for the non-treated seed planted into non-inoculated control plots was  $39.6 \pm 11.3$  plants/3.7 m row, compared to  $22.2 \pm 6.5$  plants/3.7 m row for the non-treated seed planted into *F. oxysporum* f. sp. *spinaciae*-inoculated control plots (43.9% reduction), and  $35.8 \pm 8.5$  plants/3.7 m row for the

non-treated seed planted into *P. ultimum*-inoculated control plots (9.6% reduction). Post-emergence disease 14 and 21 dap was significantly higher in the *F. oxysporum* f. sp. *spinaciae*-inoculated plots compared to the *P. ultimum*- and non-inoculated plots, but there was no significant difference in post-emergence disease among the seed and drench treatments at these ratings. Post-emergence disease 28 dap was significantly lower for seed treated with Experimental #1 ( $0.4 \pm 0.2$  plants) compared to that of all other treatments, and was significantly higher for treatments with Natural II ( $2.5 \pm 0.7$  plants/3.7 m row) and Subtilex ( $1.9 \pm 0.4$  plants/3.7 m row) compared to that of all other treatments.

Spinach biomass 42 dap was significantly higher for plants in the non-inoculated plots ( $79.5 \pm 3.3$  g) compared to that of the *P. ultimum*-inoculated plots ( $71.5 \pm 3.0$  g), which was, in turn, significantly higher than that of the *F. oxysporum* f. sp. *spinaciae*-inoculated plots ( $55.5 \pm 2.6$  g) (Fig. 3.1C). Averaged across all inoculated plots, spinach biomass 42 dap was significantly higher for seedlings that developed in plots with the conventional fungicide treatment ( $88.9 \pm 7.8$  g), and was significantly lower for seedlings that developed in plots with Experimental #2 seed treatment ( $53.2 \pm 4.3$  g), compared to all other treatments (Fig. 3.2C). Biomass was not significantly different among plots with any other treatments, ranging from  $60.2 \pm 7.5$  to  $72.7 \pm 8.7$  g.

**3.3.3. Vancouver field site.** Results from the Vancouver seed and drench treatment field trial are summarized in Fig. 3.3 and Fig. 3.4. As for the Mount Vernon trial, emergence of spinach seedlings was negligible in many of the *R. solani*-inoculated plots (averaged  $13.9 \pm 1.4$  plants/3.7 m row at 35 dap over all *R. solani* inoculated plots), compared to  $57.5 \pm 2.0$  plants/3.7 m row at 35 dap in the non-inoculated plots, apparently due to a rate of inoculation that was too high for differentiating efficacy of the seed and drench treatments. Therefore, only results from

the *P. ultimum*-, *F. oxysporum* f. sp. *spinaciae*-, and non-inoculated plots are presented. Based on the ANOVAs without the *R. solani*-inoculated plots, there was no significant interaction between the main plot factor (pathogen inoculation) and the split-plot factor (seed and drench treatments), for any variable at any of the weekly ratings. However, based on the ANOVAs, the seed and drench treatments had a significant effect on emergence for all weekly ratings (7, 14, 21, 28, and 35 dap), whereas the inoculations did not have a significant effect on emergence for any of the weekly ratings. In contrast, the main plot inoculations only had a significant effect on post-emergence disease at 28 and 35 dap, but the seed and drench treatments did not have a significant effect on post-emergence disease for any of the weekly ratings. Additionally, the main plot inoculations had a significant effect on the AUDPC<sub>post</sub> values, but not on AUEPC values, or biomass at the final rating (35 dap) (Fig. 3.3C). The seed and drench treatments had a significant effect on the AUEPC values and on biomass at the final rating (42 dap), but not on the AUDPC<sub>post</sub> values (Fig. 3.4C).

Results of the statistical analyses for the AUDPC<sub>post</sub> values for pathogen inoculations (Fig. 3.3B) were not similar to the analyses for most of the weekly ratings for post-emergence wilt, with the exception of 28 and 35 dap wilt ratings, when inoculation with *F. oxysporum* f. sp. *spinaciae* resulted in higher post-emergence wilt compared to that of the *P. ultimum*- and non-inoculated plots. The *P. ultimum*-inoculated plots also resulted in significantly higher post-emergence disease than the non-inoculated plots. Results for the AUEPC values for seed and drench treatments (Fig. 3.4A) were similar to the 28 and 35 dap ratings for emergence. There was no significant difference in emergence of spinach seedlings for any of the main plot (inoculation) treatments at any weekly rating, which ranged from  $56.5 \pm 2.2$  to  $57.5 \pm 1.9$  plants/3.7 m row 35 dap.

Seedling emergence 7 dap across all inoculations was highest for seed treated with Experimental #1 ( $5.5 \pm 1.6$  plants/3.7 m row), but was not significantly different from that of Experimental #2 or SoilGard ( $2.9 \pm 1.3$  and  $2.9 \pm 1.1$  plants/3.7 m row, respectively), and was not significantly higher than any other treatment at any subsequent ratings. Emergence observed 35 dap for the non-treated seed planted into non-inoculated control plots was  $64.8 \pm 5.8$  plants/3.7 m row, compared to  $63.2 \pm 7.2$  plants/3.7 m row for the non-treated seed planted into *F. oxysporum* f. sp. *spinaciae*-inoculated control plots (2.5% reduction), and  $58.6 \pm 5.4$  plants/3.7 m row for the non-treated seed planted into *P. ultimum*-inoculated control plots (9.6% reduction). Averaged across all inoculated plots, emergence 35 dap was significantly lower for Experimental #1, SoilGard, Micro 108, and compost tea ( $51.2 \pm 4.2$ ,  $48.7 \pm 5.4$ ,  $45.7 \pm 2.2$ , and  $44.5 \pm 4.2$  plants/3.7 m row, respectively) compared to the non-treated seed ( $62.2 \pm 3.4$  plants/3.7 m row). No other treatment resulted in significantly different emergence at 35 dap than that of the non-treated seed, including the combination conventional fungicide treatment ( $61.1 \pm 3.9$  plants/3.7 m row).

