EFFECT OF LOW DOSES OF PESTICIDES ON SOILBORNE PATHOGENS AN APPROACH TO THE HORMETIC RESPONSE

By

FRANCISCO FLORES

Bachelor of Science in Biotechnology

Escuela Politécnica del Ejército

Quito, Ecuador

2008

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2010

EFFECT OF LOW DOSES OF PESTICIDES ON

SOILBORNE PATHOGENS AN APPROACH

TO THE HORMETIC RESPONSE

Thesis Approved:

Dr. Carla Garzón

Thesis Adviser

Dr. Nathan Walker

Dr. Damon Smith

Dr. Mark E. Payton

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Carla Garzón for her most valuable guidance through the development of my project, for providing a suitable environment for me to work in; and for giving me the chance to study at Oklahoma State University. It has been a pleasure and an enriching experience to work under her supervision. Thanks to the members of my committee Dr. Nathan Walker and Dr. Damon Smith for their advice, knowledgeable suggestions and for providing some of the tools that I used in this research. Thanks to Dr. Julio Molineros for his advice on statistical analysis and to Dr. Gary Moorman for providing one of the pathogens for the investigation. I'd also like to give a very special thank to the staff and faculty of the Department of Entomology and Plant Pathology at Oklahoma State University, all the help provided during this two years is invaluable.

Thanks to everyone who was once a member of Dr. Garzón's Lab during these two years: Patty, Arif, Shefali, Yisel, Chelsea, Claudia, Kylie, Carla, Stormy and Andrea, because they were always ready to help when needed. Thanks to all my family and friends in Ecuador and Oklahoma for giving me the motivation needed to achieve my goals.

Finally I'd like to thank the Oklahoma State University Agricultural Experiment Station for providing funds for my research.

TABLE OF CONTENTS

Chapter **Page**

INTRODUCTION AND OBJECTIVES 1

General Objective: 3

Objectives: 3

REVIEW OF LITERATURE 4

Hormesis 4

Historic background 4

Hormesis in plant pathogens 5

Hormesis in other organisms 6

Possible underlying mechanisms for the hormetic response 7

Evaluation of Hormesis 9

Pythium aphanidermatum 10

Characteristics 10

Diseases caused by *Pythium aphanidermatum* 12

Control 13

Pesticides used to control *Pythium aphanidermatum* 14

Rhizoctonia zeae 17

Characteristics 17

Diseases caused by *Rhizoctonia zeae* 18

Control 19

Pesticides used to control *Rhizoctonia zeae* 19

Rhizoctonia solani 20

Characteristics 20

Diseases caused by *Rhizoctonia solani* 21

Control 23

Pesticides used to control *Rhizoctonia solani* 24

METHODOLOGY 26

Inoculum 26

In vitro testing 27

Dose response of soilborne pathogens to disinfectants 28

Dose response of soilborne pathogens to fungicides 29

Data analysis 31

Benchmark dose calculation 31

Test for hormesis and calculation of EC_{50} and NOAEL 31

RESULTS 34

Dose response of *Pythium aphanidermatum* to ethanol 34

Dose response of *Rhizoctonia zeae* to ethanol 35

Dose response of *Pythium aphanidermatum* to sodium hypochlorite 37

Dose response of *Rhizoctonia zeae* to sodium hypochlorite 38

Dose response of *Rhizoctonia zeae* to propiconazole 39

Dose response of *Pythium aphanidermatum* to cyazofamid 41

Dose response of *Pythium aphanidermatum* to propamocarb 42

Dose response of *Rhizoctonia solani* to propiconazole 44

CONCLUSIONS AND DISCUSSION 46

REFERENCES 52

APPENDIX A Tables of results 64

APPENDIX B *In vitro* testing protocol 84

APPENDIX C *In planta* testing protocol 89

LIST OF TABLES

Table **Page**

1 Parameters defining the dose response relationship between ethanol concentration and radial growth of *P. aphanidermatum in vitro*. 35

2 Parameters defining the dose response relationship between ethanol concentration and radial growth of *R. zeae in vitro*. 36

3 Parameters defining the dose response relationship between sodium hypochlorite concentration and radial growth *of P. aphanidermatum in vitro*. 38

4 Parameters defining the dose response relationship between sodium hypochlorite concentration and radial growth of *R. zeae in vitro*. 39

5 Parameters defining the dose response relationship between propiconazole concentration and radial growth of *R. zeae in vitro*. 40

6 Parameters defining the dose response relationship between cyazofamid concentration and radial growth of *P. aphanidermatum in vitro*. 42

7 Parameters defining the dose response relationship between propamocarb concentration and radial growth of *P. aphanidermatum in vitro*. 43

8 Parameters defining the dose response relationship between propiconazole concentration and radial growth of *R. zeae in vitro*. 45

LIST OF FIGURES

Figure **Page**

1 Observed values and modeled curve of the radial growth of *P. aphanidermatum* in response to low doses of ethanol. 34

2 Observed values and modeled curve of the radial growth of *R. zeae in vitro* in response to low doses of ethanol. 36

3 Observed values and modeled curve of the radial growth of *P. aphanidermatum* in response to low doses of sodium hypochlorite. 37

4 Observed values and modeled curve of the radial growth of *R. zeae in vitro* in response to low doses of sodium hypochlorite. 38

5 Observed values and modeled curve of the radial growth of *R. zeae in vitro* in response to low doses of propiconazole. 40

6 Observed values and modeled curve of the radial growth of *P. aphanidermatum in vitro* in response to low doses of cyazofamid. 41

7 Observed values and modeled curve of the radial growth of *P. aphanidermatum in vitro* in response to low doses of propamocarb. 43

8 Observed values and modeled curve of the radial growth of *R. solani in vitro* in response to low doses of propiconazole. 44

CHAPTER I

INTRODUCTION AND OBJECTIVES

The main objective of this research was to assess the effect of small doses of disinfectants and pesticides on the growth of soilborne pathogens. Low doses were tested in order to determine if the chemicals stimulate growth of pathogens *in vitro* at sub-lethal concentrations.

Growth response of living organisms to doses of chemicals below the no observed adverse effect level (NOAEL) has interested scientists since the 19th century (78). Even though the studies of such effects were limited for more than half a century, research around this issue has increased over the past three decades. This growing interest can be attributed to a phenomenon called hormesis (which can only be noticed if doses below the NOAEL are adequately assessed) and the recent linkage of dose response and adaptation concepts (35).

Hormesis is defined as "an adaptive response characterized by biphasic dose responses of generally similar quantitative features with respect to amplitude and range to the stimulatory response that are either directly induced or the result of compensatory biological processes following an initial disruption in homeostasis"(40). This adaptive response appears to be a generalizable biological phenomenon and evidence shows that it may be independent of environmental stresses, biological endpoint, and experimental model system (39).

Hormesis has been found in many fields of science where dose response relationships are present (e.g. toxicology, pharmacology, medicine, microbiology, mycology, plant pathology, and epidemiology) (35; 39; 42; 57; 132-134). For plant pathology, the knowledge about hormetic responses would be valuable when it comes to applications of pesticides. If hormesis proves to be valid for some soilborne pathogens it could dramatically change how pesticides are applied, especially in greenhouses where water is recirculated to the system along with fungicides which concentrations could get diluted to a point where hormesis may occur. When estimating the EC_{50} (effective concentration at which the growth of the pathogen is inhibited 50% compared to the control), an important parameter in plant pathology, the value obtained when taking hormesis into account may be different from the one obtained using traditional curve fitting methods (145).

There are some examples in the literature of the occurrence of hormesis for plant pathogens but studies focused on this concept are scarce. This phenomenon has allowed for new research devoted to the study of the effect of small chemical doses on biological subjects. The potential implications span from a shift in an EC_{50} to the molecular level and possible studies of biological plasticity (35).

Experimental confirmation of the phenomenon can have a great influence on the way dose response experiments are assessed. In most experiments where hormesis may occur, the expected pattern is a monotonic one. Therefore, if experimental data are non-monotonic, the enhanced responses at low doses are usually viewed as error and the data are manipulated to fit the monotonic pattern. Often the enhanced responses are pooled with the control response. In systems where hormesis occurs this pooling strategy would not be appropriate for toxicity estimation (60).

In this research, the effect of doses below the NOAEL of disinfectants and commercial pesticides on the growth of the soilborne pathogens *Rhizoctonia zeae, Rhizoctonia solani,* and

Pythium aphanidermatum in vitro was assessed. In the case of pesticides, a benchmark dose value (BMD) representing a concentration close to the NOAEL, was determined. Knowing this value, a comparative study for testing multiple pesticide doses on pathogen growth was conducted. Treatments included fungicides and disinfectants at concentrations below and above the BMD and a non-amended control. Three to five replicates of each treatment were conducted. Three to five repetitions of each experiment were performed over time. Modeling of the hormetic effect and the inference of EC_{50} and NOAEL were done using the Brain-Cousens model, modified by Schabenberger *et al*. (28; 125).

Goal:

- Determine the effect of low doses of disinfectants and fungicides on the growth of soilborne pathogens *in vitro*.

Objectives:

1. Determine the EC_{50} of disinfectants and fungicides on *Rhizoctonia zea*, *Rhizoctonia solani* and *Pythium aphanidermatum*.

2. Determine the NOAEL of disinfectants and fungicides on *R. zea*, *R. solani* and *P. aphanidermatum*.

3. Establish an experimental design that fits the correct assessment of hormetic responses in soilborne pathogens.

4. Assess the effect of the hormetic response on the growth of soilborne pathogens *in vitro* when low doses of disinfectants and pesticides are applied.

CHAPTER II

REVIEW OF LITERATURE

Hormesis

Historic background

Stebbing mentions the 16th century Renaissance man Paracelsus as the first person to acknowledge the dose response effect which allowed for the development of the concept of hormesis (133). Paracelsus said: “All things are poison and nothing is without poison, only the dose permits something not to be poisonous”. This statement has broad implications in toxicology where studies have been mainly conducted using doses for which “things are poisonous” and dose responses under the threshold of adverse effects have been, in most cases, either not assessed, ignored or regarded as background variation (35).

Recently described by Henschler (78), Rudolph Virchow appears to be the first person to describe hormesis for the effects of low doses of sodium and hydroxide potassium, on the epithelial cell ciliae of post mortem mucosa. Years later, by the end of the 19th century, Schulz (126; 127) used various agents detrimental to yeast metabolism at high doses to demonstrate their stimulatory effect at low dose levels and postulated what would later be known as the Arndt-Schulz law. This law states that “for every substance, small doses stimulate, moderate doses inhibit and large doses kill”. Unfortunately, he linked his findings with homeopathy, which many scientific groups found unacceptable, especially in the area of medicine (34).

Hormesis with yeasts continued with studies that focused on measuring CO_2 production as a means to determine a microorganism's metabolic rate. In 1929, Branham confirmed Schulz's observations using a series of different chemicals and an improved apparatus to detect CO_2 production. She reported that inhibitor compounds had an apparent stimulator effect on carbon dioxide production when diluted to very small doses (29). Further investigation on the addition of crystals of 1,2,5,6-dibenzanthracene to yeast suspensions showed that at a concentration of 9 x 10^{-4} molar yeast proliferation increased. Elton, *et al.* (64) demonstrated that proliferation was reduced for other concentrations, and total inhibition occurred when four times this amount was used.

Hormesis in plant pathogens

What was called the Arndt Schulz law has evolved throughout the years. Although currently it is not accepted as a toxicology law, it is nonetheless acknowledged as the foundation of the concept of hormesis. The term hormesis was proposed by Southam and Ehrlich when they observed that extracts of western red-cedar heartwood were stimulatory at low doses on the growth of a wood-decaying fungus (*Fomes officinalis*) in culture, while higher doses were inhibitory (132). This was the first scientific study that demonstrated hormesis on a plant pathogenic fungus. Later studies of hormesis in plant pathogens demonstrated a positive growth effect of *Fusarium oxysporum* when subjected to trichothecin (a compound produced by *Trichothecium roseum*), (79). This effect was used to assess the production of trichothecin in different soil types (80). The effect of streptomycin on fungi was assessed even though streptomycin is not toxic to fungi, the observations made suggested that some organisms may have a hormetic growth response to the antibiotic (43; 98).

Some members of another important group of plant pathogenic organisms, the oomycetes, have shown hormetic responses to low doses of pesticides. Fenn and Coffey observed

that 69 µg/ml of H_3PO_3 was stimulatory on the growth of *Pythium ultimum in vitro* and that 138 µg /ml was also stimulatory on the growth of *Pythium myriotylum* (66). The focus of these studies was on *Phytophthora* species and therefore the stimulation effects on the *Pythium* isolates was not emphasized. Recent experiments by Garzón *et al*. (74) focused on the effect of low doses of mefenoxam on the growth of *Pythium aphanidermatum in vitro* and the level of disease caused by the pathogen on geranium seedlings *in planta*. Results showed stimulation of the growth of a *P. aphanidermatum* isolate at concentrations of 1 x 10^{-14} ppm.

Hormesis in other organisms

The hormetic effect has been found in several organisms other than fungi and oomycetes. Hotchkiss found that for some bacteria, $TiCl_2$, $MgCl_2$ and, NaCl have hormetic effects on the growth of *Escherichia coli* in culture (83). The effect of low doses of penicillin on the growth of *Staphylococcus* (No. 6571 N.C.T.C.) in culture can be strong enough to double the growth compared to the non treated control (107). In plants, Jensen used $Ni(NO_3)_2$, $ZnSO_4$, $AgNO_3$, $CuSO_4$, $Fe_2(NO_3)_6$, Fe_2Cl_6, $Pb(NO_3)_2$, phenol and ethanol to assess their dose effects on the growth of wheat (*Triticum aestivum*) in soil and in nutrient solution. He observed non-monotonic responses for all the chemicals he used with stimulation at low doses and inhibition at higher doses (86). Nickell showed that low doses of some antibiotics stimulated plant growth in tissue culture, seed germination, and plant growth in soil (113). Such effect of antibiotics can also be observed in animals for which low doses of antibiotics are widely used to promote growth, even though the underlying mechanisms for this enhancement are not yet known (97). A hormetic effect of, disulfoton, dimethoate, and malathion was observed on the growth of cultured mouse liver cells (70). An increase of the total cell protein occurred for cells exposed to below toxic concentrations for organophosphorus insecticides. A different example of hormesis is the stimulatory effect of neomycin on the growth of the protozoan *Tetrahymena gelii* (26). These are

a few examples of the many investigations that have reported hormetic responses. Reviews of the subject have been conducted by both Stebbing and Calabrese (34; 38; 133).

Possible underlying mechanisms for the hormetic response

Despite the many documented studies, a lack of understanding of hormesis has prevented its widespread acceptance. It was not until 1998 that Stebbing proposed a hypothesis for hormesis. Based on the hydroid *Laomedea flexuosa* (133) and the marine yeast *Rhodotorula rubra* (136) Stebbing analyzed the biomass growth at intervals in time rather than the cumulative growth. He proposed that hormesis is a consequence of over corrections to low levels of inhibitory challenge, (i.e. overcompensation due to a disruption of homeostasis), and suggested that hormesis may be linked to an organism acquisition of tolerance to higher loads of an agent (135). Later on, based on pharmacology studies, Calabrese suggested direct stimulatory response as another possible cause of hormesis (39).

In both cases, the underlying mechanisms that generate the hormetic response have not yet been fully described. Furthermore, there's no evidence showing that the same mechanisms act both in the stimulation phase and in the inhibition phase of the biphasic curve, maintaining the skepticism towards the hormetic theory (93). Regarding the lack of a mechanistic basis for hormesis, in 2004 Conolly and Lutz hypothesized that this phenomenon may happen due to a superimposition of two monotonic dose responses: one that takes effect at low doses and other that overtakes at higher doses undermining the first one. They showed four different models that could generate a biphasic dose response: i) Membrane receptor subtypes with opposite downstream effect; ii) Androgen receptor mediated gene expression; iii) Induction of DNA repair and "co-repair" of background DNA damage; and, iv) Modulation of the cell cycle and effect on rate of mutation (48). These models were generated by computer; therefore, laboratory testing is needed to confirm one or more as the actual model for hormesis. Subsequent studies have found

evidence of hormesis and attribute the phenomenon to the presence of antagonistic membrane receptors (75) or to the induction of DNA repair (94). Hence, it is possible that multiple metabolic processes may be involved in hormetic responses.

Zhang *et al.* proposed that the altered interaction between the phase I and II of the xenobiotic homeostatic system may be the cause of the hormetic response to some mutagen/carcinogen compounds that require activation by the phase I enzymes (149). Zhang bases his hypothesis on his findings on the effect of low doses of chlorine in the increased production of antioxidant enzymes on mouse macrophages (150). Bae *et al.* suggested that hormesis may arise because of the heterogenic susceptibilities of different tissues to the same stimulus; such difference can result in the expression of a "U" shaped dose response curve. The observations that drove this conclusion were made from the response of different cell types normally present in human blood vessels to the presence of small doses of arsenic and a reactive oxygen species generator (menadione) (23). Allender *et al.* provided indirect evidence of the influence of calcium influx to the cell on hormetic responses related to plant growth (4; 5).The diversity of the models that may show a hormetic response suggests that the mechanisms acting may not be the same for different systems.

A new approach to hormesis suggests that the influence of very low doses on organisms should be linked to the enzymatic respiratory cell systems behavior since the metabolism at the molecular level appears to be regulated by the oxygen-hydrogen atomic interaction (101). This approach is closely related to homeopathy. It bases the effects of low doses on the interactions of water with the solute and on the capacity of the universal solvent to "preserve the information of the matrix-substance, showing characteristic physiological properties, often opposite to the effect of the initial dose". According to this hypothesis the dose response curve describes an oscillatory wave and knowing this wave's amplitude and length one could predict which doses of a certain agent are beneficial for an organism (101). Research that supports this hypothesis is mainly

published in homeopathic journals (25; 102; 103). When a hormesis study was published in Nature (56) it generated an abundance of correspondence by the scientific community which lead to the remaking of the experiments, and the finding that the results were not reproducible (82). Most research related to hormesis encounters opposition by the scientific community and it is argued that the basis of the resurging of this phenomenon is mainly due to *ad hoc* rather than *de novo* research (111). Therefore, studies focused specifically to demonstrate the validity of hormesis would either strengthen or weaken the argument that the phenomenon exists. According to an extensive literature review by Calabrese and Baldwin, hormetic responses often, but not always, display the following characteristics: i) Stimulation zone of the dose response within a 10-fold range; ii) Stimulatory responses 30-60% greater than the controls; and iii) NOAEL three to six-fold greater than the maximum stimulation dose (36).

Evaluation of Hormesis

When trying to prove the existence of hormesis there are some requirements that the experimental design should fulfill: i) The no observed adverse effect level (NOAEL) should be determined; ii) doses below the NOAEL need to be tested with five equally spaced doses providing enough data to detect hormesis; and iii) the separation between doses should generally be smaller than one order of magnitude since the hormetic zone is usually within a ten-fold range (37). To test for hormesis researchers must compare the effect of small doses with the response of the non-treated control. Therefore, there should always be background incidence in the control, without background incidence there is no way to detect a stimulus (48).

Evaluation of data is very important when proving hormesis. Crump suggests the criteria for evaluating hormesis as follows: strength of evidence, soundness of data, consistency and biological plausibility (52). Statistical analyses should be performed in order to differentiate a small stimulus from background occurrence. Among the analysis that can be performed to detect

hormesis, there are parametrical methods (125; 145) based on the Brain-Cousens curve model of a biphasic dose response (28), and a non parametrical method (47) based on the Mack-Wolfe K-sample rank test for umbrella alternatives (100). The Brain-Cousens model describes the dose response relationship when there is stimulation at low doses, being the commonly used sigmoidal curve, a special case of this equation when there is no stimulation at low doses (28). In the Mack-Wolfe K-sample rank test the maximum stimulation detected experimentally is compared to the response at all the other doses using Mann-Whitney counts, a test statistic is calculated and compared with simulated critical values to determine if the dose response is biphasic (100). This distribution free test doesn't have enough power to discriminate inconspicuous stimulation at low doses.

Pythium aphanidermatum

Characteristics

Pythium aphanidermatum is a plant pathogenic oomycete that causes damping off, root rots, stem rots, and blights of grasses and fruit. It has a wide host range that includes over 80 genera most of which are non-greenhouse crops, but when introduced in greenhouses can become a persistent problem (114; 116). *P. aphanidermatum* inhabits soils in five of the six continents (not present in Antarctica). Its presence is mainly concentrated in the Torrid Zone but it inhabits places as far north as Ontario Canada and as far to as southern Chile (8). It was first described by Edson in 1915 under the name of *Rheosporangium aphanidermatus* (62). In 1923, Coker established *Pythium,* a genus introduced by Pringsheim in 1858 as a *Rheosporangium* synonym. In 1923, Fitzpatric concluded that the combination *Pythium aphanidermatum* should be used over the original name *R. aphanidermatum* unless the genus *Nematosporangium*, described by Fischer in 1892, would be accepted. If so, the organism should be named *Nematosporangium*

aphanidermatum (69; 114), however it is still *P. aphanidermatum.* Current taxonomic placement is (3; 33):

Kingdom: Chromista

Phylum: Oomycota

Class: Oomycetes

Order: Pythiales

Family: Pythiaceae

Pythium aphanidermatum has a hyaline mycelium lacking septa, lobate sporangia, and aplerotic intercalary oogonia (114). It can reproduce either sexually or asexually. To undergo sexual reproduction an oogonium (female gametangia) is penetrated by the antheridium (male gametangia) and an oospore is formed after the fusion of genetic material. This is a homothallic organism which means that the same isolate can produce both male and female gametangia. Since it is a sterol auxotrophic oomycete, oosporogenesis in *P. aphanidermatum* requires the presence of these compounds (90). Oospores serve as the sole resting structure and as inoculum for future plant infections (32).

Asexual reproduction is achieved by the formation of sporangia containing zoospoes. Zoospores can form within minutes and are released in water environments where they can move freely (147). Oomycete's zoospores have two flagella, one anterior known as the tinsel flagellum and one posterior, the whiplash flagellum. The tinsel flagellum may have a sensory function (Lavesque, unpublished) while the whiplash flagellum is in charge of the movement of the zoospore (89). Once they are released, zoospores move to natural openings or wounds in the plant due to the presence of chemotactic components. When the zoospore reaches the plant tissue it encysts and forms a germination tube with an appressoria. After it penetrates the host, the former

establishes and pathogenesis proceeds (92). Other sources of inoculum are oospores and sporangia. They also form a germination tube and penetrate the host through appressoria. After infection of the plant tissue, the pathogen can grow quickly forming hyphae, oospores, sporangia and zoospores; initiating the life cycle one more time. *P. aphanidermatum* can survive in soils saprotrophically by colonizing death plant parts and forming new propagules capable of infecting plants (140).

Growth and development of *Pythium* species is directly influenced by environmental factors such as pH, temperature, moisture, and soil composition. Competition with other microorganisms also affects the behavior of *Pythium* in soil environments (105). Wet and warm conditions favor it's development, which is why it is considered a water mold. Optimum mycelial growth of *P. aphanidermatum* is achieved *in vitro* at temperatures between 35 - 40 °C and temperatures between 30 - 35 °C are ideal for infection (114). *P. aphanidermatum* is sensitive to low pH, therefore oospores do not germinate in acidic conditions (99).

Diseases caused by *Pythium aphanidermatum*

The most common diseases caused by *P. aphanidermatum* are: Damping off, stem rot, root rot, Pythium blight of turfgrasses and cottony blight. Damping off occurs at the seedling stage and can take place pre- or post-emergence. If it infects the seedling before emerging, the plant will show poor and uneven germination. In post emergence damping off, the seedling will appear collapsed and water soaked. In both cases, the plantings can easily be reduced by the microorganism. Stem rot can happen in juvenile annual and bedding plants. A water soaked lesion will appear on the stem, near the soil line. If it expands around the stem the plant may be killed. At this stage the plant can overcome the infection and recover. Root rot may happen at many stages of plant growth and even in storage. The pathogen can cause a rot of the root tips leaving the endodermis exposed. Symptoms of root rot include wilting, loss of vigor, stunting,

chlorosis and leaf drop. Pythium blight of turfgrasses, also called greasy spot, first appears as small brown spots in the grass, that will change to a dark, green color and plants will appear water soaked. When the oomycete has infected the leaf blades, cottony mycelium may be observed. Cottony blight occurs in mature plant tissue that has been in contact with soil. Cucumber and other cucurbit fruits may appear water soaked where after some time a cottony mycelial mat will form (116).

