EVALUATION OF CHEMICAL, BIOLOGICAL, AND CULTURAL CONTROLS FOR THE MANAGEMENT OF POD ROT OF LIMA BEAN CAUSED BY *PHYTOPHTHORA CAPSICI*

by

Andrew Archer Kness

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Plant and Soil Sciences

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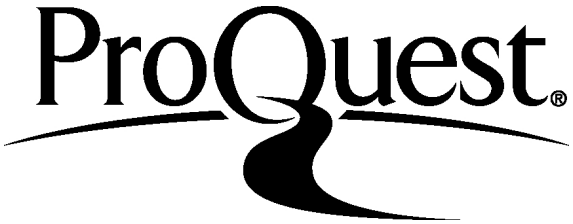

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ABSTRACT

Lima bean (*Phaseolus lunatus*) is an important crop for the Delmarva Peninsula, particularly Delaware, which plants more land area to lima bean than any other state. Yield loss due to diseases such as pod rot, caused by *Phytophthora capsici*, reduce profitability of the crop. *Phytophthora capsici* causes significant damage to lima beans during periods of high humidity and frequent rainfall. Symptoms include browning, drying, and abortion of the pod.

Current management practices for pod rot are limited, and fungicide choices are few. Mefenoxam is the most widely used fungicide and it has been registered for use against *P. capsici* on lima bean since 2000, however, repeated applications have resulted in populations of *P. capsici* with resistance. Cyazofamid was approved for control of pod rot 2013, however use remains low because of the expense.

The goal of this research was to identify new products and practices for managing pod rot of lima bean. These included identifying new fungicides with efficacy against *P. capsici* on lima bean, as well as evaluating biofumigation, biopesticides, and reduced tillage. All field experiments were conducted during the summer of 2013 and 2014 at the University of Delaware's Carvel Research and Education Center, Thurmond Adams Agricultural Research Farm located in Georgetown, Delaware.

All 12 fungicides significantly reduced pod rot disease incidence compared to controls ($P < 0.05$), and mefenoxam, cyazofamid, fluazinam, oxathiapiprolin, and potassium phosphite achieved the best control. Detached pod assays conducted in the

laboratory with the fungicides listed above support the results observed in the field, and indicate that flat pods are more susceptible to *P. capsici* infection than full pods within 72 hours post inoculation ($P = 0.0378$).

Experiments conducted to evaluate the effect of biofumigant crops (*Brassica spp.*, *Sinapis alba*, and *Sorghum bicolor*) and biopesticides (Actinovate, Double Nickel, Serenade Soil, Superzyme 1-0-4, RootShield, PlantShield, SoilGard, and *Trichoderma aperellum*) were inconclusive. Infection was low in these trials yielding no significant differences between treatments. Low levels of disease also occurred in the reduced tillage experiment conducted in 2013, as the effect of various tillage and soil surface residue practices on managing pod rot were inconclusive.

INTRODUCTION

The first description of *Phytophthora capsici* occurred in 1922 by Leon H. Leonian after he observed a novel species of *Phytophthora* causing significant damage to chili pepper (*Capsicum annuum*) plants on the Agricultural Research station in Las Cruces, New Mexico (39). Over the next several years, *P. capsici* was observed on many different vegetable crops up and down the Arkansas River Valley of Colorado, threatening vegetable production in the area (22). In 1937, cucumbers (*Cucumis sativus*) grown in Colorado and California became the first plants in the Cucurbitaceae to be known hosts for *P. capsici* (32). Three years later, eggplant (*Solanum melongena*), honeydew (*Cucumis melo*), tomato (*Solanum lycopersicum*), and summer squash (*Cucurbita pepo*) were also described as hosts (33). *Phytophthora capsici* is not just a domestic threat to the United States vegetable industry; it has reportedly caused several epidemics on several vegetable crops grown in Central and South America, Asia, and Europe (20). It is estimated that globally, *P. capsici* causes in excess of $100 million dollars in damage per year to *Capsicum* species alone (8). The widespread presence of this pathogen now threatens vegetable crops worldwide, with the potential to affect the world's food supply.

At a glance, *Phytophthora* species appear to be similar to the true fungi, however, a few key morphological differences distinguish them from the kingdom Fungi. One of the most significant differences between them and the true fungi is that their hyphae is aseptate, containing cell walls made of primarily cellulose, whereas the majority if true fungi have septate hyphae consisting mostly of chitin. DNA analysis

of *Phytophthora* species has revealed that they are more closely related to brown algae and have thus been classified as oomycetes, or water molds, in the kingdom Chromista (also referred to as Stramenopila) (17, 7). The name *Phytophthora* translates to "plant destroyer," and is a proper name for these plant pathogens as they are infamous for their ability to rapidly infect hosts and cause severe epidemics. Perhaps the infamous *Phytophthora* disease is potato late blight caused by *Phytophthora infestans*, which was responsible for the starvation of approximately 1 million people during the Irish Potato Famine (49). Diseases caused by *Phytophthora* species are particularly devastating and difficult to control due to their highly effective lifecycle.

Phytophthora capsici's lifecycle consists of two distinct reproductive stages; an asexual and a sexual stage. *Phytophthora capsici* is heterothallic and sexual reproduction can only occur when opposite mating types (designated A1 and A2) are in close enough proximity to each other that they can mate via the union of male antheridium and female oogonium (20). Sexual reproduction yields spores known as oospores. Oospores are soil-borne, bearing thick, multilayered walls containing cellulose and β-glucan. Oospores are capable of surviving in the soil without a host for many years (14). Following formation and after a dormancy period of one month, oospores may germinate directly or form sporangia to infect hosts (14). *Phytophthora capsici* also has the ability to produce thick-walled, scleritized, asexual spores called chlamydospores. These spores are highly resistant to unfavorable weather conditions, much like oospores, and may be the primary overwintering structure and the major source of primary inoculum in the absence of both mating types (6), however, many isolates of *P. capsici* do not produce chlamydospores in culture (36). *Phytophthora capsici* requires wet or saturated soil conditions to grow, and temperatures between 10

and 36 °C, with an optimal temperature between 24 and 33 °C (5). Once *P. capsici* infects a host, it produces sporangia which release asexual zoospores. Both zoospores and sporangia serve as secondary inoculum throughout the growing season and can infect susceptible hosts. Zoospores are also biflagellate and have the ability to swim for several hours (5) in free water and can locate nearby hosts by following electro and chemotactic signals in the soil (62). Under optimal conditions, sporulation from initial infection may take as little as 2-3 days (36), and the dissemination of sporangia and zoospores can quickly lead to a field epidemic and result in 100% yield loss (5).

Since Leonian's first description of *P. capsici* on chili pepper, its pathogenicity has been confirmed on more than fifty plant species (60), many of which are high-value, fresh market or processing vegetables. The list includes: tomato, eggplant, snap bean (*Phaseolus vulgaris*), beet (*Beta vulgaris*), spinach (*Spinacia oleracea*), and all species in the Cucurbitaceae family which include squash (*Cucurbita spp.*), melon (*Benincasa spp., Citrullus spp.*), pumpkin (*Cucurbita spp.*), cucumber, and zucchini (*Cucurbita pepo* var. *cylindrica*). Weed species such as Carolina geranium (*Geranium carolinianum*), purslane (*Portulaca oleracea*), American black nightshade (*Solanum americanum*) (60), and velvet-leaf (*Abutilon theophrasti*) (20) have been found to be alternate hosts for *P. capsici*. In addition, soybean (*Glycine max*) and alfalfa (*Medicago spp.*) are major agronomic crops which have been shown to be hosts under laboratory conditions (19). In 2000, *P. capsici* was confirmed for the first time on lima bean (*Phaseolus lunatus*) on five commercial cultivars from fields located in Delaware, Maryland, and New Jersey (12). Over the next several years, *P. capsici* became more prevalent in lima bean and now poses a serious threat to Delaware's processing lima bean industry.

The baby lima bean is a very important crop for Delaware, which leads the nation in planting approximately 5300 hectares of lima beans for fresh market and processing, annually, generating more than $10 million in farm revenue for Delaware farmers (13). The surrounding states of Maryland and New Jersey account for an additional 2500 hectares, for a tri-state combined total of over 7800 hectares of lima beans; accounting for over half of the total acreage grown in the United States (61). The majority of U.S. lima bean production is located in California and Delaware, and as a result of a combination of factors, including high land rent and other disease problems, California's lima bean production is in decline, presenting Delaware with an opportunity to increase its market share of baby lima bean production. Therefore, management of pod rot caused by *P. capsici* is of major importance for the industry to grow.

Phytophthora capsici has the ability to infect its host at any growth stage in crops such as pepper (5), but in lima bean, infection takes place late in the growing season when the plants are setting fruit. Droplets from rain or irrigation water splash inoculum from the soil surface onto the lima bean fruits (pods) where they germinate and infect. Zoospores, oospores, chlamydospores, and sporangia all have the ability to physically penetrate plant tissue via a germ tube and appressorium and are aided by secreted enzymes which help break down the plant's cuticle (15, 38). Once infected, the pathogen creates lesions on the pod, eventually turning it dry and brown. The plant will abort the pod, resulting in lost yield for the grower. Lima beans that are planted in an infested field during years of heavy rainfall and extended periods of standing water sustain high levels of disease, and loss of the entire crop and has been reported. Current management practices for pod rot are limited. These include

rotating lima beans with a non-host crop species, not working wet ground, avoiding moving soil between fields, reducing irrigation, and use of the commercial fungicide mefenoxam. However, mefenoxam is ineffective in many areas of the United States due to the emergence mefenoxam-resistant populations of *P. capsici* (26, 34, 44). Even if growers follow these recommendations, yield loss due to pod rot can still be significant, especially during years when weather conditions favor infection. In order to protect lima bean yields, new pest management practices must be developed for *P. capsici*.

Research on the management of *P. capsici* on lima bean production has lagged behind similar research conducted on peppers and cucurbits, because lima bean was not known as a host until 2000 and lima bean is a specialty crop grown on fewer acres than peppers and cucurbits. Many of the most effective cultural practices for the management of *P. capsici*, such as the use drip irrigation and raised beds (53), are not applicable for lima bean production and other processing vegetables due to the large scale mechanization of planting, harvesting, and management of the crop; therefore, other management tactics must be investigated.

Every good agriculturalist knows the importance of crop rotation for soil health and pest management. As for any agronomic or horticultural crop pest, it is often recommended to rotate susceptible crops with non-host crops to reduce inoculum levels, and thus reduce disease pressure in the susceptible crop. This has been effective for many soil borne diseases (1, 50). However, with *P. capsici*, this does not hold true. In 2003, Lamour and Hausbeck published the results of a rotation study and concluded that a two year crop rotation did not significantly reduce *P. capsici* disease pressure in cucurbits, likely due to the fact that oospores and chlamydospores can

survive in the soil for many years (35). In order to see a significant reduction in disease, growers may have to rotate with a non-host species for more than five years. The wide host range of *P. capsici* makes it challenging and often economically impractical for farmers to rotate away from non-host crops for any more than a couple of years. Other studies have demonstrated that modifying irrigation methods and irrigation frequency can help combat Phytophthora blight in cucurbits and peppers. It is well understood that *P. capsici* disease pressure increases with moisture levels in the soil. Multiple studies have confirmed that less frequent drip irrigation (53, 54) and furrow irrigation (10) correlate with a reduction in *P. capsici* disease incidence, which has made eliminating excessive irrigation a priority management strategy. An interesting study conducted by von Broembsen and Deacon (64) found that calcium-amended irrigation water significantly interfered with germination of *Phytophthora parisitica* zoospores. Three years later, Stanghellini *et al*. (59) discovered that amending a recirculating nutrient solution with the surfactant Naiad, resulted in complete control of *P. capsici* spread in potted pepper plants. These two studies, while promising, only concern *Phytophthora*-contaminated irrigation or nutrient solution water and have not been used in field production scale.

Other management practices which have been shown effective at managing *P. capsici* in other crops include biofumigation green manure crops (27, 47), no till and reduced till crop production (55), biopesticides (2, 3, 4, 8, 28, 38), and fungicides (23-26, 44, 58). All of these management practices were investigated in this research for their application in managing pod rot of lima bean and discussed in detail in the subsequent chapters.

Chapter 1

EVALUATION OF FUNGICIDES FOR THE MANAGEMENT OF POD ROT OF LIMA BEAN

Abstract

Management of *P. capsici* on lima bean has been largely dependent on fungicides. The only two fungicides labeled are cyazofamid (since 2013) and mefenoxam (since 2000). The main objective of this study was to identify chemicals with different active ingredients and modes of action that are effective in managing pod rot of lima bean in order to reduce the possibility of developing populations of *P. capsici* insensitive to any one chemical. Four field trials were conducted at the University of Delaware's Carvel Research and Education Center, Thurmond Adams Research Farm in Georgetown, Delaware (UD REC) during the summer of 2013 and 2014. Three of the trials were conducted in fields that had a previous history of *P. capsici* and were inoculated prior to lima bean planting. The other trial was conducted in a field with no previous history of the pathogen, however, in 2014 it was heavily infested with *P. capsici*. Twelve chemical treatments (acibenzolar, fluazinam, mandipropamid, mefenoxam, oxathiapiprolin, dimethomorph, propamocarb, fenamidone, famoxidone and cymoxanil, potassium phosphite, cyazofmid, and fluopicolide) were applied during the flat pod stage. Plots were inoculated with a *P. capsici* sporangial suspension in three of the four trials (the field with natural infection was not inoculated after treatment application). All trials were irrigated during the day to maintain a high moisture environment necessary for *P. capsici* disease

development. One field location had access to a low-pressure pump, and a mist system was installed and run during the night to further optimize environmental conditions for disease development. This location was used in 2013 and 2014. Disease incidence was rated in all four trials, and yield was evaluated from three of the trials. Disease incidence in 2013 was very low and resulted in variable data where no significant differences occurred ($P = 0.2236$). Yields did not differ among treatments in 2013 ($P = 0.8122$). Disease incidence was moderate and severe in two of the three trials in 2014. The lowest disease incidence occurred in plots sprayed with mefenoxam, cyazofamid, fluazinam, or oxathiapiprolin ($P < 0.05$). When the data was combined across all years and locations, potassium phosphite (Phostrol), in addition to the four fungicides listed previously, had significant activity against *P. capsici* on lima bean. Yields of fungicide treated plots were significantly greater than yields of control plots in the field with natural pod rot infection ($P < 0.0001$). The efficacy of these chemicals against pod rot of lima bean warrants additional research.

Introduction

Cultural practices such as irrigation management, biofumigation, and cover crops may result in some level of control of lima bean pod rot. However, the most widely used management practice is fungicide application. While *Phythophthora* species are not true fungi, fungicides have been developed with specific modes of action towards oomycete pathogens. Mefenoxam (Ridomil Gold) has been the main chemical for managing *P. capsici* diseases used in the United States. Mefenoxam has been registered for use on many vegetable crops, including cucurbits, pepper, and tomato since 1992, and a chemically similar fungicide, metalaxyl, has been registered since 1979 (45). Many growers have been forced to use mefenoxam exclusively for control of *P. capsici*, and populations of the pathogen insensitive to the chemical began appearing in fields across the United Sates as early as 2000 (34). In 2008, four *P. capsici* samples isolated from lima bean pods from grower fields in Maryland, Delaware, and New Jersey were found to be moderately insensitive to the fungicide, and an additional three isolates were completely insensitive (11). It is believed that *P. capsici*'s ability to sexually reproduce plays a key role in its epidemiology and ability to rapidly develop genetic resistance to synthetic fungicides (34). With resistance to mefenoxam and metalaxyl becoming more prevalent in the field, new chemistry has been examined with hopes of identifying alternative fungicides to provide growers with additional choices for *P. capsici* disease management and to help mitigate the development of resistant populations.

Recent research has identified many fungicides with efficacy towards *P. capsici,* which include active ingredients such as fenamidone (Reason), propamocarb (Previcur Flex), cyazofamid (Ranman), fluazinam (Omega), fluopicolide (Presidio), dimethomorph (Forum), mandipropamid (Revus), kiralaxyl, ametoctradin (Initium), and captan (26, 44). However, most of these chemistries are not approved for use on many *P. capsici* host crops, including lima bean. Currently, only mefenoxam and cyazofamid are on label for use against *P. capsici* on succulent baby limas. To date, there has been only one *P. capsici* fungicide efficacy study conducted on lima bean. Hausbeck tested four different commercial fungicides: cyazofamid, mandiproamid, fluopicolide, and the chemical standard, mefenoxam (23). The results indicated that all treatments significantly reduced disease incidence when compared to the untreated control. Mandipropamid, followed by fluopicolide, then mefenoxam, resulted in the highest yields. These data suggest that there are fungicides on the market with activity towards *P. capsici,* which warrants additional investigation and research.

With limited fungicide choices, lima bean growers in the mid-Atlantic have relied heavily on mefenoxam for control of *Phytophthora capsici*. Prior to 2013, mefenoxam was the only chemical approved for use on lima bean for *P. capsici*. Because of the presence of mefenoxam insensitive populations of *P. capsici,* it is important to identify new chemistry with different modes of action so that farmers may rotate chemicals, thus reducing the possibility of the pathogen developing resistance to any particular chemical or mode of action. In this experiment we evaluated twelve fungicides for efficacy against *P. capsici* in an effort to identify candidates for further research and eventual labelling for use in succulent lima bean production.

Materials and Methods

All trials were conducted on the University of Delaware's Carvel Rersearch and Education Center, Thurmond Adams Research Farm (UD REC) in Georgetown, Delaware. Trials were conducted in 2013 and repeated in 2014 with slight modifications to the protocols. There were a total of 4 trials in 3 separate fields (one field was used in 2013 and 2014). All trials consisted of 14 treatments (see Table 1) arranged in a randomized complete block design. Plots measured 3 meters wide (4, 76.2 cm rows) by 6 meters long with 1.5 meter aisles/buffers in between each block. Data was collected from the two inner rows of each plot. The outer rows of each plot served as buffers between adjacent treatments.

Field #1:Main Farm, East—2013

Site Selection, Field Preparation, and Plot Maintenance

A field on the main UD REC farm with a Rosedale loamy sand soil type (Loamy, siliceous, semiactive, mesic Arenic Hapludults) and a documented history of natural infestation of *P. capsici* was selected as the first trial. This field was previously planted to cucumber (*Cucumis sativus*) in 2012 and lima bean (*Phaseolus lunatus*) in 2011; both crops exhibited signs of *Phytophthora capsici* infection. This field's natural *P. capsici* infestation, coupled with its higher organic matter and water holding capacity compared to nearby soils, made it an excellent site for this trial.

The field was prepared for planting using a chisel plow and disk in early spring 2013. The cultivar Maffei 15 (source: ADM) was selected for its resistance to race F of lima bean downy mildew, caused by *Phytophthora phaseoli*, to avoid mixed infections which could affect disease rating accuracy as the two pathogens have similar signs and symptoms on lima bean pods. On July 9, 2013 lima bean was direct

seeded into conventionally tilled ground using a 4 row Monosem planter on 76.2 cm rows at 13 seeds per meter. A 30% liquid urea-ammonium nitrate (UAN) fertilizer was applied at 55 kg of nitrogen per hectare, then an additional 55 kg of nitrogen per hectare was sidedressed after planting. A pre-emergence application of 1.17 l/ha Dual II Magnum (*S*-metolachlor) + 55 ml/ha Sandea (Halosulfuron-methyl, methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl) -1-methylpyrazole-4-carboxylate) was applied for weed control. Additionally, plots were cultivated three times until canopy closure, then hand weeded as needed. Plots were irrigated using an overhead linear irrigation system with drop nozzles to provide sufficient water to maintain healthy lima bean growth.

A low-pressure mist system was installed after final cultivation in each plot to provide additional canopy and soil moisture, as well as increased humidity in an attempt to optimize environmental conditions for *P. capsici* infection. The system was installed on 6 meter centers using Rain Bird® (Azusa, CA) mister nozzles on 1 meter risers. The system was supplied with on-demand water and set to run for 20 minutes every hour between 7:00 p.m. and 6:00 a.m. daily, starting at flowering and ending at harvest.

Treatment Application

Fungicides were mixed according to the manufacturer's suggested rates (Table 1) and a single application was made on August 30, 2013 during the flat pod growth stage. Treatments were applied using a calibrated backpack CO_2 sprayer with a 2.75 meter wide, 6 nozzle boom, set in a directed spray pattern at 240 kPa to ensure proper coverage of the two middle rows of each plot.

One day after treatment application, a liquid suspension of *P. capsici* inoculum was applied to the plants in each plot (*P. capsici* isolate 32, from University of Delaware collection). Since the products tested were all systemic fungicides and not contact fungicides, the products were applied prior to pathogen inoculation. This allowed time for the chemical to be absorbed into the plant prior to inoculation and gave the chemical the best chance of working. Inoculum was prepared 2 weeks prior to inoculation by transferring a single plug of *P. capsici* from an actively growing culture onto a fresh 100 mm V-8 juice agar plate. The subcultures were then placed in the laboratory at 25 °C in a south facing window to stimulate sporangia production. After a 14 day incubation period, 5 random plates were selected and viewed under the compound light microscope for sporangia. The presence of numerous sporangia was confirmed (see Figure 1 below) and field inoculation proceeded. Each plate contained a sporangium density of approximately 10^3 ml^{-1}.

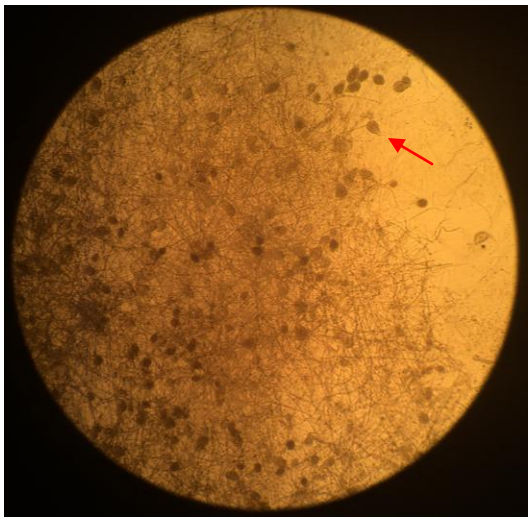


Figure 1 Sporangia production on 14 day old V-8 agar plate. Arrow pointing to a sporangium.

On August 31, 2013 the plates grown in the lab were scraped into a bucket of distilled water then sieved through a 10 over 30 mesh screen to remove large mats of mycelia. The sieved sporangial suspension was then poured into a backpack mounted, hand pressurized sprayer, and the inoculum was applied directly to the pods on the plants in the two inner treatment rows of each plot. Inoculum was applied at a rate of one plate per 3 meters of row. Prior to inoculation, the mist system was run for approximately 30 minutes to provide a favorable environment for infection. Inoculation was done in the late evening to minimize exposure to UV radiation which could kill the sporangia.

Table 1 Fungicide treatments, active ingredients, and rates used in 2013 and 2014 trials.