Post-emergence disease was first observed 14 dap in plots with all seed and/or drench treatments (*data not shown*). The number of seedlings with post-emergence disease observed 35 dap for the non-treated seed planted into non-inoculated control plots was  $0.8 \pm 0.4$  plants/3.7 m row, compared to  $2.8 \pm 1.0$  plants/3.7 m row for the non-treated seed planted into *F. oxysporum* f. sp. *spinaciae*-inoculated control plots, and  $1.0 \pm 0.8$  plants/3.7 m row for the non-treated seed planted into *P. ultimum*-inoculated control plots. Post-emergence disease measured at 28 and 35 dap was significantly higher in the *F. oxysporum* f. sp. *spinaciae*-inoculated plots compared to the *P. ultimum*- and non-inoculated plots, but there was no significant difference in post-emergence disease among the seed and drench treatments at these or any other ratings.

Pathogen inoculations had no significant effect on final spinach biomass (Fig. 3.3C). Averaged across all inoculations, biomass at 35 dap was highest for seedlings that developed in the plots with Kodiak and Experimental #1 seed treatments ( $92.1 \pm 3.7$  and  $92.0 \pm 5.3$  g, respectively), but was not significantly different than that of seedlings that developed in plots with Subtilex, Natural II, Experimental #2, and Yield Shield treatments (ranging from  $79.9 \pm 4.2$  to  $81.7 \pm 5.4$  g), and was not significantly different than that of the non-treated seed ( $80.4 \pm 5.3$  g) (Fig. 3.4C). Biomass was lowest for seedlings that developed in the plots with compost tea drench treatment ( $61.5 \pm 6.87$  g), but this was not significantly different from the biomass of seedlings that developed in plots with Micro 108, SoilGard, the combination conventional fungicide, or Natural X treatments, which ranged from  $69.4 \pm 4.8$  to  $74.4 \pm 3.9$  g (Fig. 3.4C).

**3.3.4. Sequim field site.** Results from the Sequim seed and drench treatment field trial are summarized in Fig. 3.5. Based on the ANOVAs, the seed and drench treatments had a significant effect on emergence for all weekly ratings (14, 21, 28, and 35 dap), with the exception of 7 dap. In contrast, the seed and drench treatments only had a significant effect on post-emergence disease at 7 dap, but not for any other weekly ratings. Similarly, the seed and drench treatments had a significant effect on the AUEPC values, but not the AUDPC<sub>post</sub> values. Additionally, the seed and drench treatments had a significant effect on spinach biomass at the final rating (35 dap).

Results of the statistical analysis for the AUEPC values (Fig. 3.5A) were similar to those of the emergence rating at 35 dap. Emergence at 35 dap was highest for plots with the Micro 108 treatment ( $194.4 \pm 12.4$  plants/6.1 m row), but this was not significantly different than that of the compost tea plots ( $163.6 \pm 21.8$  plants/6.1 m row). Emergence for plots with the compost tea, SoilGard, Mycostop Mix, T-22 Planter Box, PGPR Galaxy, and Yield Shield

treatments (ranging from  $106.0 \pm 7.8$  to  $163.6 \pm 21.8$  plants/6.1 m row) was not significantly different from that of the non-treated seed ( $129.2 \pm 5.8$  plants/6.1 m row). Emergence for plots with the Kodiak seed treatment ( $92.0 \pm 14.4$  plants/6.1 m row) were significantly lower than that of the non-treated seed. Seed and drench treatments only had a significant effect on post-emergence disease at 7 dap, at which time disease was highest in plots with non-treated seed and Yield Shield seed treatment ( $1.4 \pm 0.4$  and  $1.2 \pm 0.2$  plants/6.1 m row, respectively), but this was not significantly higher than that of treatments with compost tea, T-22 Planter Box, or Mycostop Mix ( $0.8 \pm 0.4$ ,  $0.6 \pm 0.4$ , and  $0.8 \pm 0.6$  plants/6.1 m row, respectively). Post-emergence disease at 7 dap in plots with Kodiak, Micro 108, SoilGard, and PGPR Galaxy (ranging from 0 to  $0.4 \pm 0.4$  plants/6.1 m row) was significantly lower than that of the non-treated seed. Spinach biomass at 35 dap was highest for seedlings from plots treated with Micro 108 ( $176.4 \pm 13.5$  g), but this was not significantly different from the biomass of plants in the compost tea-drenched plots, or from the non-treated seed ( $170.6 \pm 15.0$  and  $146.6 \pm 8.8$  g, respectively) (Fig. 3.5B). Spinach biomass was not significantly affected by any of the treatments compared to the biomass of plants that developed from non-treated seed (Fig. 3.5B).

### **3.4. DISCUSSION**

The forms of inocula used for *F. oxysporum* f. sp. *spinaciae*, *P. ultimum*, and *R. solani* in two of the three field trials in this study were effective at producing disease on spinach in plots at the Mount Vernon and Vancouver sites. However, there was a problem with the rate of inoculation used for the *R. solani* plots, as the number of emerged seedlings was negligible in those plots at each of the Mount Vernon and Vancouver field sites compared to plots inoculated with the other two pathogens and the non-inoculated plots. This prevented differentiation of

efficacy of the seed and drench treatments for control on *R. solani* of spinach. Additionally, the rate of *P. ultimum* inoculum could have been higher, or conditions made more conducive for this pathogen, e.g., by planting seed into wetter soils or under cooler temperatures to produce greater disease pressure for evaluating the seed and drench treatment products more effectively against this pathogen. However, at the Mount Vernon site, inoculations with *P. ultimum* and *F. oxysporum* f. sp. *spinaciae* had a significant effect on emergence for all weekly ratings, but only had a significant effect on post-emergence disease at 14 and 21 dap. In contrast, at the Vancouver site, inoculations did not have a significant effect on emergence for any of the weekly ratings, and only had a significant effect on post-emergence disease at 28 and 35 dap, which was later than at the Mount Vernon trial. For the Mount Vernon site, inoculation of the soil with *F. oxysporum* f. sp. *spinaciae* resulted in significantly lower emergence, higher post-emergence disease, and lower spinach biomass compared to those of the *P. ultimum*- and non-inoculated plots. At the Vancouver site, there was no significant difference in emergence or biomass of spinach among the inoculation treatments, but post-emergence disease was significantly higher in plots inoculated with *F. oxysporum* f. sp. *spinaciae* compared to that of the *P. ultimum*- and non-inoculated plots, as in Mount Vernon; and post-emergence disease was significantly higher in the *P. ultimum*-inoculated plots compared to that of the non-inoculated plots, suggesting that the *P. ultimum* inoculum was more effective at the Vancouver site than at the Mount Vernon site. The *P. ultimum* inoculum may have been more effective at the Vancouver site due to the fact that there was more precipitation/irrigation at the Vancouver site than at the Mount Vernon site, which resulted in wetter soils that are more conducive to this pathogen.