Control

Pythium aphanidermatum is hard to control due to its wide host range and its ability to resist harsh environmental conditions as oospores. Management of the oomycete can be achieved by the use or combination of cultural practices, biological, physical, and chemical agents. Resistance is not broadly deployed since there are few reported cases of cultivars resistant to *Pythium* spp. and few plants are labeled as such commercially (123). Among the cultural practices to manage *P. aphanidermatum* sanitation of the field or greenhouse, maintaining a good drainage system, growing the crop in optimal conditions for the plant, keeping the crop well aerated, manipulation of organic matter, and sowing on suppressive soils have all been used (105; 116).

Many biological agents have been reported for the control of *P. aphanidermatum. Pythium oligandrum,* a species from the same genus but nonpathogenic, may compete with *P. aphanidermatum* for niches and has shown to reduce damping off of citrus seedlings to levels similar to that attained using metalaxyl. Amongst the fungi, *Streptomyces griseoviridis, Gliocladium catenulatum* and *Trichoderma harzianum* are commercially available for suppression of diseases caused by *Pythium* spp. (105). There are also some bacteria species that might help in the management of *Pythium* diseases. Systemic induced resistance to *P. aphanidermatum* by *Pseudomonas corrugata* and *P. fluorescens* in cucumber was reported by

Zhou and Paulitz (151). Non pathogenic strains of *Burkholderia cepacia* may be used to suppress disease in the greenhouse (104). There is also a strain of this bacteria registered for seed treatment of vegetable crops (105).

Physical methods are often used to avoid propagule dissemination in recirculating nutrient systems in greenhouses. Heat treatment, membrane filtration, and UV radiation have all been used to reduce the spread of the organism. Unfortunately, these approaches also have negative effects on beneficial microorganisms present in the recirculating solutions (63).

Pesticides used to control *Pythium aphanidermatum*

Chemical seed treatment is the most common practice to control diseases caused by *Pythium* spp. (123). The pesticides commercially used to control Pythium diseases include propamocarb hydrochloride, etridiazole, fosetyl-aluminum, metalaxyl, mefenoxam, azoxystrobin, pyraclostrobin, and cyazofamid. Propamocarb hydrochloride is a carbamate that increases cell membrane permeability causing leakage of intracellular material of *Pythium* spp. (115). It affects growth, germination, and reproduction of the pathogen (19). It is a systemic fungicide that can be applied as a soil drench (0.05g a.i. x cm^{-2}), root dip (1200 ppm a.i.) or foliar spray. It was first registered in the U.S. in 1984 mainly for application on golf courses as a preventive (2.92-4.39 kg a.i. x ha^{-1}) or curative (6.83-9.27 kg a.i.ha^{-1}) treatment. In the U.S., it is manufactured by Bayer under the commercial names Banol® and Previcur®. The chemical is relatively non-persistent, with a half life of 35 days in soil (12).

Etridiazole is a thiazole compound which interferes with the lipid and cell membrane formation in fungi and oomycetes. It is used as a soil treatment to control damping off, root and stem rot in ornamental and nursery plants. It can also be used to control crown rot and root rot in turfgrass (17). It can be applied on row crops (0.14-0.42 kg a.i. x ha^{-1}), as a soil drench treatment (4.6-53.3 kg a.i. x ha^{-1}), as a potting soil treatment (42.72 ppm a.i.), on golfcourse turf (1.9 8.5 kg

a.i. x ha^{-1}), as a seed treatment (0.0078-0.0625 kg a.i. x 100 kg seed $^{-1}$), and for tobacco transplant float beds (52.4 ppm). It was first registered in the U.S. in 1964 mainly for use on cotton and ornamentals. Currently, etridiazole is also used to treat seed of barley, beans, corn, peanuts, peas, sorghum, soybeans, safflower, and wheat. Commercial products that contain this active ingredient are Terrazole®, Terraclor®, Temik® Brand TSX, Banrot®, Koban®, PCNB+ Liquid Seed Treater®, 4-Way Peanut Seed Protectant®, Terra-Coat® L-205N and Truban®. It has a half life of 25 days in water and it is considered relatively non-persistent (15).

Fosetyl-aluminium is a phosphate organometallic compound that blocks mycelial growth and spore production in oomycetes; it also inhibits spore germination and penetration into the plant. Plant defense mechanisms are also enhanced in the presence of this compound (18). It can be applied as a transplant drench or plant dip treatment; it can also be applied to foliage or incorporated into the soil before planting. It was first registered in the U.S. in 1983 for use on almonds, ginseng, asparagus, ornamental plants, turf, avocados, cranberries, pome fruits, citrus, pineapples, and stone fruits at concentrations up to 4800 ppm a.i(10; 67). Some of the trade names of products containing this active ingredient are Mikal®, Profiler® , R6 Albis®, R6 Trevi®, Rhodax®, Valiant® and Aliette®. Fosetyl-Al degrades quickly in soil under aerobic conditions due to microbial action. Its half-life is from 20 minutes to one and a half hours (9). Degradation results in the formation of phosphorous acid which is highly inhibitory to several oomycete species (49; 66).

Metalaxyl is a phenylalanine compound and it is a racemic mixture of two enantiomers. When only R-metalaxyl is present it is known as mefenoxam. It can be applied to the soil or as a foliar spray in combination with protectant fungicides. Metalaxyl is a systemic compound that inhibits protein synthesis in oomycetes (109). This active ingredient was first registered in the U.S. in 1979 for use on cotton, potatoes, and tobacco. Currently, it is also used on a wide variety of plants including agricultural crops, as a spray (0.151-8.970 kg a.i. x ha^{-1}), or seed treatment

(0.154-0.700 g a.i. x kg^{-1} seed), and to outdoor residential plants (1.00-8.07 kg a.i. x ha^{-1}) (11; 109). Trade names of products include Ridomil®, Subdue®, and Apron®. Its half life ranges from 27 days in an anaerobic (soil with water) environment to 400 days when exposed to photodegradation in water (11).

Azoxystrobin is a ß-methoxyacrylate, a compound derived from the naturally-occurring strobilurins present in wood decaying fungi. It is a systemic pesticide that inhibits mitochondrial respiration by interfering with electron transport in a wide variety of microorganisms that include both fungi and oomycetes (22). The pesticide was registered in the U.S. in 1997 for use on golf courses and turf farms. Application rates are very low, in a range of 0.02 to 1.03 kg a.i. x ha^{-1} (55). This active ingredient is present in the commercial pesticides Heritage®, Quadris®, and Abound®. Its half life by photodegradation in water is 11 days and in soil environments it ranges from 72 to 164 days (13).

Pyraclostrobin is also a ß-methoxyacrylate, a respiration inhibitor that has protective and curative action (142). The pesticide was first registered in the US in 2002 and is currently used for control of diseases of a large number of commodities including barley, berries, cole crops, citrus, corn, cotton, cucurbit vegetables, fruiting vegetables, grapes, legumes, peanuts, potatoes, rye, soybean, strawberries, stone fruits, sugar, tree nuts, and wheat, as well as residential and golf course turf. It can be applied as a spray (0.09-0.56 kg x ha^{-1}) or used as seed treatment (0.005-0.04 kg x kg $seed^{-1}$). Trade names for the pesticide include Headline®, Pristine®, and Insignia® (20). Half life of pyraclostrobin in water phase is 2 days and in soil environments 32 days (142).

Cyazofamid is a cyanoimidazol that has a highly specific activity against oomycetes. It effectively controls strains that are resistant to phenylamids and strobilurins. The pesticide inhibits all stages of oomycete development interfering with the respiration, acting specifically on the mitochondrial complex III (108). It was first registered in the U.S. in 2004 for control of

early and late blight on tomatoes and potatoes and of downy mildew on cucurbit vegetables, and is applied as a ground or aerial spray (0.08-0.1kg a.i. x ha $^{-1}$). It is a protectant fungicide with limited systemic activity. Trade names for the pesticide include Ranman® and Segway® (16). Cyazofamid has an estimated half life in soil of 5 to 8 days *in vitro* and approximately 3 to 6 days *in vivo* (137).

Rhizoctonia zeae

Characteristics

Rhizoctonia zeae is a plant pathogenic imperfect fungus that causes sclerotial diseases, damping off, and sheath and leaf spot in turfgrasses (131). Host range includes beet, aster, dahlia, wild carrot, lupine, poppy, pearl millet, bean, sesame, eggplant, potato, sorghum and wheat (144). It is widely distributed in temperate regions around the world. The pathogen has been reported in North, Central and South America, Europe, and Asia (59; 73; 84; 121; 144).

It was first described by Voorhes (146) in Florida as the causal agent of sclerotial rot of corn. It shares its teleomorph form, *Waitea circinata* with *Rhizoctonia oryzae*. Current taxonomic placement is (33):

Kingdom: Fungi

Phylum: Basidiomycota

Class: Agaricomycetes

Order: Corticales

Family: Corticiaceae

Rhizoctonia species have either multinucleate or binucleate hyphal cells. Further grouping of *Rhizoctonia* species is done based on anastomosis reaction, which is the hyphal reaction to same or different isolates grown in the presence of each other. This "manifestation of somatic or vegetative incompativility" (7) can range from fusion of the walls and membranes, occurring typically in self-anastomosis reactions, to connection (but no fusion) between members of the same anastomosis group. When isolates don't connect at all they belong to separate anastomosis groups (44). Rhizoctonia species rarely produce sexual structures and mainly reproduce through hyphae therefore they are placed within a group of fungi called mycelia sterilia. To overcome harsh environments the fungus produces small hyphae known as moniloid cells that stack together forming sclerotia (131). *R. zeae* has multinucleated hyphal cells and is confined to one anastomosis group (WAGZ) (131). When grown in corn meal agar *R. zeae* forms spherical sclerotia that are initially white, then turn to orange and finally to red or dark brown (139). Optimal growth temperature of the pathogen is 33 ºC, with humid conditions favoring disease development (124).

Diseases caused by *Rhizoctonia zeae*

Diseases caused by *R. zeae* include: sclerotial rot of corn, sheath spot of corn, brown small sclerotial disease of rice, damping off of legume seedlings, and leaf and sheath spot of turf grasses (131). Sclerotial rot of corn is characterized by wrinkling of the corn cobs in early stages of infection. A salmon pink mycelium covers the cob and turns dark gray in later stages (121). When it infects maize, *R. zeae* can also cause sheath spot of corn. Early symptoms of the disease are spots on the sheaths of flowering corn plants. The lesions begin as elliptical to irregular shaped greenish-gray areas with white or brown edges surrounded by a soft lighter halo. Lesions turn to a grayish color with well developed dark brown edges in later stages. Lesions can cover the entire leaf sheath. When the spots fuse extensive necrosis of the sheaths can occur (76).

The symptoms of brown small sclerotial disease of rice are very similar to bordered sheath spot of rice caused by *R. zeae* close relative, *R. oryzae*. The lesions are light to dark brown and irregular in shape (143). Since the occurrence of diseases caused by *R. zeae* is rather low, there is little information available about its etiology (139).

Leaf and sheath spot are turfgrass diseases caused by two *Rhizoctonia* species: *R. zeae* and *R. oryzae*. The diseases don't occur very often and have not yet been characterized thoroughly. Lesion symptoms on tall fescue are similar to the ones caused by *R. solani*. When infected, creeping bentgrass symptoms range in color from dark gray or brown to orange. A "smoke ring" similar to the one occurring in brown patch can also be associated with sheath spot. On centipedegrass and St. Augustinegrass, *R. zeae* causes leaf sheath lesions, similar to those induced by *Rhizoctonia solani*. *R. zeae* has also been associated with a diffuse foliar blight of bermudagrass (139).

Control

Effective management of diseases caused by *R. zeae* is hard to achieve due to the limited information about its etiology. Diseases caused by *R. zeae* can be controlled by cultural practices, host resistance, and fungicides. Most of the practices used to control *R. zeae* are similar to the ones described below, for *R. solani* diseases. Compounds used to control *R. zeae* include flutolanil and azoxystrobin, the later is also used to control *P. aphanidermatum* and its characteristics were discussed previously.

Pesticides used to control *Rhizoctonia zeae*

Flutolanil is a tuluanilide compound with potent and specific activity against basidiomycete fungi. It inhibits mycelial O_2 consumption as well as succinate dehydrogenase activity in the mitochondria (110). It is a systemic fungicide that forms a protective barrier on the plant, enters the plant and moves upward in the xylem. Flutolanil was first registered in the U.S.

in 1993 where it is used to control fungal diseases of both food (peanuts, potatoes, and rice) and non food crops (turf, greenhouse, field grown ornamentals, and potted ornamentals). It has protective and curative action. Pesticides containing flutolanil are Prostar®, Sysstar®, Moncut®, Moncoat® and Artisan® (21). It can be applied in furrow at concentrations from 0.4 to 1.2 kg a.i. x ha^{-1}. There are few studies about the activity of pesticides for *R. zeae* but experiments performed in Georgia show that flutolanil and azoxystrobin are more effective for suppressing disease than benzimidazole and dicarboximide chemestries (139). Half life of flutolanil in aerobic soil environment is 300 days and in an anaerobic aquatic environment its half life is over 13 years (128).

Rhizoctonia solani

Characteristics

Rhizoctonia solani is a basidiomycete first described as a pathogen of potato in 1858 by Julius Khun, the teleomorph is called *Thanatephorus cucumeris* (131), and is a very common soilborne pathogen that can infect a wide variety of crops (2). Current taxonomic placement is (33):

Kingdom: Fungi

Phylum: Basidiomycota

Class: Agaricomycetes

Order: Cantharellales

Family: Ceratobasidiaceae

The *R. solani* complex, composed by distinct strains that differ from one another genetically, biochemically, and pathogenically, is an economically important group of pathogens (54).

Isolates from *R. solani* have been classified by anastomosis groups. Currently there are 12 recognized anastomosis groups for *Rhizoctonia solani* named from AG1 to AG11 and AG BI (54). Some members of the groups are non pathogenic while others can have a wide host range.

R. solani can be isolated from the soil using baits and growing it on selective medium (45). The morphology of *R. solani* is characterized by hyphae having septa, cells are multinucleate, clear when young, and become darker with age. The hyphae typically have some shade of brown pigmentation and branches near the distal septum of young hyphal cells. Dolipore septa form in the branch near the point of origin. It doesn't form clamp connections, rhizomorphs or conidia. Mycelium is buff colored to dark brown and sclerotium cells are undifferentiated (117; 131). The fungus can survive in soil for several years in the form of sclerotia and when a susceptible host is present can germinate and infect plants. *R. solani* attaches and penetrates the host forming a specialized penetration structure called appressorium, and once inside produces several degrading enzymes that destroy plant cells providing the fungus with nutrients (45).

Diseases caused by *Rhizoctonia solani*

Due to the wide diversity of the host species, *R. solani* can cause a variety of diseases including: damping off, hypocotyl rot, seed decay, aerial blights, root canker, stem canker, wirestem, soreshin, and root and head rot (7; 24). Damping off refers to the disintegration of stem and root tissues of seedlings resulting in wilting and ultimately plant death (95). Damping off can be either pre or post emergence of the seedling. Pre-emergence damping off often occurs in infested soils when seedling emergence is delayed due to unfavorable environmental conditions, low vitality of seed, or planting depth (24). For post-emergence damping off, *R. solani* infects seedlings at or near the soil surface producing brick red to brown, sunken stem lesions; stem may become girdled if the disease progresses (95). Common bean (112) and soybean (27) hypocotyl rot is an important disease mainly caused by *R. solani* AG1 (7). Hypocotyl rot appears as red-

brown lesions on the hypocotyl or tap root of soybean seedling (27). Seedlings can become infected during emergence and if they don't die rapidly after infection, will be stunted.

Seed infection by *R. solani* can either occur while it is still in the fruit initiating a decay process that starts once the seed is planted or when it is planted in infested soils. Infected seed can rot quickly becoming an inoculum source which can spread to surrounding hosts. Seed infection serves as a means of spread to non infested areas when seed production systems are affected (24).

When warm and humid conditions are present, *R. solani* can form mycelial webs on the aerial parts of plants. The presence of these mycelia is characteristic of web blight, leaf blight, and thread blight. On fig trees, *R. solani* can survive on bark cracks and spread from tree to tree by airborne sclerotia (24). Turf brown patch producing strains can also infect by aerial hyphal growth producing diseased, light brown areas that may range from centimeters to a few meters in diameter surrounded by a purplish green margin. Mycelial webs can be observed in the mornings but desiccate during the day. Infection often occurs through stomata or mowing wounds (24).

Also know as crown and stem rot, rhizoctonia root canker is a disease of alfalfa that occurs under high soil temperature conditions (6). The disease is characterized by dark, sunken areas on the stem that usually have a brownish border. Symptoms are often visible where young roots emerged from older ones, a dead stub of an emerging root can often be found on the middle of the lesions (130).

When *R. solani* AG3 attacks potato it causes both black scurf and stem canker. Black scurf refers to the formation of sclerotial masses on the surface of potato tubers. These resting bodies accumulate and adhere tightly to the epidermis of potatoes but they don't penetrate the tuber. However, they serve as initial inoculum if infected tubers are used as seed (148). Stem canker occurs underground so it is not noticed most of the time. When the fungus attacks germinating sprouts before they emerge from the soil there may have delayed emergence,

reduction in crop vigor or no symptoms evident on the stem. When damage occurs late in the season, brown sunken lesions on underground stems and stolons can be observed and starch translocation is reduced. When carbohydrate flow from the leaves to the tubers is interrupted, small, green tubers form in the aerial part of the stem. Leaf curl, stunting, and resetting of the plant are also disease symptoms (148).

Crucifers are often attacked by *R. solani* once they are 10-15 cm high, showing symptoms of a disease known as wire stem. The pathogen damages the cortex causing deep lesions and sharply defined areas rendering stems wiry and slender. Similar symptoms in cotton are called sore shin (24). *Rizoctonia solani* also causes head rot in crucifers. After the plant has formed the head, *R. solani* invades from below spreading to the stem and leaves. Depending on the environmental conditions, the lesions may dry out becoming papery and brown (in dry weather) or the fungi can spread inside the head forming abundant sclerotia and mummifying it in as little as 10 days (24).

Control

Cultural management of *Rhizoctonia* diseases focus mainly on reducing free moisture. Irrigation should be applied in sufficient amounts to meet the water requirements of the plant, soil moisture sensors can be used to monitor soil moisture and irrigation can be planned accordingly (72). For turf, early morning irrigation encourages rapid drying which removes droplets of dew and guttation. Early morning mowing also reduces leaf wetness duration by removal of large dew droplets and guttation water from the leaves. Dew can also be removed by a practice known as "poling" that consists in dragging a pole, hose, rope, chain, or other object across the turf surface (139). Air movement can be improved by pruning or removal of surrounding trees and shrubs facilitating water evaporation. Installation of high-powered fans can also be used in high value plantations to remove water droplets helping to reduce the incidence of several diseases.

Improvement of soil drainage by installation of a tile and or modification of soil profile to increase porosity can help avoid moisture in the leaves (139).

Cultural practices such as crop rotation, the use of disease free propagation material and soil, and timing of harvest should be practiced when possible (141). Nitrogen fertilizer should be applied appropriately during the summer months since high quantities of this element enhance pathogen activity (31). *R. solani* antagonists including *Trichoderma harzianum*, fluorescent *Pseudomonas*, and *Verticillium viguttatum* may be used as a biological control but have limitations when applied on the field (141). Resistant varieties are available and can be used to manage diseases caused by *R. solani* including sheath spot, brown patch, sugarbeet root rot, and stem canker of potato (72; 139; 141). Lastly, chemical pesticides are used to control diseases caused by *R. solani.*

Pesticides used to control *Rhizoctonia solani*

In addition to flutolanil and azoxystrobin that have been described previously, there are several other fungicides used to manage disease caused by *Rhizoctonia* spp. including propiconazole and chlorothalonil. Propiconazole is a triazole and it has a broad spectrum of activity against fungal pathogens. Mode of application is typically a direct spray on plants at a rate of 0.122-0.183 kg a.i. x ha^{-1} (91). Propiconazole is a systemic fungicide that has preventive and curative activity. It acts by demethylation of C-14 during ergosterol biosynthesis, leading to accumulation of C-14 methyl sterols (50). Since biosynthesis of these ergosterols is critical to the formation of cell membrane of fungi, growth of the fungus stops, preventing further infection of host tissues. Therefore this chemical is considered fungistatic rather than fungicidal (50). There are many trade pesticide names containing propiconazole, including Banner®, Benit®, Desmel®, Orbit®, Radar®, Tilt®, Fidis®, and Feti-lome®. Depending on soil type, the half life of propiconazole ranges between 96 and 575 days (30).

Chlorothalonil is a protectant fungicide having a broad spectrum of activity. It is mainly used as a fungicide to control foliar diseases of vegetables, field, and ornamental crops. It can also be used as a wood protectant, antimold and antimildew agent, bactericide, microbiocide, algaecide, insecticide, and acaricide (14). Chlorothalonil binds to a molecule called glutathione intracellularly on which some enzymes are dependent. Enzymes that depend on glutathione are involved in cellular respiration. Binding of chlorothalonil to glutathione renders the molecule unavailable to those enzymes (138). It was first registered in the U.S. in 1966 where is used mainly on peanuts, potatoes, tomatoes, and golf courses. It can be applied as aerial, ground, spray or spreader applications. Application rates vary widely depending on the commodity from 0.0025-0.007 kg a.i. x ha^{-1} used for celery to 0.22-0.71 kg a.i. x ha^{-1} used on turfgrass (14).Trade names of chlorothalonil include Daconil® and Bravo®. Chlorothalonil is the second most widely used fungicide in terms of pounds produced per year and hectares it is applied to. Its half life in soil is one to two months (51).

CHAPTER III

METHODOLOGY

The main goal of this study was to determine the effect of low doses of disinfectants and fungicides on the growth of soilborne pathogens *in vitro* and assess the effect of the hormetic response if present. An experimental design that fits the correct assessment of hormetic responses in soilborne pathogens was established. Dose response parameters EC_{50} and NOAEL of disinfectants and fungicides on *Rhizoctonia zea*, *Rhizoctonia solani*, and *Pythium aphanidermatum* were determined. The experiments were divided in two stages: i) *In vitro* testing to assess the response of soilborne pathogens to different doses of chemicals; where hormesis may occur doses were examined further, and ii) Statistical analysis to determine EC_{50} and evaluate hormesis in the dose response curves, where the NOAEL and the maximum stimulation dose (MSD) were determined.

Inoculum

Pythium aphanidermatum (isolate P18 from Pennsylvania) was provided by Dr. Gary Moorman (Penn State University), *R. zea* and a *R. solani* (isolate Penncross 2007) with symptoms of brown patch were provided by Dr. Nathan Walker. Clean cultures were obtained either by the use of the semi-selective medium PARP (pimaricin + ampicillin + rifampicin + pentachloronitrobenzene [PCNB]) for *P. aphanidermatum* or by isolation from the edges of actively growing hyphae on corn meal agar (CMA) for *R. zeae* and *R. solani.*

Clean isolates were transferred to CMA and grown for 3 days in darkness at 28°C before transferring.

In vitro testing

Stock solutions of every chemical were prepared at 10X concentrations in sterile water and stored in amber glass bottles. Then serial dilutions were prepared from stock solutions. Solutions were mixed on a stir plate at medium speed for two minutes.

Corn meal agar (Becton Dickinson and company, Sparks, MD), Kzapeck Dox agar (KDA; Becton Dickinson and company, Sparks, MD), and potato dextrose agar (PDA; HiMedia Laboratories, India) were dispensed to 150 by 15 mm disposable petri dishes (VWR, Sugar Land, TX). Base media was prepared according to the manufacturer's instructions but at 1.1X concentration, dispensed in a number of flasks equal to the number of concentrations to be used on the experiment, and autoclaved at 121°C and 103.42 kPa for 20 min. After being autoclaved, the media was cooled down to 55°C on a water bath and then amended with the corresponding chemical solution. The agar solution was stirred for two minutes and poured into petri dishes, 25 ml of agar each, using a 25 ml disposable pipette.

Agar plugs with 5 mm of diameter were taken equidistantly from the center of a 2 to 3 day old culture of each fungus and placed on the middle of petri dishes corresponding to the different treatments. Petri dishes were sealed with parafilm. Radial growth was measured using a Kobalt 6" digital caliper after 24 h growth at 28 °C in darkness. Three replicates for each concentration were assessed for the dose response of pathogens to disinfectants, and for *R. zeae* vs. propiconazole. For the assessment of *P. aphanidermatum* vs. propamocarb, *P. aphanidermatum* vs. cyazofamid and *R. solani* vs. propiconazole, five replications were done for each concentration tested.

Dose response of soilborne pathogens to disinfectants

A series of experiments were done to assess the dose response of *P. aphanidermatum* and *R. zeae* to disinfectants in order to develop the methodology that would later be used to assess the response of pathogens to pesticides. Commercial formulation of sodium hypochlorite (Clorox, 6% active ingredient [a.i.], Oakland, CA), ethanol (99% a.i., Pharmco-AAPER, Brookfield, CT) were used.