Trade Name	Active Ingredient[z]	Rate	FRAC[y] Group	Mode of Action[x]
Actigard	acibenzolar (synthetic analogue of salicylic acid)	70.0 g/ha	P1	host plant defense induction
Omega	fluazinam	1.0 l/ha	29	respiration
Revus	mandipropamid	585 ml/ha	40	cell wall biosynthesis
Ridomil Gold	mefenoxam	2.25 kg/ha	4	nucleic acid synthesis
Zorvec	oxathiapiprolin	292 ml/ha	U15	unknown
Forum	dimethomorph	438 ml/ha	40	cell wall biosynthesis
Previcur Flex	propamocarb	1.40 l/ha	28	lipid synthesis and membrane integrity
Reason	fenamidone	600 ml/ha	11	respiration
Tanos	famoxidone, cymoxanil	730 ml/ha	11, 27	respiration, unknown (cymoxanil)
Phostrol	potassium phosphite	5.85 l/ha	33	host plant defense induction
Ranman	cyazofamid	200 ml/ha	21	respiration
Presidio	fluopicolide	292 ml/ha	43	mitosis and cell division
Water Control	N/A	90.0 l/ha	N/A	N/A
Untreated Control	N/A	N/A	N/A	N/A

[z] Also common name.
[y] Fungicide Resistance Action Committee.
[x] Information according to FRAC, a Specialist Technical Group of CropLife International.

Data Collection

After treatment application and inoculation, plots were scouted weekly for signs, disease symptoms, and phytotoxicity until harvest. Disease incidence was data was collected on September 24 and evaluated by pulling 3 plants from opposite ends of each plot and counting the total number of diseased pods on the 6 plants. Any amount of *P. capsici* on a pod was counted as an occurrence of the disease. Yield data was collected on October 4 and calculated by weighing the amount of harvestable shelled beans from 4 meters of row and later converted into kilograms per hectare. Phytotoxicity data was collected when it was observed on plants in the treatment rows. Any abnormal growth, stunting, chlorosis, or necrosis was classified as phytotoxicity.

Field #1: Main Farm, East—2014

Site Selection, Field Preparation, and Plot Maintenance

The same field location as described above was used in 2014. Prior to planting lima beans for the fungicide trial, a pickling cucumber crop (*Cucumis sativus*) was sown into conventionally tilled ground and managed for fruit production. On June 25, a liquid suspension of *P. capsici* inoculum was sprayed onto the maturing cucumber fruits using a similar protocol as above. For a 2 week time period immediately following inoculation, overhead irrigation was applied to facilitate *P. capsici* colonization and infection of the cucumbers. On July 11, the cucumbers were mowed using a tractor mounted mower and the residue immediately worked into the soil using a disk. Incorporating these infected cucumbers was used to boost natural inoculum levels in the soil.

On July 15, lima bean cv. Maffei 15 was direct seeded as described above. Fertility and weed management programs were the same as described in the 2013 trial.

A mist system was installed as described previously, however, misting duration was doubled from 20 minutes per hour to 40 minutes per hour. Plots were misted every night starting at flowering through harvest. A solid set irrigation system was also installed in order to supply more water and more splash to the plants and pods. Risers were 2 meters tall on 12 meter centers and the system was run daily for one hour in the morning and one hour in the evening, starting at flowering and ending at harvest.

Treatment Application

Treatments and inoculum were applied per the same protocol outlined above. Treatments were applied in a single application on September 1 and inoculum was applied on September 2.

Data Collection

Plots were scouted weekly following inoculation for signs of *P. capsici* and symptoms of pod rot, as well as for phytotoxicity caused by the fungicides. Disease incidence data was collected on October 7 by counting the total number of infected pods in each plot from the two inner treatment rows. Plants were harvested one week later on October 14. Yield data was collected from 4 meters of row as described above, and converted to kilograms per hectare of final harvestable yield.

Field #2: Dill Farm—2014

Site Selection, Field Preparation, and Plot Maintenance

The second field location was established in 2014 in a field with previous history of *P. capsici* and a Pepperbox loamy sand soil type (loamy, mixed, semiactive,

mesic Aquic Arenic Paleudults). This field was planted in cucumbers in 2013, and became infected with *P. capsici*. In 2014, prior to planting lima beans, a crop of pickling cucumbers was sown, inoculated with *P. capsici* and incorporated into the soil as described above for field #1—2014. Lima bean cv. Maffei 15 was direct seeded into conventionally tilled soil on July 23 using a 4 row Monosem planter as described above. Fertility and weed management programs were the same as described previously. Plots were irrigated using an overhead linear irrigation system equipped with drop nozzles but no mist system was installed. Heavy overhead irrigation was used on a daily basis from flowering until harvest (September 5 to October 10) to keep the soil and plants moist for *P. capsici* infection.

Treatment Application

Fungicides were applied in one application during the flat pod stage on September 8 using the same CO_2 sprayer and boom described above. *Phytophthora capsici* inoculum was applied on September 9, per the same protocol outlined above.

Data Collection

Plots were scouted on a weekly basis starting one week after inoculation for signs of *P. capsici*, symptoms of pod rot, and phytotoxicity. Disease incidence was determined on October 8 by counting the total number of infected pods per plot located in the two inner treatment rows. The field was not harvested due to a frost which killed the plants before they reached harvest maturity.

Field #3: Main Farm, West—2014

Site Selection, Field Preparation, and Plot Maintenance

A field on the main UD REC with a Pepperbox loamy sand soil type (loamy, mixed, semiactive, mesic Aquic Arenic Paleudults) was direct seeded into conventionally tilled ground with lima bean cv. Cypress at 13 seeds meter on June 5 using a 4 row, 76.2 cm Monosem planter. Fertility and weed management programs were the same as outlined above. This field was a dryland trial, therefore, no irrigation was used prior to infection. A mist system was not installed in this field, nor did it have any previous history of *P. capsici*. However, scouting the field on August 25 revealed a very heavy, uniform infection of *P. capsici*. The field was then irrigated daily for one hour in the morning and one hour in the evening from August 25 to September 15 to perpetuate the infection.

Treatment Application

Once the field was scouted and determined to have a heavy and uniform infection, treatments were applied in a single application on September 1 using the same CO_2 backpack sprayer and protocol as described above. The field was not inoculated following treatment since a natural infestation was present.

Data Collection

Plots were scouted weekly after treatment application for pod rot development and signs of phytotoxicity. Pod rot disease incidence was determined on September 18 by counting the total number of infected pods from 10 randomly selected plants from each plot (5 from each treatment row). Infection was too heavy for total plot counts. Since the field was past maturity, it could not be harvested with a viner,

therefore yield data was taken by conducting pod counts. On September 24, the total number of harvestable, non-infected pods, were counted per 1.5 meters of row (20 plants) from each plot. This data was then extrapolated into final yield by assuming that there are 3 harvestable beans per pod and an average 'Cypress' succulent bean weight of 0.875 grams. Lost yield was determined by counting the total number of pot rot diseased pods from 10 plants in each plot and converting to kg/ha by assuming 3 harvestable beans per pod and an average 'Cypress' succulent bean weight of 0.875 grams. Lost yield and healthy yield were added together to determine the potential yield of each plot.

Fungicide Sensitivity Lima Bean Pod Assay

Mefenoxam, cyazofamid, fluazinam, oxathiapiprolin, and potassium phosphite were tested in the laboratory for their ability to suppress growth of *P. capsici* on lima bean pods. These fungicides were selected based on their consistent performance in the field trials.

Plants of lima bean cv. C-Elite were grown in the greenhouse at the University of Delaware's main campus in Newark, Delaware. Flat pods were collected and brought back to laboratory to conduct the experiment. All pods were surface disinfested by soaking them in a 10% bleach solution for 2 minutes. Pods were removed and rinsed with sterile distilled water then treated with fungicides. Fungicides were mixed in 250 ml of distilled water (rates in Table 2) and thoroughly mixed before spraying on to pods. Pods were sprayed until runoff and then placed in the center of a 100 mm petri dish containing moist filter paper. One pod was used per plate and each treatment was replicated three times. Pods were allowed to dry before a single 5 mm plug of *P. capsici* isolate 32 (from the University of Delaware's

collection) was placed in the center of each pod. Plates were sealed with Parafilm™ to contain humidity and were placed in an incubator set at 25 °C with a 12 hour photoperiod. Controls were treated with only water.

Pods were observed for *P. capsici* infection after 72 hours and rated as either infected or not infected. A pod with any amount of *P. capsici* signs or pod rot symptoms was counted as infected. Data was entered into JMP (SAS Institute Inc.) and an analysis of means for proportions was conducted to determine if there were any differences in *P. capsici* sensitivity towards the fungicides.

Data Analysis

All disease incidence and yield data was entered into a JMP (SAS Institute Inc.) file and was square root transformed as needed to fit a normal distribution. Analysis of variance (ANOVA) was performed and means were separated using Fisher's Protected LSD. Finally, linear regression was utilized to correlate yield effects and disease incidence in trials where both disease incidence and yield data were collected.

Table 2 Amount of product added to 250 ml water for fungicide sensitivity pod assay. Controls were left untreated.

	Amount of Product added to 250 ml Water
Fluazinam (Omega)*	230.0 µl
Potassium phosphite (Phostrol)	1300.0 µl
Cyazofamid (Ranman)	46.7 µl
Mefenoxam (Ridomil Gold)	500.0 mg
Oxathiapiprolin (Zorvec)	81.5 µl
Untreated Control	N/A

*Trade names in parenthesis.

Results and Discussion

Field #1: Main Farm, East—2013

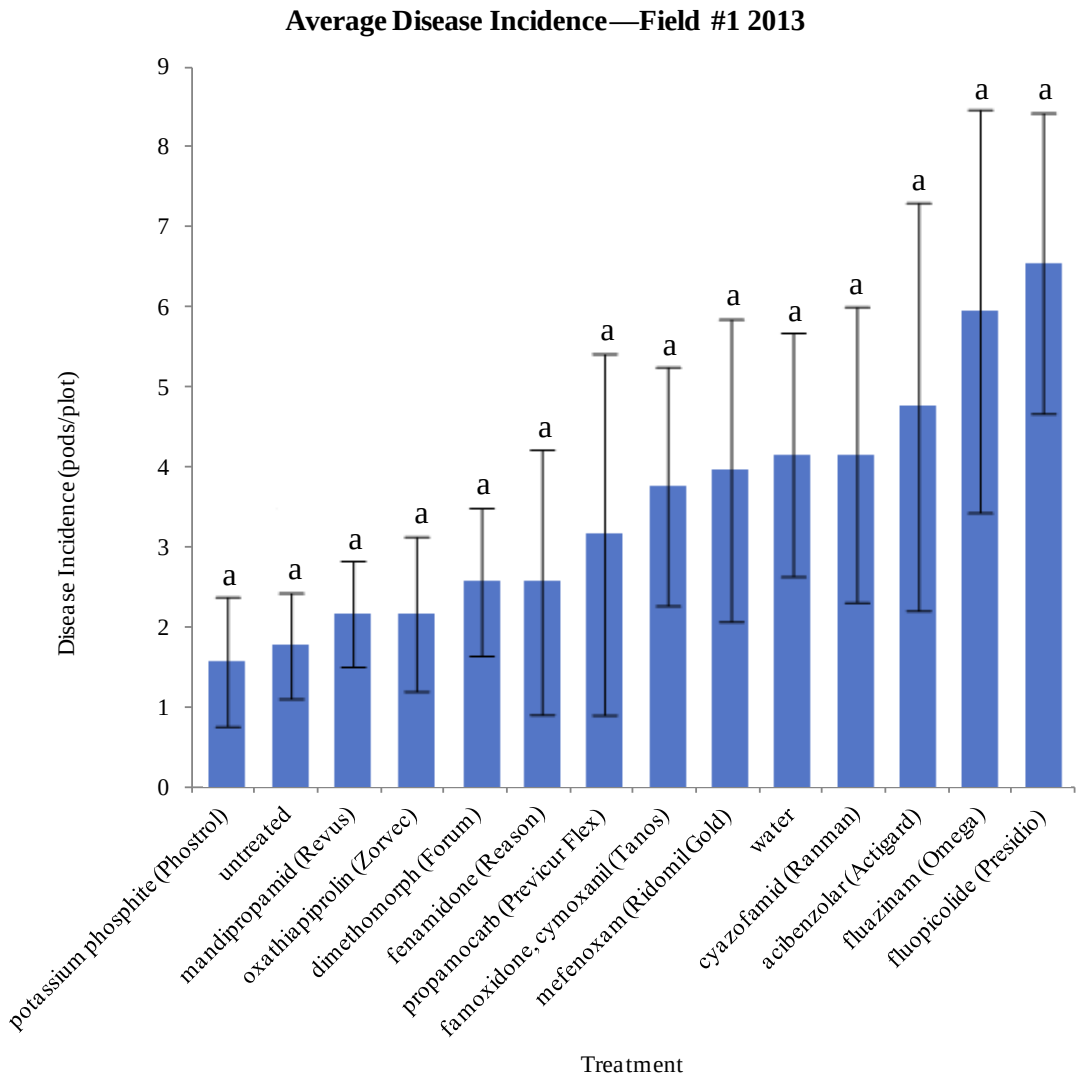
Disease incidence was low across all plots in 2013 and there was high variation across the field (Figure 2) with no significant differences among treatments (P = 0.2336). The chemical standard, mefenoxam, had an average of 4.0 infected pods per plot, which ranked higher compared to the other treatments, but was not significant (Table 3). Since we were using a mefenoxam sensitive isolate of *P. capsici*, we would have expected mefenoxam to have some of the lowest disease ratings when compared to the other treatments, especially the controls. The overall low infection levels observed in 2013 could be attributed to the hot and dry weather during August and October of 2013 (see Appendix A). Therefore although pods (host) and *P. capsici*

(pathogen) were present, a conducive environment for infection was absent. Even with a mist system misting every hour throughout the night; high daytime temperatures, low relative humidity, and low rainfall totals most likely impeded *P. capsici* sporangia germination and growth. No signs of phytotoxicity were observed in any of the plots.

Table 3 Disease incidence (diseased pods/plot) for field #1: main farm, east—2013.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Average[z]
Actibenzolar (Actigard)	11	2	0	0	11	4.8 a[y]
Dimethomorph (Forum)	1	3	0	5	4	2.6 a
Fluazinam (Omega)	12	8	0	0	10	6.0 a
Potassium phosphite (Phostrol)	3	0	0	1	4	1.6 a
Fluopicolide (Presidio)	14	4	5	4	6	6.6 a
Propamocarb (Previcur Flex)	12	3	0	1	0	3.2 a
Cyazofamid (Ranman)	10	7	2	0	2	4.2 a
Fenamidone (Reason)	0	8	0	0	5	2.6 a
Mandipropamid (Revus)	2	4	2	3	0	2.2 a
Mefenoxam (Ridomil Gold)	7	10	1	1	1	4.0 a
Famoxidone, cymoxanil (Tanos)	8	6	1	0	4	3.8 a
Oxathiapiprolin (Zorvec)	5	4	0	1	1	2.2 a
Water	9	5	0	2	5	4.2 a
Untreated	1	2	4	0	2	1.8 a
$P^{x} > F$						0.2336

[z] Average disease incidence across 5 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.
*Common names followed by trade names in parenthesis.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 2 Average disease incidence for field #1: main farm, east—2013.

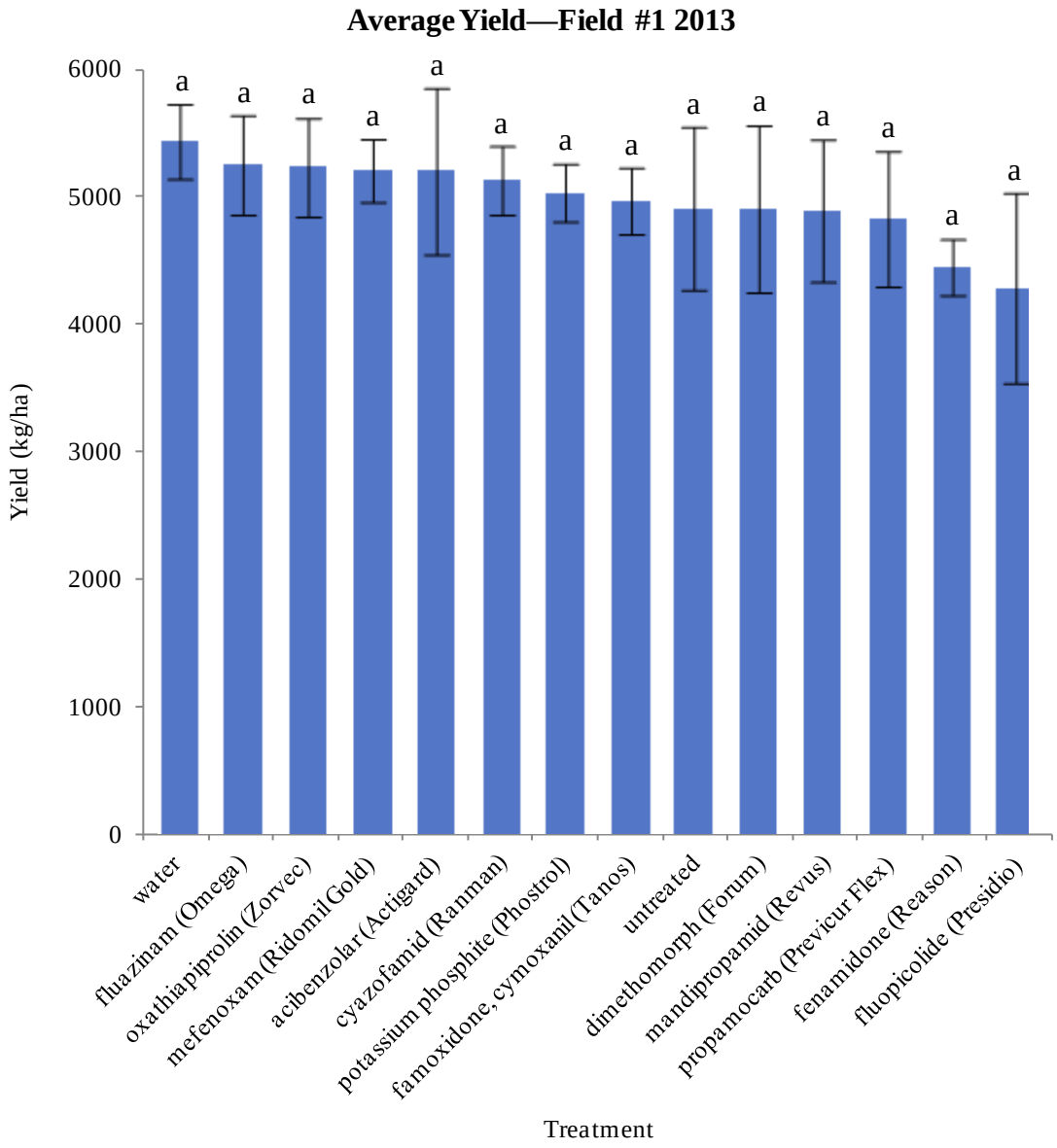
Yield's for 2013 (Table 4 and Figure 3) were relatively high, and very consistent, with only 1153 kg separating the top yielding treatment (water control)

from the lowest yielding treatment (fluopicolide), however, the differences were not significant (P = 0.8122).

Table 4 Final yield (kg/ha) for field #1: main farm, east—2013.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Average[z]
Actibenzolar (Actigard)	5997	6865	5521	2961	4976	5264 a[y]
Dimethomorph (Forum)	3223	6775	5987	3839	5011	4967 a
Fluazinam (Omega)	4994	5241	6739	4359	5234	5313 a
Potassium phosphite (Phostrol)	5158	5840	5022	4416	5040	5095 a
Fluopicolide (Presidio)	3276	6542	5628	3714	2563	4345 a
Propamocarb (Previcur Flex)	2932	5219	6130	4847	5323	4890 a
Cyazofamid (Ranman)	4420	5019	6062	5420	5040	5192 a
Fenamidone (Reason)	4044	5166	4087	4384	4865	4509 a
Mandipropamid (Revus)	5524	4560	5936	5814	2932	4953 a
Mefenoxam (Ridomil Gold)	5456	5685	5843	4739	4628	5270 a
Famoxidone, cymoxanil (Tanos)	5667	4750	5069	4201	5470	5032 a
Oxathiapiprolin (Zorvec)	4918	6230	5689	3990	5650	5295 a
Water	6166	6241	5148	5112	4821	5498 a
Untreated	3735	6643	6309	3574	4596	4971 a
P[x] $>F$						0.8122

[z] Average yield across 5 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.
*Common names followed by trade names in parenthesis.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 3 Average yield for field #1: main farm, east—2013.

Field #1: Main Farm, East—2014

Disease incidence was substantially higher in 2014 when compared to 2013, possibly due to the increased amount of precipitation, higher humidity, and more moderate temperatures during August, September, and October when susceptible plant parts (pods) were present in the field. Rainfall totals were 3.28 cm greater between August 1 and October 31, 2014, when compared to 2013 (Appendix A-D). In addition, average daily temperature was 0.4 °C cooler in 2014, and the average relative humidity was 4.26% higher in 2014 (Appendix A-D). The combined effect of slightly lower temperatures, increased rainfall, and increased relative humidity most likely contributed to a more favorable environment for *P. capsici* growth, explaining the higher infection levels observed in 2014 compared to 2013.

The water control and untreated control had the highest pod rot incidence ($P < 0.0001$), with an average number of 65.6 and 59.4 infected pods per plot, respectively. All fungicide treatments significantly reduced disease incidence compared to the controls, and oxathiapiprolin ranked lowest with an average of 11.8 infected pods per plot, followed closely by mefenoxam treated plots with an average of 12.8 infected pods. Our results indicating a significant reduction in pod rot support Ji and Csinos' findings that applications of oxathiapiprolin significantly reduced Phytophthora blight of pepper (25). To our knowledge, these are the first two studies confirming the efficacy of oxathiapiprolin on *P. capsici*.