At the Mount Vernon site, the seed and drench treatments only had a significant effect on post-emergence disease at 28 dap, but had a significant effect on emergence for all weekly

ratings. The seed and drench treatments at the Vancouver site also had a significant effect on emergence for all weekly ratings, but did not have a significant effect on post-emergence disease for any of the weekly ratings. Similarly, the seed and drench treatments at the non-inoculated Sequim site had a significant effect on emergence for all weekly ratings, but only had a significant effect on post-emergence disease at 7 dap. Although the seed and drench treatments had significant effects on emergence in each trial, this effect was not necessarily positive, as some treatments resulted in significantly lower emergence than the non-treated seed at each site. The fact that the treatments had so little effect on post-emergence disease may have been a result of inadequate disease pressure.

There was little consistency in results among the field trials. However, Experimental #1 and #2 seed treatments resulted in the highest early emergence of spinach seedlings at both the Mount Vernon and Vancouver sites; similarly, the compost tea drench resulted in significantly lower final emergence than that of the non-treated seed at both sites, and lower spinach biomass than most of the other treatments, but also low post-emergence wilt in both inoculated trials (latter confounded by poor emergence in the compost tea plots). Although seed treatment with Experimental #1 or Experimental #2 resulted in significantly higher emergence at 7 dap than other treatments, these two treatments also resulted in significantly lower final emergence compared to that of all other treatments at the Mount Vernon site, with the exception of the compost tea plots.

At the Sequim site (where Experimental #1 and Experimental #2 treatments were not included because they were not yet approved for organic production), treatment with Micro 108 resulted in significantly higher emergence at 7 dap, and treatment with Kodiak resulted in significantly lower emergence compared to that of the non-treated seed. None of the seed or

drench treatments at the Mount Vernon site resulted in higher final emergence than that of the combination conventional fungicide seed and drench treatment, and even emergence from plots with the non-treated seed was not significantly different from that of the conventional fungicide treatment. Final emergence at the Vancouver site was also not significantly different between the conventional fungicide treatment and the non-treated seed, and only treatments with Experimental #1, SoilGard, Micro 108, and compost tea resulted in significantly lower final stand counts than the conventional fungicide treatment or the non-treated seed. Results from the Sequim site revealed that only treatment with Micro 108 resulted in significantly higher final emergence, and treatment with Kodiak resulted in significantly lower final emergence, compared to that of the non-treated seed. The seed and drench treatments only had a significant effect on post-emergence damping-off at 7 dap at this site, with significantly lower post-emergence disease observed in the Kodiak, Micro 108, SoilGard, and PGPR Galaxy plots compared to the non-treated seed. In contrast, at the Mount Vernon site only treatment with Experimental #1 resulted in significantly less post-emergence disease than that of the non-treated seed or the conventional fungicide treatment at 28 dap.

For the Mount Vernon site, final spinach biomass was significantly higher for seedlings that developed in plots with the conventional fungicide treatment than for all other treatments, i.e., none of the organic seed or drench treatments was as effective as the conventional fungicide treatment; however, this was not the case at the Vancouver site. Biomass was lowest for seedlings in plots with Experimental #2 seed treatment at the Mount Vernon site. In contrast, spinach biomass at the Vancouver site was highest in plots with Kodiak seed treatment, though this was not significantly different than that of the non-treated seed. Biomass was significantly lower for seedlings that developed in plots with the compost tea treatment compared to that of

the non-treated seed at the Vancouver site. However, at the Sequim site, none of the treatments resulted in spinach biomass significantly different than that of seedlings that developed from non-treated seed.

The variation in results among trials may be attributable to differences in conditions at each field site. Each trial was planted at a slightly different time in the 2007 growing season, with different mean daily temperatures, and different amounts of precipitation and/or irrigation. These differences likely affected disease pressure from the pathogens, especially *Pythium* spp., which thrive under cooler, wetter conditions than the other two pathogens (Hendrix and Campbell, 1973). Additionally, the established levels of soilborne pathogens of spinach at each site prior to inoculation likely affected the results. For example, in almost all of the split-plots at the Mount Vernon site, there was a high incidence of post-emergence wilt starting at 28 dap that was typical of symptoms caused by *F. oxysporum* f. sp. *spinaciae*, regardless of the main-plot inoculation treatment. In contrast, there was little to no post-emergence wilt typical of that caused by *F. oxysporum* f. sp. *spinaciae* at the Vancouver site, except for the plots inoculated with that pathogen. This is a result of the fact that there had been no history of spinach production at the Vancouver site, unlike the Mount Vernon site which had been planted to a trial in 2006 that included spinach. Similarly, there was no post-emergence disease typical of symptoms caused by *F. oxysporum* f. sp. *spinaciae* at the Sequim site, even though that field had been planted with spinach in previous years.

The variable results observed among the trials for the treatments evaluated in this study are consistent with the literature on disease management with BCAs (e.g., Alabouvette et al., 1979; Baker and Paulitz, 1996; Gerhardson and Larsson, 1991; Harman, 1991; Pierson and Pierson, 2007; Roberts et al., 2005; and Taylor and Harman, 1990). In order to attain better

consistency in results for comparison among sites, it would be necessary to determine more accurate rates of inoculation of these three pathogens at each field site. To do this, inoculation rate trials should be carried out at each field site, possibly using micro-plots, to determine rates of inoculum needed to achieve a level of disease pressure for each pathogen that would optimize differentiation of seed and/or drench treatments for control of the pathogens, as was done for the greenhouse trials described in Chapter 2. Additionally, it would be necessary to determine an accurate method of quantifying *R. solani* present in the soil prior to inoculation, to assess how much inoculum of this pathogen to add to each site. A quantitative assay developed by Paulitz and Schroeder (2005) using toothpicks to bait the fungus from the soil, could be carried out to determine the population density of *R. solani* in the field plots pre- and post-inoculation, which may facilitate more efficient and effective future field work with this pathogen.