For the experiments concerning ethanol the standard concentration for surface sterilization of 7.5×10^5 ppm was used as the initial reference dose. To determine the zone where ethanol had activity against the pathogen an experiment was performed with doses of 0 ppm and concentrations of ethanol beginning at 7.5×10^4 ppm, and then 7.5×10^3 ppm diluted a 100 fold to a final concentration of 7.5×10^{-15} ppm. Then, a second experiment to estimate the no observed adverse effect level (NOAEL) was carried out with concentrations 0, 47, 120, 300, 750, 1,900, 4,700, 12,000, 30,000, and 75,000 ppm ethanol. Since the estimated NOAEL ethanol dose was similar for *P. aphanidermatum* and *R. zeae*, the same concentrations (0, 19, 47, 120, 300, 750, 1,900, 4,700, 12,000, 30,000, 75,000 ppm ethanol) were used in order to determine hormetic effects for both microorganisms. Five repetitions of the experiment were done using *P. aphanidermatum* (Two repetitions were not considered for the data analysis due to poor fungal growth), and four repetitions using *R. zeae* over time.

The standard concentration for surface sterilization of 2×10^4 ppm was used as a reference for the experiments concerning sodium hypochlorite (SH). Experiments to determine when the disinfectant was active against the organisms were performed with doses of 0 ppm and concentrations of SH beginning at 2,000 ppm, and then 200 ppm diluted a 100 fold to a final concentration of 2×10^{-16} ppm. Experiments using concentrations of 0, 0.5, 1.3, 3.2, 8.0, 20, 50, 130, 320, 800, and 2,000 ppm SH were repeated three times using *P. aphanidermatum* and three

times using *R. zeae*. All the trials made for assessing the dose response of the fungus and the oomycete to ethanol and SH were used to standardize the methodology for the assessment of the hormetic effect of fungicides on the radial growth of these organisms *in vitro*. The need of having adequate doses to test for hormesis led to the use of the benchmark dose (BMD) as a standard reference concentration.

Dose response of soilborne pathogens to fungicides

Commercial formulations of different fungicides were used for the following experiments. *Pythium aphanidermatum* was tested against cyazofamid (Segway® 34.5 % a.i., FMC Corporation, West Point, GA) and propamocarb (Previcur Flex® 66.5 % a.i., Bayer CropScience, Kansas City, MO). *Rhizoctonia zeae* and *R. solani* were tested against propiconazole (Ferti-lome® 1.55% a.i., VPG, Bonham, TX). Application rates of the compounds were used as a first reference for the dosage of the treatments. Application rates defined in kg a.i. x ha^{-1} were transformed to ppm a.i. by multiplying them by 4.17, assuming a soil bulk density of 1.2 g cm^{-3} and an effective soil depth of 2 cm (46).

In order to determine the BMD of *R. zeae* vs. propiconazole, concentrations of the active ingredient beginning at 1,000 ppm and diluted 10 fold to a final concentration of 1×10^{-6} ppm, and a 0 ppm control were evaluated on CMA. Using the BMD as a reference, a series of four experiments with concentrations 0, 1.6×10^{-4}, 4×10^{-4}, 1×10^{-3}, 2.5×10^{-3}, 6.4×10^{-3}, 1.6×10^{-2}, 4×10^{-2}, 0.1, and 1 ppm a.i. were conducted, three of these experiments were done using CMA as base medium and one using KDA. A defined medium (KDA) was used to determine if there would be any difference in the dose response when sterols where not present in the media. The data obtained from the later experiment was not used for the modeling of the dose response curve since radial growth measurements could only be taken at 72h, because of the slow growth of *R. zeae* on KDA.

An experiment with doses of 0 ppm and cyazofamid concentrations beginning at 3,300 ppm and diluted 10 fold to a final concentration of 3.3 x 10^{-5} ppm served for determining the BMD of this active ingredient on *P. aphanidermatum.* Then, six repetitions with concentrations ranging from 3.3 x 10-2 ppm to 3.3 x 10-10 ppm a.i. were done before defining a standardized protocol. Once a standardized protocol was defined, five experiments using concentrations 0, 0.0033, 0.01, 0.033, 0.1, 0.33, 1, 3.3, 33, and 330 ppb a.i. were performed.

For testing *P. aphanidermatum* vs. propamocarb and *R. solani* vs. propiconazole standardized doses based on reference concentrations were used. CMA was used as base medium for the growth of *P. aphanidermatum* and PDA for the growth of *R. solani*. Ten different doses and a control for each organism were tested with a minimum of five doses below the BMD. Five replicates for each treatment and five repetitions of the experiment were performed over time.

For determining the BMD, the minimum application rate (MAR) of each a.i. was used as a reference and doses of 0 ppm and a.i. concentrations beginning at MAR x 10^{2} and diluted 10 fold to a final concentration of MAR x 10^{-2} were tested. Concentrations of 0, 0.06, 0.6, 6, 60, and 600 ppm propamocarb and 0, 0.005, 0.05, 0.5, 5, and 50 ppm propiconazole were used on *P. aphanidermatum* and *R. solani*, respectively. Once the BMD was determined, it was used as reference for a new set of experiments with doses of 0, BMD x 10^{-2}, BMD x $10^{-1.6}$, BMD x $10^{-1.2}$, BMD x $10^{-0.8}$, BMD x $10^{-0.4}$, BMD, BMD x 10, BMD x 10^{2}, and BMD x 10^{3} ppm a.i.. Resulting concentrations of 0, 0.02, 0.051, 0.13, 0.32, 0.8, 2, 5, 50, 500, and 5000 ppm propamocarb and 0, 0.0003, 0.00075, 0.0019, 0.0048, 0.012, 0.03, 0.3, 3, 30, and 300 ppm propiconazole were used for the modeling of the dose response curves of *P. aphanidermatum* and *R. solani*, respectively.

Data analysis

Benchmark dose calculation

Mean response and standard deviation of growth at concentrations 0 ppm, MAR x 10^{-1}, MAR x 10^{-1}, MAR x 10, MAR, MAR x 10, and MAR x 10^{2} ppm a.i. were calculated using EXCEL® (Microsoft, Redmond, WA). The BMD was calculated using the US Environmental Protection Agency National Center for Environmental Assessment Software BMDS 2.1(available online at http://www.epa.gov/ncea/bmds/progreg.html). A continuous Hill model using default parameters was run following the program's guidelines (61). The benchmark response was set as a change in the mean equal to one control standard deviation from the control mean and a 0.95 confidence level was used. Global measures for continuous models defined by the program and visual examination were considered to determine the appropriateness and fit of the model. If there was no fit to the model the obtained BMD was rejected and the test was run again with fewer data or data generated in a new experiment using different doses.

Test for hormesis and calculation of EC_{50} and NOAEL

A Brain-Cousens model was used to detect the presence of hormesis and to estimate the EC_{50} and NOAEL (125). The curve model was generated using non-linear modeling procedure PROC-NLIN (SAS 9.2, SAS Institute, Cary, NC). The Brain-Cousens model is defined by equation 1 where the EC_{50} can be estimated, and γ is the rate of increase at small doses. If the 95% confidence interval for γ includes 0, then no significant hormetic response exists. If hormesis is significant, the 95% confidence interval for γ would only include positive values.

Equation 1:

$$E\left[\frac{y}{x}\right] = \delta + \frac{\alpha - \delta + \gamma x}{1 + \omega \, exp\left[\beta ln\left(\frac{x}{EC_{50}}\right)\right]}$$

where

$$\omega = 1 + \frac{2\gamma EC_{50}}{\alpha - \delta}$$

$E\left[\frac{y}{x}\right]$: average response at dosage x

α: upper bound

δ: lower bound

β: slope at the EC_{50} dose

For model building purposes, initial parameters: β, γ, and EC_{50} were estimated by visual examination of the data. Radial growth data was transformed to percent response from the control. Since the upper limit of the curve was determined by the radial growth of the control, α was fixed at 100 and the lower limit δ was fixed at 0 (total growth inhibition) on the curve modeling program (125). When hormesis was present, equation 2 was modeled to estimate the NOAEL of the compound tested.

Equation 2:

$$E\left[\frac{y}{x}\right] = \delta + \frac{\alpha - \delta + \gamma x}{1 + \omega\ exp\left[\beta ln\left(\frac{x}{NOAEL}\right)\right]}$$

where

$$\omega = \frac{\gamma NOAEL}{\alpha - \delta}$$

To estimate the dose at which the MSD occurs equation 3 was modeled.

Equation 3:

$$E\left[\frac{y}{x}\right] = \delta + \frac{\alpha - \delta + \gamma x}{1 + \omega\, exp\left[\beta ln\left(\frac{x}{MSD}\right)\right]}$$

where

$$\omega = \frac{\gamma MSD}{(\alpha - \delta)\beta - MSD\gamma(1-\beta)}$$

If the data didn't show a hormetic response (i.e. confidence interval for γ includes 0) the EC_{50} was estimated by modeling equation 4, a log-logistic model where no hormetic effects are considered.

Equation 4:

$$E\left[\frac{y}{x}\right] = \delta + \frac{\alpha - \delta}{1 + exp\left[\beta ln\left(\frac{x}{EC_{50}}\right)\right]}$$

For graphing the dose response curve, the estimated values of EC_{50} and β were replaced on either equation 1 or 2 and the $E\left[\frac{y}{x}\right]$ was calculated on EXCEL® for several doses including the estimated NOAEL and MSD. Results were plotted against the natural logarithm of the corresponding doses. Estimated parameters corresponding to concentrations are expressed on the results as the natural logarithm of the concentration in ppm, or ppb in the case of cyazofamid.

CHAPTER IV

RESULTS

Dose response of *Pythium aphanidermatum* to ethanol

The radial growth of *P. aphanidermatum* in response to low doses of ethanol *in vitro* was defined by equation 1. The modeled biphasic dose response curve shows 20% stimulation of *P. aphanidermatum* at the MSD (Fig. 1).

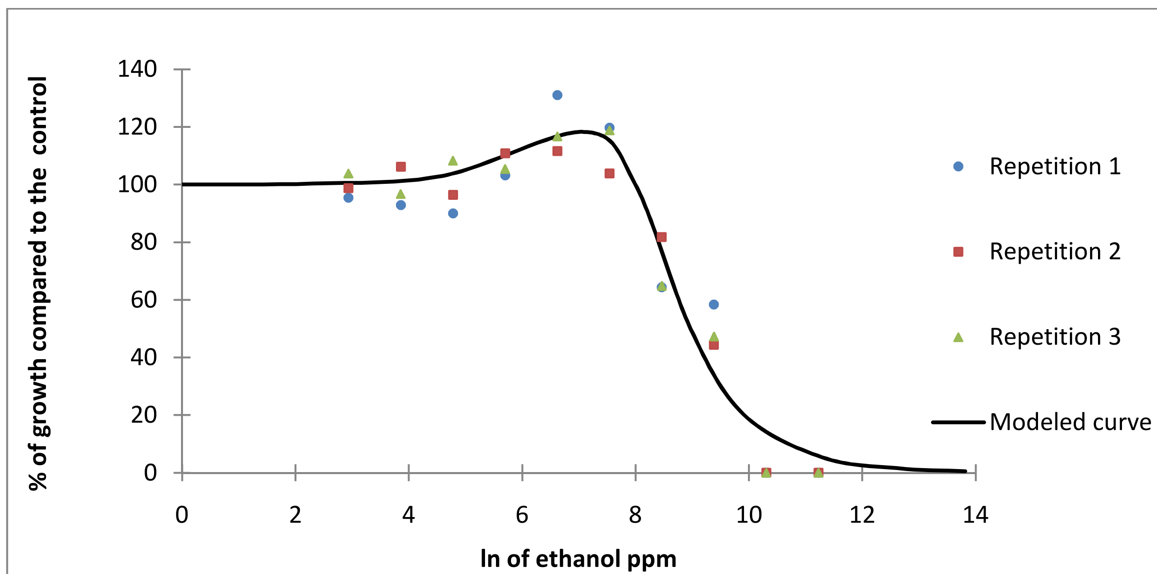

Figure 1. Observed values and modeled curve of the radial growth of *P. aphanidermatum* in response to low doses of ethanol. Each data point represents the mean value of three replicates. All replicates were used for building the curve model.

Parameter estimates and approximate 95% confidence limits are shown in table 1. According to the model the EC_{50} of ethanol on *P. aphanidermatum* was 8.97 and β was 1.9, both parameters define the shape of the curve. The 95% confidence limits of γ ranged from 0.014 to 0.049 indicating an increase in growth at low doses. When equations 2 and 3 were modeled using the *P. aphanidermatum* vs. ethanol data they rendered estimated NOAEL and MSD values of 7.99 and 7.09 respectively. From the doses tested, in addition to the control, 6 were below the NOAEL.

Table 1. Parameters defining the dose response relationship between ethanol concentration and radial growth of *P. aphanidermatum in vitro*.

Parameter	Estimated values	Approximate 95% confidence limits	
		Lower bound	Upper bound
β	1.90	1.67	2.1
EC_{50}	8.97	8.81	9.11
γ	0.032	0.014	0.049
$NOAEL$	7.99	7.78	8.17
MSD	7.09	6.80	7.32

β: slope at the EC_{50} dose; EC_{50}: dose at which radial growth is 50% of the control ; γ: rate of increase at small doses; $NOAEL$: No observed adverse effect level; MSD: Maximum stimulation dose; EC_{50}, $NOAEL$ and MSD are expressed as ln of the concentration in ppm.

Dose response of *Rhizoctonia zeae* to ethanol

The radial growth of *R. zeae* in response to ethanol *in vitro* is defined by equation 1. The biphasic dose response curve shows a maximum growth stimulation of 10% at the MSD (Fig. 2). Parameter estimates and approximate 95% confidence limits are shown in Table 3. According to the model the EC_{50} of ethanol on *R. zeae* was 9.62 and β was 2.83, both parameters define the shape of the curve. The 95% confidence limits of γ ranged from 0.00083 to 0.0063 indicating an increase in growth at low doses. When equations 2 and 3 were modeled using the *R. zeae* vs.

ethanol data they rendered estimated NOAEL and MSD values of 8.89 and 8.26, respectively. From the doses tested, besides the control, 7 were below the NOAEL.

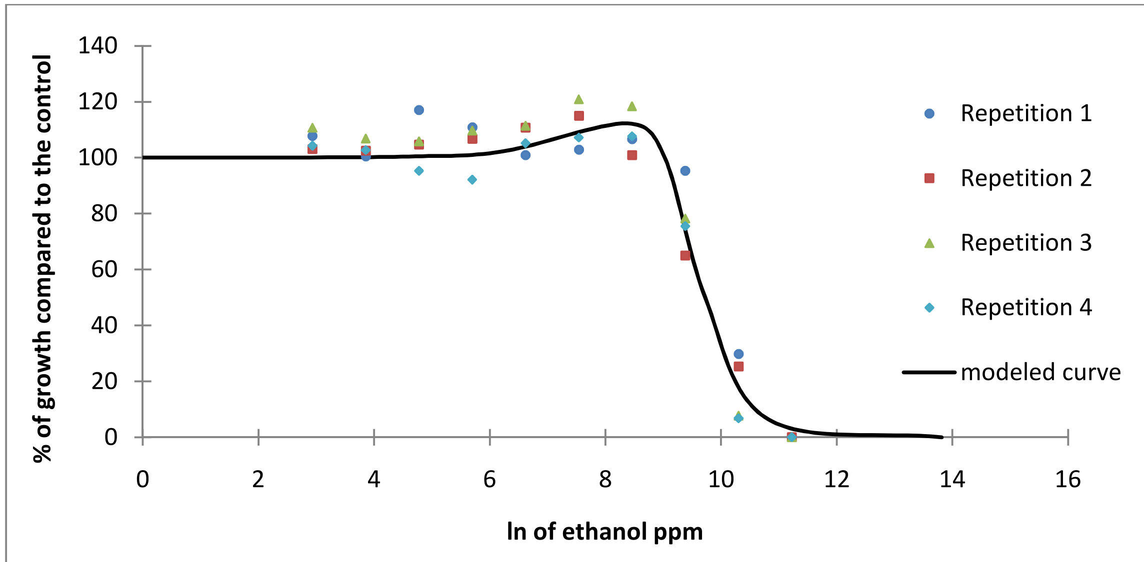

Figure 2. Observed values and modeled curve of the radial growth of *R. zeae in vitro* in response to low doses of ethanol. All observations are presented as means across replicates within each repetition. All replicates and repetitions were used in the development of the model.

Table 2. Parameters defining the dose response relationship between ethanol concentration and radial growth of *R. zeae in vitro*.

Parameter	**Estimated values**	**Approximate 95% confidence limits**	
		Upper bound	**Lower bound**
β	2.83	2.23	3.42
EC_{50}	9.62	9.53	9.71
γ	0.0036	0.00083	0.0063
$NOAEL$	8.89	8.63	9.08
MSD	8.26	7.97	8.49

β: slope at the EC_{50} dose; EC_{50}: dose at which radial growth is 50% of the control ; γ: rate of increase at small doses; $NOAEL$: No observed adverse effect level; MSD: Maximum stimulation dose; EC_{50}, $NOAEL$, and MSD are expressed as ln of the concentration in ppm.

Dose response of *Pythium aphanidermatum* to sodium hypochlorite

When the data were fit to equation 1, γ was estimated to be 0 with no standard deviation. These results indicate no significant increase in growth of *P. aphanidermatum* at low doses of SH *in vitro*, therefore the data were fit using equation 4 (Fig. 3). No increase at low doses was observed either when other below the BMD SH concentrations were tested (Appendix A).

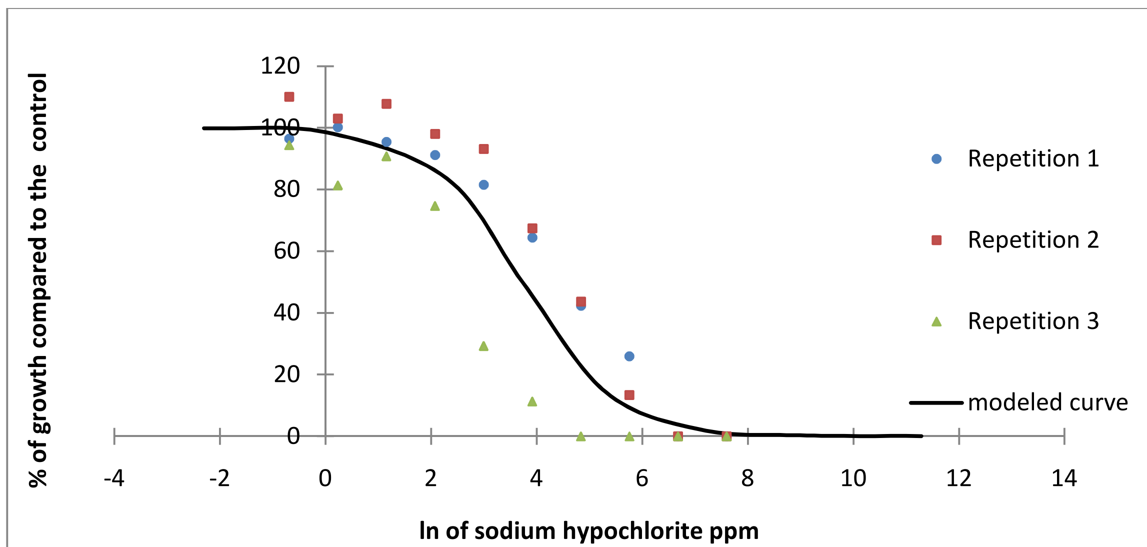

Figure 3. Observed values and modeled curve of the radial growth of *P. aphanidermatum* in response to low doses of sodium hypochlorite. All observations are presented as means across replicates within each repetition. All replicates and repetitions were used in the development of the model.

Since there wasn't a defined dose at which radial growth equaled the radial growth of the control, the NOAEL could not be determined. According to the log logistic model defined by equation 4, the EC_{50} of SH on *P. aphanidermatum* was 3.73 and β was 1.14; both parameters define the shape of the modeled curve (Table 5).

Table 3. Parameters defining the dose response relationship between sodium hypochlorite concentration and radial growth *of P. aphanidermatum in vitro*.

Parameter	Estimated values	Approximate 95% confidence limits	
		Upper bound	Lower bound
β	1.14	0.87	1.40
EC_{50}	3.73	3.46	3.94

β: slope at the EC_{50} dose; EC_{50}: dose at which radial growth is 50% of the control expressed as ln of the concentration in ppm.

Dose response of *Rhizoctonia zeae* to sodium hypochlorite

When the data were fit to equation 1, γ was estimated to be 0 with no standard deviation. These results indicate no significant increase in growth of *R. zeae* at low doses of SH *in vitro*, therefore the data were fit using equation 4 (Fig.4).

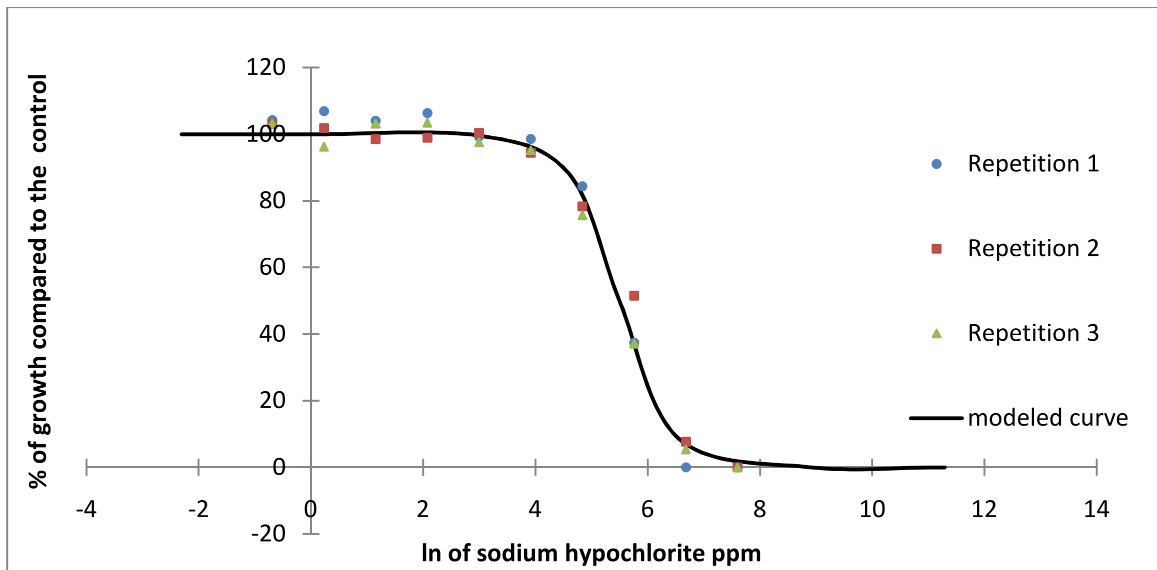

Figure 4. Observed values and modeled curve of the radial growth of *R. zeae in vitro* in response to low doses of sodium hypochlorite. All observations are presented as means across replicates within each repetition. All replicates and repetitions were used in the development of the model.

Since there wasn't a defined dose at which radial growth equaled the radial growth of the control the NOAEL could not be determined. According to the log logistic model defined by equation 4, the EC_{50} of SH on *R. zeae* was 2.23 and β was 2.23; both parameters define the shape of the modeled curve (Table 6).

Table 4. Parameters defining the dose response relationship between sodium hypochlorite concentration and radial growth of *R. zeae in vitro*.

Parameter	**Estimated values**	**Approximate 95% confidence limits**	
		Upper bound	**Lower bound**
β	2.23	1.97	2.49
EC_{50}	5.50	5.44	5.55

β: slope at the EC_{50} dose; EC_{50}: dose at which radial growth is 50% of the control expressed as ln of the concentration in ppm.

Dose response of *Rhizoctonia zeae* to propiconazole

The MAR to assess the dose response of *P. aphanidermatum* to propiconazole was set at 1 ppm. The determined BMD was 0.0064 MAR (0.0064 ppm). When the data were fit to equation 1, γ 95% confidence lower bound was negative. The data were then fit to the model defined by equation 4 (Fig. 5). When KD was used as base medium a similar dose response was observed with no stimulation at low doses (Appendix A).