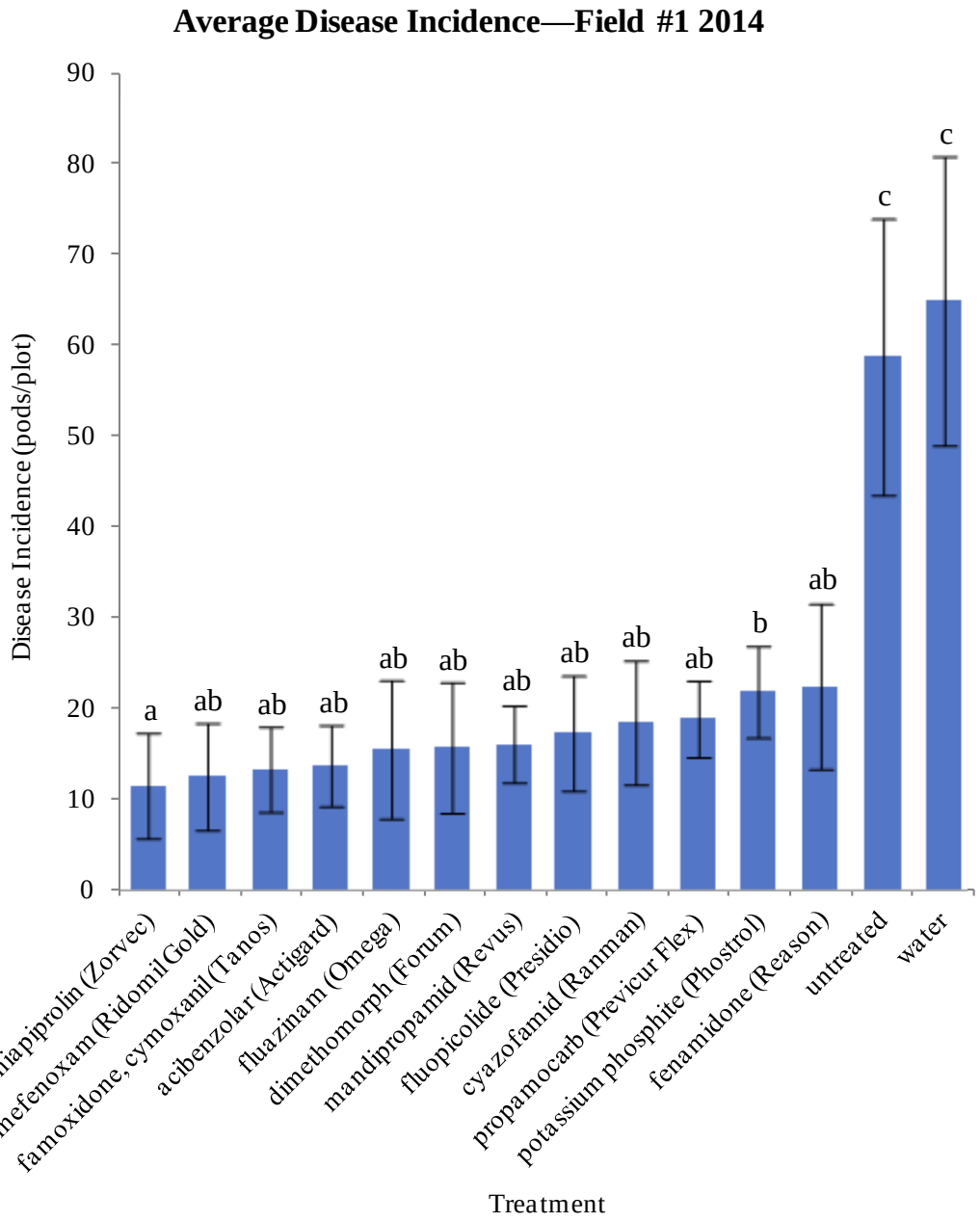
There was also a significant difference in disease incidence between blocks ($P < 0.0001$). Blocks 3 and 4 had the highest disease incidence with an average of 34.07 and 34.36 infected pods per plot, respectively. Block 1 had the lowest disease incidence across treatments with an average of 6.07 infected pods per plot. There was a slight slope to the field, and the experiment was blocked with replication 1 being the

highest elevation and 5 being the lowest. Therefore, block 1 would be the driest which may explain why the lowest disease incidence was observed in block 1, and greater amounts in blocks 2, 3, 4, and 5. There was no phytotoxicity observed in any of the plots.

Table 5 Disease incidence (diseased pods/plot) for field #1: main farm, east—2014.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Average[z]
Actibenzolar (Actigard)	12	5	14	31	8	14.0 ab[y]
Dimethomorph (Forum)	0	15	32	33	0	16.0 ab
Fluazinam (Omega)	0	12	18	44	5	15.8 ab
Potassium phosphite (Phostrol)	30	21	33	28	8	22.2 b
Fluopicolide (Presidio)	2	26	30	28	2	17.6 ab
Propamocarb (Previcur Flex)	5	17	31	21	2	19.2 ab
Cyazofamid (Ranman)	2	29	31	30	2	18.8 ab
Fenamidone (Reason)	2	9	54	31	18	22.8 ab
Mandipropamid (Revus)	0	23	21	22	16	16.4 ab
Mefenoxam (Ridomil Gold)	4	16	10	34	0	12.8 ab
Famoxidone, cymoxanil (Tanos)	4	21	17	25	1	13.6 ab
Oxathiapiprolin (Zorvec)	0	20	9	30	0	11.8 a
Water	15	48	106	68	91	65.6 c
Untreated	9	50	85	56	97	59.4 c
P[x] $>F$						< 0.0001

[z] Average disease incidence across 5 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicate significant difference among treatments.
*Common names followed by trade names in parenthesis.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

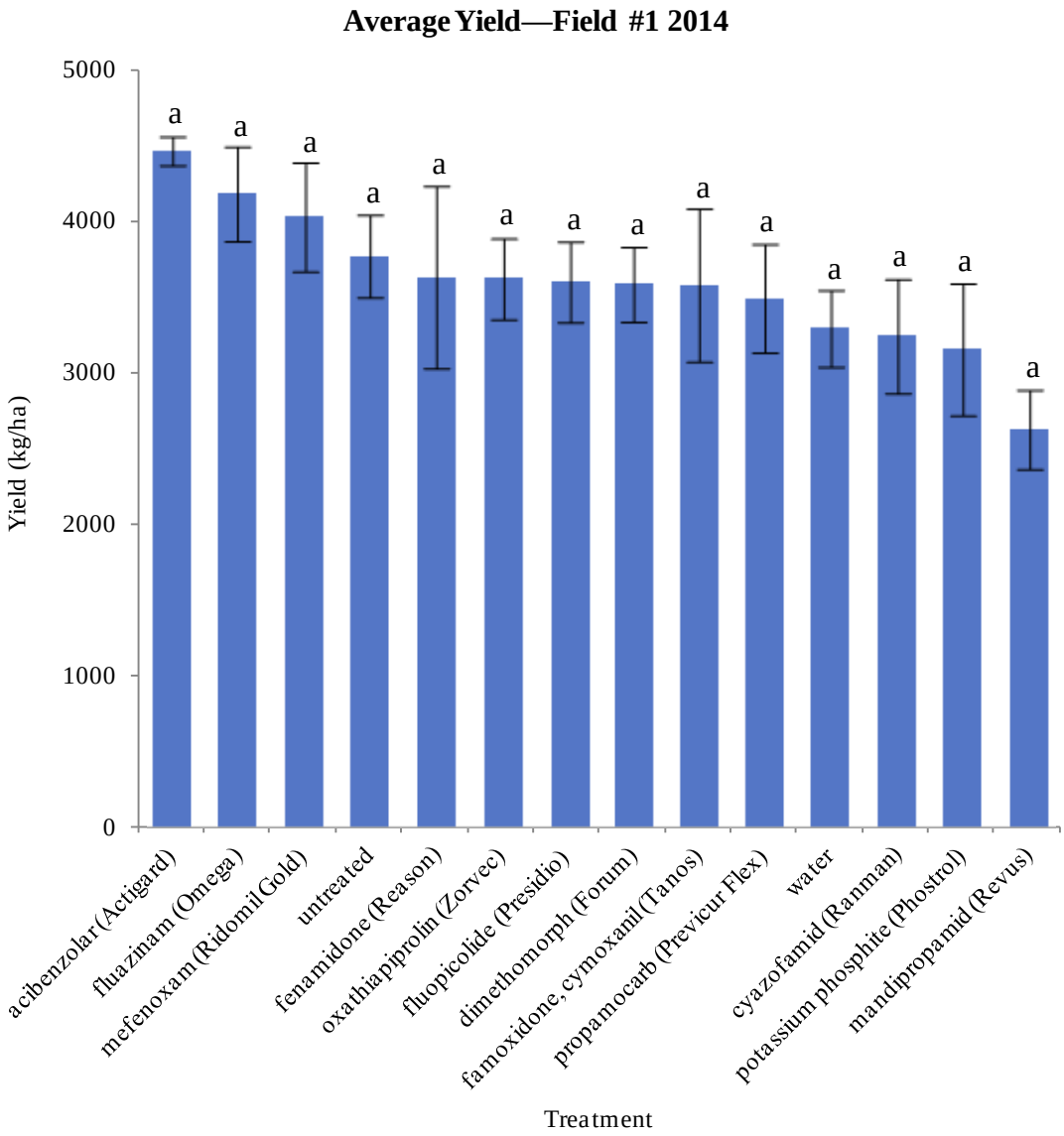
Figure 4 Average disease incidence for field #1: main farm, east—2014.

Yields were fairly consistent across all treatments in the trial (Table 6 and Figure 5). The top yielding treatment was actibenzolar with an average of 4497 kg/ha and the lowest yielding treatment was mandipropamid averaging 2650 kg/ha, however there were no significant differences in yield between treatments ($P = 0.0587$) or between blocks.

Table 6 Final yield (kg/ha) for field #1: main farm, east—2014.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Average[z]
Actibenzolar (Actigard)	4481	4302	4359	4496	4847	4497 a[y]
Dimethomorph (Forum)	2761	3369	4173	3893	3857	3610 a
Fluazinam (Omega)	4660	5055	3800	3270	4273	4211 a
Potassium phosphite (Phostrol)	2381	4273	4158	2896	2194	3180 a
Fluopicolide (Presidio)	3857	3736	2854	4424	3270	3628 a
Propamocarb (Previcur Flex)	3491	2875	3778	2717	4732	3519 a
Cyazofamid (Ranman)	3649	3284	4330	2029	3048	3268 a
Fenamidone (Reason)	2896	5750	2560	4287	2803	3659 a
Mandipropamid (Revus)	1871	3363	2402	3082	2531	2650 a
Mefenoxam (Ridomil Gold)	4230	5305	3312	3363	4080	4058 a
Famoxidone, cymoxanil (Tanos)	2545	5090	3477	2516	4402	3606 a
Oxathiapiprolin (Zorvec)	3829	4437	3592	2767	3614	3648 a
Water	3778	3004	2696	3069	4051	3320 a
Untreated	3721	3713	4639	2940	3979	3799 a
P[x] $>F$						0.0587

[z] Average yield across 5 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value $\leq$ 0.05 indicate significant difference among treatments.
*Common names followed by trade names in parenthesis.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 5 Average yield for field #1: main farm, east—2014.

A regression of diseased pods against final yield was performed (Figure 6). Incidence of pod rot accounted for 0.6% of the variation in yield, which was not statistically significant ($P = 0.5092$).

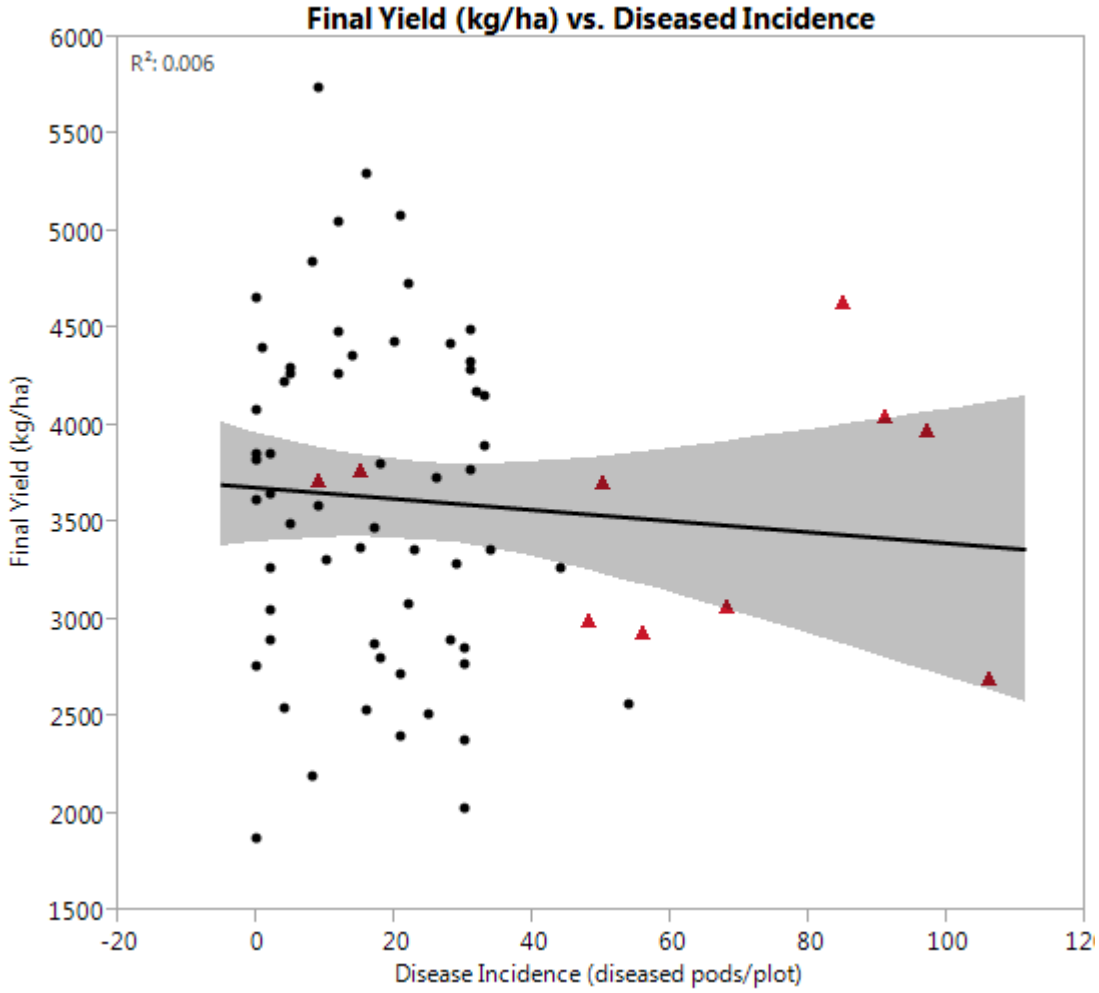


Figure 6 Regression analysis of average disease incidence vs. average final yield for field #1: main farm, east—2014. Control treatments are represented by red triangles. Shaded region represents 95% confidence of fit.

Field #1: Main Farm, East—2013 & 2014 Combined Data

Disease incidence data from 2013 and 2014 were collated and square root transformed to fit a normal distribution before running statistics. Means were reverse transformed for presentation in Tables 7 and 8. The disease incidence data from both years for field location #1 were combined and a Dunnett's test compared treatments to the untreated control. All fungicide treatments, except for fluopicolide and the water control, resulted in significantly better control of pod rot of lima bean compared to the untreated control (α=0.05). Fluopicolide has been demonstrated as an effective fungicide for managing diseases caused by *P. capsici* (24, 29), but insensitive isolates have been identified (24) and may explain why floupicolide was not significantly different than the untreated control in the Dunnett's test. Sensitivity of our isolate of *P. capsici* to fluopicolide is unknown. Fisher's Protected LSD indicates that all treatments are significantly different than both control treatments, with oxathiapiprolin achieving the best control of pod rot of lima bean.

Table 7 Dunnett table for field #1: main farm—2013 & 2014 comparing average disease incidence to the untreated control.

LS Means Dunnett

Treatment*	Disease Incidence LS MEAN[z]	Ho:LS Mean=Control $P^{y}r < t$
Untreated Control	18.0209	
Water Control	22.8188	0.9964
Acibenzolar (Actigard)	6.7003	0.0155
Dimethomorph (Forum)	4.9475	0.0020
Fluazinam (Omega)	6.7761	0.0166
Potassium phosphite (Phostrol)	7.5834	0.0342
Fluopicolide (Presidio)	9.6864	0.1424
Propamocarb (Previcur Flex)	7.5054	0.0321
Cyazofamid (Ranman)	7.7813	0.0402
Fenamidone (Reason)	7.0958	0.0224
Mandipropamid (Revus)	6.0659	0.0080
Mefenoxam (Ridomil Gold)	5.6649	0.0051
Famoxidone, cymoxanil (Tanos)	6.2420	0.0097
Oxathiapiprolin (Zorvec)	3.6806	0.0003

[z] Average disease incidence across 10 replications.
[y] Treatments with *P*-values ≤ 0.05 are significantly different than the untreated control.
*Common names followed by trade names in parenthesis.

Table 8 Connected letters report for field #1: main farm, east—2013 & 2014 combined disease incidence data.

Grouping[z]		Mean[y]	N[x]	Treatment*
	A	22.8188	10	water Control
	A	18.0209	10	untreated Control
	B	9.6864	10	fluopicolide (Presidio)
C	B	7.7813	10	cyazofamid (Ranman)
C	B	7.5834	10	potassium phosphite (Phostrol)
C	B	7.5054	10	propamocarb (Previcur Flex)
C	B	7.0958	10	fenamidone (Reason)
C	B	6.7761	10	fluazinam (Omega)
C	B	6.7003	10	acibenzolar (Actigard)
C	B	6.2420	10	famoxidone, cymoxanil (Tanos)
C	B	6.0659	10	mandipropamid (Revus)
C	B	5.6649	10	mefenoxam (Ridomil Gold)
C	B	4.9475	10	dimethomorph (Forum)
C		3.6806	10	oxathiapiprolin (Zorvec)

[z] Treatment groupings with different letters are significantly different according to Fisher's Protected LSD (α=0.05).
[y] Average disease incidence across 10 replications.
[x] Number of replications.
*Common names followed by trade names in parenthesis.

Field #2: Dill Farm—2014

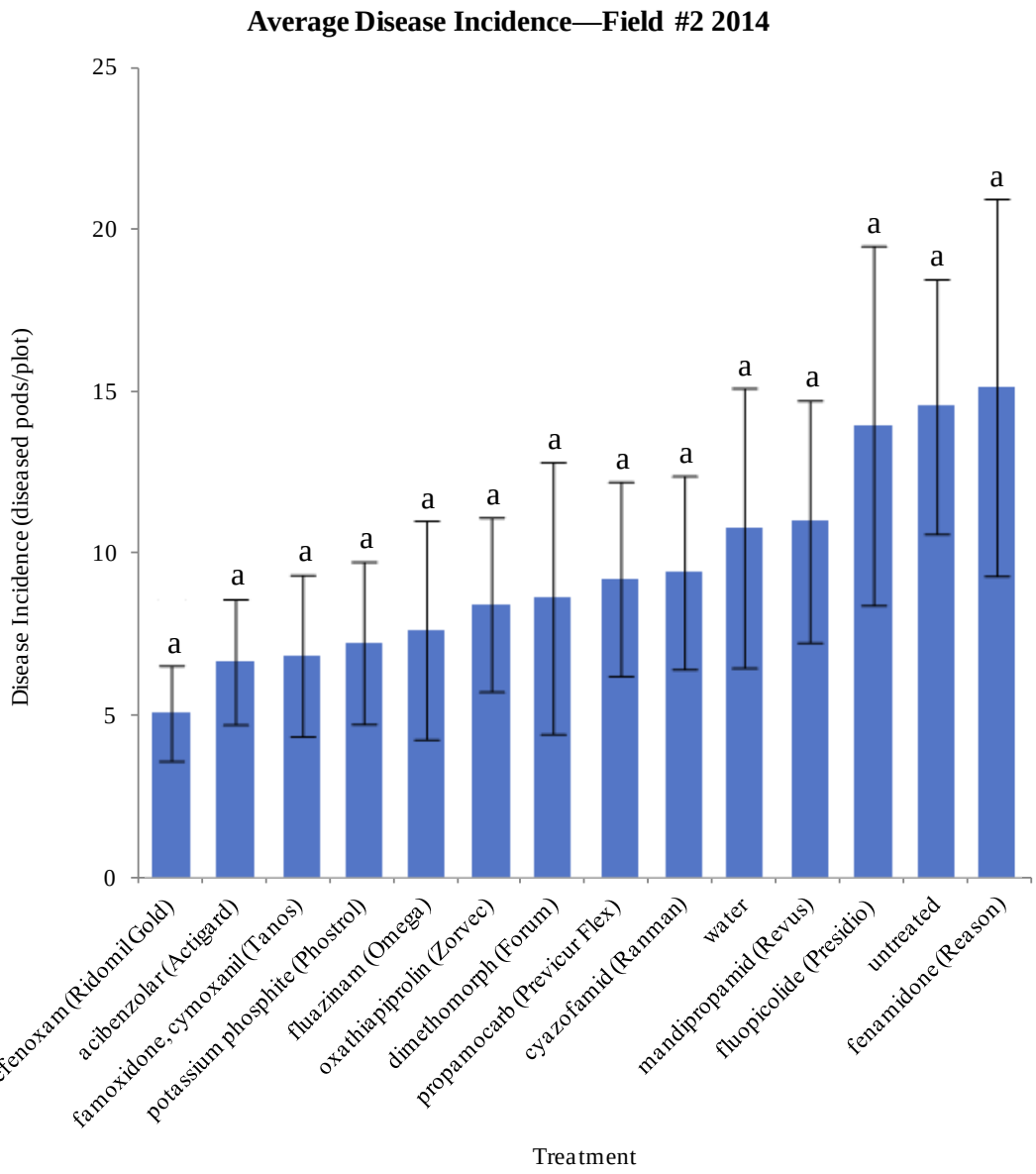
Disease incidence was low in field #2 (Table 9) and there was no significant difference between treatments in the trial ($P = 0.1249$), and large amounts of variability existed within treatments as evident by the large error bars in Figure 7. Low infection levels in this field may be attributed to the lack of consistent moisture during pod set and pod development. This field did not have access to a low pressure, on demand water source, therefore, a mist system was not installed. Heavy overhead irrigation was used twice daily during pod development, however, it may have not been enough to facilitate an epidemic. This field was also planted later than the other trials, resulting in pod set during a slightly drier time of year. As a result of the later planting date, this trial received approximately 2.5 cm less rain than the other trial planted just 7 days prior (Appendix C). This disparity in rainfall between the two fields may help explain why field #1 had higher infection levels than field #2.

Yield data was not collected from this field due to the late planting date and an early frost which killed the plants prior to harvest maturity. No phytotoxicity was observed in any of the treatments.

Table 9 Disease incidence (diseased pods/plot) for field #2: Dill farm—2014.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Average[z]
Actibenzolar (Actigard)	3	11	12	5	3	6.8 a[y]
Dimethomorph (Forum)	1	5	12	24	2	8.8 a
Fluazinam (Omega)	3	19	5	12	0	7.8 a
Potassium phosphite (Phostrol)	2	15	7	11	2	7.4 a
Fluopicolide (Presidio)	15	14	8	34	0	14.2 a
Propamocarb (Previcur Flex)	5	13	12	17	0	9.4 a
Cyazofamid (Ranman)	5	15	13	15	0	9.6 a
Fenamidone (Reason)	12	12	15	37	1	15.4 a
Mandipropamid (Revus)	8	16	8	23	1	11.2 a
Mefenoxam (Ridomil Gold)	4	8	6	8	0	5.2 a
Famoxidone, cymoxanil (Tanos)	1	13	9	11	1	7.0 a
Oxathiapiprolin (Zorvec)	7	8	9	18	1	8.6 a
Water	4	10	14	26	1	11.0 a
Untreated	20	14	8	27	5	14.8 a
P [x]$>F$						0.1249

[z] Average disease incidence across 5 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value $\leq$ 0.05 indicate significant difference among treatments.
*Common names followed by trade names in parenthesis.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 7 Average disease incidence for field #2: Dill farm—2014.