For the compost tea drench treatment used in these field trials, a diluted rate of  $5.4 \times 10^3$  liters compost tea in  $1.1 \times 10^4$  liters water/ha was used. However, in the greenhouse trials (see Chapter 2) a rate of 646 liters/100 m<sup>2</sup> potting mix was used. The rate/volume used in the greenhouse trials would have been impractical to attain under field conditions ( $= 6.5 \times 10^4$  liters compost tea/ha). Therefore, the rate was reduced approximately 12-fold to make application feasible under the conditions of these field trials. Additionally, application methods for many of the drench treatments evaluated at the rates/volumes recommended on the labels may only be practical on a commercial scale through an irrigation system. Therefore, a farm would have to be set up for irrigation in order to utilize these organic drench products on a large scale. Drench treatments may also be more labor intensive to apply than seed treatments, but typically apply a larger amount of the BCA over a larger area of the soil than do seed treatments, which may enhance the ability of the BCA to colonize the soil and compete with, antagonize, or parasitize

soilborne pathogen propagules. Additionally, drench treatments may be applied before planting, allowing more time for the BCAs to colonize the soil compared to seed treatments. However, seed treatments have the benefits of being typically inexpensive, easier to apply than drench treatments, and provide direct protection of the seed as an infection court or for the BCA to colonize the root system to protect the developing seedlings against pathogens (Baker and Paulitz, 1996; Gindrat, 1979; Taylor and Harman, 1990). However, many seed treatments are only intended to protect the seed and germinating seedlings for a limited duration (Gindrat, 1979). Results from this study were variable among trials and did not indicate obvious differences in efficacy between seed or drench formulations of treatments, except that drench treatments with compost tea and Micro 108 resulted in significantly lower emergence in two of the trials, and the SoilGard drench treatment resulted in significantly lower emergence in one of the trials compared to that of the non-treated seed.

The formulation of a BCA can have an important role in the efficacy of a treatment, e.g., mycelial vs. conidial preparations, or liquid vs. dry coating seed treatments (Lewis and Papavizas, 1984 and 1985; Taylor et al., 1991). Additionally, BCAs applied individually may not perform consistently against all pathogens challenging a specific crop (e.g., Roberts et al., 2005; Spadaro and Gullino, 2005). Therefore, using combinations of BCAs may enhance efficacy of organic seed and/or drench treatments against a wider range of pathogens under a wider range of environmental conditions than using individual BCAs. However, few BCAs have proven consistently successful enough to be used on a large scale, often due to difficulties in production, storage, and application (Alabouvette et al., 1979). Nonetheless, recent advancements in the production, formulation, and storage of BCAs for disease management products offer promise

for improving the efficacy and consistency of BCAs in disease management (Spadaro and Gullino, 2005).

Some of the treatments offered promising results in greenhouse trials (see Chapter 2). However, results from field trials were highly variable, and did not offer strong differentiation among treatments against the three pathogens. These trials would need to be repeated with more accurate rates of inoculation for each pathogen in order to determine whether this variability was due to the conditions of the trials.

### **3.5. LITERATURE CITED**

- Alabouvette, C., Rouxel, F., and Louvet, J. 1979. Characteristics of *Fusarium* wilt-suppressive soils and prospects for their utilization in biological control. Pages 165-182, in: *Soil-Borne Plant Pathogens*. B. Schippers and W. Gams, editors. Academic Press, New York, NY.
- Baker, R., and Paulitz, T. C. 1996. Theoretical basis for microbial interaction leading to biological control of soilborne plant pathogens. Pages 50-79, in: *Principles and Practice of Managing Soilborne Plant Pathogens*. R. Hall, editor. APS Press, St. Paul, MN.
- Bassi, A., Jr., and Goode, M. J. 1978. *Fusarium oxysporum* f. sp. *spinaciae* seedborne in spinach. *Plant Dis. Rep.* 62:203-205.
- Campbell, C. L., and Neher, D. A. 1996. Challenges, opportunities, and obligations in root disease epidemiology and management. Pages 20-49, in: *Principles and Practice of Managing Soilborne Plant Pathogens*. R. Hall, editor. APS Press, St. Paul, MN.
- du Toit, L. J., Inglis, D. A., and Miles, C. M. 2005. USDA Organic Cropping Research for the Northwest: Organic Seed Treatments: 2005 Progress Report. Washington State University-Mount Vernon NWREC, Mount Vernon, WA.

- Gerhardson, B., and Larsson, M. 1991. Effects of *Trichoderma* and other fungal antagonists on the incidence of fungal pathogens. Pages 121-128, in: Biotic Interaction and Soilborne Diseases. A. B. R. Beemster, G. J. Bollen, M. Gerlagh, M. A. Ruissen, B. Schippers, and A. Tempel, editors. Elsevier Science Publishing Co. Inc., New York, NY.
- Gindrat, D. 1979. Side effects of pesticides: Concluding remarks. Pages 531-534, in: Soil-Borne Plant Pathogens. B. Schippers and W. Gams, editors. Academic Press, New York, NY.
- Groot, S., van der Wolf, J. M., Jalink, H., Langerak, C. J., and van den Bulk, R. W. 2004. Challenges for the production of high quality organic seeds. Seed Testing Internat. ISTA News Bull. No. 127:12-15.
- Harman, G. E. 1991. Seed treatments for biological control of plant disease. Crop Protec. 10:166-171.
- Hendrix, F. F., and Campbell, W. A. 1973. Pythiums as plant pathogens. Annu. Rev. Phytopath. 11:77-98.
- Irish, B. M., Correll, J. C., Koike, S. T., and Morelock, T. E. 2007. Three new races of the spinach downy mildew pathogen identified by a modified set of spinach differentials. Plant Dis. 91:1392-1396.
- Koenig, R. L., and Baker, B. 2002. U.S. National Organic Program Standards: Implications for researchers. APSnet <http://www.apsnet.org/online/feature/organic/>
- Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. Rev. Plant Prot. Res. 8:114-125.