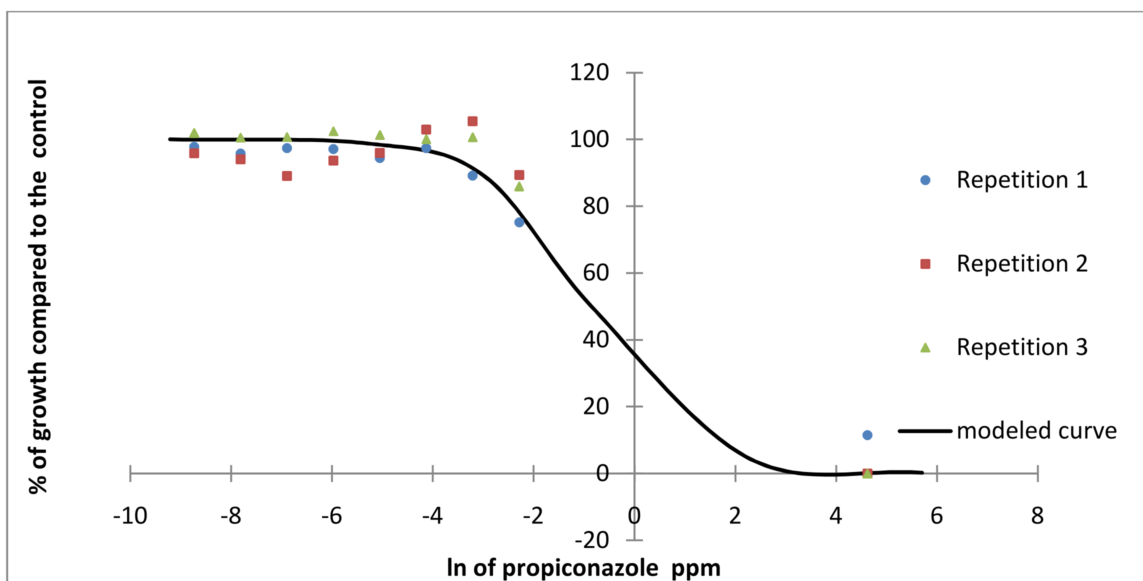

Figure 5. Observed values and modeled curve of the radial growth of *R. zeae in vitro* in response to low doses of propiconazole. All observations are presented as means across replicates within each repetition. All replicates and repetitions were used in the development of the model.

No stimulation of the growth of *R. zeae in vitro* in response to low doses of propiconazole was shown. Since there wasn't a defined dose at which radial growth equals the radial growth of the control the NOAEL could not be determined. According to the log logistic model defined by equation 4, the EC_{50} of propiconazole on *R. zeae* was -0.84 and β was 0.98; both parameters define the shape of the modeled curve (Table 7).

Table 5. Parameters defining the dose response relationship between propiconazole concentration and radial growth of *R. zeae in vitro*.

Parameter	Estimated values	Approximate 95% confidence limits	
		Upper bound	Lower bound
β	0.98	0.61	1.36
EC_{50}	-0.84	-1.96	-0.33

β: slope at the EC_{50} dose; EC_{50}: dose at which radial growth is 50% of the control expressed as ln of the concentration in ppm.

Dose response of *Pythium aphanidermatum* to cyazofamid

Based on a minimum application rate of 0.08 kg a.i. x ha^{-1} the MAR to assess the dose response of *P. aphanidermatum* to cyazofamid was set at 0.33ppm. The BMD was determined to be 0.1 MAR (0.033 ppm). The radial growth of *P. aphanidermatum* in response to low doses of cyazofamid *in vitro* is defined by equation 1 (Fig. 6). The biphasic dose response curve shows a maximum growth stimulation of 6% at the MSD.

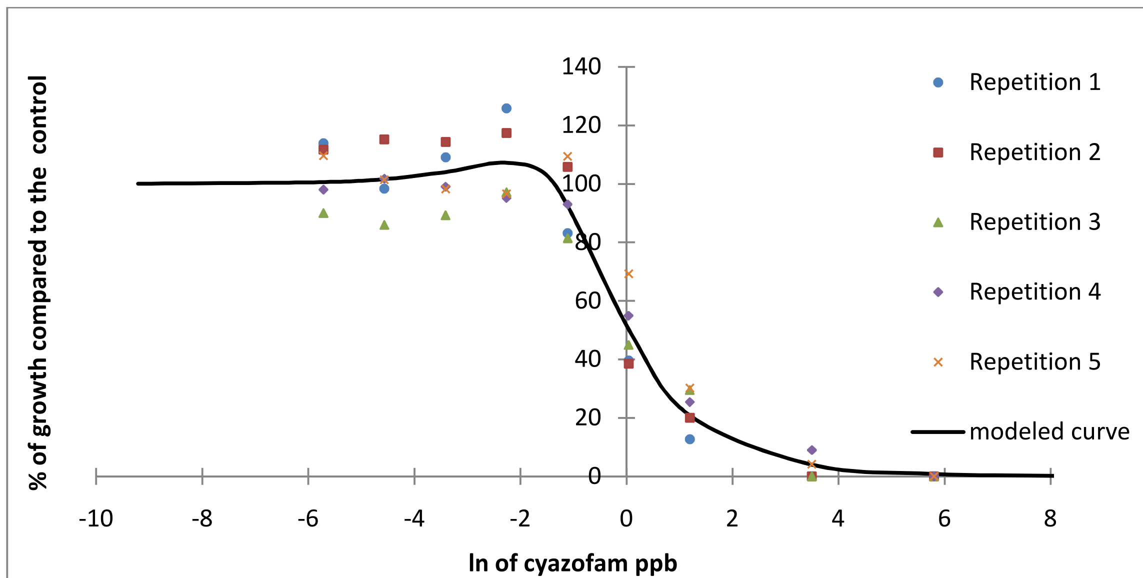

Figure 6. Observed values and modeled curve of the radial growth of *P. aphanidermatum in vitro* in response to low doses of cyazofamid. All observations are presented as means across replicates within each repetition. All replicates and repetitions were used in the development of the model.

According to the model the EC_{50} of cyazofamid on *P. aphanidermatum* was 0.58 and β was 1.67, both parameters define the shape of the curve. The 95% confidence limits of γ ranged from 54.28 to 279.1 indicating an increase in growth at low doses. Radial growth of *P.*

aphanidermatum at -1.34 was equal to the radial growth on the control and the estimated MSD was -2.21 (Table 8). From the doses tested, besides the control, 4 were below the NOAEL.

Table 6. Parameters defining the dose response relationship between cyazofamid concentration and radial growth of *P. aphanidermatum in vitro*.

Parameter	Estimated values	Approximate 95% confidence limits	
		Lower bound	Upper bound
β	1.67	1.53	1.79
EC_{50}	0.58	-0.83	0.17
γ	166.7	54.28	279.1
$NOAEL$	-1.34	-1.61	-1.14
MSD	-2.21	-2.52	-2.04

β: slope at the EC_{50} dose; EC_{50}: dose at which radial growth is 50% of the control ; γ: rate of increase at small doses; $NOAEL$: No observed adverse effect level; MSD: Maximum stimulation dose; EC_{50}, $NOAEL$ and MSD are expressed as ln of the concentration in ppb.

Dose response of *Pythium aphanidermatum* to propamocarb

Based on a minimum application rate of 1.44 kg a.i. x ha^{-1} the MAR to assess the dose response of *P. aphanidermatum* to propamocarb was set at 6 ppm. The BMD was determined to be 0.08 MAR (0.5 ppm). The radial growth of *P. aphanidermatum* in response to low doses of propamocarb *in vitro* is defined by equation 1 (Fig. 7). The biphasic dose response curve shows a maximum growth stimulation of 6% at the MSD.

According to the model the EC_{50} of propamocarb on *P. aphanidermatum* was 2.70 and β was 1.37, both parameters define the shape of the curve. The 95% confidence limits of γ ranged from 14.08 to 56.26 suggesting an increase in growth at low doses. At 0.57 the growth of *P.*

aphanidermatum was equal to the growth on the non amended control and the estimated MSD was -0.45 (Table 10). From the doses tested, besides the control, 5 were below the NOAEL.

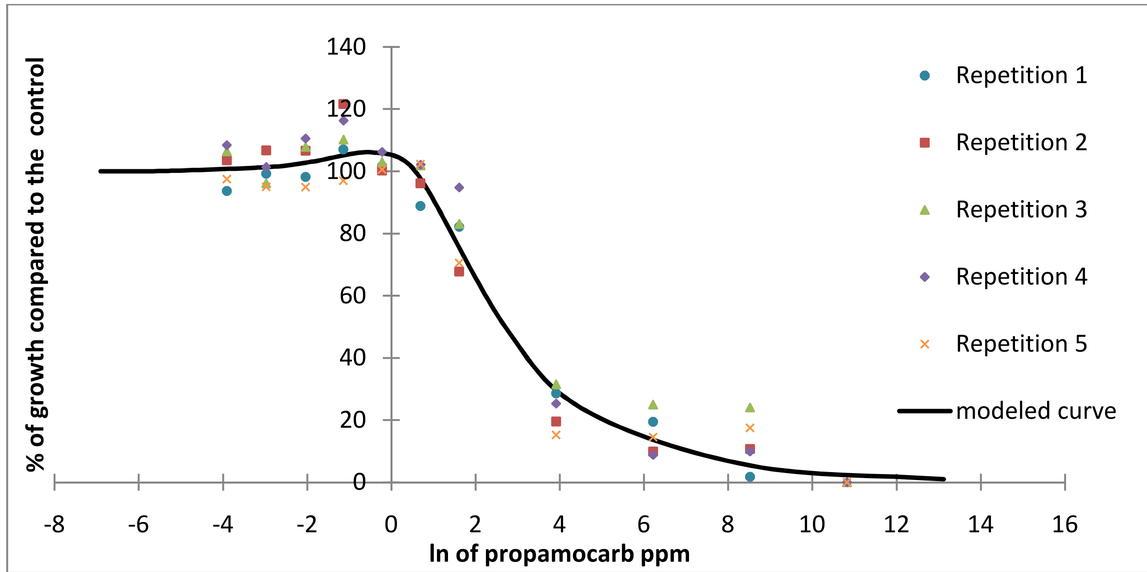

Figure 7. Observed values and modeled curve of the radial growth of *P. aphanidermatum in vitro* in response to low doses of propamocarb. All observations are presented as means across replicates within each repetition. All replicates and repetitions were used in the development of the model.

Table 7. Parameters defining the dose response relationship between propamocarb concentration and radial growth of *P. aphanidermatum in vitro*.

Parameter	Estimated values	Approximate 95% confidence limits	
		Lower bound	Upper bound
β	1.37	1.32	1.42
EC_{50}	2.70	2.50	2.87
γ	35.17	14.08	56.26
$NOAEL$	0.57	0.30	0.78
MSD	-0.45	-0.69	-0.24

β: slope at the EC_{50} dose; EC_{50}: dose at which radial growth is 50% of the control ; γ: rate of increase at small doses; $NOAEL$: No observed adverse effect level; MSD: Maximum stimulation dose; EC_{50}, $NOAEL$ and MSD are expressed as ln of the concentration in ppm.

Dose response of *Rhizoctonia solani* to propiconazole

The MAR to assess the dose response of *R. solani* to propiconazole was set at 0.5 ppm. The determined BMD was 0.06 MAR (0.03 ppm). When the data were fit to equation 1, γ 95% confidence lower bound was negative; therefore no stimulation at low doses was inferred. The data were then fit to the model defined by equation 4 (Fig. 8). When KD was used as base medium a similar dose response was observed with no stimulation at low doses (Appendix A).

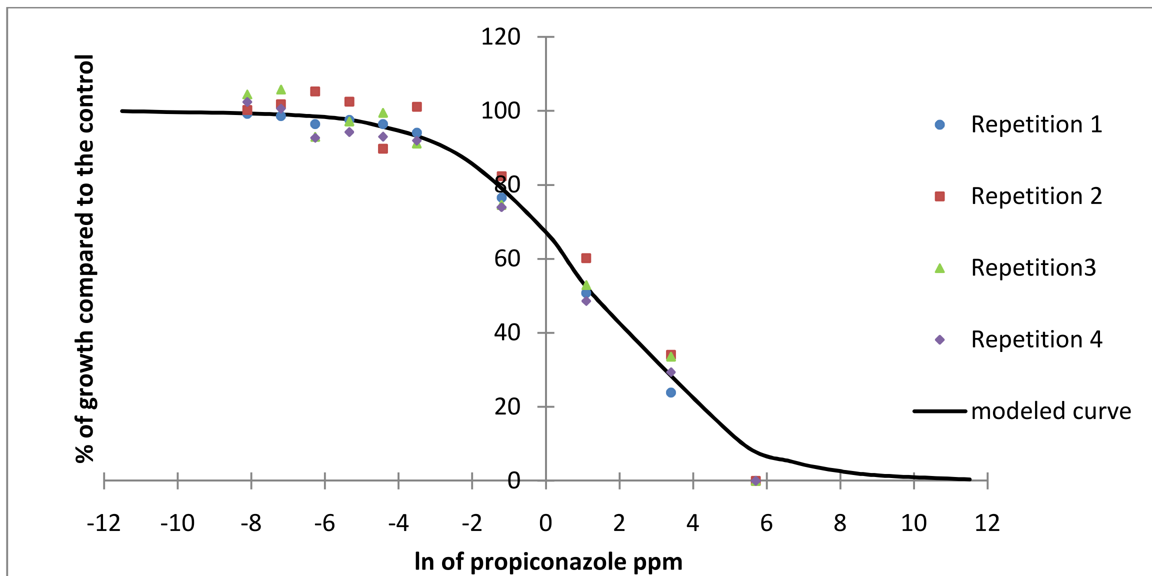

Figure 8. Observed values and modeled curve of the radial growth of *R. solani in vitro* in response to low doses of propiconazole. All observations are presented as means across replicates within each repetition. All replicates and repetitions were used in the development of the model.

Since there wasn't a defined dose at which radial growth equals the radial growth of the control the NOAEL could not be determined. According to the log logistic model defined by equation 4, the EC_{50} of propiconazole on *R. solani* was 1.33 and β was 0.54; both parameters define the shape of the modeled curve (Table 12).

Table 8. Parameters defining the dose response relationship between propiconazole concentration and radial growth of *R. zeae in vitro*.

Parameter	Estimated values	Approximate 95% confidence limits	
		Upper bound	Lower bound
β	0.54	0.51	0.57
EC_{50}	1.33	1.18	1.46

β: slope at the EC_{50} dose; EC_{50}: dose at which radial growth is 50% of the control expressed as ln of the concentration in ppm.

When the standardized protocol was used, this was the case of *R. solani* vs. propiconazole (Table 12), the standard error for beta decreased near to 7 fold and the standard error for EC_{50} decreased almost 5 fold, compared with the assessment of *R. zeae* vs. propiconazole (Table 7).

CHAPTER V

CONCLUSIONS AND DISCUSSION

In the dose response of *P. aphanidermatum* to ethanol the estimated NOAEL was 2.5 times greater than the MSD which was consistent with what Calabrese and Baldwin described as one of the general attributes of the hormetic dose response (36). In the model of the dose response of *R. zeae* to ethanol the estimated NOAEL was 1.9 times greater than the MSD. This feature was not consistent with Calabrese and Baldwin descriptions, putting in evidence the variability of the phenomenon. Even though hormetic responses are described as generally having a 30-60% maximum stimulation, the positive values of the 95% confidence limits for γ show that stimulation at low doses of ethanol was significant for the growth of both *P. aphanidermatum* and *R. solani* which had maximum stimulations of 20% and 10% respectively.

Ethanol inhibited radial growth in *R. zeae* and *P. aphanidermatum* at concentrations 10 times less than the surface sterilization concentration (75,000 ppm) but had hormetic effects on both organisms at concentrations below 3,000 ppm. Since ethanol is a highly energetic carbohydrate, stimulation of growth could be attributed to the use of the chemical as a carbon source. However, with cornmeal is more than 70% starch and sugar, (96) and the use of ethanol over more abundant and readily available carbon sources is unlikely (58).

There are various mechanisms for ethanol toxicity including NAD/NADH imbalances, acetaldehyde accumulation and deactivation of replication processes (87). For cells that are

metabolically active at high ethanol concentrations, water stress is another factor that may account for more than 30% of growth inhibition (77). Ethanol exposure can also cause cellular membrane disorder and protein denaturation (119). As ethanol toxicity happens in different ways, there are also different means in which organisms react to its presence. Chemical hormesis is commonly observed and highly generalizeable in the case of ethanol (41). On animals, hormetic effects of ethanol on motor activity, electro-encephalographic activation and gastric acid secretion are well documented (120). For yeasts, responses to sublethal doses of ethanol are identical to sublethal heat exposure. Heat shock proteins including Hsp104 that contributes to ethanol tolerance and anti-oxidant enzymes are induced (119). When exposed to ethanol, plasma membrane H^+-ATPase (protein responsible for maintaining the proton gradient across the membrane) levels decrease dramatically but the remaining H^+-ATPase is stimulated (119). This phenomenon causes a proton influx to the cell followed by an enhanced proton efflux catalyzed by the remaining H^+-ATPase when ethanol concentrations are low. A transient increase in proton concentration inside the cell can stimulate growth factor formation (106). On the other hand, when severe stress is present proton gradient can't be restored and high proton concentration inside the cell may cause activation of proteases, production of oxygen radicals and ATP depletion damaging and ultimately killing the cell (106; 118).

For rats, the effect of prenatal ethanol exposure on birth weight is biphasic (1) and one of the speculative explanations of the increase in weight at low doses is the higher rate of protein synthesis at low dose ethanol exposure (68). It is likely that there's not one single mechanism that is acting on the increased radial growth of the organisms at low doses of ethanol, but rather a combination of processes that are triggered once homeostatic conditions are lost. The observed stimulation at low doses of ethanol highlights the importance of keeping adequate concentrations of the disinfectant when sanitizing working surfaces and tools.

No hormetic response to sodium hypochlorite was detected on either *P. aphanidermatum* or *R. zeae*. Sodium hypochlorite acts as a solvent on fatty acids, neutralizing and degrading amino acids by hydrolysis, forming chloramines that interfere in cell metabolism and inhibiting enzymes acting as a strong base or by the strong oxidant activity of chlorine (65). Even though there have been reports of sodium hypochlorite being stimulatory at low doses for the growth of dermal fibroblasts (81), there appeared to be no hormetic effect on the growth of either *R. solani* and *P. aphanidermatum*. This probably has to do with the diverse mode of action of the chemical that may overwhelm the fungal and oomycete mechanism for reaching homeostasis even at low concentrations of the disinfectant. These results suggest that hormesis may not be a generalizable phenomenon independent of environmental stressor as it has been proposed (39).

No hormetic effect of propiconazole was observed on either of the *Rhizoctonia* species tested. Propiconazole acts by preventing ergosterol biosynthesis, essential for cell wall formation (50). The fact that compensation mechanisms to overcome this mode of action may not result in increased growth, does not mean that such mechanisms do not exist. Further experimentation on the effect of small doses of propiconazole on *Rhizoctonia* virulence *in planta* is recommended.

Propiconazole dose response experiments of *R. zeae* and *R. solani* with Kzapeck Dox as base medium showed slower growth compared to the use of either CMA or PDA as base, but in both cases, a similar behavior, with no significant hormetic response was observed when growth data were transformed to a percentage of the control. This suggests that media composition does not alter the radial growth dose response of *R. zeae* or *R. solani* to propiconazole *in vitro*. When the standardized protocol was used, which was the case of *R. solani* vs. propiconazole, the confidence limits for EC_{50} and beta narrowed down greatly compared with the assessment of *R. zeae* vs. propiconazole. This shows that the standardized protocol has a better reproducibility rendering results that are more reliable.

A biphasic dose response of cyazofamid on *P. aphanidermatum* was observed when adequate doses were tested. Cyazofamid specifically inhibits complex III (bc1 complex; ubiquinol:cytochrome c oxidoreductase) activity of the respiratory pathway on oomycetes (108). It is well known that many phytopathogenic eukaryotes can use an alternative pathway that enables respiration to continue even in the presence of complex III inhibitors (88). When *Pythium spinosum* was exposed to 3.25 ppm cyazofamid it recovered respiration after one hour. When exposed to potassium cyanide (KCN), an inhibitor of the standard respiratory pathway, it showed resistance to the chemical, while the same treated oomycete was sensitive to presence of Salicylhydroxamic Acid (SHAM), an inhibitor of the alternative pathway (108). These results suggest a degree of adaptation of the oomycete to the challenge of a respiratory chain inhibitor. Furthermore, in plants, whose mitochondria display a similar array of respiratory pathways as in plant pathogenic eukaryotes, low doses of respiration inhibitors (KCN, azide, and SHAM) showed stimulation in oxygen uptake when present individually but KCN and SHAM together are inhibitory (129). This shows an increased activity of either pathway when the other is inhibited.

According to the experimental data, the zone of stimulation of cyazofamid on *P. aphanidermatum* had a 3 fold range; the estimated NOAEL was 2.4 times greater than the MSD. Both of these characteristics were consistent with what Calabrese and Baldwin described as general attributes of the hormetic dose response (36). Values for γ in the 95% confidence range didn't include 0 therefore hormesis was significant with a maximum growth stimulation of 6%. After five repetitions of the experiment the 95% confidence limits for γ were still widely spaced, this is probably due to the fact that maximum stimulation was achieved at different doses on different repetitions. Inconsistence in the maximum stimulation dose was probably a consequence of cyazofamid being active against the P18 isolate at part per billion (ppb) concentrations, making experimental error more likely.

Hormesis was detected on the dose response of *P. aphanidermatum* to propamocarb. Propamocarb acts on *Pythium* by disrupting cell membrane structure producing a leak of cytoplasmic materials (115). A possible explanation for the hormetic effect of propamocarb on *P. aphanidermatum* is the stimulation produced of augmented Ca^{2+} influx to the cell due to a transient increased permeability at low doses. Calcium influx regulates several intracellular events (71) including tip growth on hyphal cells (85). High Ca^{2+} concentration inside the cell can induce the synthesis of heat shock proteins (HSP) that regulate homeostasis under stressful conditions by the activation of different mechanisms (53). Acting mostly as chaperones, HSP aid in the correct folding of damaged proteins (53); an over expression of these HSP may also result in a hormetic effect on the hyphae radial growth. Furthermore, there is evidence that calcium influx is involved in growth hormesis on plant systems (4; 5).

The zone of stimulation of propamocarb on *P. aphanidermatum* that had a 6 fold range and the estimated NOAEL which was 2.8 times greater than the MSD were consistent with what Calabrese and Baldwin described as general attributes of the hormetic dose response (36). Values for γ in the 95% confidence range didn't include 0 therefore hormesis was significant with a maximum growth stimulation of 6%. For the modeled dose response of *P. aphanidermatum* against propamocarb the 95% confidence intervals for all parameters were narrower compared to the ones obtained from the curve modeling of *P. aphanidermatum* against cyazofamid. This is probably due to the use of higher concentrations in the experiments involving propamocarb. *P. aphanidermatum* (isolate P18) was characterized as resistant to both mefenoxam and propamocarb by Dr. Gary Moorman. Resistance was evident showing that the activity of propamocarb against *P. aphanidermatum* (P18) was 10,000 times lower than the activity of cyazofamid against the same isolate in terms of EC_{50}.

Previous studies of the dose response of the *P. aphanidermatum* (P18) to mefenoxam, to which the oomycete is resistant, showed *in vitro* growth stimulation of up to 22% at low doses

(74). While stimulation at small concentrations of propamocarb (pesticide with which the isolate might have had previous contact) and cyazofamid (pesticide with which the isolate hasn't have previous contact) was of 10% and 7% respectively. These results support the hypothesis that hormesis may be linked to organism plasticity (35). Considering that under stressful conditions there is the possibility for an organism to undergo an adaptive mutation, which is induced by the stressor rather than just being selected by it (122); there is the chance that the stress caused by pesticide exposure may induce mutations that render the pathogen resistant.

The statistical significance of hormetic responses can be determined by curve modeling and can only be assessed if adequate doses are tested. If dose spacing is too wide stimulant concentrations may be overlooked. The BMD proved to be a valuable reference for determining doses that would fall in the hormetic zone. When standardized doses based on the BMD were tested and hormesis was present, a minimum of four doses fell below the NOAEL. Calabrese recommends a minimum of five equally spaced doses below the NOAEL to test for hormesis (37) so an extra dose below the BMD could be included in further experiments to meet this condition. Other requirements for an experimental design for assessing hormesis include the determination of the NOAEL and the dose separation smaller than one order in magnitude (37); both of these requisites are fulfilled by the protocol developed on this study. Furthermore, Crump's criteria for evaluating hormesis that include strength of evidence, soundness of data, consistency and biological plausibility are met by the protocol. It is also important to emphasize that hormesis should be taken into account when modeling dose effect relationships between pesticides and plant pathogens since there is a shift on the EC_{50} value (that may be significant) when hormetic effects are considered in the model (125). This study shows statistical evidence that stimulation occurs in the growth of oomycete and fungal plant pathogens *in vitro* in response to low doses of disinfectants or pesticides; it also provides tools to test for the occurrence of hormesis in other systems.