Field #3: Main Farm, West—2014

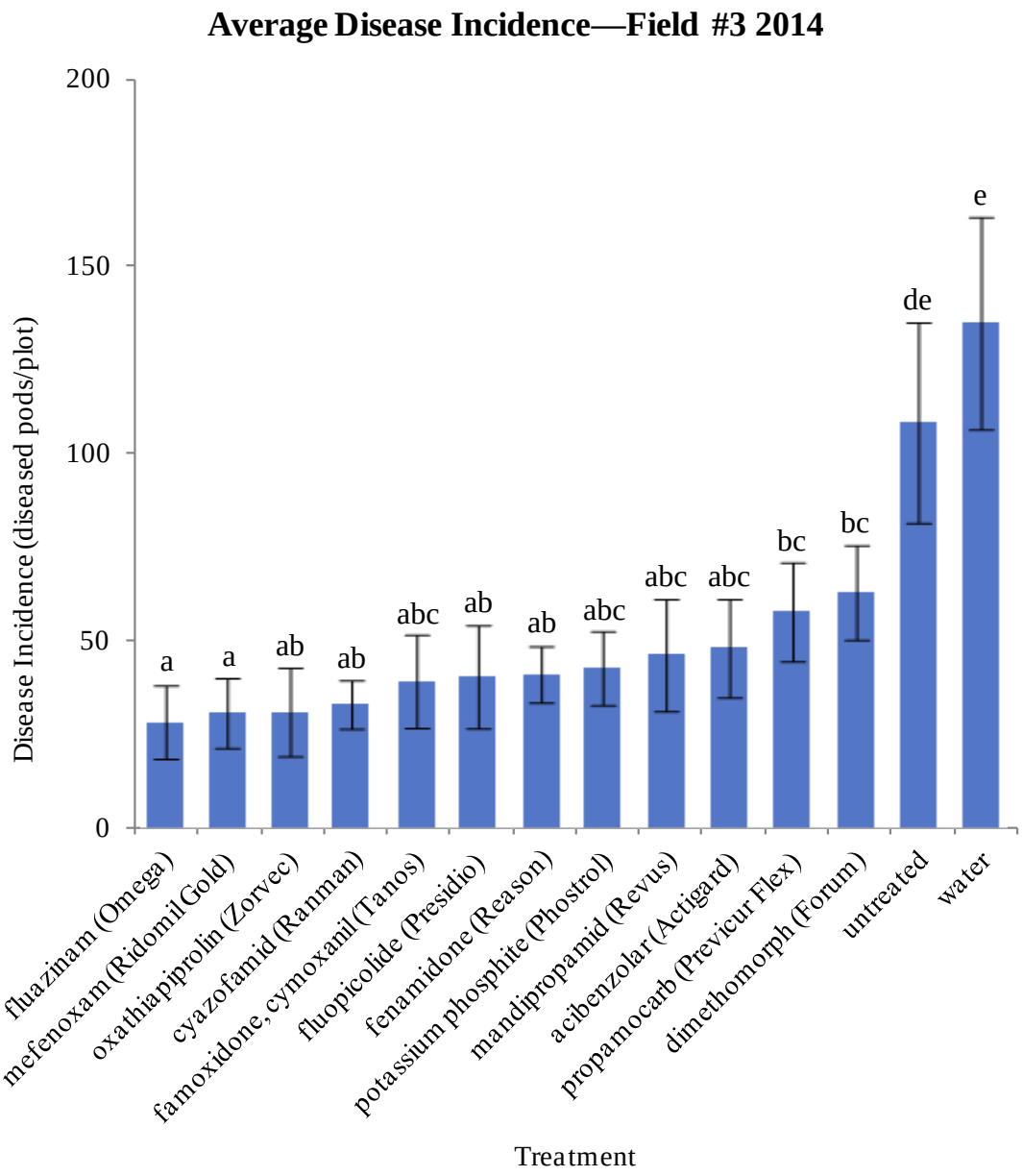
Fungicide treatments significantly reduced pod rot disease incidence in this trial ($P < 0.0001$, Table10 and Figure 8). The water control treatment had the highest average disease incidence with 131.2 diseased pods per plot, significantly higher than all other treatments, except for the untreated control (105.4 infected pods/plot). Dimethomorph was not significantly different than the untreated control, averaging 61.4 infected pods per plot. Applications of fluazinam and mefenoxam reduced pod rot disease incidence the most, with an average of 27.8 and 30.2 infected pods per plot, respectively. The remaining treatments, actibenzolar, potassium phosphite, fluopicolide, propamocarb, fenamidone, mandipropamid, cyazofamid, oxathiapiprolin, and famoxidone and cymoxanil (Tanos), all reduced pod rot disease incidence significantly when compared to the controls. Average disease incidence for treatments in blocks 1 and 3 were 72.6 and 70.1 diseased pods per plot, respectively. However, not significantly greater than the average disease rating for treatments in blocks 2 (53.4 infected pods/plot), 4 (28.4 infected pods/plot), or 5 (35.3 infected pods/plot). No phytotoxicity was observed in any of the plots.

The two control treatments yielded significantly higher disease ratings, as expected, and fungicide treatments significantly reduced *P. capsici* disease incidence. However, we may have observed better control with these treatments if the fungicides were applied earlier, before onset of disease and if the treatments were sprayed on a 5-7 day schedule throughout pod development. Still, significant reduction in disease incidence was achieved with all treatments, excluding dimethomorph, through just one application when compared to the controls. A single fungicide application in this trial arrested pod rod disease development.

Table 10 Disease incidence (diseased pods/plot) for field #3: main farm, west—2014.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Average[z]
Actibenzolar (Actigard)	84	59	47	6	39	47.0 cde[y]
Dimethomorph (Forum)	88	94	36	41	48	61.4 bc
Fluazinam (Omega)	20	22	63	6	28	27.8 e
Potassium phosphite (Phostrol)	70	50	42	11	36	41.8 cde
Fluopicolide (Presidio)	64	52	66	0	16	39.6 de
Propamocarb (Previcur Flex)	97	67	54	45	19	56.4 cd
Cyazofamid (Ranman)	57	30	24	28	23	32.4 de
Fenamidone (Reason)	34	30	69	31	37	40.2 de
Mandiproamid (Revus)	86	22	73	12	33	45.2 cde
Mefenoxam (Ridomil Gold)	51	47	19	2	32	30.2 e
Famoxidone, cymoxanil (Tanos)	13	42	83	26	28	38.4 cde
Oxathiapiprolin (Zorvec)	3	10	50	63	26	30.4 de
Water	181	109	207	56	103	131.2 a
Untreated	169	114	148	70	26	105.4 ab
P[x]$>F$						< 0.0001

[z] Average disease incidence across 5 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value $\leq$ 0.05 indicates significant differences among treatments.
*Common names followed by trade names in parenthesis.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 8 Average disease incidence for field #3: main farm, west—2014.

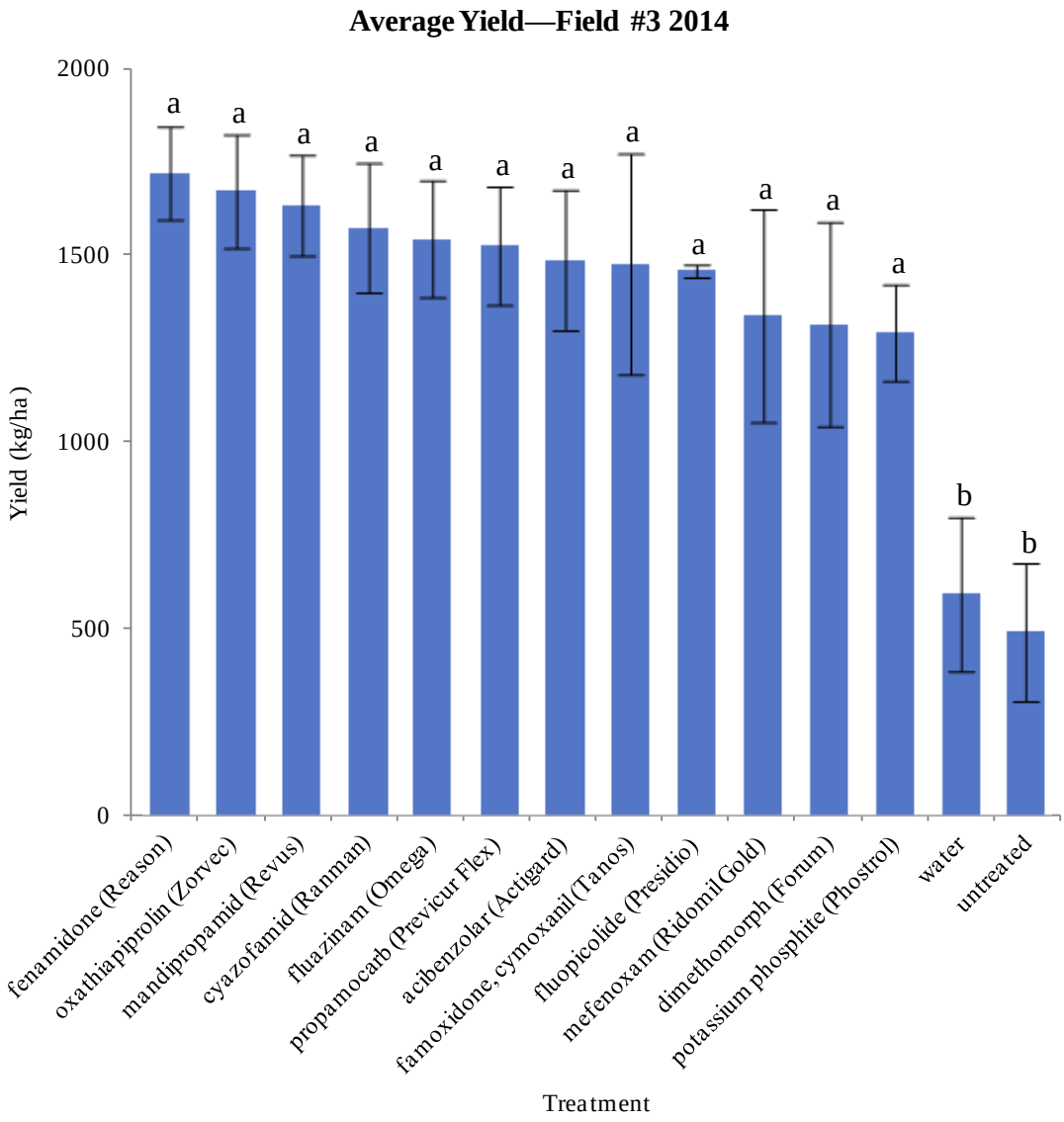
Table 11 and Figure 9 show that final yields calculated through pod counts significantly differed by treatment ($P < 0.0001$). The untreated control treatment (497 kg/ha) and the water control treatment at (599 kg/ha) yielded the lowest. Fenamidone yielded the most with an average of 1733 kg/ha, but not significantly different than the other fungicide treatments, which ranged in average from 1326 kg/ha (dimethomorph) to 1684 kg/ha (oxathiapiprolin). This is consistent with field research on other *P. capsici* vegetable diseases where fungicide application often significantly increases yields compared to controls (16, 57). Yields across treatments between blocks were not significantly different, with block 1 yielding an average of 1158 kg/ha, block 2 yielding an average of 1420 kg/ha, and block 3 yielding an average of 1552 kg/ha.

Yield data was only collected from the first 3 blocks due to the accelerated maturity in blocks 4 and 5, in which pods were past maturity at the time of yield data collection. Still, a single fungicide application increased yield, and using these chemicals on a 5-7 day spray schedule, or in a spray rotation, would likely further improve yields.

Table 11 Final yield (kg/ha) for field #3: main farm, west—2014.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Average[z]
Actibenzolar (Actigard)	1232	1401	1865	1499 a[y]
Dimethomorph (Forum)	961	1153	1865	1326 a
Fluazinam (Omega)	1345	1865	1458	1556 a
Potassium phosphite (Phostrol)	1379	1051	1481	1304 a
Fluopicolide (Presidio)	1503	1447	1458	1469 a
Propamocarb (Previcur Flex)	1412	1854	1345	1537 a
Cyazofamid (Ranman)	1243	1707	1808	1586 a
Fenamidone (Reason)	1560	1661	1978	1733 a
Mandipropamid (Revus)	1436	1605	1899	1647 a
Mefenoxam (Ridomil Gold)	972	1165	1910	1349 a
Famoxidone, cymoxanil (Tanos)	1243	2079	1141	1488 a
Oxathiapiprolin (Zorvec)	1412	1944	1696	1684 a
Water	327	463	1005	599 b
Untreated	180	486	825	497 b
P [x]$>F$				< 0.0001

[z] Average yield across 3 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.
*Common names followed by trade names in parenthesis.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 9 Average yield for field #3: main farm, west—2014.

There was a strong correlation between disease incidence and final yield (Figure 10). Incidence of pod rot accounted for over 49% in the variation in yield ($P < 0.0001$). The correlation is strong and negative, indicating that yield decreases as disease incidence increases. The graph in Figure 10 has two distinct groupings. The 12 chemical fungicide treatments clustered in the upper left corner of the graph were not significantly different from each other, and the 6 triangular points clustered in the lower right corner of the graph are the two control treatments, which had significantly lower yields than the fungicide treatments. Reduced yield of untreated control plots compared to fungicide treated plots is consistent with multiple studies conducted on *Phytophthora spp.* plant diseases (16, 24, 53, 57).

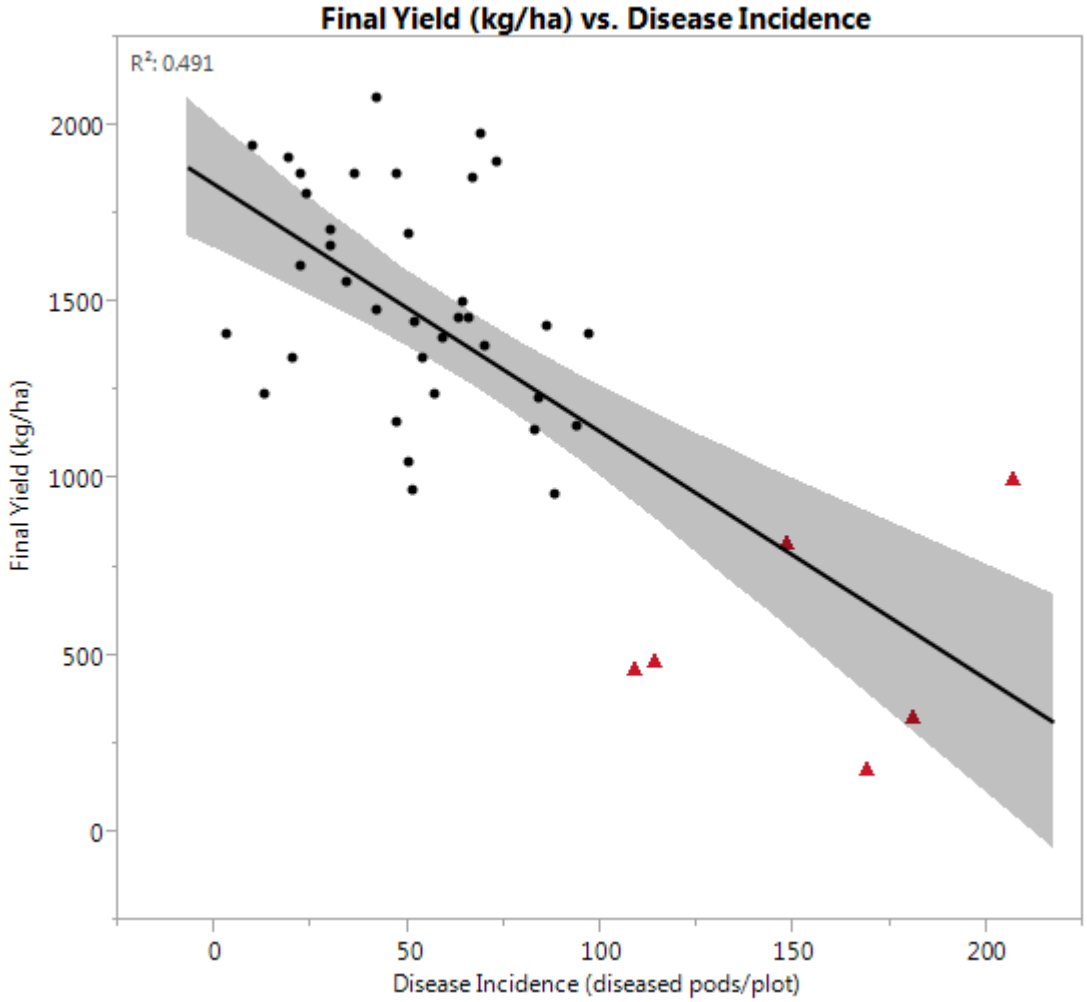


Figure 10 Regression analysis of disease incidence vs. final yield for field #3: main farm, west—2014. Controls are represented by red triangles. Shaded region represents 95% confidence of fit.

Yields were low in these plots compared to a 5 year historical average of 2600 kg/ha (13). This is likely due to the heavy *P. capsici* infestation and its quick onset. The entire first set of pods was aborted by the plants, effectively reducing yield by half before the plots were treated. If the infection was detected earlier, treatments could

have been applied sooner and the likelihood of attaining better disease control and higher yields would have increased.

Yield loss represented as a percent of total potential yield revealed over an 86% yield loss in both control treatments (Table 12 and 13). Yield loss was still high in the fungicide treatments, ranging from a low of 21.99% of total yield potential (oxathiapiprolin) to a high of 55.33% of total yield potential (dimethomorph). These low yields are likely due to the early development and heavy *P. capsici* infection present in this field, causing the first set of pods to abort, thus severely limiting the yield potential. The data does indicate, however, that a single fungicide application could potentially save 30-60% of the crop's yield. Spraying more frequently throughout the year would likely further reduce pod rot disease incidence and reduce yield loss.

Table 12 Lost yield (kg/ha) for field #3: main farm, west—2014.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Average[z]
Actibenzolar (Actigard)	1694	1190	948	1277 a[y]
Dimethomorph (Forum)	1775	1896	726	1465 a
Fluazinam (Omega)	403	444	1271	706 a
Potassium phosphite (Phostrol)	1412	1008	847	1089 a
Fluopicolide (Presidio)	1291	1049	1331	1223 a
Propamocarb (Previcur Flex)	1956	1351	1089	1465 a
Cyazofamid (Ranman)	1150	605	484	746 a
Fenamidone (Reason)	1392	1482	1765	894 a
Mandipropamid (Revus)	1734	444	1472	1217 a
Mefenoxam (Ridomil Gold)	1029	948	383	787 a
Famoxidone, cymoxanil (Tanos)	262	847	1674	928 a
Oxathiapiprolin (Zorvec)	61	202	1008	424 a
Water	3650	2198	4175	3341 b
Untreated	3408	2299	2985	2897 b
P[x] $>F$				<0.0001

[z] Average lost yield across 3 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.
*Common names followed by trade names in parenthesis.

Table 13 Potential yield (kg/ha) and lost yield for field #3: main farm, west—2014.

Treatment*	Rep. 1	Rep. 2	Rep. 3	Yield Loss (% of Potential Yield)[z]
Actibenzolar (Actigard)	2793	2440	2612	48.84
Dimethomorph (Forum)	2632	2924	2390	55.33
Fluazinam (Omega)	1603	2107	2571	33.71
Potassium phosphite (Phostrol)	2642	1946	2168	48.36
Fluopicolide (Presidio)	2632	2339	2632	48.28
Propamocarb (Previcur Flex)	3217	3005	2289	51.66
Cyazofamid (Ranman)	2259	2128	2097	34.53
Fenamidone (Reason)	2077	2087	3156	36.64
Mandipropamid (Revus)	3015	1876	3166	45.31
Mefenoxam (Ridomil Gold)	1896	1986	2087	39.53
Famoxidone, cymoxanil (Tanos)	1371	2702	2692	41.13
Oxathiapiprolin (Zorvec)	1321	1936	2521	21.99
Water	3943	2612	5072	86.21
Untreated	3570	2733	3721	86.72

[z] Yield loss calculated by counting the number of pod rot diseased pods on 10 plants per plot (0.762 m of row) and converting to kg/ha assuming 'Cypress' succulent bean weight of 0.875 g.
*Common names followed by trade names in parenthesis.

Combined Data, 2013 & 2014

Combining disease incidence data across all four trials and comparing them to the untreated control treatment using a Dunnett's test (Table 14) reveals that dimethomorph, potassium phosphite, cyazofamid, mefenoxam, and oxathiapiprolin are all significantly different than the untreated control ($P < 0.05$). All treatments were significantly better at reducing disease incidence across all trials according to Fisher's Protected LSD than both control treatments, with mefenoxam achieving the lowest disease incidence (Table 15).

Table 14 Dunnett's test comparing disease incidence across all trials in 2013 and 2014 to the untreated control.

LS Means Dunnett

Treatment*	Disease Incidence LS MEAN[z]	Ho:LS Mean=Control $P^{y}r < t$
Untreated Control	31.0667	
Water Control	38.5667	0.9983
Acibenzolar (Actigard)	18.0000	0.1504
Dimethomorph (Forum)	22.0333	0.3858
Fluazinam (Omega)	12.2333	0.0210
Potassium phosphite (Phostrol)	13.5000	0.0343
Fluopicolide (Presidio)	18.3000	0.1634
Propamocarb (Previcur Flex)	20.3333	0.2715
Cyazofamid (Ranman)	12.9667	0.0280
Fenamidone (Reason)	16.0333	0.0831
Mandipropamid (Revus)	17.1667	0.1181
Mefenoxam (Ridomil Gold)	11.6667	0.0167
Famoxidone, cymoxanil (Tanos)	14.7667	0.0542
Oxathiapiprolin (Zorvec)	12.1333	0.0202

[z] Average disease incidence across 10 replications.
[y] Treatments with *P*-values ≤ 0.05 are significantly different than the untreated control.
*Common names followed by trade names in parenthesis.

Table 15 Connected letters report for all fungicide trials combined disease incidence data—2013 and 2014.

Grouping[z]		Mean[y]	N[x]	Treatment*
	A	53.00	20	Water Control
	A	45.35	20	Untreated Control
	B	22.20	20	fluazinam (Forum)
C	B	22.05	20	propamocarb (Previcur Flex)
C	B	20.25	20	fenamidone (Reason)
C	B	19.50	20	fluopicodide (Presidio)
C	B	18.75	20	mandipropamid (Revus)
C	B	18.25	20	potassium phosphite (Phostrol)
C	B	18.15	20	acibenzolar (Actigard)
C	B	16.25	20	cyazofamid (Ranman)
C	B	15.70	20	famoxidone, cymoxanil (Tanos)
C	B	14.35	20	fluazinam (Omega)
C	B	13.25	20	oxathiapiprolin (Zorvec)
C		13.05	20	mefenoxam (Ridomil Gold)

[z] Treatment groupings with different letters are significantly different according to Fisher's Protected LSD (α=0.05).
[y] Average disease incidence across 10 replications.
[x] Number of replications.
*Common names followed by trade names in parenthesis.