- Lewis, J. A., and Papavizas, G. C. 1984. A new approach to stimulate population proliferations of *Trichoderma* species and other potential biocontrol fungi introduced into natural soils. *Phytopathology* 74:1240-1244.
- Lewis, J. A., and Papavizas, G. C. 1985. Effect of mycelial preparations of *Trichoderma* and *Gliocladium* on populations of *Rhizoctonia solani* and the incidence of damping-off. *Phytopathology* 75:812-816.
- Mircetich, S. M., and Kraft, J. M. 1973. Efficiency of various selective media in determining *Pythium* populations in soil. *Mycopathol. Mycol. Appl.* 50:151-161.
- National Organic Program. 2005. Organic Foods Production Act of 1990. Section 2109: Prohibited Crop Production Practices and Materials.  
<http://www.ams.usda.gov/nop/NOP/NOPhome.html>
- Paulitz, T. C., and Schroeder, K. L. 2005. A new method for the quantification of *Rhizoctonia solani* and *R. oryzae* from soil. *Plant Dis.* 89:767-772.
- Pierson, L. S. III, and Pierson, E. A. 2007. Roles of diffusible signals in communication among plant-associated bacteria. *Phytopathology* 97:227-232.
- Roberts, D. P., Lohrke, S. M., Meyer, S. L. F., Buyer, J. S., Bowers, J. H., Baker, C. J., Li, W., de Souza, J. T., Lewis, J. A., and Chung, S. 2005. Biocontrol agents applied individually and in combination for suppression of soilborne diseases of cucumber. *Crop Protec.* 24:141-155.
- Scheuerell, S., and Mahaffee, W. 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. *Phytopathology* 94:1156-1163.

- Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67:1051-1056.
- Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* Species. APS Press, St. Paul, MN.
- Spadaro, D., and Gullino, M. L. 2005. Improving the efficacy of biocontrol agents against soilborne pathogens. *Crop Protec.* 24:601-613.
- Sumner, D. R., and Bell, D. K. 1982. Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zea*. *Phytopathology* 72:86-91.
- Steele, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach, 2<sup>nd</sup> ed. McGraw Hill, New York, NY.
- Taylor, A. G., and Harman G. E. 1990. Concepts and technologies of selected seed treatments. *Annu. Rev. Phytopathology* 28:321-339.
- Taylor, A. G., Min, T. G., Harman, G. E., and Jin, X. 1991. Liquid coating formulation for the application of biological seed treatments of *Trichoderma harzianum*. *Biol. Control* 1:16-22.
- United States Department of Agriculture. Natural Resources Conservation Service Web Soil Survey. <http://websoilsurvey.nrcs.usda.gov/app/WebSoilSurvey.aspx>.

**Table 3.1.** Seed and drench treatments evaluated in field trials in Mount Vernon and Vancouver, WA for efficacy against damping-off and vascular wilt of spinach caused by *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum* f. sp. *spinaciae*<sup>a</sup>

Treatment <sup>b</sup>	Active ingredient (rate in product)	Registrant or manufacturer	Rate of application <sup>c</sup>	Method of application	OMRI-listed in 2007 <sup>d</sup>	Registered for spinach in WA state in 2007 <sup>e</sup>
Compost tea	High bacterial diversity compost tea <sup>f</sup>	Washington State University, Pullman, WA	50 liters tea/100 liters water	Drench immediately after planting	Yes	No
Experimental #1	Proprietary organic disinfectant	Proprietary	Proprietary	Seed treatment	Not yet applied	No
Experimental #2	Proprietary organic disinfectant + <i>Trichoderma harzianum</i> T-22	Proprietary	Proprietary	Seed treatment	Not yet applied	No
Kodiak Concentrate Biological Fungicide	<i>Bacillus subtilis</i> (1.37%)	Bayer CropScience, Research Park Triangle, NC	31.2 g/100 kg seed	Slurry seed treatment	Yes	No
Micro 108 Seed Inoculant + Actinovate AG	<i>Streptomyces lydicus</i> (10 <sup>8</sup> cfu/g) + <i>S. lydicus</i> (10 <sup>7</sup> cfu/g)	Natural Industries, Houston, TX	1.76 kg/100 kg seed + 2.58 g/100 liters water	Dry seed coating + drench immediately after planting	Yes	Yes
Natural II	Actinomycete (0.6%)	Agricoat LLC, Soledad, CA	750.7 g/100 kg seed	Seed treatment	No	No
Natural X	Actinomycete (0.6%)	Agricoat LLC	750.7 g/100 kg seed	Seed treatment	No	No
SoilGard 12G	<i>Gliocladium virens</i> (12%)	Certis USA, Columbia, MD	239.7 g/100 liters water	Drench >24 h before planting	Yes	No
Subtilex	<i>Bacillus subtilis</i> (2.75%)	Becker Underwood, Ames, IA	15.6 g/100 kg seed	Slurry seed treatment	No	No
Yield Shield	<i>Bacillus pumilus</i> (0.28%)	Bayer CropScience	6.26 g/100 kg seed	Slurry seed treatment	Yes	No

Treatment <sup>b</sup>	Active ingredient (rate in product)	Registrant or manufacturer	Rate of application <sup>c</sup>	Method of application	OMRI-listed in 2007 <sup>d</sup>	Registered for spinach in WA state in 2007 <sup>e</sup>
Combination conventional fungicide treatment included the following three treatments						
Apron XL LS	Mefenoxam (33%)	Syngenta Crop Protection, Greensboro, NC	20.8 ml/100 kg seed	Slurry seed treatment	No	Yes
Mertect 340F	Thiabendazole (42.3%)	Syngenta Crop Protection	122.4 ml/100 kg seed	Slurry seed treatment	No	No
Terraclor 75% WP	Pentachloro-nitrobenzene (75%)	Crompton Uniroyal Chemical, Middlebury, CT	59.9 g/100 liters water or 30.0 g/100 liters water <sup>g</sup>	Drench immediately after planting	No	No
Non-treated seed	-	-	-	-	-	-

<sup>a</sup> Each trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. The four main plot inoculation treatments included: inoculation of the soil with *Pythium ultimum*, *Fusarium oxysporum* f. sp. *spinaciae*, or *Rhizoctonia solani*, and non-inoculated soil. The split-plot treatments included 12 seed or drench treatments. Each split-plot was 3.00 m long x 0.76 m wide, with five rows of the spinach hybrid ‘Lazio’ (Pop Vriend Seeds BV, Andijk, The Netherlands) planted at a 15 cm spacing between rows and with 250 seed in each 3 m of row.

<sup>b</sup> Products were selected for evaluation against the three pathogens based on results from greenhouse seed and drench treatment evaluations described in Chapter 2. Not all products were EPA registered, reviewed for compliance with the USDA National Organic Program (NOP), or reviewed by the Organic Materials Review Institute (OMRI) in 2007. The combination conventional fungicide treatment with Apron XL LS, Mertect 340F, and Terraclor 75% WP was included for control of *Pythium* spp., *Fusarium* spp., and *Rhizoctonia* spp., respectively. Non-treated seed was included as a control treatment.