REFERENCES

1. Abel, E. L. 1995. Effects of prenatal alcohol exposure on birth weight in rats: Is there an inverted u-shaped function? Alcohol (1):99-102.

2. Adams, G. C. 1988. *Thanatephorus cucumeris (Rhizoctonia solani)*, a species complex of wide host range. Advances in plant pathology (6):535-552.

3. Adl, S. M., A. G. B. Simpson, M. A. Farmer, R. A. Andersen, O. R. Anderson, J. R. Barta, S. S. Bowser, G. U. Y. Brugerolle, R. A. Fensome, S. Fredericq, T. Y. James, S. Karpov, P. Kugrens, J. Krug, C. E. Lane, L. A. Lewis, J. Lodge, D. H. Lynn, D. G. Mann, R. M. McCourt, L. Mendoza, Ø. Moestrup, S. E. Mozley-Standridge, T. A. Nerad, C. A. Shearer, A. V. Smirnov, F. W. Spiegel, and M. F. J. R. Taylor. 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. Journal of Eukaryotic Microbiology (5):399-451.

4. Allender, W. J. 1997. Effect of trifluoperazine and verapamil on herbicide stimulated growth of cotton. Journal of Plant Nutrition (1):69-80.

5. Allender, W. J., G. C. Cresswell, J. Kaldor, and I. R. Kennedy. 1997. Effect of lithium and lanthanum on herbicide induced hormesis in hydroponically-grown cotton and corn. Journal of Plant Nutrition (1):81-95.

6. Anderson, J. R., S. Bentley, J. A. G. Irwin, J. M. Mackie, S. Neate, and J. A. Pattemore. 2004. Characterization of *Rhizoctonia solani* isolates causing root canker of lucerne in Australia. Australasian Plant Pathology (2):241-247.

7. Anderson, N. A. 1982. The Genetics and Pathology of *Rhizoctonia solani*. Annual Review of Phytopathology (1):329-347.

8. Anonymous. 1978. *Pythium aphanidermatum*. Distribution map. in: Distribution Maps of Plant Diseases. CABI UK, Wallingford, UK.

9. Anonymous. 1983. Fosetyl-al (Aliette). in: Chemical Fact Sheet. Environmental Protection Agency, United States.

10. Anonymous. 1991. Fosetyl-Al (Alliete). in: R.E.D. Facts. Environmental Protection Agency, United States.

11. Anonymous. 1994. Metalaxyl. in: R.E.D. Facts. Environmental Protection Agency, United States.

12. Anonymous. 1995. Propamocarb Hydrochoride. in: R.E.D. Facts. Environmental Protection Agency, United States.

13. Anonymous. 1997. Azoxystrobin. in: Pesticide Fact Sheet. Environmental Protection Agency, United States.

14. Anonymous. 1999. Chlorothalonil. in: Reregistration Elegibility Decision (RED). Environmental Protection Agency, United States.

15. Anonymous. 2000. Etridiazole (Terrazole). in: R.E.D. Facts. Environmental Protection Agency, United States.

16. Anonymous. 2004 a. Cyazofamid. in: Pesticide Fact Sheet. Environmental Protection Agency, United States.

17. Anonymous. 2004 b. Etridiazole. in: Pesticide Info. Ministry of Agriculture Food and Fisheries, British Columbia, CA.

18. Anonymous. 2004 c. Fosetyl aluminium. in: Pesticide Info. Ministry of Agriculture Food and Fisheries, British Columbia, CA.

19. Anonymous. 2004 d. Propamocarb hydrochloride. in: Pesticide Info. Ministry of Agriculture Food and Fisheries, British Columbia, CA.

20. Anonymous. 2008 a. EPA-HQ-OPP-2007-0906-0004. Environmental Protection Agency, United States.

21. Anonymous. 2008 b. Flutolanil summary document registration review: Initial docket. Environmental Protection Agency, United States.

22. Anonymous. 2009. Azoxystrobin. in: Pesticide Info. Ministry of Agriculture Food and Fisheries, British Columbia, CA.

23. Bae, O. N., K. M. Lim, J. Y. Han, B. I. Jung, J. Y. Lee, J. Y. Noh, S. M. Chung, M. Y. Lee, and J. H. Chung. 2008. U-shaped dose response in vasomotor tone: A mixed result of heterogenic response of multiple cells to xenobiotics. Toxicological Sciences (1):181-190.

24. Baker, K. F. 1970. Types of *Rhizoctonia* diseases and their occurrece. in: *Rhizoctonia solani,* biology and pathology. J. R. Parmeter, ed. University of California Press.

25. Betti, L., L. Lazzarato, G. Trebbi, M. Brizzi, G. L. Calzoni, F. Borghini, and D. Nani. 2003. Effects of homeopathic arsenic on tobacco plant resistance to tobacco mosaic virus. Theoretical suggestions about system variability, based on a large experimental data set. Homeopathy (4):195-202.

26. Blumberg, A. J., and J. B. Loefer. 1952. Effect of neomycin on two species of free-living protozoa. Physiological Zoology (3):276-282.

27. Bradley, C. A., G. L. Hartman, L. M. Wax, and W. L. Pedersen. 2002. Influence of herbicides on Rhizoctonia root and hypocotyl rot of soybean. Crop Protection (8):679-687.

28. Brain, P., and R. Cousens. 1989. An equation to describe dose responses where there is stimulation of growth at low doses. Weed Research (2):93-96.

29. Branham, S. E. 1929. The effects of certain chemical compounds upon the course of gas production by baker's yeast. J. Bacteriol. (4):247-264.

30. Bromilow, R. H., A. A. Evans, and P. H. Nicholls. 1999. Factors affecting degradation rates of five triazole fungicides in two soil types: 2. Field studies. Pesticide Science (12):1135-1142.

31. Burpee, L. L. 1995. Interactions among mowing height, nitrogen fertility, and cultivar affect the severity of rhizoctonia blight of tall fescue. Plant Disease (7):721-726.

32. Burr, T. J., and M. E. Stanghel. 1973. Propagule nature and density of *Pythium aphanidermatum* in field soil. Phytopathology (12):1499-1501.

33. CABI, CBS, and L. Research. Species Fungorum [database on the Internet]. 2010 [cited 2010 Nov 17]. Available from: http://www.speciesfungorum.org. (English).

34. Calabrese, E. J. 2005. Toxicological awakenings: the rebirth of hormesis as a central pillar of toxicology. Toxicology and Applied Pharmacology (1):1-8.

35. Calabrese, E. J. 2008. What Is Hormesis? Pages 5-19. in: Mild Stress and Healthy Aging. E. L. Bourg, and S. I. S. Rattan, eds. Springer, Netherlands.

36. Calabrese, E. J., and L. A. Baldwin. 1997. The dose determines the stimulation (and poison): Development of a chemical hormesis database. International Journal of Toxicology (6):545-559.

37. Calabrese, E. J., and L. A. Baldwin. 1997. A quantitatively-based methodology for the evaluation of chemical hormesis. Human and Ecological Risk Assessment (4):545-554.

38. Calabrese, E. J., and L. A. Baldwin. 2000. Chemical hormesis: its historical foundations as a biological hypothesis. Human & Experimental Toxicology (1):2-31.

39. Calabrese, E. J., and L. A. Baldwin. 2001. Hormesis: A generalizable and unifying hypothesis. Critical Reviews in Toxicology (4-5):353-424.

40. Calabrese, E. J., and L. A. Baldwin. 2002. Defining hormesis. Human & Experimental Toxicology (2):91-97.

41. Calabrese, E. J., and L. A. Baldwin. 2003. Ethanol and hormesis. Critical Reviews in Toxicology (3-4):407-424.

42. Calabrese, E. J., and R. B. Blain. 2009. Hormesis and plant biology. Environmental Pollution (1):42-48.

43. Campbell, C. C., and S. Saslaw. 1949. Enhancement of growth of certain fungi by streptomycin. Proceedings Of The Society For Experimental Biology And Medicine (3):562-562.

44. Carling, D. E., and D. R. Sumner. 1992. *Rhizoctonia*. Pages 157-165. in: Methods for Research on Soilborne Phytopathogenic Fungi. L. L. Singleton, J. D. Mihail, and C. M. Rush, eds. APS Press, St. Paul, MN.

45. Ceresini, P. *Rhizoctonia solani* [monograph on the Internet]. Raleigh, NC: NC State University, 1999 [cited 2010 Apr 29]. [about 4 screens] Available from: http://www.cals.ncsu.edu/course/pp728/Rhizoctonia/Rhizoctonia.html. (English).

46. Chen, S. K., C. A. Edwards, and S. Subler. 2001. Effects of the fungicides benomyl, captan and chlorothalonil on soil microbial activity and nitrogen dynamics in laboratory incubations. Soil Biology & Biochemistry (14):1971-1980.

47. Chen, Y. I. 1999. Rank-based tests for dose finding in nonmonotonic dose-response settings. Biometrics (4):1258-1262.

48. Conolly, R. B., and W. K. Lutz. 2004. Nonmonotonic dose-response relationships: Mechanistic basis, kinetic modeling, and implications for risk assessment. Toxicological Sciences (1):151-157.

49. Cook, P. J., P. J. Landschoot, and M. J. Schlossberg. 2009. Inhibition of *Pythium spp*. and suppression of Pythium blight of turfgrasses with phosphonate fungicides. Plant Disease (8):809-814.

50. Cornell University, M. S. U., Oregon State University, and University of California at Davis. Propiconazole [monograph on the Internet]. [place unknown]: Extoxnet 1997 Oct [cited 2009 Nov 28]. [about 6 screens] Available from:

http://pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/propiconazole-ext.html. (English).

51. Cox, C. 1997. Fungicide factsheet: Chlorothalonil. Journal of pesticide reform. A publication of the Northwest Coalition for Alternatives to Pesticides (4):14-20.

52. Crump, K. 2001. Evaluating the evidence for hormesis: A statistical perspective. Human & Ecological Risk Assessment (4):781.

53. Csermely, P., and I. Yahara. 2003. Heat shock proteins. Pages 67-75. in: Molecular pathomechanisms and new trends in drug research. G. Kéri, and I. Tóth, eds. Taylor & Francis, London, UK.

54. Cubeta, M. A., and R. Vilgalys. 1997. Population biology of the *Rhizoctonia solani* complex. Phytopathology (4):480-484.

55. Cush, R., K. Parker, J. Dipaola, and D. Ross. 2009. Azoxystrobin formulations. Pages 7. Syngenta Crop Protection, United States.

56. Davenas, E., F. Beauvais, J. Amara, M. Oberbaum, B. Robinzon, A. Miadonnai, A. Tedeschi, B. Pomeranz, P. Fortner, P. Belon, J. Sainte-Laudy, B. Poitevin, and J. Benveniste. 1988. Human basophil degranulation triggered by very dilute antiserum against IgE. Nature (6176):816-818.

57. Davis, J. M., and D. J. Svendsgaard. 1990. U-shaped dose-response curves, their occurrence and implications for risk assessment. Journal of Toxicology and Environmental Health (2):71-83.

58. Deacon, J. 2006. Fungal nutrition. Pages 110-121. in: Fungal Biology. Wiley Blackwell, Malden, MA.

59. Demirci, E., and C. Eken. 1999. First report of *Rhizoctonia zeae* in Turkey. Plant Disease (2):200-200.

60. Deng, C., Q. Zhao, and R. Shukla. 2000. Detecting hormesis using a non-parametric rank test. Human & Experimental Toxicology (12):703-708.

61. E.P.A. Benchmark dose (BMD) methodology Version 2.1. [place unknown]: US Environmental Protection Agency, 2008 Sept [updated 2009 Jul; cited 2009 Nov 26]. [about 13 screens] Available from: http://www.epa.gov/ncea/bmds/bmds_training/methodology/intro.htm#Continuous. (English).

62. Edson, H. A. 1915. *Rheosporangium Aphanidermatus*, a new genus and species of fungus parasitic on sugar beets and radishes. Journal Agricultural Research (4):279-292.

63. Ehret, D. L., B. Alsanius, W. Wohanka, J. G. Menzies, and R. Utkhede. 2001. Disinfestation of recirculating nutrient solutions in greenhouse horticulture. Agronomie (4):323-339.

64. Elton, S. C., M. J. Hart, and R. A. Joly. 1938. The effect of 1,2,5,6-dibenzanthracene on the growth and respiration of yeast. Science (2258):331.

65. Estrela, C., C. R. A. Estrela, E. L. Barbin, J. C. E. Spanó, M. A. Marchesan, and J. D. Pécora. 2002. Mechanism of action of sodium hypochlorite. Brazilian Dental Journal (2):113-117.

66. Fenn, M. E., and M. D. Coffey. 1984. Studies on the *in vitro* and *in vivo* antifungal activity of fosetyl-Al and phosphorous acid. Phytopathology:606-611.

67. Fenske, R. A., S. J. Hamburger, and C. L. Guyton. 1987. Occupational exposure to fosetyl-Al fungicide during spraying of ornamentals in greenhouses. Archives of Environmental Contamination and Toxicology (5):615-621.

68. Fisher, S. E., M. A. Barnicle, B. Steis, I. Holzman, and D. H. Vanthiel. 1980. Acute ethanol exposure and protein synthesis in the fetal rat dose response. Alcoholism-Clinical and Experimental Research (2):214-214.

69. Fitzpatrick, H. M. 1923. Generic Concepts in the Pythiaceae and Blastocladiaceae. Mycologia (4):166-173.

70. Gabliks, J., M. Bantug-Jurilla, and L. Friedman. 1967. Responses of cell cultures to insecticides. IV. Relative toxicity of several organophosphates in mouse cell cultures. Proceedings Of The Society For Experimental Biology And Medicine (3):1002-1005.

71. Gadd, G. M. 1994. Signal transduction in fungi. Pages 183-210. in: The Growing Fungus. N. A. R. Gow, and G. M. Gadd, eds. Springer Netherlands.

72. Gallian, J. J. Management of sugarbeet root rots Twin Falls ID: University of Idaho College of Agriculture, 2003 [12 Feb 2009; cited 12 Dec 2010]. [about 4 screens] Available from: http://www.uiweb.uidaho.edu/sugarbeet/Disease/ rtdspnw.htm. (English).

73. Garcia, M. G., E. R. Ramos, and R. Ramirez. 2007. First report of *Rhizoctonia zeae* in *Cynodon dactylon* in Cuba. Phytopathology (7):S174.

74. Garzon, C. D., G. W. Moorman, J. M. Yanez, J. E. Molineros, R. C. Leonard, and M. Jimenez-Gasco. 2008. Low-doses of fungicides have a stimulatory effect on Pythium spp. in vitro and in planta. Phytopathology (6):S58.

75. Gómez-Icazbalceta, G., L. Huerta, L. E. Soto-Ramirez, and C. Larralde. Extracellular HIV-1 Nef protein modulates lytic activity and proliferation of human CD8+ T lymphocytes. Cellular Immunology (1-2):85-90.

76. Gutiérrez, S. A., M. A. Cúndom, V. Barrera, and L. Gasoni. 2007. First record of *Rhizoctonia zeae* on corn in Argentina. Australasian Plant Disease Notes (1):137-138.

77. Hallsworth, J. E., Y. Nomura, and M. Iwahara. 1998. Ethanol-induced water stress and fungal growth. Journal of Fermentation and Bioengineering (5):451-456.

78. Henschler, D. 2006. The origin of hormesis: historical background and driving forces. Human & Experimental Toxicology (7):347-351.

79. Hessayon, D. G. 1951. Double-action of trichothecin and its production in soil. Nature (4284):998-999.

80. Hessayon, D. G. 1953. Fungitoxins in the soil: II Trichothecin, its production and inactivation in unsterilized soils. Soil Science (5):395-404.

81. Hidalgo, E., and C. Dominguez. 2000. Growth-altering effects of sodium hypochlorite in cultured human dermal fibroblasts. Life Sciences (11):1331-1344.

82. Hirst, S. J., N. A. Hayes, J. Burridge, F. L. Pearce, and J. C. Foreman. 1993. Human basophil degranulation is not triggered by very dilute antiserum against human IgE. Nature (6455):525-527.

83. Hotchkiss, M. 1923. Studies on salt action VI. The stimulating and inhibitive effect of certain cations upon bacterial growth. J. Bacteriol. (2):141-162.

84. Hsiang, T., and P. Masilamany. 2007. First report of *Rhizoctonia zeae* on turfgrass in Ontario. Plant Pathology (2):350.

85. Jackson, S. L., and I. B. Heath. 1993. Roles of calcium ions in hyphal tip growth. Microbiological Reviews (2):367-382.

86. Jensen, G. H. 1907. Toxic limits and stimulation effects of some salts and poisons on wheat. Pages 11-44 in: Contributions from the Hull botanical laboratory. University of Chicago Press, Chicago.

87. Jones, R. P. 1989. Biological principles for the effects of ethanol. Enzyme and Microbial Technology (3):130-153.

88. Joseph-Horne, T., D. W. Hollomon, and P. M. Wood. 2001. Fungal respiration: a fusion of standard and alternative components. Biochimica et Biophysica Acta (BBA) - Bioenergetics (2-3):179-195.

89. Judelson, H. S., and F. A. Blanco. 2005. The spores of *Phytophthora*: weapons of the plant destroyer. Nature Reviews Microbiology (1):47-58.

90. Kerwin, J. L., and N. D. Duddles. 1989. Reassessment of the role of phospholipids in sexual reproduction by sterol-auxotrophic fungi. Journal of Bacteriology (7):3831-3839.

91. Kim, I. S., L. A. Beaudette, J. Han Shim, J. T. Trevors, and Y. Tack Suh. 2002. Environmental fate of the triazole fungicide propiconazole in a rice-paddy-soil lysimeter. Plant and Soil (2):321-331.

92. Kim, S. H., J. G. Kantzes, and L. O. Weaver. 1974. Infection of aboveground parts of bean by *Pythium aphanidermatum*. Phytopathology:373-380.

93. Kitchin, K. T., and J. W. Drane. 2005. A critique of the use of hormesis in risk assessment. Human & Experimental Toxicology (5):249-253.

94. Kushida, M., T. Sukata, S. Uwagawa, K. Ozaki, A. Kinoshita, H. Wanibuchi, K. Morimura, Y. Okuno, and S. Fukushima. 2005. Low dose DDT inhibition of hepatocarcinogenesis initiated by diethylnitrosamine in male rats: Possible mechanisms. Toxicology and Applied Pharmacology (3):285-294.

95. Laemmlen, F. 2001. Damping-Off diseases. University of California, Division of Agricultural and Natural Resources, Oakland, CA.

96. Langworthy, C. F., and C. L. Hunt. 1914. Corn meal as a food and ways of using it in: Farmers' bulletin 565. U.S. Dept. of Agriculture, Washington, D.C.

97. Levy, S. B. 1998. The challenge of antibiotic resistance. Scientific American (3):46.

98. Loefer, J. B., F. W. Bieberdorf, and R. G. Weichlein. 1950. Effect of streptomycin on growth of certain fungi. Texas Reports on Biology and Medicine (1):105.

99. Lumsden, R. D., W. A. Ayers, P. B. Adams, R. L. Dow, J. A. Lewis, G. C. Papavizas, and J. G. Kantzes. 1976. Ecology and Epidemiology of *Pythium* species in field soil. Phytopathology (10):1203-1209.

100. Mack, G. A., and D. A. Wolfe. 1981. K-Sample rank tests for umbrella alternatives. Journal of the American Statistical Association (373):175-181.

101. Malarczyk, E. 2008. How living organisms detect very low doses? A new approach to homeopathy and hormesis. Pages 127-135. in: Signals and Images. L. V. Bonamin, ed. Springer, Netherlands.

102. Malarczyk, E. 2008. Kinetic changes in the activity of HR-peroxidase induced by very low doses of phenol. International Journal of High Dilution Research (23):3-11.

103. Malarczyk, E., A. Jarosz-Wilkołazka, and J. Kochmanska-Rdest. 2003. Effect of low doses of guaiacol and ethanol on enzymatic activity of fungal cultures. Nonlinearity In Biology, Toxicology, Medicine (2):167-178.

104. Mao, W., J. A. Lewis, P. K. Hebbar, and R. D. Lumsden. 1997. Seed treatment with a fungal or a bacterial antagonist for reducing corn damping-off caused by species of *Pythium* and *Fusarium.* Plant Disease (7):824-824.

105. Martin, F. N., and J. E. Loper. 1999. Soilborne plant diseases caused by *Pythium* spp.: Ecology, epidemiology, and prospects for biological control. Critical Reviews in Plant Sciences (2):111-181.

106. Mattson, M. P. 2008. Hormesis and disease resistance: activation of cellular stress response pathways. Human & Experimental Toxicology (2):155-162.

107. Miller, W. S., C. A. Green, and H. Kitchen. 1945. Biphasic action of Penicillin and other sulphonamide similarity. Nature (3929):210-211.

108. Mitani, S., S. Araki, Y. Takii, T. Ohshima, N. Matsuo, and H. Miyoshi. 2001. The biochemical mode of action of the novel selective fungicide cyazofamid: Specific inhibition of mitochondrial complex III in *Phythium spinosum*. Pesticide Biochemistry and Physiology (2):107-115.

109. Monkiedje, A., and M. Spiteller. 2005. Degradation of metalaxyl and mefenoxam and effects on the microbiological properties of tropical and temperate soils. International Journal of Environmental Research and Public Health (2):272-285.

110. Motoba, K., M. Uchida, and E. Tada. 1988. Mode of antifungal action and selectivity of flutolanil (pesticide chemistry). Agricultural and Biological Chemistry (6):1445-1449.

111. Mushak, P. 2009. Ad hoc and fast forward: The science of hormesis growth and development. Environmental Health Perspectives (9):1333-1338.

112. Nerey, Y., J. Pannecoucque, H. P. Hernandez, M. Diaz, R. Espinosa, S. DeVos, S. VanBeneden, L. Herrera, and M. Hofte. 2010. *Rhizoctonia* spp. causing root and hypocotyl rot in *Phaseolus vulgaris* in Cuba. Journal of Phytopathology:236-243.

113. Nickell, L. G. 1952. Stimulation of plant growth by antibiotics. Proceedings of the Society for Experimental Biology and Medicine (4):615-617.

114. Niterink, V. D. P. 1981. Monograph of the genus *Pythium*, *Studies in Mycology*. Centraalbureau voor Schimmelcultures. Baarn, NL.

115. Papavizas, G. C., N. R. Oneill, and J. A. Lewis. 1978. Fungistatic activity of propyl-N-(gamma-dimethylaminopropyl) carbamate on *Pythium* spp. and its reversal by sterols. Phytopathology (11):1667-1671.

116. Parker, K. *Pythium aphanidermatum* Raleigh, NC: NC State University, 2009 [cited 2009 Nov 11]. Available from: http://www.cals.ncsu.edu/course/pp728/Pythium/Pythium_aphanidermatum.html. (English).

117. Parmeter, J. R., and H. S. Whitney. 1970. Taxonomy and nomenclature of the imperfect state. in: Rhizoctonia solani, biology and pathology. J. R. Parmeter, ed. University of California Press, Berkeley, CA.

118. Paschen, W. 2000. Role of calcium in neuronal cell injury: which subcellular compartment is involved? Brain Research Bulletin (4):409-413.

119. Piper, P. W. 1995. The heat shock and ethanol stress responses of yeast exhibit extensive similarity and functional overlap. FEMS Microbiology Letters (2-3):121-127.

120. Pohorecky, L. A. 1977. Biphasic action of ethanol. Biobehavioral Reviews (4):231-240.

121. Poltronieri, L. S., D. R. Trindade, and F. C. Albuquerque. 2002. Rot of corn caused by *Rhizoctonia zeae* in the State of Para, Brazil. Fitopatologia Brasileira (4):423.

122. Rosenberg, S. M. 2001. Evolving responsively: adaptive mutation. Nature Reviews Genetics (7):504-515.

123. Rosso, M. L., J. C. Rupe, P. Chen, and L. A. Mozzoni. 2008. Inheritance and genetic mapping of resistance to Pythium damping-off caused by *Pythium aphanidermatum* in 'Archer' Soybean. Crop Sci (6):2215-2222.

124. Ryker, T. C., and F. S. Gooch. 1938. Rhizoctonia sheath spot of rice. Phytopathology (4):233-246.

125. Schabenberger, O., B. E. Tharp, J. J. Kells, and D. Penner. 1999. Statistical tests for hormesis and effective dosages in herbicide dose response. Agronomy Journal (4):713-721.

126. Schulz, H. 1887. Zur Lehre von der Arzneiwirdung. Virchows Archiv fur Pathologische Anatomie und Physiologie fur Klinische Medizin (3):423-445.

127. Schulz, H. 1888. Ueber Hefegifte. Pflügers Archiv European Journal of Physiology (1):517-541.

128. Serafini, M. P. 2005. Registration of a major change in labeled use pattern for flutolanil contained in the pesticide product Moncut® 70-DF fungicide. Department of Environmental Conservation. Albany, NY.