Combined Data—2014 Trials

Combining the data from all three trials in 2014 reveals that all treatments were significantly better at controlling pod rot when compared to the two controls (Table 16) with mefenoxam achieving the best control (Table 17). The disease incidence data was square root transformed to fit a normal distribution to run the tests, then back transformed for presentation in Tables 16 and 17.

Table 16 Dunnett's test comparing treatments with untreated control for all fungicide trials in 2014.

LS Means Dunnett

Treatment*	Disease Incidence LS MEAN[z]	Ho:LS Mean=Control $P^{y}r < t$
Untreated Control	48.4467	
Water Control	53.5814	0.9897
Acibenzolar (Actigard)	17.4810	<.0001
Dimethomorph (Forum)	19.7700	<.0001
Fluazinam (Omega)	12.5826	<.0001
Potassium phosphite (Phostrol)	19.7756	<.0001
Fluopicolide (Presidio)	17.2531	<.0001
Propamocarb (Previcur Flex)	22.4540	0.0003
Cyazofamid (Ranman)	16.4617	<.0001
Fenamidone (Reason)	22.2296	0.0003
Mandipropamid (Revus)	18.9157	<.0001
Mefenoxam (Ridomil Gold)	11.3464	<.0001
Famoxidone, cymoxanil (Tanos)	15.0622	<.0001
Oxathiopiprolin (Zorvec)	11.8647	<.0001

[z] Average disease incidence across 10 replications.
[y] Treatments with *P*-values ≤ 0.05 are significantly different than the untreated control.
*Common names followed by trade names in parenthesis.

Table 17 Connected letters report for all fungicide trials in 2014.

Grouping[z]			Mean[y]	N[x]	Treatment*
	A		53.5809	15	Water Control
	A		48.4472	15	Untreated Control
	B		22.4543	15	propamocarb (Previcur Flex)
	B		22.2293	15	fenamidone (Reason)
C	B		19.7758	15	potassium phosphite (Phostrol)
C	B		19.7705	15	dimethomorph (Forum)
C	B	D	18.9155	15	mandipropamid (Revus)
C	B	D	17.4808	15	acibenzolar (Actigard)
C	B	D	17.2532	15	fluopicolide (Presidio)
C	B	D	16.4617	15	cyazofamid (Ranman)
C	B	D	15.0622	15	famoxidone, cymoxanil (Tanos)
C		D	12.5826	15	fluazinam (Omega)
C		D	11.8646	15	oxathiapiprolin (Zorvec)
		D	11.3461	15	mefenoxam (Ridomil Gold)

[z] Treatment groupings with different letters are significantly different according to Fisher's Protected LSD (α=0.05).
[y] Average disease incidence across 10 replications.
[x] Number of replications.
*Common names followed by trade names in parenthesis.

Fungicide Sensitivity Lima Bean Pod Assay

All control pods were infected with *P. capsici*, as well as one pod from fluazinam and potassium phosphite treatments. All pods that were treated with

cyazofamid, mefenoxam, or oxathiapiprolin were not infected. According to an analysis of means (Figure 11), the untreated control treatment had a significantly higher number of infected pods than the fungicide treatments. These data are similar to our results obtained through field experimentation in that fungicide treatments significantly reduce pod rot disease incidence compared to the controls.

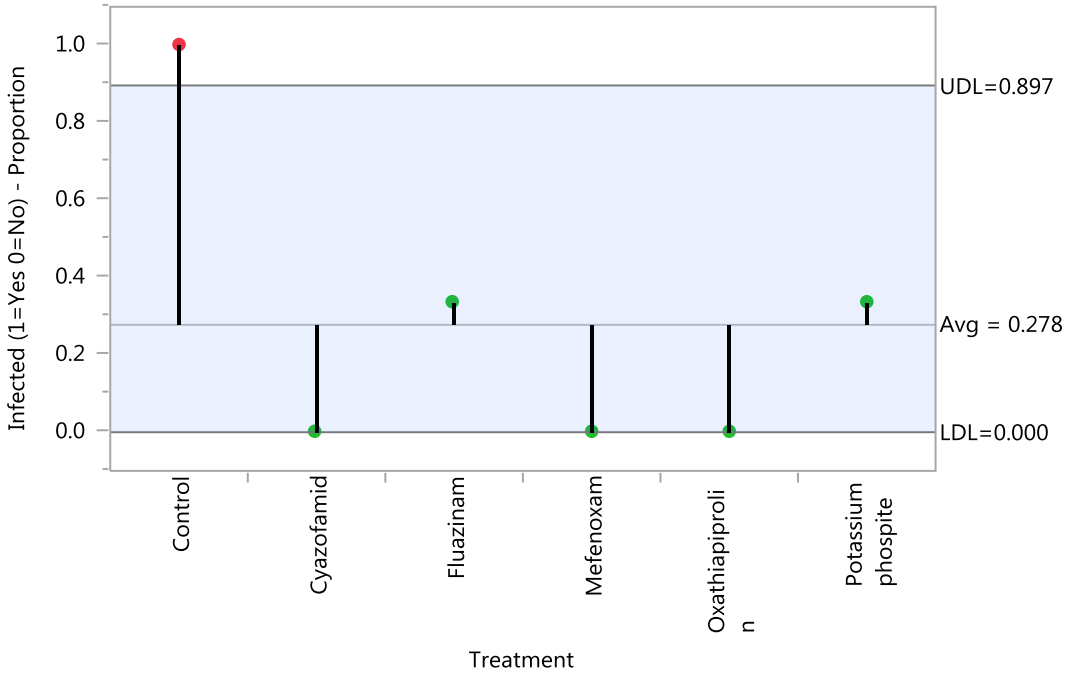


Figure 11 Analysis of means for the proportion of infected pods vs. non-infected pods for fungicide sensitivity pod assay. Treatments within the shaded region are not significantly different from each other. Red dot of control treatment indicates a significant difference at α=0.05.

Summary and Conclusions

Mefenoxam (Ridomil Gold) has been used extensively for the control of *P. capsici* on lima bean in Delaware since the early 2000's, and recently, cyazofamid (Ranman), has been been approved by the EPA treat pod rot. With documented resistance to mefenoxam (11, 34), the objective of this study was to identify additional fungicides that are effective at managing pod rot and that are in different FRAC (Fungicide Resistance Action Committee) groups to provide alternative products to mitigate resistance to any single fungicide.

Our trials indicate that there are fungicides currently on the market that have significant efficacy against *P. capsici* on lima. Of the 12 fungicides tested, fluazinam (Omega), mefenoxam (Ridomil Gold), cyazofamid (Ranman), and oxathiapiprolin (Zorvec) reduced pod rot of lima bean the most while maintaining high yields. Mefenoxam and cyazofamid are currently labeled for use against *P. capsici* on lima bean, however, oxathiapiprolin and fluazinam are not. Cyazofamid is a relatively expensive product, therefore widespread use for managing pod rot of lima bean is not likely. Fluazinam, however, is a commercially available product that may have a significant benefit for growers if it is eventually registered for use against *P. capsici* on lima bean. Fluazinam is already registered for the management of two other diseases of lima bean; downy mildew caused by *Phytophthora phaseoli*, and white mold caused by *Sclerotinia sclerotiorum*. The potential for control of pod rot of lima bean, as well as white mold and downy mildew via a single product would be a significant benefit for growers, as one product could be used for control of three common diseases of lima bean, saving them time and money.

Another potentially valuable product for the industry is potassium phosphite (Phostrol), or other chemically similar phosphite-based fungicides. These are

relatively inexpensive products and are often sprayed preventatively for the management of downy mildew of lima bean. Combined trial analyses show that potassium phosphite offers significant control of *P. capsici*, which would be beneficial for the industry as growers could achieve control of both downy mildew and pod rot with a single, relatively inexpensive product. Our results show that additional research with phosphite-based fungicides for controlling pod rot of lima bean is warranted in order to confirm our results. Furthermore, the combined data reveals that all fungicides were effective treatments for managing pod rot when compared to the controls. More research should be conducted in the future with these fungicides to help confirm these findings, with additional research focusing on determining optimal spray timings for the best control of pod rot of lima bean.

Additionally, according to the Fungicide Resistance Action Committee (FRAC), all five of the fungicides discussed above are in different FRAC groups, which is beneficial for managing fungicide resistant populations. Fluazinam is in FRAC group 29, affecting respiration. Cyazofamid also affects respiration, but is in FRAC group 21 because of its novel mode of action. Mefenoxam is in FRAC group 4 and interferes with RNA polymerase 1. Potassium phosphite is grouped in 33, being an activator of plant defenses, but research suggests that it may have an additional direct mode of action (58). Finally, oxathiapiprolin is a relatively new fungicide and its mode of action has not been determined, therefore it is in FRAC group U15. The information from this research will hopefully lead to IR-4 (agency which supports new pesticide labels for specialty crops in the United States) support and additional product labelling, giving growers more management options and reducing the possibility of developing resistant populations of *P. capsici* to any single fungicide.

Chapter 2

USING BIOFUMIGANTS AND BIOLOGICAL SOIL INOCULANTS FOR THE CONTROL OF POD ROT OF LIMA BEAN

Abstract

In recent years there has been increasing interest and use of biofumigation and biopesticides for the management of many soilborne plant diseases. We tested the efficacy of biofumigant mustards (*Brassica juncea* 'Caliente 199'; *Brassica juncea* 'Kodiak'; *Brassica juncea* 'Pacific Gold'; and *Sinapis alba* 'IdaGold'), rapeseed (*Brassica napus* 'Dwarf Essex'), *Sorghum spp.*(*Sorghum bicolor* 'Wilder Game Food, *Sorghum bicolor* 'Piper), and sorghum-sudangrass hybirds (*Sorghum bicolor x sudanese* 'F1OG', *Sorghum bicolor x sudanese* 'SS130', *Sorghum bicolor x sudanese* 'SS220'), and several different biopesticides (Actinovate, Double Nickel, Serenade Soil, Superzye 1-0-4, RootShield, PlantShield, and SoilGard), and an experimental *Trichoderma asperellum* isolate, for managing pod rot of lima bean caused by *Phytophthora capsici*. Trials were conducted in the summer of 2013 and repeated in 2014 on the University of Delaware's Carvel Research and Education Center, Thurmond Adams Agricultural Research Farm (UD REC) located in Georgetown, Delaware. Disease incidence in 2013 and 2014 was very low, and no significant treatment effects were observed with biofumigation cover crops or biopesticides.

Introduction

In recent years, agriculture has come under intense public scrutiny and the safety and sustainability of some of its practices have been questioned. Concerns include the environmental safety of synthetic pesticides, which has lead to the research and development of biological agents to control many agricultural pests.

Biological fumigation using biofumigant crops is one potential biological control method shown to be effective against *P.capsici* (27). Common biofumigant crops are mustard and rapeseed in the family Brassicaceae (30). Many plants in the Brassicaseae family can suppress the growth and spread of soilborne plant pathogens (30). Mustards and rapeseed produce abundant amounts of glucosinolates (31), which are released into the soil upon cell disruption and hydrolyse to form isothiocyanates (ITCs) and are toxic to many soil microorganisms (56). Research has confirmed that Brassicaceous plants are efficacious against many soilborne plant pathogens such as *Fusarium sp., Bipolaris sp., Pythium sp., Rhizoctonia sp.,* and *Gaeumannomyces sp.* (30). Monfort *et al*. demonstrated that some *Brassica* species also have a moderate level of nematicidal activity against root-knot nematode (48). Additionally, *Brassica*'s efficacy against *P. capsici* has been confirmed by Ji *et al*. in production squash fields in Georgia (27). Their study demonstrated that mustard (*Brassica juncea*) and Canola rapeseed (*Brassica napus*) reduced Phytophthora blight incidence to as low as 20-30%, being more effective than the chemical standard fungicide mefenoxam (27).

Additional crops which have biofumigation properties are *Sorghum* species, sudangrass cultivars (*Sorghum bicolor* subsp. *drummondii*), and sorghum-sudangrass hybrids. *Sorghum* species are warm-season grasses commonly grown as a forage, green manure crop, or summer cover crop. Some cultivars of *Sorghum* and sudangrass have been shown to suppress weed seed germination (65), root-knot nematode (47, 63, 66) and the plant pathogenic fungus, *Verticillium dahliae* (65). *Sorghum* and sudangrass' biofumigation ability has been attributed to its production of the cyanogenic glucoside, dhurrin, which is degraded and hydrolyzed to form hydrogen cyanide when cells are ruptured and incorporated into the soil (67). Hydrogen cyanide is toxic to many organisms, thus the use of *Sorghum* and sudangrass green manures as biofumigant crops has the potential for being an effective biological control agent for managing *P. capsici*, although this hypothesis has yet to be tested.

Antagonistic soil microorganisms, or biopesticides, are another form of biological control with potential efficacy towards *P. capsici*. Antagonistic soil microbes are bacteria and fungi which have the ability to suppress the growth of soilborne plant pathogens through a variety of mechanisms, such as the production of suppressive or toxic chemicals (21), parasitism (41), induction of host plant resistance (2, 3), or by out-competing the pathogen for vital nutrients (3). Akgul and Mirik planted susceptible pepper varieties in *P. capsici* infested fields treated with multiple strains of *Bacillus megaterium*, which significantly reduced disease severity (4). Two of the strains also increased yields by nearly 50% when compared to untreated controls (4). Just one year prior, Lee *et al*. discovered that *Bacillus subtilis* had an antagonistic affect on *P. capsici*, reducing disease severity by as much as 86.8% *in vivo* (38). In addition to *Bacillus* species, another bacterial species, *Streptomyces*

halstedii, was demonstrated to have a severe antifungal effect towards *P. capsici* (28). *Phytophthora capsici* spores were spread out on a potato dextrose agar (PDA) plate along with a paper disk containing a *S. halstedii* culture. Antifungal activity was determined by measuring the growth and spread of *P. capsici* through the PDA. Their research revealed that *S. halstedii* significantly inhibited *P. capsici* spore germination and growth, resulting in less than 1% *P. capsici* survival after just 12 hours of exposure (28).

In this experiment we tested a variety of biofumigants and biopesticides for their ability to reduce *P. capsici* disease incidence in lima bean, including biofumigant mustards (*Brassica juncea* 'Caliente 199'; *Brassica juncea* 'Kodiak'; *Brassica juncea* 'Pacific Gold'; and *Sinapis alba* 'IdaGold'), rapeseed (*Brassica napus* 'Dwarf Essex'), *Sorghum spp.*(*Sorghum bicolor* 'Wilder Game Food, *Sorghum bicolor* 'Piper), and sorghum-sudangrass hybirds (*Sorghum bicolor x sudanese* 'F1OG', *Sorghum bicolor x sudanese* 'SS130', *Sorghum bicolor x sudanese* 'SS220'), and several different biopesticides (Actinovate, Double Nickel, Serenade Soil, Superzye 1-0-4, RootShield, PlantShield, and SoilGard), and an experimental *Trichoderma asperellum* isolate.

Materials and Methods

All trials were conducted on the University of Delaware's Carvel Research and Education Center, Thurmond Adams Agricultural Research Farm (UD REC) located in Georgetown, Delaware. Trials were conducted in micro-plots constructed from 23 cm wide aluminum flashing. The flashing was installed approximately 15 cm deep in the soil in a one meter diameter circle. The micro-plots served as barriers to contain the treatments within the plot area. All trials were arranged in a randomized complete block design with four replications. The experiments were conducted in 2013 and

repeated in 2014 in the same field location. All plots were misted with Rain Bird® (Azusa, CA) mister nozzles constructed on 1 meter risers and 6 meter centers for 20 minutes in 2013 and 40 minutes in 2014, on an hourly schedule between 7:00 p.m. and 6:00 a.m., starting at flowering and ending at harvest.

Biofumigation—2013

Site Selection, Field Preparation, and Plot Maintenance

Trials were conducted in micro-plots located in a field on the UD REC in with a previous history of *Phytophthora capsici* on cucumber (*Cucumis sativus*) in 2012 and lima bean (*Phaseolus lunatus*) in 2011. The soil was a Rosedale loamy sand (Loamy, siliceous, semiactive, mesic Arenic Hapludults) with relatively high organic matter content and water holding capacity when compared to other fields on the farm, making it an excellent candidate for this work. Each micro-plot was inoculated with a 100 mm, 21 day old, V-8 juice agar plate of *P. capsici* isolate 32 culture (from University of Delaware collection). Each plate was cut into approximately 5 mm squares and mixed in a bucket containing 4 liters of water. The suspension was poured evenly over the soil surface of each micro-plot and worked into the soil approximately 5-8 cm deep.

Treatment Application

Five mustard and rapeseed varieties (Table 18); *Brassica juncea* 'Caliente 199'; *Brassica napus* 'Dwarf Essex'; *Brassica juncea* 'Kodiak'; *Brassica juncea* 'Pacific Gold'; *Sinapis alba* 'IdaGold'; were grown in one large planting outside of the micro-plots, but in the same field. A clean seedbed was prepared using a rototiller and 135 kg of nitrogen per hectare in the form of urea was incorporated prior to

planting. Mustards were seeded on May 22 at a rate of 11 kg/ha. An additional 34 kg/ha of nitrogen was applied at first bloom on Jun 19 to help maximize biofumigation potential.

The mustards were chopped and incorporated into the micro-plots two weeks after first bloom on July 3. A section of plants equivalent to the area of each micro-plot (0.80 m^2) was pulled by hand and chopped into approximately 2.5 cm pieces. The chopped foliage was then weighed and immediately incorporated into the soil in the appropriate micro-plot. Approximately 1 cm of water was applied after incorporation which was needed to synthesize the ITC gasses and to seal the soil surface, preventing volatilization of the biofumigant gasses. Control plots were left untreated.

Five locally available *Sorghum spp.*, sudangrass, and sorghum-sudangrass hybrids (Table 18) were grown adjacent to the mustards (seed source: Clark Seed Inc., Kenton, Delaware). The varieties were: brown midrib sudangrass F1OG, *Sorghum bicolor x sudanese*; SS130, *Sorghum bicolor x sudanese*; SS220, *Sorghum bicolor x sudanese*; Wilder Game Food, *Sorghum bicolor*; and Piper, *Sorghum bicolor var. sudanense*. A clean seedbed was prepared using a rototiller and 135 kg of nitrogen per hectare in the form of urea was incorporated prior to planting. The grasses were directed seeded on May 29 using a push planter at the rate of 28 kg/ha.

The varieties were chopped and incorporated into the micro-plots on July 3 as described above. Control plots were left untreated.

Seeds of lima bean cv. Eastland (source: Park Seed Co. Hodges, SC) were planted on July 17 in a circle parallel to the edge of the micro-plot in each plot and spaced 7.5 cm apart. Weeds were managed by hand. Straw was spread around the

perimeter of each micro-plot to prevent the splashing of *P. capsici* inoculum from outside the treatment area onto the lima beans inside the micro-plots.

Table 18 Biofumigant cultivars and biomass (kg) added to each micro-plot—2013.

Cultivar	Rep 1	Rep 2	Rep 3	Rep 4	Average*
Mustards					
Caliente 199	1.17	1.16	1.19	1.17	1.17
Dwarf Essex	1.54	1.45	1.50	1.39	1.47
Kodiak	1.67	2.18	1.70	1.87	1.86
IdaGold	1.14	1.13	1.06	0.95	1.07
Pasific Gold	1.32	1.16	1.08	1.05	1.15
Sorghum, Sudangrass, and Sorghum-Sudangrass Hybrids					
BMR Sudangrass F1OG	0.41	0.44	0.52	0.49	0.47
SS130 Hybrid	0.23	0.21	0.18	0.23	0.21
Wilder Game Food	0.24	0.28	0.36	0.25	0.28
SS220 Hybrid	0.28	0.36	0.35	0.33	0.33
Piper Sudangrass	0.24	0.24	0.26	0.25	0.25

*Average biomass across 4 replications.

Data Collection and Analysis

Disease incidence was evaluated on October 21 from five random plants in each plot. Any amount of *P. capsici* present on a pod was counted as an occurrence of the disease.

Data was analyzed using JMP statistical software (SAS Institute Inc.). An analysis of variance (ANOVA) was performed, and means were separated using

Fisher's Protected LSD. Disease incidence data was square root transformed as needed to fit a normal distribution.

Biofumigation—2014

Site Selection, Field Preparation, and Plot Maintenance

Trials were conducted in micro-plots just as in 2013, in the same field location on the UD REC farm. Micro-plots were inoculated prior to treatment application using the same protocol outlined previously.

In addition to installing a low pressure mist system as in 2013, a solid set irrigation system was installed in the field constructed on 2 meter risers and 12 meter centers in order to create more soil surface disturbance necessary for splashing *P. capsici* inoculum from the soil up on to the pods. The system was run for approximately one hour twice daily (9 a.m. and 3 p.m.) starting at flowering and ending after disease rating data collection on October 7.

Treatment Application

Mustards and rapeseed (same varieties as 2013) were seeded into previously rototilled ground on April 30 in the same plot area as in 2013 at 11 kg/ha. Nitrogen was applied at 135 kg/ha of in the form of urea at planting and followed by an additional 34 kg/ha at flowering. On June 26, the mustards were pulled by hand, chopped, weighed, and incorporated into the micro-plots per the protocol above (refer to Table 19 for biomass). Control plots remained untreated.

Sorghum spp., sudangrass, and sorghum-sudangrass hybrids (same varieties as 2013) were direct seeded into clean tilled ground on May 26 at 28 kg/ha using a push planter. 135 kg/ha of nitrogen in the form of urea was applied at planting. The

varieties were manually pulled, chopped, weighed, and incorporated into the micro-plots on June 30 per the same protocol used for the mustards (refer to Table 19 for biomass). Control plots were left untreated.

Seeds of lima bean cv. Eastland were planted on July 14 in a circular pattern as described previously. Weeds were managed by hand. Straw was spread around the perimeter of each micro-plot to prevent the splashing of *P. capsici* inoculum from outside the plot area onto the lima beans inside the micro-plots.

Table 19 Biofumigant cultivars and biomass (kg) added to each micro-plot—2014.

Cultivar	Rep 1	Rep 2	Rep 3	Rep 4	Average*
Mustards					
Caliente 199	0.93	1.17	1.30	1.22	1.16
Dwarf Essex	5.28	6.00	5.35	4.94	5.39
Kodiak	1.32	1.05	1.26	1.16	1.20
IdaGold	1.13	1.26	1.22	1.24	1.21
Pasific Gold	1.56	1.73	1.52	1.42	1.56
Sorghum, Sudangrass, and Sorghum-Sudangrass Hybrids					
BMR Sudangrass F1OG	0.20	0.21	0.30	0.37	0.27
SS130 Hybrid	0.10	0.12	0.11	0.08	0.10
Wilder Game Food	0.12	0.14	0.18	0.16	0.15
SS220 Hybrid	0.38	0.35	0.39	0.36	0.37
Piper Sudangrass	0.10	0.11	0.11	0.10	0.11

*Average biomass across 4 replications.