<sup>c</sup> Each product was evaluated at the highest recommended label rate for spinach and/or a crop with similar-sized seed, or according to registrant recommendations. Drench treatments were applied according to the label based on surface area or volume of soil treated.

- <sup>d</sup> The OMRI provides organic certifiers, growers, manufacturers, and suppliers an independent review of products intended for use in organic production, handling, and processing in the U.S. (<http://www.omri.org/>). “Not yet applied” means registrant had not yet applied for OMRI approval at the time of this study.
- <sup>e</sup> Approved by the Washington State Department of Agriculture for use on organic spinach crops in Washington State in 2007 (<http://agr.wa.gov/FoodAnimal/Organic/MaterialsLists.htm>).
- <sup>f</sup> Ingredients of the compost tea included vermicompost (50 ml), seaweed powder (1 ml), liquid humic acids (2 ml), and Azomite rock dust (Scheuerell and Mahaffee, 2004). The compost tea was aerated for 24 h prior to application.
- <sup>g</sup> The same amount of Terraclor 75% WP was added to the split-plots at each site, but was diluted with twice as much water at the Vancouver site than at the Mount Vernon site to facilitate adequate distribution over the split-plots.

**Table 3.2.** Seed and drench treatments evaluated in a field trial in Sequim, WA for efficacy against damping-off and vascular wilt of spinach<sup>a</sup>

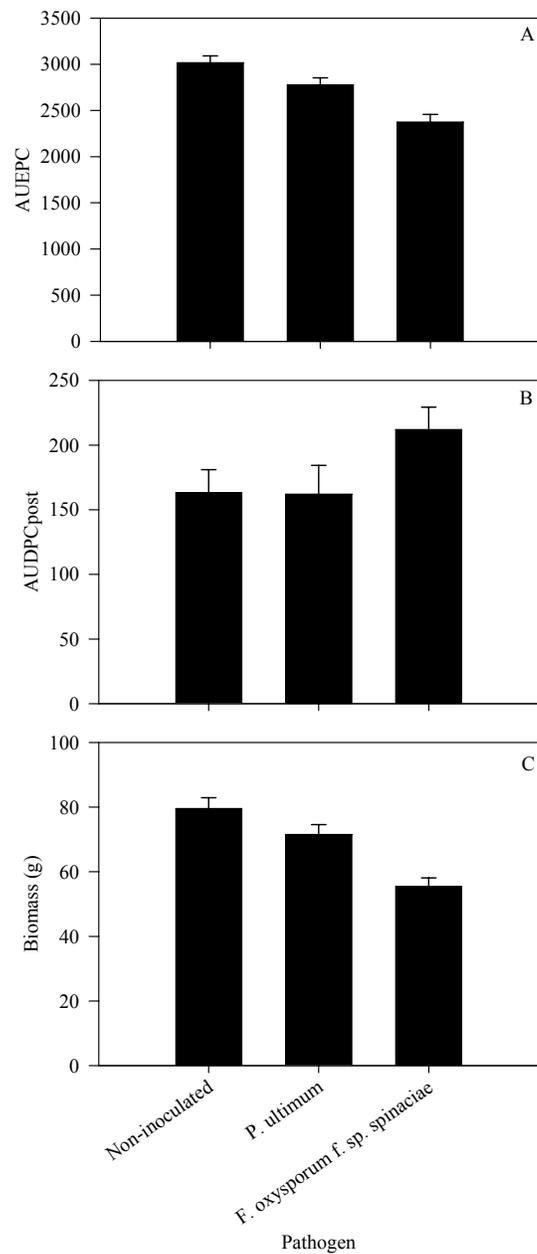
Treatment <sup>b</sup>	Active ingredient (rate in product)	Registrant or manufacturer	Rate of application <sup>c</sup>	Method of application	OMRI-listed in 2007 <sup>d</sup>	Registered for spinach in WA state in 2007 <sup>e</sup>
Compost tea	High bacterial diversity compost tea <sup>f</sup>	Washington State University, Pullman, WA	50 liters tea/100 liters water	Drench immediately after planting	Yes	No
Kodiak Concentrate Biological Fungicide	<i>Bacillus subtilis</i> (1.37%)	Bayer CropScience, Research Park Triangle, NC	31.2 g/100 kg seed	Slurry seed treatment	Yes	No
Micro 108 Seed Inoculant + Actinovate AG	<i>Streptomyces lydicus</i> (10 <sup>8</sup> cfu/g) + <i>S. lydicus</i> (10 <sup>7</sup> cfu/g)	Natural Industries, Houston, TX	1.76 kg/100 kg seed + 2.58 g/100 liters water	Dry seed coating + drench immediately after planting	Yes	Yes
Mycostop Mix	<i>Streptomyces griseoviridis</i> (4%)	Verdera Oy, Luoteisrinne, Finland	625.7 g/100 kg seed	Dry seed coating	Yes	Yes
PGPR Galaxy	Bacterial mixture <sup>g</sup>	Holmes ENVIRO, LLC, Philomath, OR	7 liters/100 kg seed	Slurry seed treatment	Yes	No
SoilGard 12G	<i>Gliocladium virens</i> (12%)	Certis USA, Columbia, MD	239.7 g/100 liters water	Drench >24 h before planting	Yes	No
T-22 Planter Box	<i>Trichoderma harzianum</i> T-22 (1.15%)	BioWorks, Inc., Victor, NY	250 g/100 kg	Dry seed coating	Yes	Yes
Yield Shield	<i>Bacillus pumilus</i> (0.28%)	Bayer CropScience	6.26 g/100 kg seed	Slurry seed treatment	Yes	No
Non-treated seed	-	-	-	-	-	-

<sup>a</sup> The trial was set up as a randomized complete block design with five replications of nine treatments. Each plot was 3.0 m long x 1.5 m wide, with four rows of the spinach hybrid ‘Lazio’ planted at a 38 cm spacing between rows and with 100 seed in each 3 m of row.