129. Sesay, A., R. M. Shibles, and C. R. Stewart. 1986. Effects of KCN and salicylhydroxamic acid on respiration of soybean leaves at different ages. Plant Physiology (2):443-447.

130. Smith, O. F. 1943. Rhizoctonia root canker of alfalfa (*Medicago sativa*). Phytopathology (11):1081-1085.

131. Sneh, B., L. Burpee, and A. Ogoshi. 1991. Identification of *Rhizoctonia* Species. APS Press, St. Paul, MN.

132. Southam, C. M., and J. Ehrlich. 1943. Effects of extract of western red-cedar heartwood on certain wood-decaying fungi in culture. Phytopathology (6):517-524.

133. Stebbing, A. R. 1982. Hormesis--the stimulation of growth by low levels of inhibitors. The Science of the Total Environment (3):213-234.

134. Stebbing, A. R. D. 1987. Growth hormesis a by-product of control. Health Physics (5):543-547.

135. Stebbing, A. R. D. 1998. A theory for growth hormesis. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis (1-2):249-258.

136. Stebbing, A. R. D., and M. D. Brinsley. 1985. The effects of load and stress induced by cadmium on the growth of yeast. Pages 577-583. in: Marine Biology of Polar Regions and Effects of Stress on Marine Organisms. J. S. Gray, and M. E. Christiansen, eds. Wiley, London, UK.

137. Suzuki, K., and M. Hirose. 2004. Cyazofamid. in: Evaluation Report. Food Safety Commission, Japan.

138. Tillman, R. W., M. R. Siegel, and J. W. Long. 1973. Mechanism of action and fate of the fungicide chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) in biological systems. Pesticide biochemistry and physiology (3):160-167.

139. Tredway, L., and L. Burpee. Rhizoctonia diseases of turfgrass [monograph on the Internet]. Athens, GA APS net, 2001 Nov [2006; cited 2009 Nov 27]. [about 13 screens] (Plant Disease Lessons). Available from: http://www.apsnet.org/edcenter/intropp/lessons/fungi/Basidiomycetes/Pages/Rhizoctonia.aspx. (English).

140. Trujillo, E. E., and R. B. Hine. 1965. Role of papaya residues in papaya root rot caused by *Pythium aphanidermatum* and *Phytophthora parasitica*. Phytopathology (12):1293-1298.

141. Tsror, L. 2010. Biology, Epidemiology and Management of Rhizoctonia solani on Potato. Journal of Phytopathology (10):649-658.

142. University of Hertfordshire, A. E. R. U. Pyraclostrobin (Ref: BAS 500F) [monograph on the Internet]. Hatfield, UK: 2007 Mar [cited 2009 Nov 13]. [about 6 screens] (Pesticide

Properties DataBase). Available from: http://sitem.herts.ac.uk/aeru/iupac/Reports/564.htm. (English).

143. Uyeda, A., Y. Ishikawa, K. Inagaki, J. Chujo, and T. Ogasawara. 2000. Comparison of vitamin effects on growth of *Rhizoctonia zeae, Rhizoctonia oryzae* and *Waitea circinata*. Scientific Reports of the Faculty of Agriculture Meijo University (36):67-73.

144. Vajna, L., and G. Oros. 2005. First report of *Rhizoctonia zeae* on *Lolium perenne* and *Festuca* sp. in Hungary. Plant Pathology (2):250.

145. Vanewijk, P. H., and J. A. Hoekstra. 1993. Calculation of the EC_{50} and its confidence interval when subtoxics stimulus is present Ecotoxicology and Environmental Safety (1):25-32.

146. Voorhees, R. K. 1934. Sclerotial rot of corn caused by *Rhizoctonia zeae*, n sp. Phytopathology (11):1290-1303.

147. Walker, C. A., and P. van West. 2007. Zoospore development in the oomycetes. Fungal Biology Reviews (1):10-18.

148. Wharton, P., K. William, B. Devan, and S. Sieglinde. Rhizoctonia stem canker and black scurf of potato [monograph on the Internet]. East Lansing, MI: Michigan State University Extension, 2007 May [cited 2009 Nov 18]. 6 p. (Michigan Potato Diseases). Available from: http://www.potatodiseases.org/pdf/rhizoctonia-bulletin.pdf. (English).

149. Zhang, Q., J. Pi, C. G. Woods, and M. E. Andersen. 2009. Phase I to II cross-induction of xenobiotic metabolizing enzymes: A feedforward control mechanism for potential hormetic responses. Toxicology and Applied Pharmacology (3):345-356.

150. Zhang, Q., J. B. Pi, C. G. Woods, A. M. Jarabek, H. J. Clewell, and M. E. Andersen. 2008. Hormesis and adaptive cellular control systems. Dose-Response (2):196-208.

151. Zhou, T., and T. C. Paulitz. 1994. Induced resistance in biocontrol of *Pythium aphanidermatum* by *Pseudomonas* spp. on cucumber Journal of Phytopathology-Phytopathologische Zeitschrift (1):51-63.

APPENDIX A

Data obtained in all the experiments performed during the course of the research. The concentration, mean radial growth of each plate (a, b, c), the total mean and the standard deviation are presented. Concentrations are presented in terms of either standard concentration for surface sterilization (SCSS), MAR or BMD. SCSS: Standard concentration for surface sterilization. MAR: Minimum application rate. BMD: Bench mark dose.

Pathogen: *Pythium aphanidermatum*
Desinfectant: Ethanol
SCSS 75%

Results:04-03-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean (mm)	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c		
0	0	15.8	13.3	14.5	14.5	1.3
1.0E-20	7.5E-15	16.0	14.3	14.0	14.8	1.1
1.0E-18	7.5E-13	14.8	13.0	15.5	14.4	1.3
1.0E-16	7.5E-11	14.5	12.0	14.3	13.6	1.4
1.0E-14	7.5E-09	14.5	14.0	15.0	14.5	0.5
1.0E-12	7.5E-07	13.8	14.0	15.0	14.3	0.7
1.0E-10	7.5E-05	11.0	13.8	13.8	12.8	1.6
1.0E-08	7.5E-03	15.3	14.5	13.8	14.5	0.8
1.0E-06	7.5E-01	14.5	14.8	15.3	14.8	0.4
1.0E-04	7.5E+01	17.0	14.8	13.5	15.1	1.8
1.0E-02	7.5E+03	11.8	11.0	11.8	11.5	0.4
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Results:04-14-2009 Incubation time: 24h

Means: Conc./SCSS	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	12.8	9.5	12.5	11.6	1.8
6.3E-05	4.7E+01	16.3	14.5	16.0	15.6	0.9
1.6E-04	1.2E+02	14.5	15.3	15.8	15.2	0.6
4.0E-04	3.0E+02	14.5	16.3	15.8	15.5	0.9
1.0E-03	7.5E+02	15.0	16.0	14.5	15.2	0.8
2.5E-03	1.9E+03	13.8	15.8	17.3	15.6	1.8
6.3E-03	4.7E+03	14.3	13.0	15.8	14.3	1.4
1.6E-02	1.2E+04	10.0	10.8	10.8	10.5	0.4
4.0E-02	3.0E+04	0.0	4.8	0.0	1.6	2.7
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Results:04-23-2009 Incubation time: 24h

Means: Conc./SCSS	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	10.9	11.8	11.2	11.3	0.5
2.5E-05	1.9E+01	11.9	10.6	10.7	11.1	0.7
6.3E-05	4.7E+01	11.3	11.2	12.3	11.6	0.6
1.6E-04	1.2E+02	13.5	10.6	11.8	12.0	1.4
4.0E-04	3.0E+02	11.2	12.2	10.8	11.4	0.7
1.0E-03	7.5E+02	11.3	11.9	13.2	12.1	1.0
2.5E-03	1.9E+03	10.6	11.7	8.7	10.3	1.6
6.3E-03	4.7E+03	10.1	9.2	8.9	9.4	0.7
1.6E-02	1.2E+04	7.4	7.6	6.7	7.2	0.5
4.0E-02	3.0E+04	0.0	0.0	0.0	0.0	0.0
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Results:04-25-2009 Incubation time: 30h

Means: Conc./SCSS	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	12.7	15.8	13.4	14.0	1.6
2.5E-05	1.9E+01	11.0	14.1	14.8	13.3	2.0
6.3E-05	4.7E+01	10.5	16.3	12.1	13.0	3.0
1.6E-04	1.2E+02	16.2	10.7	10.8	12.6	3.1
4.0E-04	3.0E+02	15.8	15.3	12.0	14.4	2.1
1.0E-03	7.5E+02	17.0	19.3	18.6	18.3	1.1
2.5E-03	1.9E+03	17.5	17.5	15.1	16.7	1.4
6.3E-03	4.7E+03	5.8	8.2	12.9	9.0	3.6
1.6E-02	1.2E+04	14.3	2.2	8.0	8.2	6.0
4.0E-02	3.0E+04	0.0	0.0	0.0	0.0	0.0
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Results:04-30-2009 Incubation time: 30h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	17.4	17.1	15.5	16.7	1.0
2.5E-05	1.9E+01	17.7	16.3	15.4	16.5	1.2
6.3E-05	4.7E+01	20.6	16.8	15.7	17.7	2.6
1.6E-04	1.2E+02	15.9	16.6	15.7	16.1	0.5
4.0E-04	3.0E+02	16.9	19.7	18.8	18.5	1.4
1.0E-03	7.5E+02	19.7	17.8	18.3	18.6	1.0
2.5E-03	1.9E+03	14.6	18.7	18.6	17.3	2.3
6.3E-03	4.7E+03	14.8	12.4	13.7	13.6	1.2
1.6E-02	1.2E+04	9.1	5.9	7.2	7.4	1.6
4.0E-02	3.0E+04	0.0	0.0	0.0	0.0	0.0
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Results:07-15-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	16.8	7.4	15.0	13.1	5.0
6.3E-05	4.7E+01	10.8	14.5	12.8	12.7	1.8
1.6E-04	1.2E+02	14.0	10.9	12.7	12.5	1.6
4.0E-04	3.0E+02	9.7	13.9	13.5	12.4	2.3
1.0E-03	7.5E+02	10.3	15.6	11.0	12.3	2.9
2.5E-03	1.9E+03	12.8	16.6	11.0	13.5	2.9
6.3E-03	4.7E+03	0.0	0.0	0.0	0.0	0.0
1.6E-02	1.2E+04	0.0	0.0	0.0	0.0	0.0
4.0E-02	3.0E+04	0.0	0.0	0.0	0.0	0.0
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Results:07-17-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	14.6	19.4	17.1	17.0	2.4
2.5E-05	1.9E+01	20.2	15.6	17.2	17.7	2.4
6.3E-05	4.7E+01	18.4	17.6	13.5	16.5	2.6
1.6E-04	1.2E+02	17.5	19.2	18.6	18.4	0.9
4.0E-04	3.0E+02	18.7	16.4	18.7	17.9	1.4
1.0E-03	7.5E+02	19.7	20.2	19.7	19.9	0.3
2.5E-03	1.9E+03	20.7	20.9	19.1	20.2	1.0
6.3E-03	4.7E+03	10.7	12.0	10.5	11.0	0.8
1.6E-02	1.2E+04	7.7	9.0	7.5	8.0	0.8
4.0E-02	3.0E+04	0.0	0.0	0.0	0.0	0.0
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Microorganism: *Pythium aphanidermatum*
Disinfectant: Sodium Hypochlorite
SCSS 2%

Results:04-03-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	15.8	13.2	14.5	14.5	1.3
1.0E-20	2.0E-16	15.2	14.5	14.0	14.6	0.6
1.0E-18	2.0E-14	14.5	14.5	14.8	14.6	0.1
1.0E-16	2.0E-12	16.5	13.8	17.0	15.8	1.8
1.0E-14	2.0E-10	14.0	14.5	13.8	14.1	0.4
1.0E-12	2.0E-08	14.5	14.0	14.8	14.4	0.4
1.0E-10	2.0E-06	15.2	14.5	15.0	14.9	0.4
1.0E-08	2.0E-04	14.8	15.8	15.8	15.4	0.6
1.0E-06	2.0E-02	16.0	15.2	16.0	15.8	0.4
1.0E-04	2.0E+00	15.2	14.2	16.0	15.2	0.9
1.0E-02	2.0E+02	16.2	15.0	15.5	15.6	0.6
1.0E-01	2.0E+03	17.0	14.5	16.8	16.1	1.4

Results:04-14-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	16.5	18.8	16.2	17.2	1.4
6.3E-03	1.3E+02	13.2	13.8	12.5	13.2	0.6
1.6E-02	3.2E+02	12.8	11.2	11.2	11.8	0.9
4.0E-02	8.0E+02	8.0	8.5	9.0	8.5	0.5
1.0E-01	2.0E+03	5.8	6.0	7.8	6.5	1.1
2.5E-01	5.0E+03	0.0	0.0	0.0	0.0	0.0

Results:04-15-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	12.5	14.0	13.8	13.4	0.8
1.0E-16	2.0E-12	12.2	10.2	12.8	11.8	1.3
1.0E-14	2.0E-10	10.0	12.5	10.8	10.9	1.4
1.0E-12	2.0E-08	12.8	13.8	13.2	13.3	0.5
1.0E-10	2.0E-06	11.2	13.5	12.5	12.4	1.1
1.0E-08	2.0E-04	12.2	12.8	13.2	12.8	0.5
1.0E-06	2.0E-02	13.2	12.8	13.5	13.2	0.4
1.0E-04	2.0E+00	12.0	11.2	11.5	11.6	0.4
1.0E-02	2.0E+02	5.0	4.5	4.2	4.6	0.4

Results:04-17-2009 Incubation time: 30h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	18.8	22.2	21.0	20.7	1.8
1.0E-16	2.0E-12	20.2	20.5	22.5	21.1	1.2
1.0E-14	2.0E-10	21.8	18.2	19.2	19.8	1.8
1.0E-12	2.0E-08	21.5	21.0	18.8	20.4	1.5
1.0E-10	2.0E-06	18.5	20.2	20.2	19.7	1.0
1.0E-08	2.0E-04	19.0	21.0	21.5	20.5	1.3
1.0E-06	2.0E-02	18.2	20.8	18.5	19.2	1.4
1.0E-04	2.0E+00	18.8	21.8	18.0	19.5	2.0
1.0E-02	2.0E+02	8.5	7.8	8.0	8.1	0.4

Results:05-02-2009 Incubation time: 30h

Means: Conc./SCSS	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	18.4	20.0	18.3	18.9	1.0
2.5E-05	5.0E-01	18.2	18.0	18.5	18.2	0.3
6.3E-05	1.3E+00	21.0	16.7	19.1	18.9	2.2
1.6E-04	3.2E+00	18.2	17.6	18.3	18.0	0.4
4.0E-04	8.0E+00	17.3	17.0	17.4	17.2	0.2
1.0E-03	2.0E+01	15.0	15.3	15.9	15.4	0.5
2.5E-03	5.0E+01	12.4	11.8	12.3	12.2	0.3
6.3E-03	1.3E+02	8.9	7.0	8.1	8.0	1.0
1.6E-02	3.2E+02	5.4	4.1	5.2	4.9	0.7
4.0E-02	8.0E+02	0.0	0.0	0.0	0.0	0.0
1.0E-01	2.0E+03	0.0	0.0	0.0	0.0	0.0

Results:05-04-2009 Incubation time: 24h

Means: Conc./SCSS	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	12.5	13.0	12.0	12.5	0.5
2.5E-05	5.0E-01	14.1	13.3	13.9	13.8	0.4
6.3E-05	1.3E+00	13.3	12.2	13.2	12.9	0.6
1.6E-04	3.2E+00	13.6	13.4	13.5	13.5	0.1
4.0E-04	8.0E+00	12.6	11.8	12.4	12.3	0.4
1.0E-03	2.0E+01	11.8	11.4	11.7	11.6	0.2
2.5E-03	5.0E+01	8.5	7.9	8.9	8.4	0.5
6.3E-03	1.3E+02	5.0	6.3	5.0	5.5	0.7
1.6E-02	3.2E+02	1.0	2.0	2.0	1.7	0.6
4.0E-02	8.0E+02	0.0	0.0	0.0	0.0	0.0
1.0E-01	2.0E+03	0.0	0.0	0.0	0.0	0.0

Incubation time: 24h
Results:07-06-2009

Means: Conc./SCSS	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	19.0	20.4	19.0	19.5	0.8
2.5E-05	5.0E-01	18.1	17.8	19.2	18.4	0.7
6.3E-05	1.3E+00	15.7	16.0	15.8	15.8	0.2
1.6E-04	3.2E+00	17.4	17.7	17.9	17.7	0.3
4.0E-04	8.0E+00	13.3	14.6	15.7	14.5	1.2
1.0E-03	2.0E+01	4.6	3.1	9.4	5.7	3.3
2.5E-03	5.0E+01	3.3	0.0	3.3	2.2	1.9
6.3E-03	1.3E+02	0.0	0.0	0.0	0.0	0.0
1.6E-02	3.2E+02	0.0	0.0	0.0	0.0	0.0
4.0E-02	8.0E+02	0.0	0.0	0.0	0.0	0.0
1.0E-01	2.0E+03	0.0	0.0	0.0	0.0	0.0

Results:07-09-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	14.7	13.4	9.4	12.5	2.8
2.5E-05	5.0E-01	13.9	13.0	14.8	13.9	0.9
6.3E-05	1.3E+00	12.4	12.2	11.6	12.1	0.4
1.6E-04	3.2E+00	12.0	9.4	13.5	11.6	2.1
4.0E-04	8.0E+00	3.5	0.0	0.0	1.2	2.0
1.0E-03	2.0E+01	0.0	0.0	0.0	0.0	0.0
2.5E-03	5.0E+01	0.0	0.0	0.0	0.0	0.0
6.3E-03	1.3E+02	0.0	0.0	0.0	0.0	0.0
1.6E-02	3.2E+02	0.0	0.0	0.0	0.0	0.0
4.0E-02	8.0E+02	0.0	0.0	0.0	0.0	0.0
1.0E-01	2.0E+03	0.0	0.0	0.0	0.0	0.0

Incubation time: 24h

Results:07-10-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	20.6	22.4	17.4	20.1	2.5
6.3E-08	1.3E-03	20.7	20.0	20.8	20.5	0.4
1.6E-07	3.2E-03	22.6	22.1	20.6	21.8	1.0
4.0E-07	8.0E-03	20.9	17.0	20.2	19.4	2.1
1.0E-06	2.0E-02	20.6	20.5	20.5	20.5	0.1
2.5E-06	5.0E-02	19.3	20.8	17.5	19.2	1.7
6.3E-06	1.3E-01	18.6	18.9	16.5	18.0	1.3
1.6E-05	3.2E-01	20.4	20.9	18.2	19.8	1.4
4.0E-05	8.0E-01	16.7	20.0	19.5	18.7	1.8
1.0E-04	2.0E+00	0.0	0.0	0.0	0.0	0.0

Incubation time: 24h

Results:07-14-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	13.9	12.6	15.5	14.0	1.5
2.5E-06	5.0E-02	13.2	9.9	8.3	10.5	2.5
4.0E-06	8.0E-02	13.3	15.0	4.7	11.0	5.5
6.3E-06	1.3E-01	2.4	13.8	14.1	10.1	6.7
1.0E-05	2.0E-01	13.5	14.6	15.4	14.5	1.0
1.6E-05	3.2E-01	11.1	12.1	2.7	8.6	5.2
2.5E-05	5.0E-01	12.0	10.1	11.3	11.1	1.0
4.0E-05	8.0E-01	11.5	14	13.5	13.0	1.3
6.3E-05	1.3E+00	0.0	1.6	14.5	5.4	8.0
1.0E-04	2.0E+00	14.5	10	0.0	8.2	7.4

Results:07-17-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	14.6	15.6	13.6	14.6	1.0
2.5E-06	5.0E-02	14.4	14.5	13.4	14.1	0.6
4.0E-06	8.0E-02	12.7	12.3	16.9	14.0	2.6
6.3E-06	1.3E-01	12.5	12.0	14.6	13.0	1.4
1.0E-05	2.0E-01	13.1	14.2	13.5	13.6	0.6
1.6E-05	3.2E-01	13.9	11.7	14.5	13.4	1.5
2.5E-05	5.0E-01	10.8	14.8	12.4	12.7	2.1
4.0E-05	8.0E-01	12.4	14.6	12.3	13.1	1.3
6.3E-05	1.3E+00	11.8	10.0	11.2	11.0	0.9
1.0E-04	2.0E+00	9.0	12.3	13.3	11.5	2.3

Microorganism: *Rhizoctonia solani*
Disinfectant: Ethanol
SCSS 75%

Results:06-11-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	13.8	14	15.1	14.3	0.7
1.0E-18	7.5E-13	15.0	14.9	14.2	14.7	0.4
1.0E-16	7.5E-11	15.9	13.9	15.8	15.2	1.1
1.0E-14	7.5E-09	13.6	14.3	14.9	14.3	0.7
1.0E-12	7.5E-07	14.8	14.3	14.7	14.6	0.3
1.0E-10	7.5E-05	14.7	15.2	14.1	14.7	0.6
1.0E-08	7.5E-03	15.7	16.0	13.5	15.1	1.4
1.0E-06	7.5E-01	15.4	15.0	14.5	15.0	0.5
1.0E-04	7.5E+01	14.4	14.3	15.1	14.6	0.4
1.0E-02	7.5E+03	15.5	15.9	15.6	15.7	0.2
1.0E-01	7.5E+04	3.0	4.9	7.5	5.1	2.3

Results:06-17-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	14.0	13.4	14.9	14.1	0.8
2.5E-05	1.9E+01	13.8	17.0	14.8	15.2	1.6
6.3E-05	4.7E+01	14.0	14.3	14.2	14.2	0.2
1.6E-04	1.2E+02	18.4	15.7	15.4	16.5	1.7
4.0E-04	3.0E+02	16.2	15.4	15.3	15.6	0.5
1.0E-03	7.5E+02	14.0	14.4	14.3	14.2	0.2
2.5E-03	1.9E+03	14.3	14.2	15.0	14.5	0.4
6.3E-03	4.7E+03	14.7	14.7	15.7	15.0	0.6
1.6E-02	1.2E+04	14.0	12.6	13.7	13.4	0.7
4.0E-02	3.0E+04	4.5	4.2	3.9	4.2	0.3
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Results:06-19-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	14.4	14.9	15.3	14.9	0.5
2.5E-05	1.9E+01	15.3	15.5	15.2	15.3	0.2
6.3E-05	4.7E+01	15.2	15.2	15.3	15.2	0.1
1.6E-04	1.2E+02	15.0	16.2	15.5	15.6	0.6
4.0E-04	3.0E+02	15.7	17.1	14.8	15.9	1.2
1.0E-03	7.5E+02	16.6	16.4	16.4	16.5	0.1
2.5E-03	1.9E+03	17.1	17.1	17.1	17.1	0.0
6.3E-03	4.7E+03	15.9	14.8	14.3	15.0	0.8
1.6E-02	1.2E+04	9.3	9.2	10.5	9.7	0.7
4.0E-02	3.0E+04	1.7	2.6	7.0	3.8	2.8
1.0E-01	7.5E+04	0.0	00	0.0	0.0	0.0

Incubation time: 24h

Results:06-24-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	13.0	12.9	13.2	13.0	0.2
2.5E-05	1.9E+01	14.0	14.5	14.8	14.4	0.4
6.3E-05	4.7E+01	13.7	14.0	14.1	13.9	0.2
1.6E-04	1.2E+02	13.3	13.5	14.6	13.8	0.7
4.0E-04	3.0E+02	14.3	14.3	14.3	14.3	0.0
1.0E-03	7.5E+02	13.8	15.0	14.8	14.5	0.6
2.5E-03	1.9E+03	16.0	15.8	15.5	15.8	0.3
6.3E-03	4.7E+03	15.0	16.0	15.3	15.4	0.5
1.6E-02	1.2E+04	8.6	10.8	11.2	10.2	1.4
4.0E-02	3.0E+04	1.0	1.0	1.0	1.0	0.0
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Incubation time: 24h

Results:06-27-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	Conc./ppm	a	b	c	(mm)	
0	0	14.5	16.0	14.0	14.8	1.0
2.5E-05	1.9E+01	15.9	15.2	15.3	15.5	0.4
6.3E-05	4.7E+01	15.3	15.4	15.0	15.2	0.2
1.6E-04	1.2E+02	13.5	14.0	14.9	14.1	0.7
4.0E-04	3.0E+02	13.8	13.5	13.7	13.7	0.2
1.0E-03	7.5E+02	15.5	16.1	15.2	15.6	0.5
2.5E-03	1.9E+03	14.8	16.4	16.5	15.9	1.0
6.3E-03	4.7E+03	16.6	15.4	15.9	16.0	0.6
1.6E-02	1.2E+04	11.8	10.6	11.2	11.2	0.6
4.0E-02	3.0E+04	1.0	1.0	1.0	1.0	0.0
1.0E-01	7.5E+04	0.0	0.0	0.0	0.0	0.0