Data Collection and Analysis

Disease incidence data was collected on October 7 from all plants in each plot. Any amount of *P. capsici* present on a pod was counted as an occurrence of the disease.

Data was analyzed using JMP statistical software (SAS Institute Inc.). An analysis of variance (ANOVA) was performed and means were separated using Fisher's Protected LSD. Disease incidence data was square root transformed as needed to fit a normal distribution.

Biopesticides—2013

Site Selection, Field Preparation, and Plot Maintenance

Biopesticide trials were conducted in micro-plots in the same field location as the biofumigation trials described previously. Each micro-plot was inoculated with a 100 mm plate of *P. capsici* isolate 32 as described for biofumigant trials. A mist system was installed and run as described above.

Treatment Application

The 9 treatments and application rates for the study are listed in Table 20. Treatments were applied on July 9 by thoroughly mixing each product into the top 3-8 cm of soil, or per the manufacturer's recommendations. Actinovate was applied as two separate treatments. Both treatments had a single soil application at planting. The second treatment had an additional foliar application of Actinovate at flowering. The remaining products were all applied as single applications at planting as a soil drench, except for the USDA treatment, which was broadcasted dry then incorporated into the top 3-8 cm of soil and watered in. Control plots were drenched with only water.

Seeds of lima bean cv. Eastland were planted on July 10 in a circular pattern as described previously. Weeds were managed by hand. Straw was spread around the perimeter of each micro-plot as described above.

Table 20 Biopesticide treatment active ingredients and application rates—2013 and 2014.

Treatment	Active Ingredient	Rate
Actinovate	*Streptomyces lydicus*	840 g/ha
Actinovate 2nd Application	*Streptomyces lydicus*	840 g/ha at planting & 840 g/ha foliar at flowering
Double Nickel	*Bacillus amyloliquefaciens*	1.12 kg/ha
Serenade Soil	*Bacillus subtilis*	14.0 l/ha
Superzyme	*Bacillus subtilis, Pseudomonas putida, Trichoderma koningii, Trichoderma harzianum*	4.5 kg/ha
RootShield	*Trichoderma harzianum*	2.25 kg/ha
USDA	*Trichoderma asperellum*	3700 kg/ha
SoilGard	*Gliocladium virens*	2.5 kg/ha
PlantShield	*Trichoderma harzianum*	4.5 kg/ha

Data Collection and Analysis

Disease incidence data was collected on October 21 from five random plants in each plot. Any amount of *P. capsici* present on a pod was counted as an occurrence of the disease.

Data was analyzed using JMP statistical software (SAS Institute Inc.). An analysis of variance (ANOVA) was performed and means were separated using Fisher's Protected LSD. Disease incidence data was square root transformed as needed to fit a normal distribution.

Biopesticides—2014

Site Selection, Field Preparation, and Plot Maintenance

The 2014 trials were conducted in the same field and micro-plots as in 2013. Plots were inoculated prior to treatment application with *P. capsici* isolate 32 per the protocol described for the 2013 trial.

In addition to the low pressure mist system, a solid set irrigation system was installed in the field constructed on 2 meter risers and 12 meter centers in order to create more soil surface disturbance necessary for splashing *P. capsici* inoculum from the soil up on to the pods. The system was run for approximately one hour twice daily (9 a.m. and 3 p.m.) starting at flowering and ending after disease rating data collection on October 7.

Treatment Application

Treatments (Table 20) were the same and applied per the same protocol outlined in the 2013 trial except they were applied on July 7.

Lima bean cv. Eastland were planted in to clean tilled ground on July 17 in a circular pattern on 7.5 cm spacing as described prior. Weeds were managed by hand. Straw was spread around the perimeter of the micro-plots to prevent inoculum from outside of the plot area splashing up and infecting pods in the plot area.

Data Collection and Analysis

Disease incidence data was collected on October 7 from all plants in each plot. Any amount of *P. capsici* present on a pod was counted as an occurrence of the disease.

All data was compiled and entered into a JMP (SAS Institute Inc.) file, an analysis of variance (ANOVA) was performed, and means were separated using Fisher's Protected LSD. Disease incidence data was square root transformed as needed to fit a normal distribution.

Results and Discussion

Biofumigation—2013

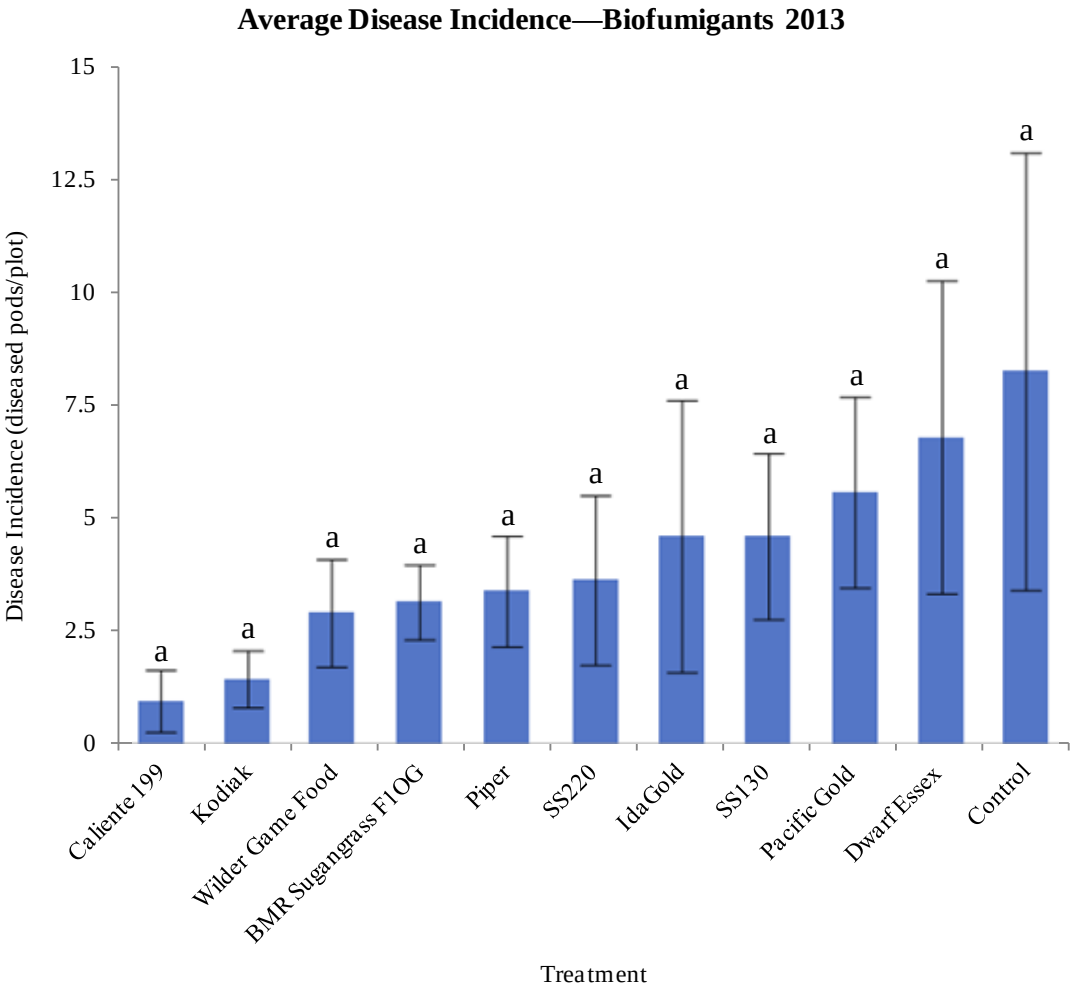
No significant differences were observed in the number of diseased pods per plot between any of the treatments ($P = 0.3166$, Table 21). Little disease occurred and there was high variability across the field (Figure 12). The low level of *P. capsici* infection in the trial was likely due to the lack of rain and relatively low humidity experienced during pod set and pod development. Only 1.07 cm of rain fell between September 13 and October 6 when pods were present in the field (Appendix A). The sparse rainfall, coupled with a relatively low average humidity of 76%, likely made for an unfavorable environment for *P. capsici*, which may explain the lack of disease development in the trial. *Phytophthora phaseoli*, the causal organism of downy

mildew of lima bean, was prevalent in this trial, which could have further inhibited *P. capsici* growth by competing for space and resources. The competitive exclusion principal states that "no two species can indefinitely occupy the same ecological niche" (18), and models have been proposed which indicate that hosts have their own carrying capacity (9). Therefore, *P. phaseoli* could have impeded *P. capsici* infection on pods heavily infected with downy mildew and thus contributing to the low disease incidence.

Table 21 Disease incidence (diseased pods/plot), biofumigant trial—2013.

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average[z]
BMR Sudangrass F1 OG	5	3	4	1	3.25 a[y]
IdaGold	14	2	2	1	4.75 a
Pacific Gold	10	2	9	2	5.75 a
Kodiak	1	3	2	0	1.50 a
Dwarf Essex	17	7	3	1	7.00 a
Wilder Game Food	2	5	5	0	3.00 a
Hybrid SS220	1	6	8	0	3.75 a
Caliente 199	0	3	1	0	1.00 a
SS130 Hybrid	10	4	4	1	4.75 a
Piper	3	1	7	3	3.05 a
Untreated Control	21	12	0	1	8.50 a
P[x] $>F$					0.3166

[z] Average disease incidence across 4 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 12 Average disease incidence for biofumigant trial—2013.

Biofumigation—2014

Disease incidence in 2014 (Table 22 and Figure 13) was even lower than that of 2013, with no *P. capsici* infection in 4 of the 11 treatments, and an average between

0.25 and 1.25 infected pods per plot in the remaining 7 treatments. The highest disease incidence (1.25 infected pods per plot) was observed in the untreated control treatment, however, because of the overall low levels of *P. capsici* infection, there were no significant differences among any of the means ($P = 0.2380$).

Rainfall amounts between September 6 and October 2, when susceptible flat pods were present in the field, were 10.54 cm; up 9.47 cm compared to 2013 (see Appendix A and C). Average relative humidity during this timeframe was 81%, up 5% over 2013 (Appendix A and C). However, even with high rainfall and heavy overhead irrigation, pod rot disease incidence was not increased over 2013 observations. Low disease incidence in these trials under conducive conditions suggest that an insufficient amount primary inoculum existed in the soil, or the primary inoculum was not splashed up on to the pods. The solid set irrigation system should have provided adequate splashing to move inoculum from the soil to the pods; however, wind velocity and wind direction may have interfered with irrigation water dispersal.

In both 2013 and 2014, survival of inoculum in the soil most likely played a significant role in the lack of pod rot. There was approximately 80 days between inoculation of the micro-plots with *P. capsici* and pod set in which susceptible plant parts were present in the field. The survival of this inoculum, which was primarily sporangia, was crucial for the development of pod rot later in the season, and the sporangia could have died in the 80 days between inoculation and flat pod. The survival of the sporangia is dependent on a wide array of variables, both biotic and abiotic, with ultraviolet radiation being a major factor. Eduardo *et. al.* found that just 1 hour of UV exposure on a sunny day (solar irradiance > 600 W/m^2) was enough to

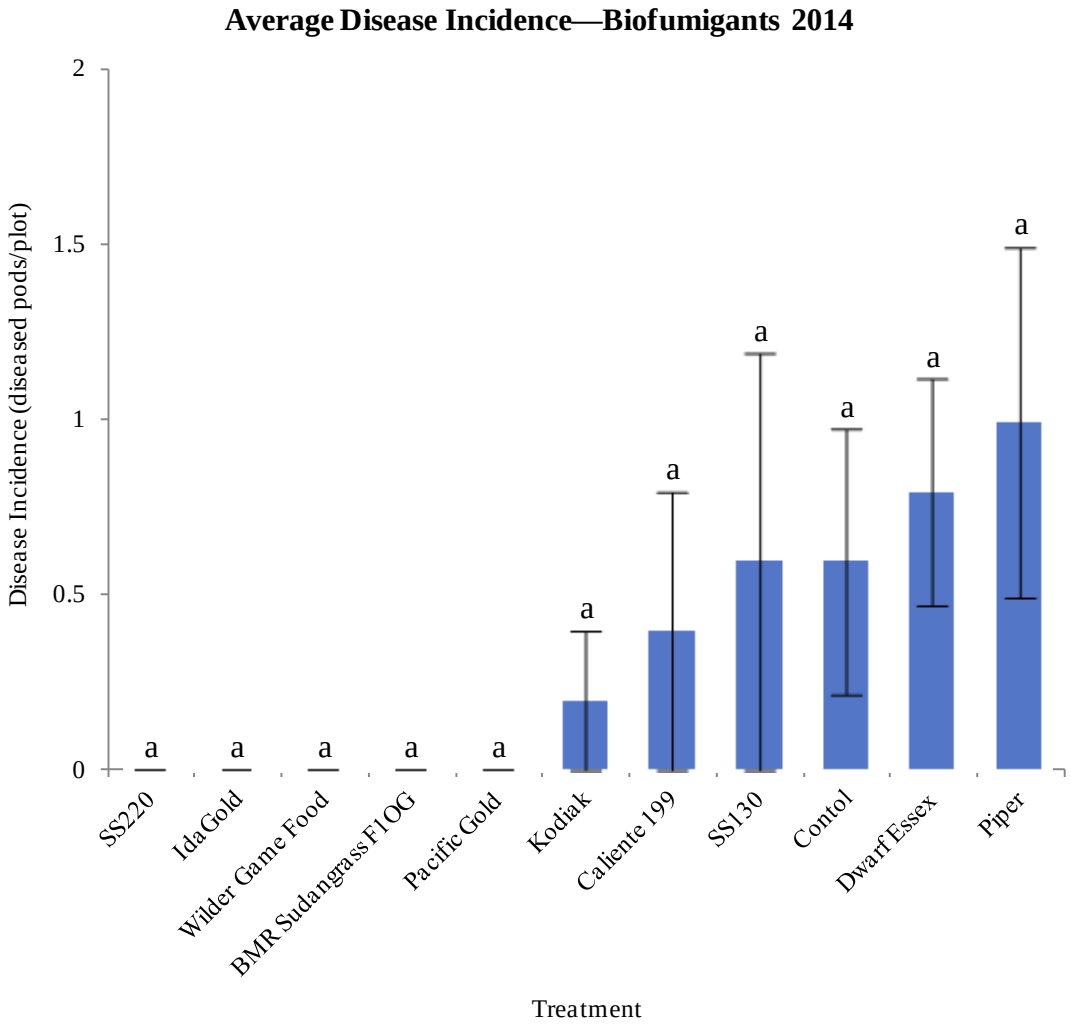
decrease the viability of *Phytophthora infestans* sporangia by 95% (46). The inoculum should have been protected from UV radiation since it was incorporated into the soil; however, subsequent tillage of the soil for treatment application and planting could have exposed a large portion of the inoculum to direct UV radiation, killing the *P. capsici* sporangia which would have served as primary inoculum.

An additional contributing factor to the low pod rot disease pressure could have come from competition from *P. phaseoli*, the downy mildew of lima bean pathogen. The 2014 trial was heavily infested with *P. phaseoli*, which established itself early in the plots, potentially occupying the niche *P. capsici* would have fulfilled as discussed in earlier sections.

Table 22 Disease incidence (diseased pods/plot), biofumigant trial—2014.

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average[z]
BMR Sudangrass F1 OG	0	0	0	0	0.00 a[y]
Idagold	0	0	0	0	0.00 a
Pacific Gold	0	0	0	0	0.00 a
Kodiak	1	0	0	0	0.25 a
Dwarf Essex	1	0	1	2	1.00 a
Wilder Game Food	0	0	0	0	0.00 a
Hybrid SS220	1	0	0	2	0.75 a
Caliente 199	0	0	0	0	0.00 a
SS130 Hybrid	0	2	0	0	0.50 a
Piper	0	3	0	0	0.75 a
Untreated Control	1	0	1	3	1.25 a
P[x] $>F$					0.2380

[z] Average disease incidence across 4 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 13 Average disease incidence for biofumigant trial—2014.

Biopesticides—2013

Disease incidence in 2013 (Table23 and Figure 14) was very low across all treatments, including controls, which may have been due to the low rainfall amounts

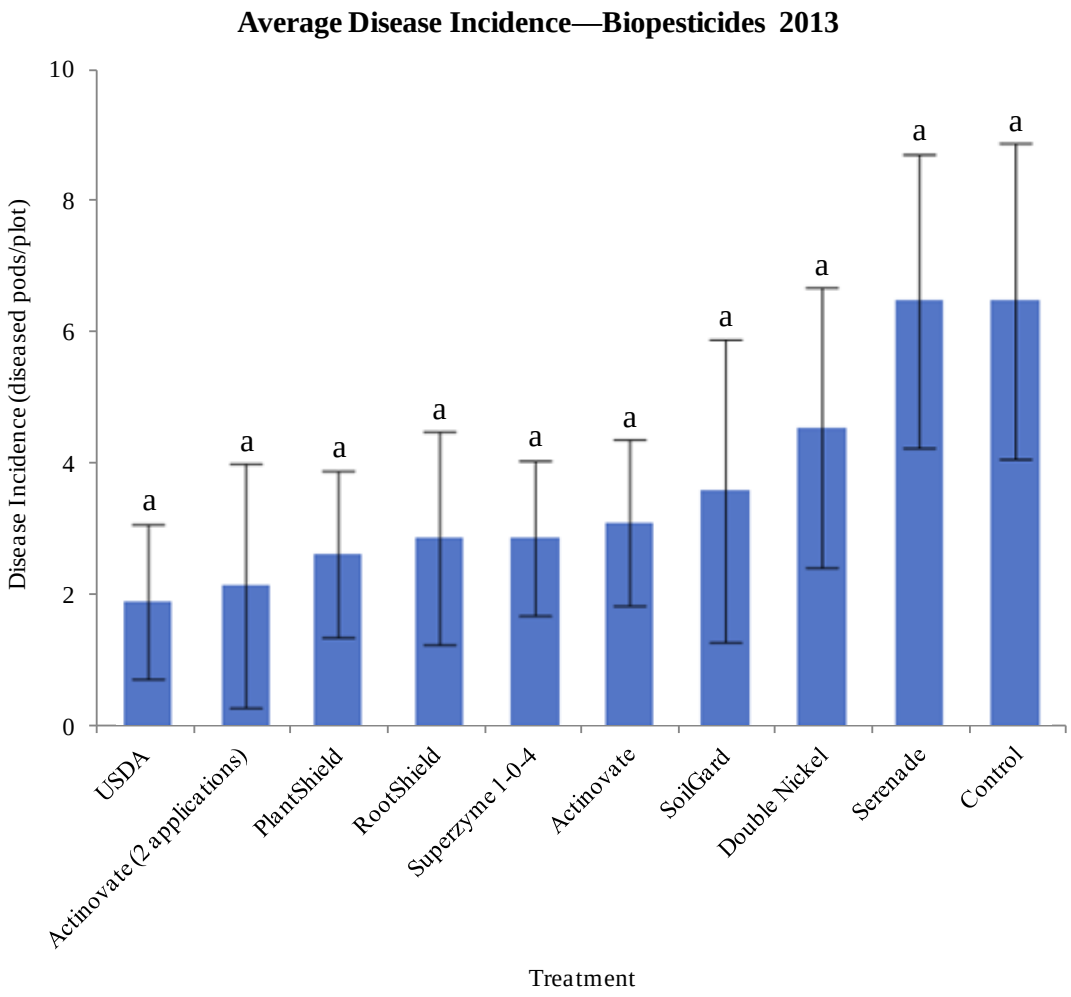
between September 13 and October 6 (see appendix A), during which time the lima beans were at the flat-pod growth stage. During this timeframe, the research station received a total of 1.07 cm of rain and average relative humidity was 76%.

There were no significant differences in disease incidence among treatments ($P = 0.5274$). It should also be noted that the prevalence and disease pressure from *P. phaseoli* was great in this trial, which could have adversely affected *P. capsici* disease development as discussed above.

Table 23 Disease incidence (diseased pods/plot), biopesticide trial—2013.

Treatment	**Rep. 1**	**Rep. 2**	**Rep. 3**	**Rep. 4**	**Average[z]**
Serenade	5	11	10	1	6.75 a[y]
USDA	0	5	3	0	2.00 a
Superzyme 1-0-4	6	3	0	3	3.00 a
SoilGard	0	10	5	0	3.75 a
Double Nickel	2	9	8	0	4.75 a
Actinovate	7	2	3	1	3.25 a
Actinovate (2 applications)	0	8	0	1	2.25 a
RootShield	1	1	8	2	3.00 a
PlantShield	0	5	5	1	2.75 a
Water Control	7	8	12	0	6.75 a
P[x] > F					0.5274

[z] Average disease incidence across 4 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 14 Average disease incidence for biopesticide trial—2013.

Biopesticides—2014

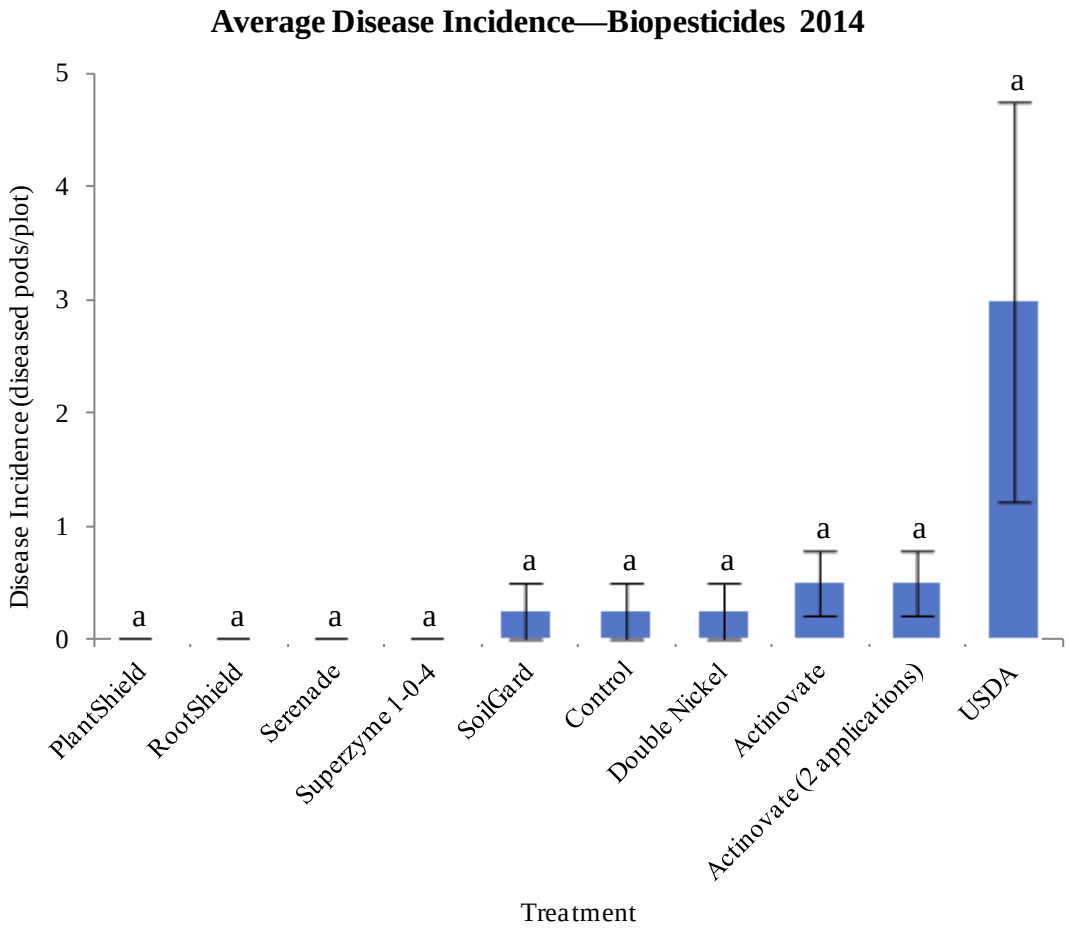
Disease incidence in the 2014 biopesticide trial was also very low, as seen in Table 24 and Figure 15. The water control treatment, which we would expect to have

a very high disease incidence rating, only averaged 0.25 diseased pods per plot (only one diseased pod across all four replications). No pod rot occurred in Superzyme 1-0-4, RootShield, PlantShield, and Serenade Soil plots. A single diseased pod across all four replications was observed in SoilGard and Double Nickel plots, and both Actinovate treatments had two diseased pods across all replications. There were no significant differences in pod rot incidence between treatments ($P = 0.0518$). Low disease incidence may be explained by the lack of viable primary inoculum present in the field during the flat pod stage, as described previously. These trials were also heavily infested with *P. phaseoli*, which could have adversely affected *P. capsici* growth by competing for space and nutrients as discussed previously.