<sup>b</sup> All products selected for this trial were previously evaluated against the three pathogens *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium oxysporum* f. sp. *spinaciae* in greenhouse seed and drench treatment trials described in Chapter 2. All products were EPA registered and approved for use in organic production by the

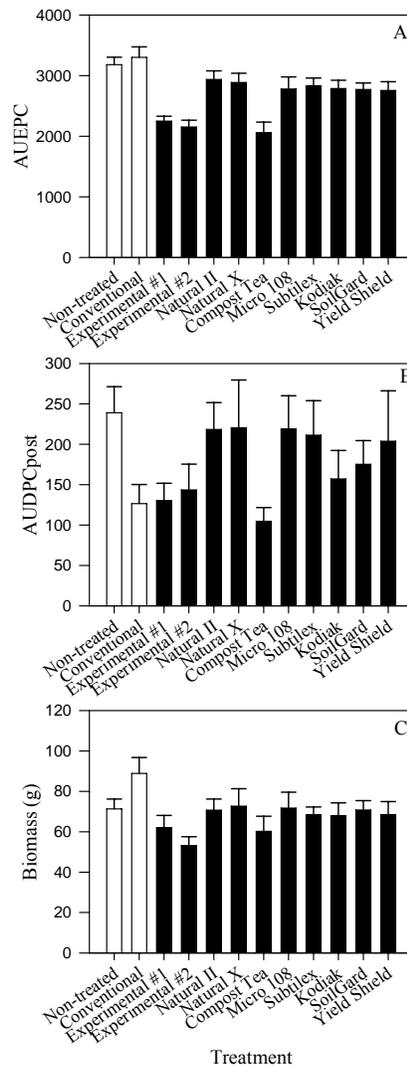
USDA National Organic Program (NOP), and Organic Materials Review Institute (OMRI) listed at the time of this study. Non-treated seed was included as a control treatment.

- <sup>c</sup> Each product was evaluated at the highest recommended label rate for spinach and/or a crop with similar-sized seed, or according to registrant recommendations. Drench treatments were applied according to the label based on surface area or volume of soil treated.
- <sup>d</sup> The OMRI provides organic growers, manufacturers, and suppliers an independent review of products intended for use in organic production, handling, and processing in the U.S. (<http://www.omri.org/>).
- <sup>e</sup> Approved by the Washington State Department of Agriculture for use on organic spinach crops in Washington State in 2007 (<http://agr.wa.gov/FoodAnimal/Organic/MaterialsLists.htm>).
- <sup>f</sup> Ingredients of the compost tea included vermicompost (50 ml), seaweed powder (1 ml), liquid humic acids (2 ml), and Azomite rock dust (Scheuerell and Mahaffee, 2004). The compost tea was aerated for 24 h prior to application.
- <sup>g</sup> PGPR Galaxy contains *Bacillus azotofixans* ( $304 \times 10^9$  cells/liter), *Azotobacter chroococcum* ( $304 \times 10^9$  cells/liter), *Pseudomonas putida* ( $304 \times 10^9$  cells/liter), and *Pseudomonas fluorescens* ( $304 \times 10^9$  cells/liter).



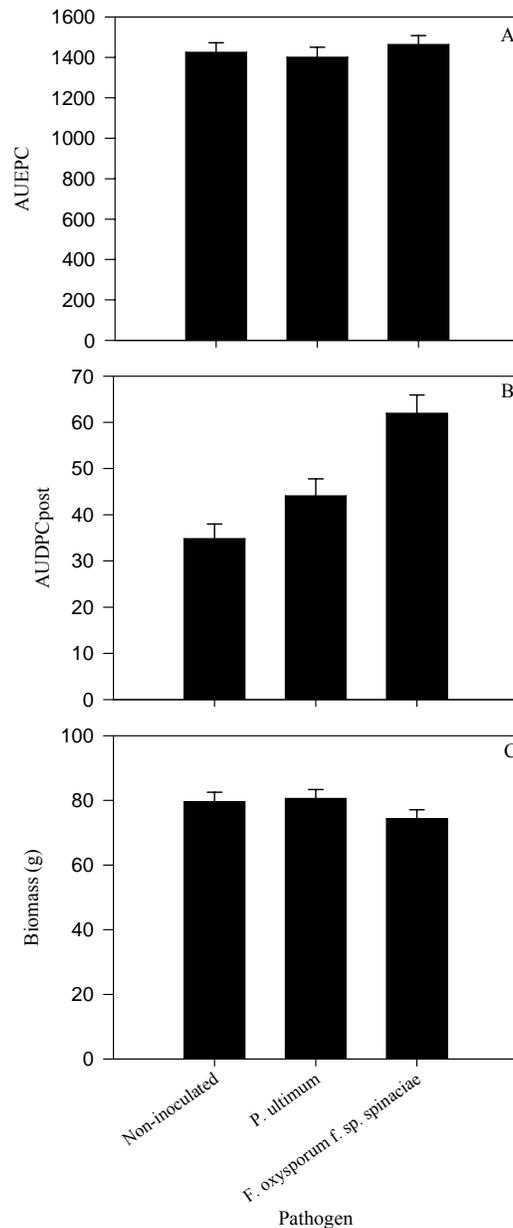
**Fig. 3.1.** Area under progress curve (AUPC) values for A) emergence (AUEPC) and B) post-emergence disease (AUDPC<sub>post</sub>), and C) above-ground dry biomass for spinach plants in main plots at the Mount Vernon, WA field trial in which 12 seed and/or drench treatments were evaluated for control of seedling blight and damping-off of spinach. The trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. The main plot treatments included inoculation of the soil with *Fusarium oxysporum* f.

*sp. spinaciae*, *Pythium ultimum*, or *Rhizoctonia solani*, or non-inoculated soil for the control treatment. However, only results for *F. oxysporum* f. *sp. spinaciae*, *P. ultimum*, and the non-inoculated treatment are presented because of low emergence/excessive damping-off in the *R. solani*-inoculated plots (see text for explanation). Refer to Table 3.1 for details of the seed and drench treatments. The duration of the trial was 35 d. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications.