Microorganism: *Rhizoctonia solani*
Disinfectant: Clorox
SCSS 2%

Results:06-20-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0	16.8	16.3	16.1	16.4	0.4
2.5E-05	5.0E-01	17.6	17.3	16.4	17.1	0.6
6.3E-05	1.3E+00	17.9	17.9	16.8	17.5	0.6
1.6E-04	3.2E+00	17.2	17.6	16.4	17.1	0.6
4.0E-04	8.0E+00	18.2	17.4	16.7	17.4	0.8
1.0E-03	2.0E+01	15.8	17.3	15.7	16.3	0.9
2.5E-03	5.0E+01	17.0	15.3	16.2	16.2	0.9
6.3E-03	1.3E+02	14.6	13.7	13.2	13.8	0.7
1.6E-02	3.2E+02	6.3	5.9	6.2	6.1	0.2
4.0E-02	8.0E+02	0.0	0.0	0.0	0.0	0.0
1.0E-01	2.0E+03	0.0	0.0	0.0	0.0	0.0

Results:06-26-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0	15.1	16.3	17.1	16.2	1.0
2.5E-05	5.0E-01	16.5	16.8	16.7	16.7	0.2
6.3E-05	1.3E+00	16.0	16.9	16.5	16.5	0.5
1.6E-04	3.2E+00	15.3	16.5	16.0	15.9	0.6
4.0E-04	8.0E+00	16.7	15.9	15.4	16.0	0.7
1.0E-03	2.0E+01	16.3	16.7	15.7	16.2	0.5
2.5E-03	5.0E+01	14.7	16.4	14.7	15.3	1.0
6.3E-03	1.3E+02	12.5	11.9	13.6	12.7	0.9
1.6E-02	3.2E+02	9.0	7.4	8.6	8.3	0.8
4.0E-02	8.0E+02	2.2	0.0	1.5	1.2	1.1
1.0E-01	2.0E+03	0.0	0.0	0.0	0.0	0.0

Results:07-03-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./SCSS	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0	12.5	12.4	12.5	12.5	0.1
2.5E-05	5.0E-01	12.7	13.2	12.7	12.9	0.3
6.3E-05	1.3E+00	12.6	12.1	11.3	12.0	0.7
1.6E-04	3.2E+00	12.9	13.0	12.7	12.9	0.2
4.0E-04	8.0E+00	12.7	13.0	13.0	12.9	0.2
1.0E-03	2.0E+01	12.3	11.9	12.3	12.2	0.2
2.5E-03	5.0E+01	12.2	11.8	11.6	11.9	0.3
6.3E-03	1.3E+02	9.0	9.1	10.2	9.4	0.7
1.6E-02	3.2E+02	4.8	4.8	4.3	4.6	0.3
4.0E-02	8.0E+02	1.0	0.0	1.0	0.7	0.6
1.0E-01	2.0E+03	0.0	0.0	0.0	0.0	0.0

Microorganism: *Rhizoctonia zeae*
Disinfectant: Ferti-lome (propiconazole 1.55%)
Minimum Application Rate (MAR) 1ppm a.i. (really 0.5 ppm)

Results:07-14-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./MAR	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0	11.3	12.3	12.6	12.1	0.7
1.0E-06	1.0E-06	11.0	12.2	11.8	11.7	0.6
1.0E-05	1.0E-05	11.2	11.8	12.2	11.8	0.5
1.0E-04	1.0E-04	11.2	10.9	11.9	11.3	0.5
1.0E-03	1.0E-03	11.7	11.3	12.4	11.8	0.5
1.0E-02	1.0E-02	11.6	10.6	10.9	11.0	0.5
1.0E-01	1.0E-01	7.6	6.9	7.1	7.2	0.4
1.0E+00	1.0E+00	3.3	3.8	3.1	3.4	0.4
1.0E+01	1.0E+01	1.4	1.6	1.2	1.4	0.2
1.0E+02	1.0E+02	1.3	0.9	1.7	1.3	0.4
1.0E+03	1.0E+03	1.2	1.4	1.3	1.3	0.1

BMD 0.0064 MAR

Results:09-18-2009a Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0	8.8	8.7	8.7	8.7	0.1
2.5E-02	1.6E-04	8.8	8.5	8.2	8.5	0.3
6.3E-02	4.0E-04	8.3	8.0	8.7	8.3	0.3
1.6E-01	1.0E-03	8.7	8.4	8.4	8.5	0.2
4.0E-01	2.5E-03	8.8	8.3	8.3	8.5	0.3
1.0E+00	6.4E-03	8.0	8.4	8.2	8.2	0.2
2.5E+00	1.6E-02	8.1	8.5	8.8	8.5	0.3
6.3E+00	4.0E-02	7.5	7.7	8.0	7.8	0.2
1.6E+01	1.0E-01	6.9	6.3	6.4	6.5	0.3
1.6E+04	1.0E+02	1.0	1.0	1.0	1.0	0.0

Results:09-18-2009b Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0.00	8.2	8.3	7.6	8.0	0.3
2.5E-02	1.6E-04	7.5	7.9	7.6	7.7	0.2
6.3E-02	4.0E-04	8.0	7.5	7.1	7.5	0.4
1.6E-01	1.0E-03	7.4	6.3	7.7	7.1	0.8
4.0E-01	2.5E-03	7.1	7.7	7.7	7.5	0.3
1.0E+00	6.4E-03	7.9	7.5	7.7	7.7	0.2
2.5E+00	1.6E-02	8.7	7.8	8.3	8.3	0.5
6.3E+00	4.0E-02	8.5	8.4	8.4	8.5	0.1
1.6E+01	1.0E-01	7.3	7.0	7.3	7.2	0.2
1.6E+04	1.0E+02	0.0	0.0	0.0	0.0	0.0

Results:10-01-2009 Incubation time: 24h

Means: Conc./BMD	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	8.3	8.4	8.7	8.4	0.2
2.5E-02	1.6E-04	9.3	7.7	8.8	8.6	0.8
6.3E-02	4.0E-04	8.7	8.0	8.8	8.5	0.5
1.6E-01	1.0E-03	7.9	9.0	8.6	8.5	0.6
4.0E-01	2.5E-03	8.7	8.3	8.9	8.7	0.3
1.0E+00	6.4E-03	8.3	8.8	8.6	8.6	0.3
2.5E+00	1.6E-02	8.1	8.9	8.4	8.5	0.4
6.3E+00	4.0E-02	8.6	8.3	8.5	8.5	0.2
1.6E+01	1.0E-01	7.4	7.2	7.2	7.3	0.1
1.6E+04	1.0E+02	0.0	0.0	0.0	0.0	0.0

Results:10-02-2009 Czapec Dox Incubation time: 24h

Means: Conc./BMD	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	13.5	14.9	15.2	14.5	0.9
2.5E-02	1.6E-04	13.6	15.1	15.4	14.7	1.0
6.3E-02	4.0E-04	15.0	13.4	14.6	14.4	0.8
1.6E-01	1.0E-03	15.3	15.0	14.0	14.8	0.7
4.0E-01	2.5E-03	14.8	13.9	14.3	14.4	0.5
1.0E+00	6.4E-03	13.9	12.7	14.4	13.7	0.9
2.5E+00	1.6E-02	13.7	13.2	12.5	13.1	0.6
6.3E+00	4.0E-02	10.8	11.7	10.8	11.1	0.5
1.6E+01	1.0E-01	9.6	10.3	9.3	9.7	0.5
1.6E+04	1.0E+02	0.0	0.0	0.0	0.0	0.0

Microorganism: *Pythium aphanidermatum*
Disinfectant: Segway (cyazofamid 34.5%)
Minimum Application Rate(MAR) 0.33 ppm a.i.

Results:10-19-2009 Incubation time: 72h

Means: Conc./MAR	Conc./ppm	Replicates (mm) a	b	c	tot. mean (mm)	sd (mm)
0	0	14.8	15.9	14.2	15.0	0.9
1.0E-04	3.30E-05	19.6	15.8	17.2	17.5	1.9
1.0E-03	3.30E-04	13.5	14.7	3.0	10.4	6.5
1.0E-02	3.30E-03	1.9	0.0	4.6	2.2	2.3
1.0E-01	3.30E-02	2.9	0.0	2.7	1.8	1.6
1.0E+00	0.33	1.0	1.5	2.0	1.5	0.5
1.0E+01	3.30	0.0	0.0	7.5	2.5	4.3
1.0E+02	3.30E+01	0.0	0.0	0.0	0.0	0.0
1.0E+03	3.30E+02	0.0	0.0	0.0	0.0	0.0
1.0E+04	3.30E+03	0.0	0.0	0.0	0.0	0.0

Results:10-30-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	Conc./ppm	a	b	c	(mm)	
0	0	3.4	3.6	2.4	3.1	0.6
3.2E-05	1.04E-06	3.2	3.9	3.8	3.6	0.4
1.0E-04	3.30E-06	3.9	2.5	2.6	3.0	0.8
3.2E-04	1.04E-05	2.9	2.4	3.3	2.9	0.5
1.0E-03	3.30E-05	3.4	4.6	2.6	3.5	1.0
3.2E-03	1.04E-04	3.2	2.5	3.3	3.0	0.4
1.0E-02	3.30E-04	3.0	2.3	3.4	2.9	0.5
3.2E-02	1.04E-03	2.3	2.0	1.4	1.9	0.5
1.0E-01	3.30E-03	1.7	2.8	1.9	2.1	0.6
3.2E-01	1.04E-02	0.0	0.0	0.0	0.0	0.0

Incubation time: 24h

Results:10-31-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	Conc./ppm	a	b	c	(mm)	
0	0	10.6	10.1	10.0	10.2	0.3
3.16E-05	1.04E-06	11.6	10.3	11.9	11.2	0.8
1.00E-04	3.30E-06	9.1	13.1	10.9	11.0	2.0
3.16E-04	1.04E-05	9.9	9.0	11.0	10.0	1.0
1.00E-03	3.30E-05	12.2	8.3	7.6	9.4	2.5
3.16E-03	1.04E-04	8.2	7.9	10.1	8.7	1.2
1.00E-02	3.30E-04	7.2	9.6	7.0	7.9	1.4
3.16E-02	1.04E-03	7.8	8.0	7.4	7.7	0.3
1.00E-01	3.30E-03	5.3	6.4	3.8	5.2	1.3
3.16E-01	1.04E-02	2.5	2.9	2.5	2.6	0.2
1.0	3.30E-02	1.9	2.4	1.2	1.8	0.6

Incubation time: 24h

Results:11-03-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	Conc./ppm	a	b	c	(mm)	
0	0	2.8	4.2	4.1	3.7	0.8
3.16E-05	1.04E-06	5.5	5.5	4.2	5.1	0.8
1.00E-04	3.30E-06	5.7	3.8	3.0	4.2	1.4
3.16E-04	1.04E-05	3.0	5.7	2.6	3.8	1.7
1.00E-03	3.30E-05	6.1	5.6	2.5	4.7	2.0
3.16E-03	1.04E-04	3.7	3.7	5.5	4.3	1.0
1.00E-02	3.30E-04	5.5	2.0	2.1	3.2	2.0
3.16E-02	1.04E-03	1.4	4.2	3.5	3.0	1.4
1.00E-01	3.30E-03	1.7	2.2	1.2	1.7	0.5
3.16E-01	1.04E-02	0.5	0.5	0.9	0.6	0.2
1.0	3.30E-02	0.0	0.5	1.0	0.5	0.5

Results:11-04-2009 Incubation time: 24h

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BDM	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0	5.0	5.5	4.7	5.0	0.4
3.16E-05	1.04E-06	6.4	6.6	8.0	7.0	0.9
1.00E-04	3.30E-06	10.0	6.4	5.3	7.2	2.5
3.16E-04	1.04E-05	6.2	7.1	5.3	6.2	0.9
1.00E-03	3.30E-05	6.2	4.4	6.1	5.5	1.0
3.16E-03	1.04E-04	5.5	5.0	5.7	5.4	0.4
1.00E-02	3.30E-04	6.4	3.4	6.1	5.3	1.7
3.16E-02	1.04E-03	7.8	4.3	5.7	5.9	1.8
1.00E-01	3.30E-03	7.2	5.8	6.3	6.4	0.7
3.16E-01	1.04E-02	5.7	5.9	4.0	5.2	1.0
1.0	3.30E-02	2.6	4.4	4.3	3.8	1.0

Results:11-21-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0	8.6	10.6	12.8	10.7	2.1
1.00E-08	3.30E-10	9.5	8.5	13.4	10.4	2.6
3.16E-08	1.04E-09	12.2	12.4	13.1	12.5	0.5
1.00E-07	3.30E-09	10.6	12.2	10.1	10.9	1.1
3.16E-07	1.04E-08	14.0	14.0	13.9	14.0	0.1
1.00E-06	3.30E-08	14.0	9.6	12.5	12.0	2.2
3.16E-06	1.04E-07	11.1	10.1	12.1	11.1	1.0
1.00E-05	3.30E-07	9.9	10.0	10.5	10.1	0.3
1.00E-04	3.30E-06	5.9	8.2	13.3	9.1	3.8
1.00E-03	3.30E-05	7.3	5.8	13.6	8.9	4.1
1.00E-02	3.30E-04	10.3	8.7	9.6	9.5	0.8
1.0	3.30E-02	0.0	0.0	0.0	0.0	0.0

Results: 12-18-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**(mm)**	
0	0	27.1	13.4	14.6	18.4	7.6
1.0E-04	3.3E-06	13.4	26.0	25.8	21.7	7.2
3.2E-04	1.0E-05	23.4	17.6	11.9	17.6	5.8
1.0E-03	3.3E-05	21.1	22.8	22.9	22.2	1.0
3.2E-03	1.0E-04	21.8	25.7	25.7	24.4	2.3
1.0E-02	3.3E-04	15.9	17.0	15.1	16.0	1.0
3.2E-02	1.0E-03	6.4	5.2	9.1	6.9	2.0
0.10	3.3E-03	3.3	0.5	3.8	2.5	1.8
1.00	3.3E-02	0.0	0.0	0.0	0.0	0.0
10.00	3.3E-01	0.0	0.0	0.0	0.0	0.0
100.00	3.3	0.0	0.0	0.0	0.0	0.0

Results: 12-18-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	Conc./ppm	a	b	c	(mm)	
0	0	17.5	21.8	21.9	20.4	2.5
1.0E-04	3.3E-06	21.8	21.9	25.8	23.1	2.3
3.2E-04	1.0E-05	18.5	21.5	17.6	19.2	2.0
1.0E-03	3.3E-05	17.6	18.6	22.8	19.6	2.8
3.2E-03	1.0E-04	19.9	25.7	25.7	23.8	3.3
1.0E-02	3.3E-04	17.0	13.6	20.0	16.8	3.2
3.2E-02	1.0E-03	8.5	8.3	7.3	8.0	0.6
0.10	3.3E-03	1.9	2.5	2.3	2.2	0.3
1.00	3.3E-02	0.0	0.0	0.0	0.0	0.0
10.00	3.3E-01	0.0	0.0	0.0	0.0	0.0
100.00	3.3	0.0	0.0	0.0	0.0	0.0

Results: 12-18-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./BMD	Conc./ppm	a	b	c	(mm)	
0	0	5.2	8.2	12.6	8.6	3.7
1.0E-05	3.3E-07	7.6	7.4	7.2	7.4	0.2
3.2E-05	1.0E-06	10.6	8.2	10.1	9.6	1.3
1.0E-04	3.3E-06	3.0	7.6	11.7	7.4	4.4
3.2E-04	1.0E-05	7.1	6.7	7.0	6.9	0.2
1.0E-03	3.3E-05	10.2	14.0	11.1	11.8	2.0
3.2E-03	1.0E-04	11.6	9.5	4.8	8.6	3.5
0.01	3.3E-04	4.4	6.4	4.9	5.2	1.0
0.03	1.0E-03	4.3	2.2	2.6	3.0	1.1
0.10	3.3E-03	0.9	0.0	0.0	0.3	0.5
1.00	3.3E-02	0.0	0.0	0.0	0.0	0.0

Microorganism: *Pythium aphanidermatum*
Disinfectant: Ridomil Gold (mefenoxam 47.6%)
Minimum Application Rate(MAR) 0.63 ppm a.i.

Results: 12-18-2009

Means:		Replicates (mm)			tot. mean	sd (mm)
Conc./MAR	Conc./ppm	a	b	c	(mm)	
0	0	14.9	17.0	16.8	16.3	1.1
1.0E-05	6.3E-06	20.7	20.5	15.1	18.8	3.2
1.0E-04	6.3E-05	11.6	22.7	15.3	16.5	5.7
1.0E-03	6.3E-04	14.8	13.7	20.7	16.4	3.7
1.0E-02	6.3E-03	12.6	17.6	17.9	16.0	3.0
1.0E-01	6.3E-02	23.0	21.2	16.8	20.4	3.2
1.0E+00	6.3E-01	17.3	18.2	18.1	17.9	0.5
1.0E+01	6.3E+00	11.4	14.5	10.7	12.2	2.1
1.0E+02	6.3E+01	3.9	5.5	5.2	4.9	0.9

Microorganism: *Pythium aphanidermatum*
Disinfectant: Segway (cyazofamid 34.5%)
Minimum Application Rate(MAR) 0.33 ppm a.i.

Results: 12-18-2009 Incubation time: 24h

Means:		Replicates (mm)						tot. mean	sd
Conc./BMD	Conc./ppm	a	b	c	d	e	f	(mm)	(mm)
0	0	27.0	13.4	14.6	17.4	21.7	21.9	18.9	5.6
1.0E-04	3.3E-06	13.1	26.0	25.8	20.6	21.9	25.8	21.5	5.2
3.2E-04	1.0E-05	23.4	17.5	11.9	18.5	21.4	17.6	18.6	4.4
1.0E-03	3.3E-05	21.0	22.7	22.9	17.5	18.5	22.8	20.6	2.4
3.2E-03	1.0E-04	21.7	25.6	25.7	19.9	25.7	25.7	23.7	2.7
1.0E-02	3.3E-04	15.8	17.0	15.1	16.9	13.5	20.0	15.7	1.4
3.2E-02	1.0E-03	6.3	5.2	9.1	8.4	8.2	7.3	7.5	1.6
0.10	3.3E-03	3.3	0.5	3.8	1.9	2.4	2.3	2.4	1.3
1.00	3.3E-02	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10.00	3.3E-01	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results: 01-18-2010 Incubation time: 24h

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	Conc./ppm	a	b	c	d	e	(mm)	(mm)
0	0	15.6	20.4	21.9	21.0	17.7	19.4	2.6
1.0E-04	3.3E-06	23.4	22.1	16.9	24.9	20.7	21.6	3.1
3.2E-04	1.0E-05	21.3	22.8	21.8	24.2	21.4	22.3	1.2
1.0E-03	3.3E-05	27.2	20.0	18.2	21.9	23.2	22.1	3.4
3.2E-03	1.0E-04	24.8	24.5	20.4	20.7	23.2	22.7	2.1
0.01	3.3E-04	17.3	26.3	19.8	21.9	17.0	20.5	3.8
0.03	1.0E-03	6.8	8.1	7.4	7.1	7.8	7.5	0.5
0.10	3.3E-03	3.8	3.8	2.7	4.0	4.9	3.9	0.8
1.00	3.3E-02	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10.00	3.3E-01	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results: 01-24-2010 Incubation time: 24h

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	Conc./ppm	a	b	c	d	e	(mm)	(mm)
0	0	24.0	18.4	17.0	29.4	25.7	22.9	5.2
1.0E-04	3.3E-06	23.6	18.0	21.6	18.7	21.2	20.6	2.3
3.2E-04	1.0E-05	22.1	20.2	17.8	19.0	19.4	19.7	1.6
1.0E-03	3.3E-05	22.4	20.4	19.5	17.4	22.7	20.5	2.2
3.2E-03	1.0E-04	20.4	21.3	21.0	27.6	21.0	22.3	3.0
0.01	3.3E-04	17.5	15.4	24.8	12.4	23.4	18.7	5.3
0.03	1.0E-03	11.4	9.4	8.8	11.5	10.4	10.3	1.2
0.10	3.3E-03	6.6	6.4	8.0	6.6	6.2	6.8	0.7
1.00	3.3E-02	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10.00	3.3E-01	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results: 02-13-2010 Incubation time: 24h

Means:		Replicates (mm)					tot. mean (mm)	sd (mm)
Conc./BMD	Conc./ppm	a	b	c	d	e		
0	0	31.5	39.7	41.0	38.8	41.8	38.5	4.1
1.0E-04	3.3E-06	43.4	39.8	42.8	32.5	30.5	37.8	5.9
3.2E-04	1.0E-05	42.8	29.4	39.4	41.4	43.1	39.2	5.7
1.0E-03	3.3E-05	43.9	43.4	41.0	30.8	31.8	38.2	6.4
3.2E-03	1.0E-04	41.4	42.1	40.3	28.3	31.3	36.7	6.4
0.01	3.3E-04	29.2	36.5	40.2	39.9	33.5	35.8	4.6
0.03	1.0E-03	20.0	18.9	18.8	23.0	25.2	21.2	2.8
0.10	3.3E-03	8.5	8.7	11.5	11.6	8.8	9.8	1.6
1.00	3.3E-02	0.0	1.6	2.5	5.0	8.3	3.5	3.2
10.00	3.3E-01	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results: 02-20-2010 Incubation time: 24h

Means:		Replicates (mm)					tot. mean (mm)	sd (mm)
Conc./BMD	Conc./ppm	a	b	c	e	f		
0	0	26.6	24.7	22.6	35.9	26.6	27.3	5.1
1.0E-04	3.3E-06	35.3	40.4	22.9	24.4	26.6	29.9	7.6
3.2E-04	1.0E-05	28.5	27.6	27.9	25.3	29.0	27.7	1.4
1.0E-03	3.3E-05	26.8	27.9	27.6	25.3	26.4	26.8	1.0
3.2E-03	1.0E-04	21.7	24.2	28.1	32.5	25.3	26.3	4.1
0.01	3.3E-04	37.9	25.5	23.4	24.0	38.5	29.8	7.7
0.03	1.0E-03	17.6	20.6	16.9	21.9	17.6	18.9	2.2
0.10	3.3E-03	9.3	9.6	8.3	7.9	6.3	8.2	1.3
1.00	3.3E-02	0.0	0.0	0.9	2.2	2.7	1.1	1.2
10.00	3.3E-01	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Microorganism: *Pythium aphanidermatum*
Disinfectant: Previcur (propamocarb 66.5%)
Minimum Application Rate(MAR) 6 ppm

Results:02-27-2010

Means:		Replicates (mm)			tot. mean (mm)	sd (mm)
Conc./MAR	Conc./ppm	a	b	c		
0.0	0.0	45.8	40.5	42.1	42.8	2.7
0.0	0.1	42.5	42.2	44.3	43.0	1.1
0.1	0.6	41.0	38.1	41.4	40.1	1.8
1.0	6.0	14.8	23.5	15.6	18.0	4.8
10.0	60.0	7.0	4.7	5.3	5.7	1.2
100.0	600.0	0.5	2.5	1.5	1.5	1.0

BMD **0.5 ppm**

Results:03-06-2010 24h @ 26 degrees

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	Conc./ppm	a	b	c	d	e	(mm)	(mm)
0	0	29.4	28.1	26.8	23.5	29.0	27.4	2.4
4.0E-02	0.02	28.7	25.7	23.6	23.4	26.9	25.6	2.2
1.0E-01	0.051	24.6	29.8	24.6	25.9	30.9	27.1	3.0
2.5E-01	0.13	23.6	26.0	29.6	22.2	33.0	26.9	4.4
6.3E-01	0.32	29.9	29.6	24.1	28.1	34.9	29.3	3.9
1.6E+00	0.8	33.7	28.2	21.7	26.6	29.1	27.8	4.4
4.0E+00	2	24.7	27.8	16.1	25.3	27.7	24.3	4.8
10	5	20.7	22.7	23.6	22.0	23.7	22.5	1.2
1.0E+02	50	9.0	7.6	8.2	9.4	5.0	7.8	1.7
1.0E+03	500	4.3	2.9	5.9	6.1	7.6	5.3	1.8
1.0E+04	5000	1.2	0.0	0.0	1.3	0.0	0.5	0.7