Table 24 Disease incidence (diseased pods/plot), biopesticide trial—2014.

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average[z]
Serenade	0	0	0	0	0.00 a[y]
USDA	3	8	1	0	3.00 a
Superzyme 1-0-4	0	0	0	0	0.00 a
SoilGard	1	0	0	0	0.25 a
Double Nickel	1	0	0	0	0.25 a
Actinovate	1	0	1	0	0.50 a
Actinovate (2 applications)	0	0	1	1	0.50 a
RootShield	0	0	0	0	0.00 a
PlantShield	0	0	0	0	0.00 a
Water Control	0	0	0	1	0.25 a
P[x] $>F$					0.0518

[z] Average disease incidence across 4 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.



Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 15 Average disease incidence for biopesticide trial—2014.

Summary and Conclusions

The effectiveness of biopesticides and biofumigants as tools for managing pod rot of lima bean is inconclusive from these trials. Low infection levels in 2013 and 2014, stemming from unfavorable weather conditions and the possible lack of primary

inoculum, made it impossible to evaluate the true control potential of the treatments. To effectively evaluate the treatments, future research is needed with modifications to the protocols to ensure increased disease incidence, including an improved inoculation technique. Inoculation with oospores rather than sporangia may improve inoculum viability and result in greater disease incidence. In addition, conducting these trials in a larger plot may expose more soil area and increase the odds of rain or irrigation water splashing inoculum onto pods. With adequate disease incidence, differences between treatments may be observed and effective treatments may be identified.

Chapter 3

EVALUATION OF REDUCED TILLAGE PRACTICES FOR THE MANAGEMENT OF POD ROT OF LIMA BEAN

Abstract

Reduced tillage cropping systems have become more common in many agricultural crops because of their added benefits to soil health and environmental safety. No-till and reduced tillage systems leave a significant amount of the previous crop's residue on the soil surface, protecting the soil from wind and water erosion. Since *Phytophthora capsici* is a soilborne pathogen, rain or irrigation water splash play a role in introducing the pathogen to the infection court since *P. capsici* must contact lima bean (*Phaseolus lunatus*) pods in order to cause infection. The amount of mulch on the soil surface could impact disease incidence by reducing splash and inhibiting the flow of water throughout the field. Here we test 9 different combinations of stubble cut height, chaff spread or removal, and tillage practices to evaluate the potential for a reduced tillage system for the management of pod rot of lima bean. A field on the University of Delaware Carvel Research and Education Center, Thurmond Adams Research Farm (UD REC) located in Georgetown, Delaware, with a previous history of *P. capsici* was chosen for this study. The field was also inoculated with *P. capsici* prior to planting an oat (*Avena sativa*) cover crop in early spring 2013. Oats were grown to physiological maturity and harvested in July. Tillage and residue treatments were applied after oat harvest and prior to lima bean planting. We observed a very low level of *P. capsici* infection across all

treatments, including the clean tilled controls and there were no significant differences in disease incidence ($P = 0.6219$). In addition, the different tillage treatments had no effect in yield ($P = 0.5915$). Based on this experiment, we cannot make any conclusions on the effect of a reduced tillage cropping system for the management of pod rot of lima bean.

Introduction

In lima bean (*Phaseolus lunatus*), *Phytophthora capsici* can only infect the maturing pods, and primary inoculum in the soil must come in contact with the pod to cause infection. If *P. capsici* inoculum cannot come in contact with maturing pods, the disease cannot occur, therefore, the amount of mulch over the soil surface could affect incidence and spread of the disease. The more mulch over the soil surface, the less the chance of *P. capsici* inoculum can come in contact with susceptible pods.

The impact of plant stubble and crop residue on the spread of Phytophthora blight in pepper was studied at North Carolina State University. They were able to reduce disease incidence to 2.5% in bell pepper with a fall sown winter wheat cover crop (55). Wheat (*Triticum aestivum*) was planted in the fall, killed and mowed in the spring before it reached physiological maturity. The wheat straw was left in the field and acted as mulch over the soil surface, preventing rain and irrigation water from splashing *P. capsici* inoculum from the soil on to the transplanted pepper plants. The no-till system was also more effective than black plastic mulch at limiting the spread of *P. capsici* within the field (55). Plastic mulch may facilitate the spread of sporangia and zoospores down the row as the flow of water on plastic is largely unrestricted. The straw mulch, however, may interfere with the free flow of water through the field, inhibiting the travel of sporangia and zoospores. We hypothesized that planting lima bean into no-till or reduced tillage small grain stubble may have a similar effect, reducing the splash of inoculum on to pods and reducing the spread of sporangia and zoospores throughout the field, significantly reducing incidence of the disease.

In order to test this hypothesis, we planted lima beans behind a small grain cover crop and treated the stubble and chaff with a combination of varying stubble cut lengths, chaff spread or removal, vertical and conventional tillage, to achieve a wide range of soil surface residue from clean (conventional tillage) to no-till.

Materials and Methods

The trial was conducted in Georgetown, Delaware at the University of Delaware Research and Education Center, Thurmond Adams Research Farm (UD REC) in 2013 and repeated in 2014. Plots were 3 meters wide (4 rows) by 6 meters long and were arranged in a split plot design with four replications. Due to a poor initial stand and a subsequent late replanting, and the lack of pod rot, we could not collect any disease data in the 2014 plots. Therefore, only the 2013 trial is reported below.

Site Selection, Field Preparation, and Plot Maintenance

The field had a Rosedale loamy sand soil type (Loamy, siliceous, semiactive, mesic Arenic Hapludults) and a previous history of *Phytophthora capsici* on cucumbers (*Cucumis sativus*) in 2012 and lima bean (*Phaseolus lunatus*) in 2011. The Rosedale loamy sand soil series is a heavier soil, and this particular field had a higher organic matter content and water holding capacity compared to other fields on the farm. Its high water holding capacity coupled with history of *P. capsici* infection made it an excellent site for this experiment.

Inoculum was prepared on vermiculite in the lab slightly modified from Ji *et al.* (27). Briefly, 12, 4 liter Nalgene bottles containing 2 liters of vermiculite and V-8 juice (200 ml V-8 juice, 8 g $CaCO_3$, and 1800 ml of distilled water) were autoclaved

for 30 minutes on consecutive days and allowed to cool for at least 24 hours. Each vermiculite-V8 juice Nalgene bottle was inoculated with a single 100 mm, 14 day old V-8 *P. capsici* isolate 32 (from University of Delaware collection) plate cut into 5 mm squares. The bottles were shaken to thoroughly incorporate the *P. capsici* and placed in a south facing window of the lab at 25 °C. Bottles were shaken daily. After 14 days of incubation the inoculum was combined and mixed in a large, surface disinfested tub and taken to the field for inoculation. The inoculum was placed 2-6 cm deep in the two inner rows of each plot on April 2 at the rate of 65 ml per meter of row.

Spring oats (*Avena sativa*) were direct seeded into clean-tilled ground on April 4 using a Great Plains grain drill on 19 cm row spacing. After oats were harvested and tillage treatments were applied (see next section), lima bean cv. Maffei 15 (source: ADM) was direct seeded on July 18 using a 4 row Monosem no-till planter on 76.2 cm rows at the rate of 13 seeds per meter. A 30% liquid urea-ammonium nitrate (UAN) fertilizer was applied at planting at 55 kg of nitrogen per hectare, then an additional 55 kg of nitrogen per hectare was sidedressed after planting. A pre-emergence application of 1.17 l/ha Dual II Magnum (*S*-metolachlor) + 55 ml/ha Sandea (Halosulfuron-methyl, methyl 3-chloro-5-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl) -1-methylpyrazole-4-carboxylate) was made for weed control. Since this was a no-till trial, plots were hand weeded.

Overhead irrigation was used on an as-needed basis to maintain healthy lima bean growth. A mist system equipped with Rain Bird® (Azusa, CA) mister nozzles was installed on 1 meter risers and 6 meter centers and run for 20 minutes every hour between 7:00 p.m. and 6:00 a.m., starting at flowering and ending at harvest. Misting

was used to increase soil and plant wetness and to increase canopy humidity in an attempt to optimize environmental conditions necessary for *P. capsici* infection.

Treatment Application

Oats were harvested on July 17 using a Massey Ferguson small plot combine with a 3 meter wide grain head. The straw chopper was detached and chaff was dropped in a windrow. Two different cut heights were used; short (approximately 15 cm) and tall (approximately 40 cm tall). After harvest, straw was either spread or removed based on the treatment (Table 25). After the straw was spread or removed, plots were either left alone or vertical tilled using a Great Plains vertical tillage machine depending on the treatment (Table 25). Finally, control plots were clean tilled using a disk and chisel plow. Lima bean was then planted as described in the previous section.

Data Collection and Analysis

Disease incidence data was collected on October 15 from 6 random plants from the two inner rows of each plot. Any amount of *P. capsici* or symptoms of pod rot present on a pod was counted as an occurrence of the disease. Yield data was collected from 4 meters of row (two inner rows) on October 14 by recording the amount of shelled bean weight and converting to kg/ha.

All data was entered into a JMP (SAS Institute Inc.) file, an analysis of variance (ANOVA) was performed and means were separated using Fisher's Protected LSD. Disease incidence data was square root transformed as needed to fit a normal distribution.

Results and Discussion

The conventional tillage (clean tilled) plots had the highest disease incidence rating with an average of 3.25 infected pods; however, not significantly different than any of the other treatments (α=0.05). See Table 25 and Figure 16 for complete results. Due to the large variability in the data and the overall low level of *P. capsici* infection, there were no significant differences in disease incidence between treatments (P = 0.6219).

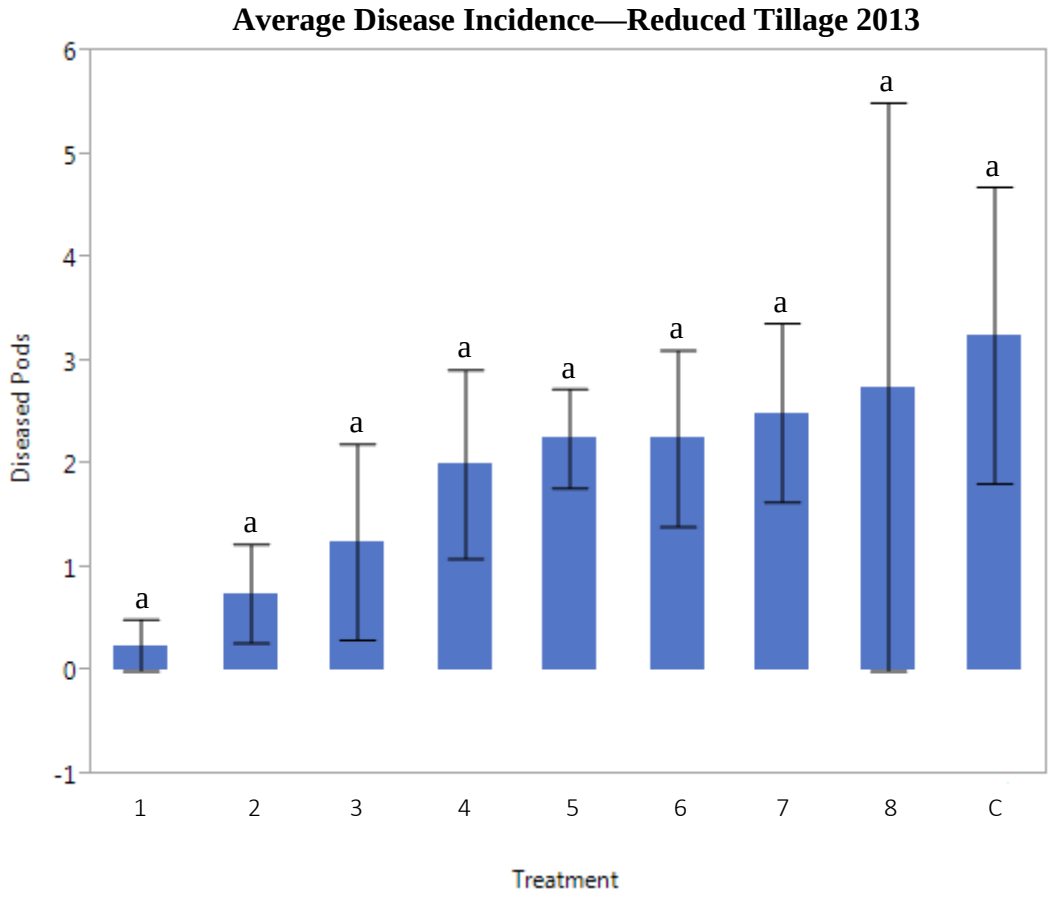
As described in the previous chapters, environmental conditions during pod set in 2013 were not conducive for pod rot disease development and supplemental misting throughout the night did not result in disease development. Furthermore, the possibility of a lack of primary inoculum may have also contributed to the low infection levels in this experiment. As described in Chapter 2, the survival of sporangia in the soil is largely dependent on UV radiation; just 1 hour of exposure to strong UV radiation can reduce sporangia viability by 95% (46). Lab prepared inoculum was in the field for approximately 140 days before susceptible pods were present in the field. During this period of time, any number of environmental variables, including UV radiation, could have reduced inoculum viability, thus resulting in low disease incidence.

Additionally, weed pressure in this trial was heavy. Since this was a no-till trial, cultivation was not performed and chemical products for the management of broadleaf weeds are limited since lima bean is a broadleaf crop. As a result, heavy broadleaf weed pressure was observed in many of the plots and may have impeded splash dispersal of *P. capsici* inoculum.

Table 25 Disease incidence (diseased pods/plot), tillage trial—2013.

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average[z]
High cut stubble, straw spread	3	4	0	1	2.00 a[y]
High cut stubble, straw removed	2	1	3	3	2.25 a
High cut stubble, straw spread followed by vertical tillage	0	11	0	0	2.75 a
High cut stubble, straw removed followed by vertical tillage	1	0	4	0	1.25 a
Low cut stubble, straw spread	0	4	3	3	2.50 a
Low cut stubble, straw removed	2	1	0	0	0.75 a
Low cut stubble, straw spread followed by vertical tillage	0	1	0	0	0.25 a
Low cut stubble, straw removed followed by vertical tillage	3	2	4	0	2.25 a
Control (conventional)	3	7	0	3	3.25 a
P[x] > F					0.6219

[z] Average disease incidence across 4 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.



Key:
C = Control (conventional tillage)
1 = Low cut stubble, straw spread, vertical tillage
2 = Low cut stubble, straw removed
3 = High cut stubble, straw removed, vertical tillage
4 = High cut stubble, straw spread
5 = High cut stubble, straw removed
6 = Low cut stubble, straw spread
8 = High cut stubble, straw spread, vertical tillage

Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

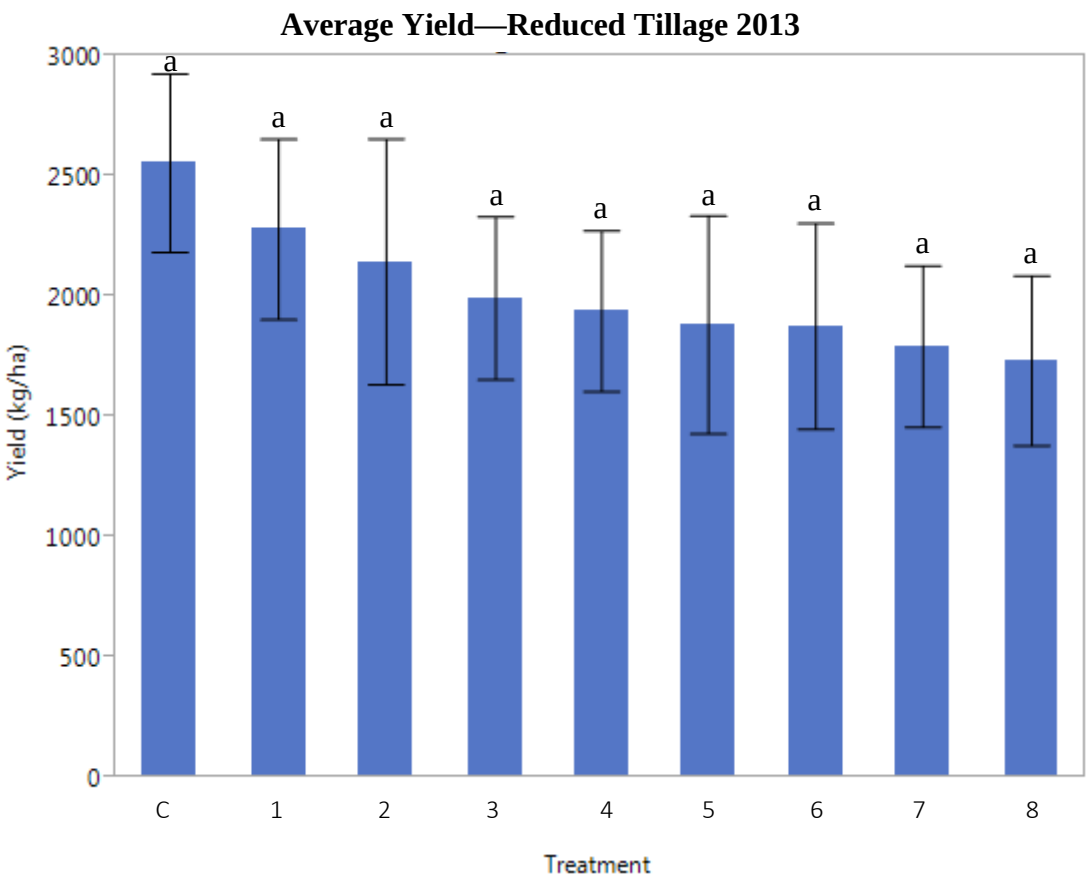
Figure 16 Average disease incidence for tillage trial—2013.

Tillage treatments did affect yield (*P* = 0.5915). See Table 26 and Figure 17 for yield data.

Table 26 Final yield (kg/ha), tillage trial—2013.

Treatment	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average[z]
High cut stubble, straw spread	810	2524	1728	1879	1735 a[y]
High cut stubble, straw removed	1111	1821	2581	2466	1995 a
High cut stubble, straw spread followed by vertical tillage	1348	2789	1613	1427	1794 a
High cut stubble, straw removed followed by vertical tillage	1147	3105	1771	1491	1878 a
Low cut stubble, straw spread	1685	3082	1598	2761	2281 a
Low cut stubble, straw removed	3656	1541	1491	1900	2147 a
Low cut stubble, straw spread followed by vertical tillage	1513	1692	1133	3198	1884 a
Low cut stubble, straw removed followed by vertical tillage	2545	1527	1218	2474	1941 a
Control (conventional)	3030	1448	2860	2890	2557 a
P[x] > F					0.5915

[z] Average yield across 4 replications.
[y] Treatment averages connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).
[x] P-value ≤ 0.05 indicates significant differences among treatments.



Key:
C = Control (conventional tillage)
1 = Low cut stubble, straw spread
2 = Low cut stubble, straw removed
3 = High cut stubble, straw removed
4 = Low cut stubble, straw removed, vertical tillage
5 = Low cut stubble, straw spread, vertical tillage
6 = High cut stubble, straw removed, vertical tillage
8 = High cut stubble, straw spread

Each error bar is constructed using 1 standard error from the mean.
Treatments connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Figure 17 Average yield for tillage trial—2013.

Summary and Conclusions

The goal of this study was to determine if a reduced tillage lima bean cropping system where lima beans sown into small grain stubble had the ability to reduce the incidence and spread of pod rot of lima bean. A secondary objective of the study was to determine if there was any effect on yield due to a reduced till system when compared to conventionally tilled controls.

In order to determine the effects of a reduced tillage system for the management of pod rot of lima bean, the disease must be established in the field during the growing season. Pod rot infection in the trials was low and disease incidence was highly variable, resulting in no significant differences in disease incidence between any of the treatments.

Likewise, there were no significant differences in yield among any of the treatments. Based on this experiment, we cannot conclude the effects of a reduced tillage cropping system on *P. capsici* for the management of pod rot of lima bean, nor their effect on yield. The experiment needs to be conducted again with modifications to the protocols to ensure greater disease pressure. With access to opposite mating types, it may be advantageous to inoculate with oospores rather than sporangia. Oospores are less ephemeral than sporangia, and may increase disease incidence as their chances of being viable over time are greater than that of sporangia.

Chapter 4

EVALUATING THE SUCSEPTIBILITY OF DIFFERENT POD STAGES TO *PHYTOPHTHORA CAPSICI* ON LIMA BEAN

Abstract

Phytophthora capsici only infects the maturing pods of lima bean (*Phaseolus lunatus*). However, observations are that not all pods are equally susceptible; flat pods may be more susceptible than pin pods and full pods. The goal of this experiment was to determine if there were any differences in pod susceptibility to *P. capsici* based on their age. Flat pods and full pods of lima bean breeding lines DE0407907, DE0505002A, and DE0505002B were detached from plants, surface sterilized, and inoculated with a 5 mm plug of *P. capsici*. Growth on the pods was monitored every 48 hours for one week after inoculation. Growth of *P. capsici* on flat pods was significantly greater than growth on full pods from 48 to 72 hours, with an average of 70.4% infection for flat pods versus 40.3% for full pods after 72 hours ($P = 0.0378$). However, growth was similar on full pods and flat pods after 120 hours. The data shows that flat pods are more susceptible than full pods to *P. capsici* infection, and pod age should be considered for successful management of pod rot of lima bean.