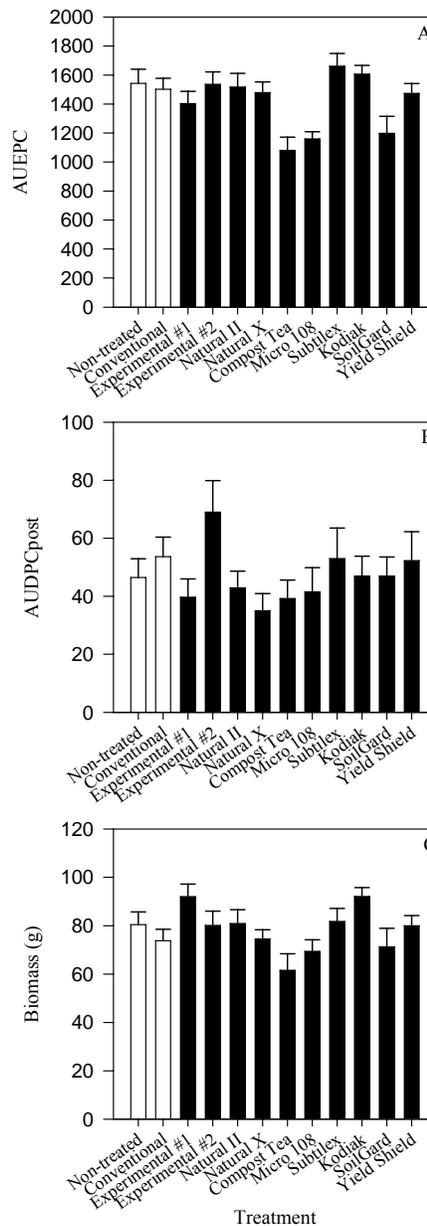
**Fig. 3.2.** Area under progress curve (AUPC) values for A) emergence (AUEPC) and B) post-emergence disease (AUDPC<sub>post</sub>), and C) above-ground dry biomass of spinach plants for the split-plot factor of seed or drench treatments for the Mount Vernon, WA field trial evaluating seed and/or drench treatments against seedling blight or damping-off pathogens of spinach. The trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. Main plots were inoculated with *Fusarium oxysporum* f. sp. *spinaciae*, *Pythium ultimum*, or *Rhizoctonia solani*, or not inoculated. Split-plot treatments included 12 seed and/or drench treatments. Refer to Table 3.1 for details of the seed and drench

treatments. The duration of the trial was 35 d. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the two control treatments (non-treated seed and a combination conventional fungicide seed and drench treatment).



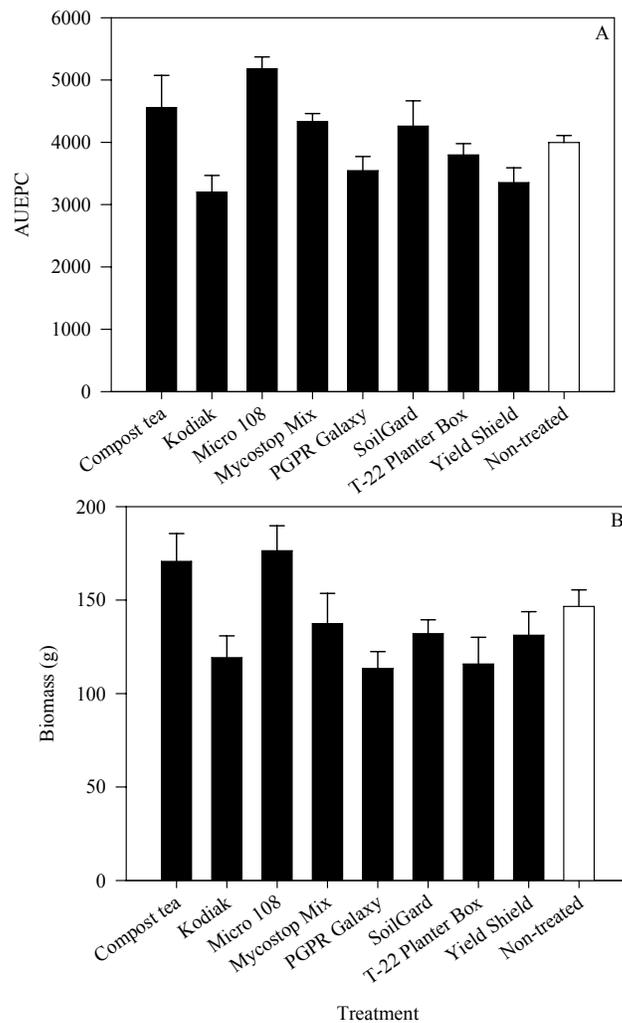
**Fig. 3.3.** Area under progress curve (AUPC) values for A) emergence (AUEPC) and B) post-emergence disease (AUDPC<sub>post</sub>), and C) above-ground dry biomass of spinach plants in the main plots at the Vancouver, WA field trial in which 12 seed and/or drench treatments were evaluated for control of seedling blight and damping-off pathogens of spinach. The trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. The main plot treatments included inoculation of the soil with *Fusarium oxysporum* f.

sp. *spinaciae*, *Pythium ultimum*, or *Rhizoctonia solani*, or a non-inoculated soil for the control treatment. However, only results for *F. oxysporum* f. sp. *spinaciae*, *P. ultimum*, and the non-inoculated treatment are presented because of low emergence/excessive damping-off in the *R. solani*-inoculated plots (see text for explanation). Refer to Table 3.1 for details of the seed and drench treatments. The duration of the trial was 35 d. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications.



**Fig. 3.4.** Area under progress curve (AUPC) values for A) emergence (AUEPC) and B) post-emergence disease (AUDPC<sub>post</sub>), and C) above-ground dry biomass for spinach plants for the split-plot factor of seed or drench treatments for the Vancouver, WA field trial evaluating seed and/or drench treatments against seedling blight and damping-off pathogens of spinach. The trial was set up as a split-plot, randomized complete block design with five replications of a 4 x 12 factorial treatment design. Main plots were inoculated with *Fusarium oxysporum* f. sp.

*spinaciae*, *Pythium ultimum*, or *Rhizoctonia solani*, or not inoculated. The split-plot treatments included 12 seed and/or drench treatments. Refer to Table 3.1 for details of the seed and drench treatments. The duration of the trial was 35 d. Refer to the text for the formula used to calculate the AUPC values. Each bar shows the mean and standard error of five replications. The white bars represent the two control treatments (non-treated seed and a combination conventional fungicide seed and drench treatment).



**Fig. 3.5.** A) Area under emergence progress curve (AUEPC) values and B) above-ground biomass of spinach plants for the Sequim, WA field trial evaluating seed and drench treatments for control of seedling blight and damping-off pathogens of spinach in a non-inoculated field. The trial was set up as a randomized complete block design with five replications of nine treatments, on a certified organic farm. The duration of the trial was 35 d. Refer to Table 3.2 for details of the seed and drench treatments, and to the text for the formula used to calculate the AUEPC values. Each bar shows the mean and standard error of five replications. The white bar represents the non-treated seed (control treatment).