Results:03-13-2010 24h @ 28 degrees

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	Conc./ppm	a	b	c	d	e	(mm)	(mm)
0	0	39.8	40.9	34.4	21.2	24.6	32.2	8.9
4.0E-02	0.02	36.5	31.0	35.4	21.4	42.3	33.3	7.8
1.0E-01	0.051	37.0	41.7	34.5	31.7	26.8	34.3	5.6
2.5E-01	0.13	35.6	37.4	40.5	24.0	34.0	34.3	6.3
6.3E-01	0.32	37.2	39.2	41.7	39.7	37.9	39.1	1.7
1.6E+00	0.8	36.9	22.6	34.7	40.5	26.5	32.2	7.4
4.0E+00	2	25.3	22.5	35.9	34.4	36.5	30.9	6.5
10	5	21.9	18.8	22.7	22.7	23.0	21.8	1.8
1.0E+02	50	3.4	6.6	9.3	6.9	5.5	6.3	2.2
1.0E+03	500	2.9	1.3	8.6	1.9	1.4	3.2	3.1
1.0E+04	5000	2.8	2.1	5.9	1.9	4.6	3.4	1.7

Results:04-06-2010 24h @ 28 degrees

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	Conc./ppm	a	b	c	d	e	(mm)	(mm)
0	0	21.6	48.0	53.8	45.8	46.4	43.1	12.4
4.0E-02	0.02	45.4	43.4	33.4	49.0	48.2	43.9	6.3
1.0E-01	0.051	44.0	45.8	50.2	49.9	47.4	47.5	2.7
2.5E-01	0.13	21.3	47.9	48.4	48.3	45.5	42.3	11.8
6.3E-01	0.32	10.9	15.2	15.0	16.1	15.8	14.6	2.1
1.6E+00	0.8	39.5	43.8	25.9	52.3	47.2	41.7	10.0
4.0E+00	2	24.7	46.2	23.5	49.2	25.0	33.7	12.8
10	5	37.8	42.9	23.3	44.8	46.2	39.0	9.3
1.0E+02	50	19.5	7.7	19.1	17.0	12.1	15.0	5.1
1.0E+03	500	12.1	2.7	8.3	5.1	7.2	7.1	3.5
1.0E+04	5000	0.8	10.1	7.5	7.0	12.9	7.6	4.5

Results:04-13-2010 24h @ 28 degrees

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**d**	**e**	**(mm)**	**(mm)**
0	0	34.4	38.3	29.8	30.1	28.4	32.2	4.1
4.0E-02	0.02	38.9	37.9	33.9	30.9	29.7	34.3	4.1
1.0E-01	0.051	36.4	34.0	30.1	26.6	27.8	30.9	4.1
2.5E-01	0.13	38.7	37.1	39.2	26.7	31.9	34.7	5.3
6.3E-01	0.32	38.2	38.5	37.1	33.3	30.4	35.5	3.5
1.6E+00	0.8	35.8	35.9	36.4	28.2	29.5	33.2	4.0
4.0E+00	2	38.5	37.3	25.8	31.4	31.1	32.8	5.2
10	5	32.2	31.6	26.9	19.9	23.2	26.7	5.3
1.0E+02	50	13.1	8.0	9.7	10.3	9.7	10.1	1.9
1.0E+03	500	8.6	6.1	8.8	10.1	6.7	8.0	1.6
1.0E+04	5000	10.3	8.5	3.8	8.4	7.9	7.7	2.4
1.0E+05	50000	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results:04-20-2010 24h @ 28 degrees

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**d**	**e**	**(mm)**	**(mm)**
0	0	37.1	38.9	37.9	28.4	27.0	33.8	5.7
4.0E-02	0.02	45.6	29.1	43.8	34.7	30.3	36.7	7.6
1.0E-01	0.051	37.4	32.1	33.7	38.0	30.5	34.3	3.3
2.5E-01	0.13	39.4	38.9	43.6	34.0	31.3	37.4	4.8
6.3E-01	0.32	40.9	34.5	42.9	43.4	35.3	39.4	4.2
1.6E+00	0.8	39.8	38.9	42.8	27.4	30.9	36.0	6.5
4.0E+00	2	35.7	37.2	35.4	38.6	26.1	34.6	4.9
10	5	38.5	33.2	31.7	34.1	23.0	32.1	5.7
1.0E+02	50	14.3	4.4	16.0	3.3	5.0	8.6	6.0
1.0E+03	500	4.1	1.8	3.4	3.3	2.2	3.0	0.9
1.0E+04	5000	4.1	2.3	4.9	4.8	0.8	3.4	1.8
1.0E+05	50000	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results:04-27-2010 24h @ 28 degrees C

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**d**	**e**	**(mm)**	**(mm)**
0	0	40.4	39.1	40.0	36.0	34.3	38.0	2.7
4.0E-02	0.02	39.0	32.3	40.1	35.6	38.2	37.0	3.1
1.0E-01	0.051	37.1	35.2	37.7	31.2	39.1	36.0	3.0
2.5E-01	0.13	37.6	36.0	37.3	32.7	36.6	36.0	2.0
6.3E-01	0.32	35.4	38.1	39.3	32.4	38.9	36.8	2.9
1.6E+00	0.8	40.0	36.6	41.5	36.6	36.3	38.2	2.4
4.0E+00	2	37.9	43.0	40.1	33.0	40.3	38.8	3.7
10	5	27.4	32.1	29.3	23.2	21.9	26.8	4.2
1.0E+02	50	5.6	6.2	4.0	6.1	7.1	5.8	1.1
1.0E+03	500	3.2	6.3	5.1	6.1	7.0	5.5	1.5
1.0E+04	5000	7.7	6.5	5.3	5.3	8.6	6.7	1.5
1.0E+05	50000	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Microorganism: *Rhizoctonia solani*
Pesticide: Ferti-lome (Propiconazole 1.55%) PDA Hi Media
Minimum Application Rate(MAR) 0.5ppm

Results:02-27-2010

Means:		Replicates (mm)			tot. mean	sd
Conc./MAR	Conc./ppm	a	b	c	(mm)	(mm)
0	0	27.3	24.7	28.0	26.7	2.7
0.01	0.005	26.9	26.0	24.8	25.9	1.1
0.1	0.05	22.9	25.2	21.5	23.2	1.8
1	0.5	18.55	17.9	15.7	17.4	4.8
10	5	12.65	13.9	11.2	12.6	1.2
100	50	5.7	5.1	6.3	5.7	1.0

BMD **0.03ppm**

Medium: PDA

Results:06-23-2010 24h @ 21 degrees C+24h @ 28 degrees C

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	Conc./ppm	a	b	c	d	e	(mm)	(mm)
0	0	45.8	45.0	45.7	44.0	42.0	44.5	1.6
1.0E+02	0.0003	43.5	45.6	43.4	44.3	43.7	44.1	0.9
4.0E+01	0.00075	44.0	46.1	41.0	44.9	43.4	43.9	1.9
1.6E+01	0.0019	42.4	42.6	41.3	43.7	44.6	42.9	1.3
6.3E+00	0.0048	42.7	38.2	44.0	42.7	49.3	43.4	4.0
2.5E+00	0.012	45.1	40.9	45.9	40.9	41.6	42.9	2.4
1.0E+00	0.03	40.8	46.8	40.5	40.5	40.7	41.8	2.8
10	0.3	35.8	34.7	34.7	34.8	30.3	34.0	2.2
1.0E+02	3	22.4	23.9	23.2	22.4	21.0	22.6	1.1
1.0E+03	30	10.4	11.5	9.1	12.3	10.1	10.6	1.2
1.0E+04	300	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results:06-26-2010 24h @ 28 degrees C **Medium:** PDA

Means:		Replicates (mm)					tot. mean	sd
Conc./BMD	Conc./ppm	a	b	c	d	e	(mm)	(mm)
0	0	31.0	37.3	30.6	32.6	33.0	32.9	2.7
1.0E+02	0.0003	31.0	34.9	30.0	32.5	36.4	32.9	2.7
4.0E+01	0.00075	31.4	36.2	32.8	33.6	33.4	33.5	1.7
1.6E+01	0.0019	33.9	37.7	31.6	34.7	35.1	34.6	2.2
6.3E+00	0.0048	32.4	34.9	33.2	33.0	34.9	33.7	1.1
2.5E+00	0.012	29.2	30.6	29.2	28.3	30.3	29.5	0.9
1.0E+00	0.03	32.1	36.1	32.0	32.9	33.1	33.2	1.7
10	0.3	25.4	26.4	29.1	25.8	28.7	27.1	1.7
1.0E+02	3	19.9	19.5	19.9	19.1	20.5	19.8	0.5
1.0E+03	30	11.5	11.1	11.2	10.8	11.5	11.2	0.3
1.0E+04	300	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results:06-29-2010 24h @ 28 degrees C **Medium:** PDA

Means:		**Replicates (mm)**					**tot. mean (mm)**	**sd (mm)**
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**d**	**e**		
0	0	31.9	34.6	29.9	31.2	29.9	31.5	1.9
1.0E+02	0.0003	32.6	35.2	31.6	32.6	32.4	32.9	1.4
4.0E+01	0.00075	33.2	33.2	31.7	35.8	32.7	33.3	1.5
1.6E+01	0.0019	28.5	29.4	28.5	31.8	28.4	29.3	1.4
6.3E+00	0.0048	31.6	28.6	28.9	33.3	30.7	30.6	1.9
2.5E+00	0.012	31.7	31.0	29.0	32.8	32.3	31.3	1.5
1.0E+00	0.03	29.1	27.6	28.3	30.4	28.2	28.7	1.1
10	0.3	24.7	23.1	22.5	24.2	23.0	23.5	0.9
1.0E+02	3	15.0	15.7	19.1	17.5	16.1	16.7	1.6
1.0E+03	30	10.0	11.2	9.9	11.1	10.8	10.6	0.6
1.0E+04	300	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results:07-02-2010 24h @ 28 degrees C **Medium:** PDA

Means:		**Replicates (mm)**					**tot. mean (mm)**	**sd (mm)**
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**	**d**	**e**		
0	0	34.6	36.6	33.6	31.5	36.6	34.6	2.2
1.0E+02	0.0003	37.6	34.1	34.2	37.6	33.6	35.4	2.0
4.0E+01	0.00075	34.3	35.4	33.9	34.6	35.7	34.8	0.8
1.6E+01	0.0019	31.8	30.6	31.3	33.4	33.2	32.0	1.2
6.3E+00	0.0048	33.2	31.9	31.3	33.8	32.8	32.6	1.0
2.5E+00	0.012	30.6	32.6	31.8	33.6	32.3	32.1	1.1
1.0E+00	0.03	31.5	31.3	30.3	32.9	33.0	31.8	1.1
10	0.3	25.6	24.5	24.8	25.7	27.3	25.6	1.1
1.0E+02	3	16.3	16.0	15.8	18.1	17.9	16.8	1.1
1.0E+03	30	10.3	10.3	9.9	10.2	10.2	10.2	0.2
1.0E+04	300	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Results:07-08-2010 24h @ 28 degrees C **Medium:** Kzapeck Dox

Means:		**Replicates (mm)**			**tot. mean (mm)**	**sd (mm)**
Conc./BMD	**Conc./ppm**	**a**	**b**	**c**		
0	0	14.6	18.1	16.3	16.3	1.8
1.0E+02	0.0003	14.4	14.2	13.0	13.8	0.7
4.0E+01	0.00075	13.3	16.0	15.4	14.9	1.4
1.6E+01	0.0019	15.0	18.1	16.7	16.6	1.6
6.3E+00	0.0048	16.0	17.0	14.2	15.7	1.4
2.5E+00	0.012	15.3	16.9	15.3	15.8	0.9
1.0E+00	0.03	13.9	16.5	14.7	15.0	1.3
10	0.3	10.4	10.5	10.3	10.4	0.1
1.0E+02	3	5.2	5.9	5.6	5.5	0.4
1.0E+03	30	1.7	2.3	1.8	1.9	0.3
1.0E+04	300	0.0	0.0	0.0	0.0	0.0

APPENDIX B

Standardized protocol for in vitro testing

The BMD was calculated and with the knowledge of this value, a comparative randomized block design was applied for each microorganism tested. The endpoint was the mycelia radial growth. The experimental units were petri dishes containing solid growing media amended with different doses of the pesticide. Ten different pesticide doses and a control for each pathogen were tested with a minimum of five doses below the BMD. Five replicates for each treatment and five repetitions of the experiment over time were performed. The modeling of the hormetic effect and the inference of EC_{50} and NOAEL were done using the Brain-Cousens model (28; 125).

Stock solution preparation

All solutions were prepared as described in the following protocol:

1. Autoclave distilled water, measurement cylinders, and amber glass bottles (each one with a magnetic stirrer inside).

2. Label each one of the previously autoclaved glass bottles with the letter corresponding to the different concentrations and one as control.

3. In the amber bottle labeled A prepare a solution with the highest concentration. To prepare this solution use distilled autoclaved water and two autoclaved cylinders, one to measure the water and one to measure the chemical.

4. Mix the solution on a stir plate at medium speed for two minutes.

5. Prepare serial dilutions and bottle in individual labeled flasks. Wash the measurement cylinder used for the chemical at least three times with sterile water before preparing a more diluted sample. Fill the bottle labeled as control with autoclaved water.

Determining parameter doses

Use the concentration of the minimum application rate (MAR) of the chemical to be used as a reference. If the application rate is in terms of kg a.i. x ha^{-1} transform this concentration to ppm a.i. by multiplying it by 4.17. We are assuming a soil bulk density of 1.2 g cm^{-3} and an effective soil depth of 2cm (46).

1. Use the set of dilutions shown in table 1a in the dose response assessment protocol in order to obtain data to determine the BMD.
2. If the obtained datasets are not appropriate for a BMD analysis (explained in the BMD calculation protocol) try spacing each dilution by two orders of magnitude.
3. Prepare a second set of dilutions with the concentrations shown in table 2a using the BMD as a reference.
4. Use this second set of dilutions in the dose response assessment protocol to obtain data to test for hormesis and to determine the NOAEL and EC_{50}.

Table 1a Concentrations of chemical agents to be used to determine an approximate no observed adverse effect level (NOAEL) dose for radial growth of soilborne pathogens *in vitro*.

Stock solution	Stock Concentration	Concentration in plate
A	MAR x 10^{3}	MAR x 10^{2}
B	MAR x 10^{2}	MAR x 10
C	MAR x 10	MAR
D	MAR	MAR x 10^{-1}
E	MAR x 10^{-1}	MAR x 10^{-2}
Control	0	0

MAR: Minimum application rate of the chemical agent

Table 2a Concentrations of chemical agents to be used to evaluate the hormetic response on radial growth of soilborne pathogens to pesticides *in vitro*

Stock solution	Stock Concentration	Concentration in plate
A	BMD x 10^4	BMD x 10^3
B	BMD x 10^3	BMD x 10^2
C	BMD x 10^2	BMD x 10
D	BMD x 10	BMD
E	BMD x $10^{0.6}$	BMD x $10^{-0.4}$
F	BMD x $10^{0.2}$	BMD x $10^{-0.8}$
G	BMD x $10^{-0.2}$	BMD x $10^{-1.2}$
H	BMD x $10^{-0.6}$	BMD x $10^{-1.6}$
I	BMD x 10^{-1}	BMDL x 10^{-2}
J	BMD x $10^{-1.4}$	BMD x $10^{-2.4}$
Control	0	0

BMD: Benchmark dose

Dose response assessment

To determine the dose response of *P. aphanidermatum* and *R. zeae* to different chemical agents the following protocol was developed:

1. Prepare Corn Meal Agar (CMA) using 10% less water to get the recommended concentration after the chemical was added.

2. Dispense CMA on flasks containing 112.5 ml of medium each. Label flasks with the letter corresponding to the different concentrations and one as control and autoclave with magnetic a stirrer inside each flask.

3. Place flasks with agar on a 55 °C water bath.

4. Once agar has cooled to the water bath temperature add 12.5 ml of the corresponding stock solution; starting with the control then continuing from the most diluted to the most concentrated. Stir the agar solution for two minutes. Maintain sterile conditions at all time.

5. Use a 25 ml pipette to pour 23 ml of agar on five plates for each concentration. Start with the control and then continue from the minimum to the maximum concentration.

6. Once the agar solidifies on the Petri dishes, plate 5mm diameter plugs from a 2 to 3 days old culture (inoculum) in the middle of the plate. Try to plate plugs that are equidistant from the center of growth of the inoculum culture (Fig. 1a). And seal plates with parafilm.

7. Incubate in the dark at 28 C for one day.

8. After incubation record the growth on each plate measuring two diameters in a 90 angle using a caliper (Fig. 2a).

Note: Hormetic responses are often minimal so keeping all the possible variables (e.g. volume of agar on the Petri dish, distance of the inoculum plug from the center of growth) as constants is very important for detecting it. Room temperature can also be used for incubation.

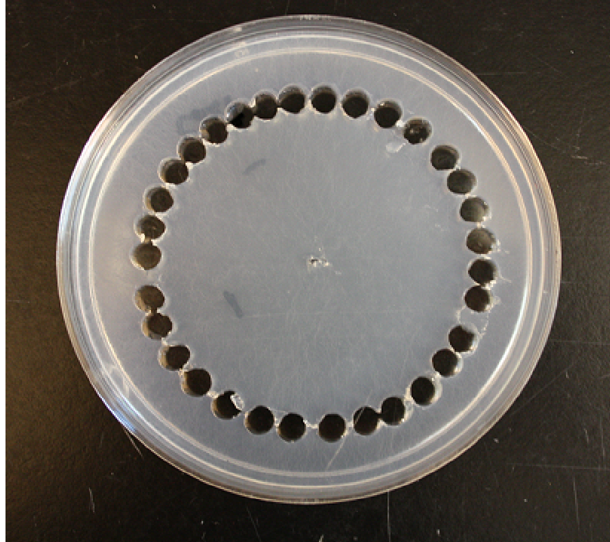

Figure 1a Equidistant plugs from the center of growth of *Pythium aphanidermatum* on corn meal agar.

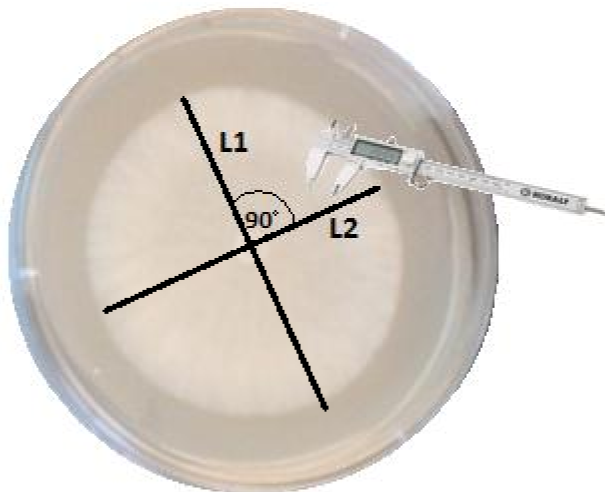

Figure 2a Measurement of two diameters to determine radial growth.

APPENDIX C

In planta testing

To standardize the conditions in which the seeds were germinated and infected a series of preliminary experiments regarding plant species, fertilizer concentration and growth chamber level were performed. Using the results of such experiments as reference, the following protocol was developed.

1. Place a 60x15mm dish on the top of a 100x15mm petri dish to hold a filter paper embedded on a 400ppm fertilizer (20-20-20) solution to create a container that will sustain geranium seeds for germination (Fig 1a).

2. Arrange five seeds and use 20 ml of fertilizer solution per petri dish array.

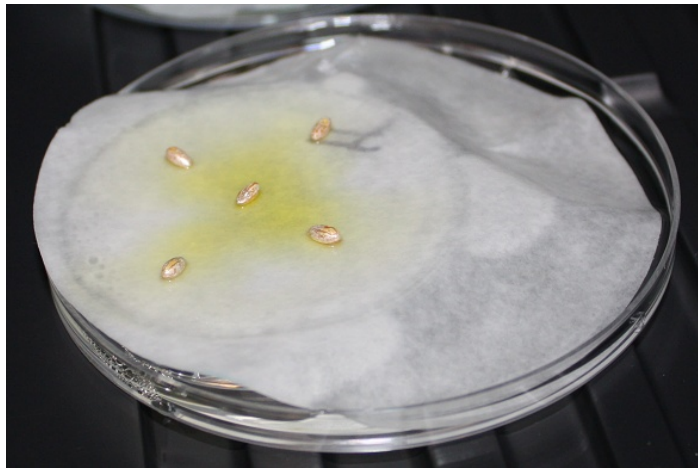

Figure 1a Petri dish array for seed germination

3. Place a total of 18 containers into plastic trays and label them randomly from A to E and Control by triplicate.

4. Cover trays with a dark lid and incubate at 26°C until the formation of the first true leaves.

5. Replace nutrient solution with pesticide solutions at different concentrations as shown in Table 1a. The pesticide concentrations are determined using the data obtained from the *in vitro* testing. The solutions need to be prepared using the stock solution preparation guidelines shown above.

6. After 48 hours of adding the pesticide solution inoculate the seedling with a 5mm diameter plug containing the pathogen. Place the inoculum on the seedling root, 5mm away from the beginning of the stem.

7. Incubate at 26 °C for 24 hours and use a caliper to record the disease development as the length of the seedling tissue that has turned dark due to the infection by the pathogen.

Table 1a. Pesticide concentrations to be used for in planta assessment of the effect of small doses of pesticides on disease development

Solution	**Concentration**
A	Complete control
B	EC_{50}
C	MSD
D	MSD 95% confidence upper limit
E	MSD $x10^{-1}$
Control	0

All concentrations are determined by *in vitro* testing of the effect of pesticide dose on the radial growth of the pathogen EC_{50}: Effective concentration at which the radial growth is inhibited by 50% compared to the control MSD: Maximum stimulation dose.

VITA

Francisco Javier Flores

Candidate for the Degree of

Master of Science

Thesis: EFFECT OF LOW DOSES OF PESTICIDES ON SOILBORNE PATHOGENS AN APPROACH TO THE HORMETIC RESPONSE

Major Field: Plant Pathology

Biographical:

Education:

Completed the requirements for the Master of Science in Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in December, 2010

Completed the requirements for the Bachelor of Science in Biotechnology at Escuela Politécnica del Ejército, Sangolquí, Ecuador in 2008

Experience:

Graduate Research Assistant and Teaching Assistant, Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater OK, January 2009 to December 2010

Research Assistant, Microbiology Laboratory, Escuela Politécnica del Ejército, Sangolquí, Ecuador, August 2007 to August 2008

Intern, International Potato Center, Cutuglagua, Ecuador, August to September 2006

Intern, Asociacion de Ganaderos de la Sierra y Oriente Genes, Quito, Ecuador, February to March 2006

Intern, Izquieta Pérez National Hygiene Center, Quito, Ecuador, August to September 2005.

Professional Memberships:

American Phytopathological Society (APS)
Oklahoma Academy of Science (OAS)
Phi Kappa Phi Honor Society
Golden Key Honor Society
American Association for the Advancement of Science (AAAS)

Name: Francisco Javier Flores — Date of Degree: December, 2010

Institution: Oklahoma State University — Location: Stillwater, Oklahoma

Title of Study: EFFECT OF LOW DOSES OF PESTICIDES ON SOILBORNE PATHOGENS AN APPROACH TO THE HORMETIC RESPONSE

Pages in Study: 90 — Candidate for the Degree of Master of Science

Major Field: Plant Pathology

Scope and Method of Study:
The effects of doses below the no observed adverse effect level (NOAEL) of disinfectants and commercial fungicides on the growth of *Rhizoctonia zeae, Rhizoctonia solani*, and *Pythium aphanidermatum in vitro* were assessed. A benchmark dose value (BMD), was determined for each chemical. Each organism was grown on solid growing media amended with two disinfectants and fungicides at different doses, with a minimum of five doses below the BMD, and a non amended control. The modeling of the hormetic effect and the inference of EC_{50} and NOAEL were done using a Brain-Cousens model.

Findings and Conclusions:

An increase of growth at low doses of ethanol was observed for both *R. zeae* and *P. aphanidermatum,* while sodium hypochlorite showed no stimulation. Non-monotonic responses were also observed on the dose effect of cyazofamid and of propamocarb on *P. aphanidermatum*. In contrast, propiconazole didn't show a hormetic effect on either *R. zeae* or *R. solani.* Due to the different modes of action of the chemical agents that rendered hormetic responses on the radial growth the organisms *in vitro*, multiple factors are suspected to be responsible for this phenomenon. An accurate experimental design and a sensitive data analysis tool were necessary to detect the hormetic response consistently. Results suggest that hormesis may not be a generalizable phenomenon in biology but it could have a relationship with the plasticity of an organism. This study provides, for the first time, evidence that the phenomenon of hormesis occurs in oomycete and fungal plant pathogens in response to doses of disinfectants or pesticides.

ADVISER'S APPROVAL: Carla Garzón