Introduction

Phytophthora capsici has the ability to infect roots, shoots, leaves, and fruits in many of its hosts such as pepper and cucurbits, however, it only infects the maturing pods on lima bean (*Phaseolus lunatas*) (5). Pod development was broken down into three stages; pin pods, where the pods are only a few millimeters to a couple centimeters long; flat pods, where the pods are fully elongated but seed fill has yet to start (seed diameter 3-5mm); and full pods, where seeds are full but still succulent (seed diameter 10-15mm). During the progression from pin pod to flat pod to full pod, tissues develop from young and succulent to mature and lignified (51). Lignification and nutrient content of the pod presumably play a large role in *P. capsici*'s infection ability and pod preference during infection (51). Observations in the field led to the hypothesis that the flat pod stage of development is the most susceptible to *P. capsici* infection. This experiment was conducted to test that hypothesis.

Materials and Methods

Flat (seed diameter of 3-5 mm) and full (seed diameter of 10-15 mm) lima bean pods were collected from the University of Delaware's breeding lines. Pin pods (approximately 5-20 mm in length) were not available, therefore not used in this experiment. The three lines used were: DE0407907, DE0505002A, and DE0505002B. Four flat pods and four full pods were collected from each breeding line and divided into four replications. The detached pods were surface sterilized in a 10% bleach solution for 2 minutes. The pods were rinsed with sterile distilled water and placed in

the center of a 100 mm petri dish containing moist filter paper. Pods were inoculated with a 5 mm plug of *P. capsici* isolate 32 (from the University of Delaware's collection) taken from the margin of an actively growing culture on V-8 juice agar and placed in the center of each pod. Plates were then sealed with Parafilm™ to retain moisture and humidity necessary for *P. capsici* infection. The plates were placed in an incubator at 25 °C with a 12 hour photoperiod.

Growth was monitored every 48 hours for a total of one week. Lesion expansion was measured by assessing the percentage of the pod infected with *P. capsici* and the data analyzed with JMP (SAS Institute Inc.). An analysis of variance (ANOVA) was performed, and means were separated using Fisher's Protected LSD.

Results and Discussion

At 48 and 72 hours after infection, the percent of pod infected was significantly greater on flat than full pods ($P = 0.0378$, Figure 18). Average pod infection on flat pods after 48 hours post inoculation was 24.2% versus 7.5% for full pods. Average pod infection after 72 hours was 70.4% for flat pods and 43.3% for full pods. After 120 hours there were no differences, and flat pods averaged 87.5% infection and full pods averaged 90.4%. Similarly, no differences remained after 168 hours with flat pods averaging 91.3% infection and full pods averaging 96.7% infection. Nearly all pods, full and flat, were 100% infected after 120 post inoculation. Figure 19 shows that lesion expansion on full and flat pods are similar (similar slopes to the lines between 48 and 72 hours), but initial infection on full pods is significantly delayed compared to flat pods.

The results indicate that flat pods are more susceptible to initial lesion expansion than full pods. Perhaps the higher lignin content in the full pods makes for

more difficult penetration and entry for *P. capsici* (51), and flat pods may have a greater nutritional value for the pathogen as maturing pods are sinks for photosynthetic sugars (52).

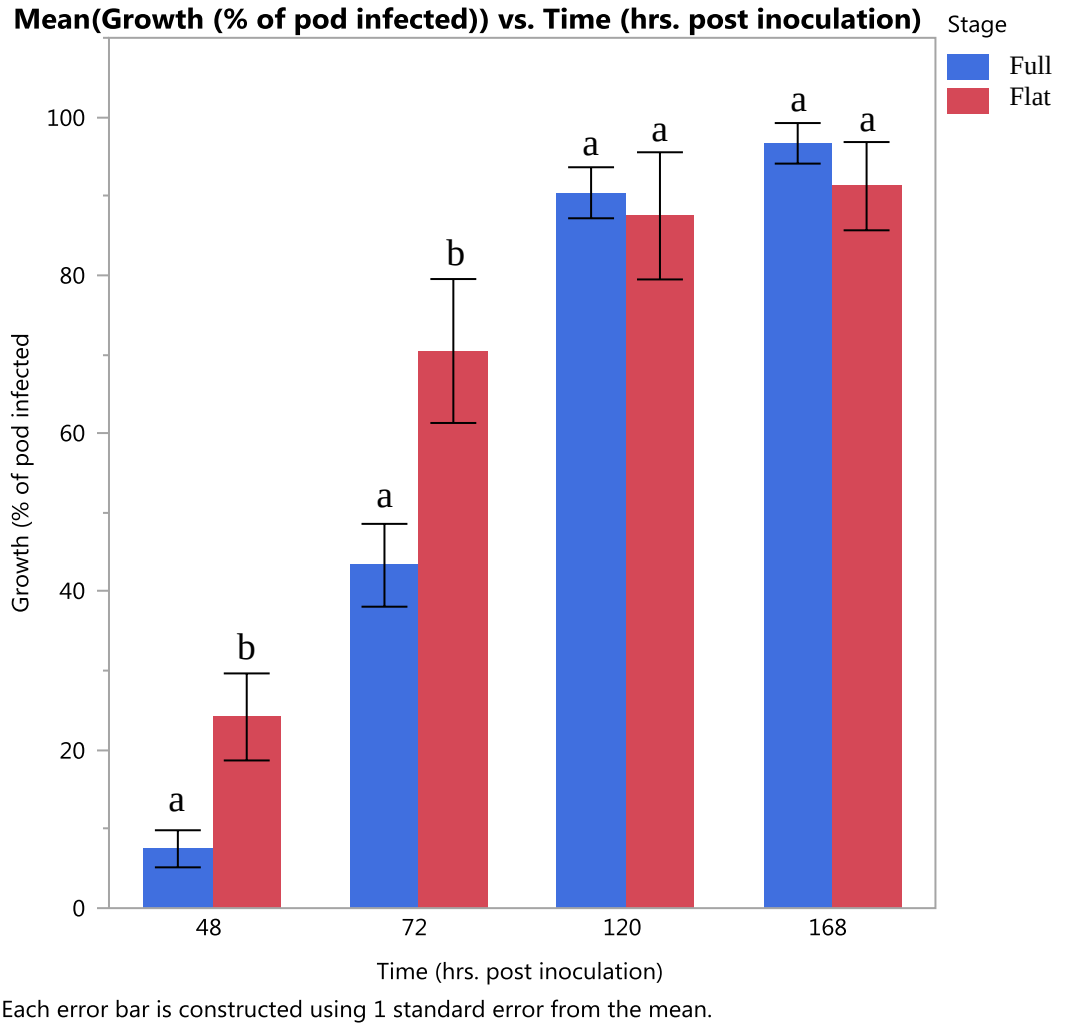


Figure 18 Growth of *P. capsici* on flat and full at 48 hour intervals. Pod growth stages within the same rating period connected by the same letter are not significantly different according to Fisher's Protected LSD (α=0.05).

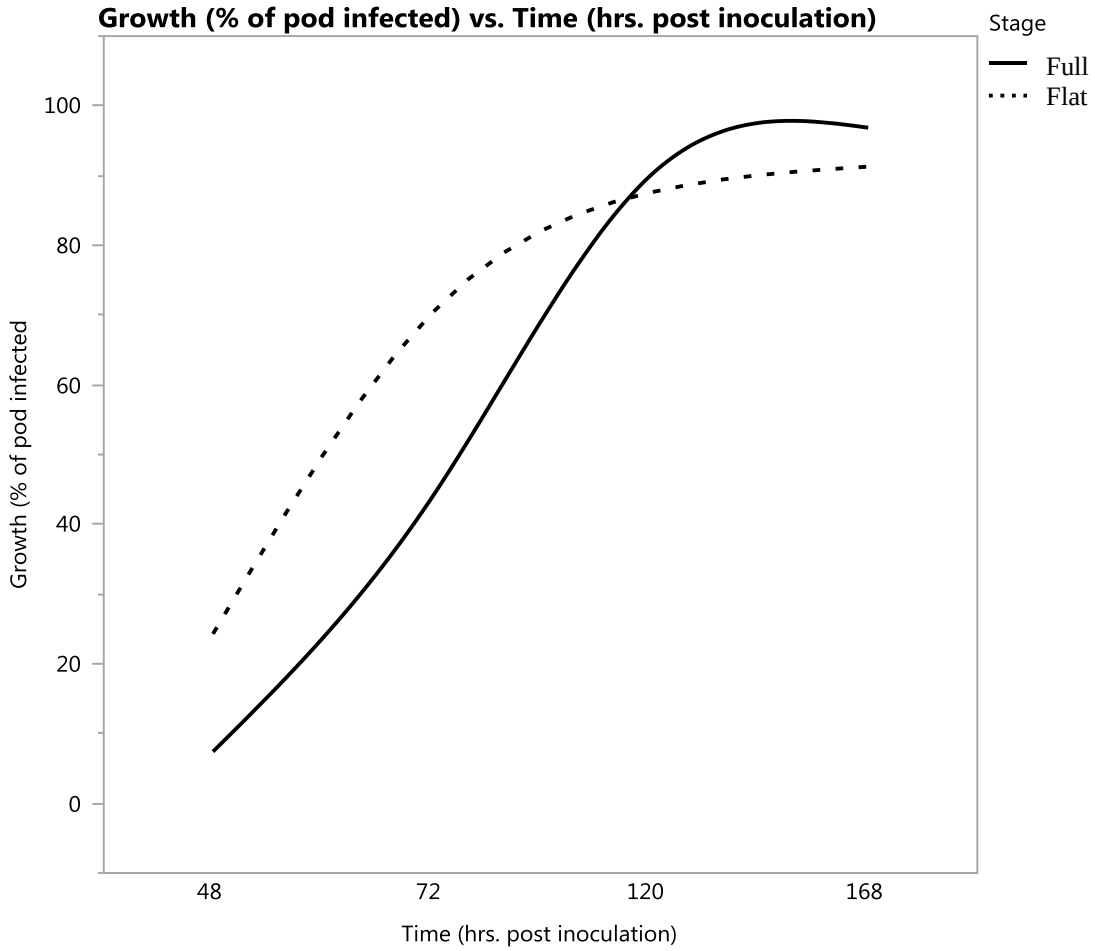


Figure 19 Growth of *P. capsici* on flat and full pods over time.

Summary and Conclusions

There was a significant difference in lesion expansion between flat pods and full pods at 48 and 72 hours post inoculation, with average growth on flat pods being significantly greater than growth on full pods. This experiment indicates that flat pods are more susceptible to *P. capsici* infection early on (< 72 hours post inoculation). This information could be particularly valuable for farmers from a management

standpoint, helping them target specific growth stages for disease scouting and to optimize fungicide treatment application timing. For example, if a grower has flat pods in the field and environmental conditions become favorable for *P. capsici* disease development (i.e. a large rainfall event), it is critical to apply a product (fungicide) within the first 48 hours to protect pods and prevent infection. This study needs to be repeated to confirm these findings.

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Appendix A

DAILY WEATHER CONDITIONS FOR UNIVERSITY OF DELAWARE CARVEL RESEARCH AND EDUCATION CENTER (AUG 1 – OCT 31 2013)

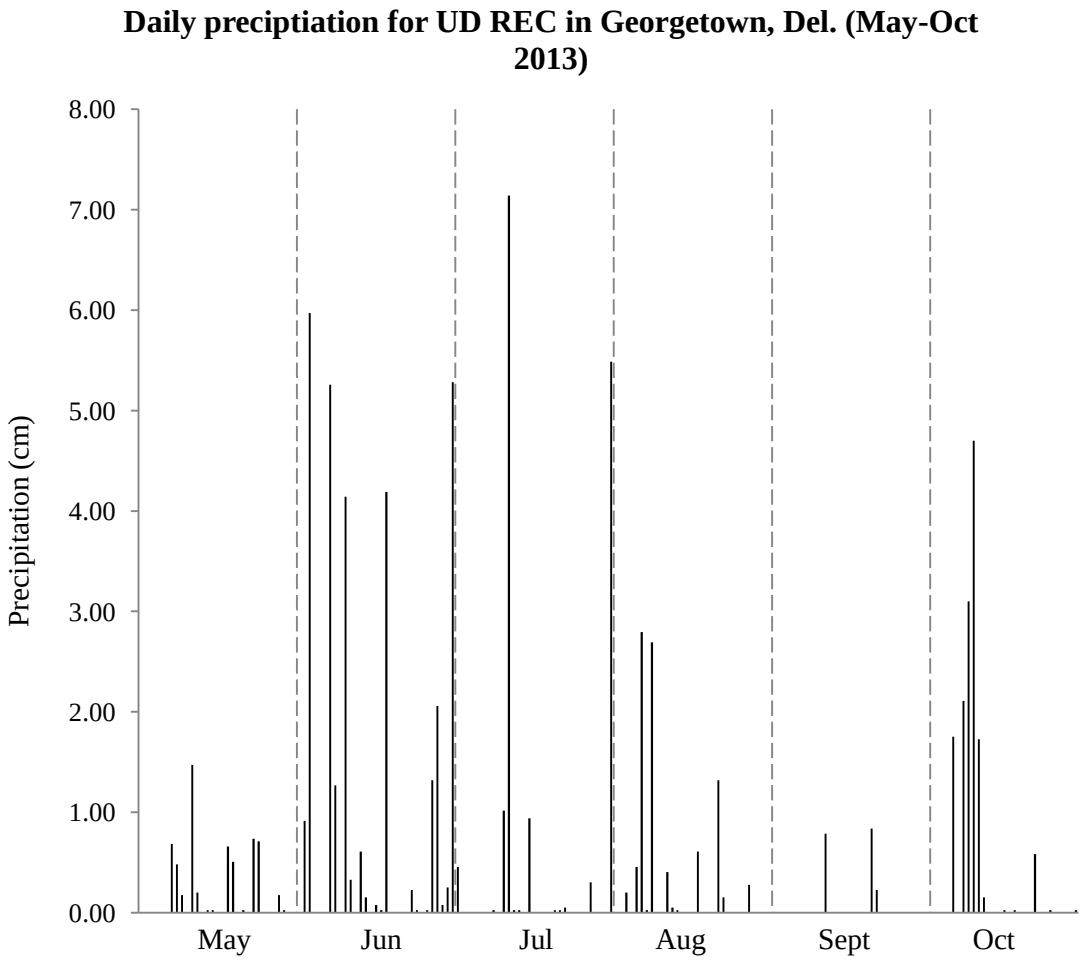
Day	Month	Av. Temp. (°C)	Av. RH (%)	Precip. (cm)
1	Aug	21.1	92.8	5.49
2	Aug	23.9	75.3	0.00
3	Aug	23.3	81.2	0.00
4	Aug	22.0	72.1	0.20
5	Aug	19.9	70.0	0.00
6	Aug	19.0	91.2	0.46
7	Aug	21.8	88.1	2.79
8	Aug	24.8	81.9	0.03
9	Aug	26.2	84.7	2.69
10	Aug	24.2	78.7	0.00
11	Aug	22.4	86.0	0.00
12	Aug	24.7	84.4	0.41
13	Aug	24.9	84.1	0.05
14	Aug	19.6	70.4	0.03
15	Aug	17.9	72.9	0.00
16	Aug	18.1	76.3	0.00
17	Aug	19.9	77.2	0.00
18	Aug	19.7	87.8	0.61
19	Aug	20.0	89.1	0.00
20	Aug	22.6	82.8	0.00
21	Aug	24.2	80.6	0.00
22	Aug	24.3	84.3	1.32
23	Aug	22.7	81.0	0.15
24	Aug	20.4	81.5	0.00
25	Aug	18.8	74.4	0.00
26	Aug	20.8	75.2	0.00
27	Aug	24.7	73.8	0.00
28	Aug	23.6	89.1	0.28
29	Aug	22.7	85.7	0.00
30	Aug	21.7	84.6	0.00

31	Aug	24.6	79.2	0.00
1	Sept	26.2	81.1	0.00
2	Sept	25.4	83.9	0.00
3	Sept	24.7	75.1	0.00
4	Sept	20.3	69.7	0.00
5	Sept	21.7	71.5	0.00
6	Sept	18.1	66.6	0.00
7	Sept	18.2	70.7	0.00
8	Sept	22.6	72.7	0.00
9	Sept	20.3	74.3	0.00
10	Sept	24.4	80.0	0.00
11	Sept	26.3	74.0	0.00
12	Sept	25.1	77.6	0.79
13	Sept	21.3	75.1	0.00
14	Sept	15.2	72.1	0.00
15	Sept	15.4	73.4	0.00
16	Sept	17.9	83.1	0.00
17	Sept	13.3	74.0	0.00
18	Sept	13.1	76.0	0.00
19	Sept	15.2	74.5	0.00
20	Sept	16.6	75.6	0.00
21	Sept	18.6	80.6	0.84
22	Sept	17.9	77.9	0.23
23	Sept	12.6	73.2	0.00
24	Sept	12.3	73.6	0.00
25	Sept	14.1	77.6	0.00
26	Sept	15.7	83.4	0.00
27	Sept	15.6	78.4	0.00
28	Sept	16.3	76.0	0.00
29	Sept	15.1	78.8	0.00
30	Sept	14.5	78.9	0.00
1	Oct	17.8	77.4	0.00
2	Oct	21.0	72.4	0.00
3	Oct	21.0	78.5	0.00
4	Oct	21.7	77.3	0.00
5	Oct	22.7	78.1	0.00
6	Oct	22.7	77.8	0.00
7	Oct	20.7	88.5	1.75
8	Oct	14.1	77.6	0.00

9	Oct	15.2	82.4	2.11
10	Oct	16.9	95.7	3.10
11	Oct	18.6	96.3	4.70
12	Oct	17.5	94.7	1.73
13	Oct	16.9	86.6	0.15
14	Oct	15.4	85.8	0.00
15	Oct	14.4	85.3	0.00
16	Oct	17.6	87.4	0.00
17	Oct	18.9	86.7	0.03
18	Oct	14.9	67.5	0.00
19	Oct	12.6	90.8	0.03
20	Oct	11.4	65.6	0.00
21	Oct	10.7	74.7	0.00
22	Oct	13.4	77.7	0.00
23	Oct	9.1	82.1	0.58
24	Oct	6.4	69.7	0.00
25	Oct	5.4	74.9	0.00
26	Oct	6.3	70.7	0.03
27	Oct	10.1	59.2	0.00
28	Oct	9.6	77.3	0.00
29	Oct	10.7	83.2	0.00
30	Oct	11.2	93.1	0.00
31	Oct	16.9	82.8	0.03

Appendix B

DAILY PRECIPITATION TOTALS FOR UNIVERSITY OF DELAWARE CARVEL RESEARCH AND EDUCATION CENTER (MAY-OCT 2013)



Appendix C

DAILY WEATHER CONDITIONS FOR UNIVERSITY OF DELAWARE CARVEL RESEARCH AND EDUCATION CENTER (AUG 1 – OCT 31 2014)

Day	Month	Av. Temp (°C)	Av. RH (%)	Precip. (cm)
1	Aug	22.1	85.6	0.46
2	Aug	19.7	92.2	3.43
3	Aug	21.7	91.5	4.06
4	Aug	23.0	83.2	0.00
5	Aug	23.5	78.4	0.03
6	Aug	22.8	77.1	0.00
7	Aug	21.6	73.4	0.00
8	Aug	20.3	75.0	0.00
9	Aug	21.1	74.4	0.00
10	Aug	21.7	72.2	0.00
11	Aug	20.8	78.4	0.00
12	Aug	22.9	89.4	4.24
13	Aug	23.0	78.0	0.00
14	Aug	20.6	66.6	0.00
15	Aug	18.7	68.8	0.00
16	Aug	21.2	75.1	0.03
17	Aug	22.0	78.4	0.00
18	Aug	21.2	76.8	0.00
19	Aug	22.7	79.2	0.03
20	Aug	21.4	77.2	0.00
21	Aug	22.1	87.1	0.48
22	Aug	21.4	87.8	3.18
23	Aug	19.5	85.9	0.18
24	Aug	19.4	76.9	0.00
25	Aug	19.0	77.5	0.00
26	Aug	19.8	76.4	0.00
27	Aug	20.7	75.6	0.00
28	Aug	21.7	74.6	0.00
29	Aug	18.3	74.8	0.00
30	Aug	20.1	80.3	0.00

31	Aug	24.8	79.6	0.00
1	Sept	25.3	81.7	0.20
2	Sept	26.4	74.1	0.10
3	Sept	23.4	78.0	0.20
4	Sept	21.9	79.6	0.00
5	Sept	23.7	82.9	0.00
6	Sept	25.2	79.0	1.65
7	Sept	20.2	82.3	0.15
8	Sept	18.4	86.3	0.51
9	Sept	20.8	84.6	0.00
10	Sept	20.3	81.5	0.00
11	Sept	22.4	83.1	0.00
12	Sept	20.3	75.0	0.00
13	Sept	17.3	91.7	0.33
14	Sept	14.8	77.6	0.00
15	Sept	15.2	72.5	0.00
16	Sept	18.3	75.3	0.08
17	Sept	16.0	71.2	0.00
18	Sept	15.9	75.3	0.00
19	Sept	16.2	84.2	0.00
20	Sept	17.9	83.3	0.00
21	Sept	20.4	85.5	0.00
22	Sept	18.6	60.6	0.00
23	Sept	12.5	72.8	0.00
24	Sept	16.5	82.3	0.89
25	Sept	17.6	95.5	6.68
26	Sept	15.7	82.4	0.00
27	Sept	16.3	78.9	0.00
28	Sept	17.3	77.5	0.00
29	Sept	17.4	88.5	0.13
30	Sept	17.4	83.9	0.13
1	Oct	16.1	88.0	0.00
2	Oct	14.5	87.4	0.00
3	Oct	14.8	84.7	0.08
4	Oct	16.9	74.4	0.30
5	Oct	9.4	64.9	0.00
6	Oct	13.4	67.7	0.00
7	Oct	18.2	70.8	0.00
8	Oct	18.8	69.5	0.05

9	Oct	14.1	65.4	0.00
10	Oct	14.3	83.9	0.97
11	Oct	12.7	96.8	1.55
12	Oct	11.9	81.5	0.00
13	Oct	15.1	92.4	0.41
14	Oct	19.5	82.6	0.03
15	Oct	20.7	88.1	0.48
16	Oct	15.4	83.0	0.97
17	Oct	14.1	77.9	0.00
18	Oct	15.2	69.9	0.00
19	Oct	9.2	66.9	0.00
20	Oct	8.9	72.1	0.00
21	Oct	14.6	76.3	1.47
22	Oct	11.6	92.3	0.36
23	Oct	11.6	76.1	0.03
24	Oct	13.7	65.0	0.00
25	Oct	12.8	66.0	0.00
26	Oct	14.1	54.2	0.00
27	Oct	10.9	62.8	0.00
28	Oct	18.1	66.4	0.00
29	Oct	16.9	76.0	0.03
30	Oct	10.2	76.6	0.00
31	Oct	7.5	78.9	0.00

Appendix D

DAILY PRECIPITATION TOTALS FOR UNIVERSITY OF DELAWARE CARVEL RESEARCH AND EDUCATION CENTER (MAY-OCT 2014)