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Biological Control Potential of *Streptomyces* Isolates on Pathogens (*Helminthosporium solani* and *Pythium ultimum*) of Potatoes

Shae Jamison Taylor

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

Biological Control Potential of *Streptomyces* Isolates on Pathogens (*Helminthosporium solani* and *Pythium ultimum*) of Potatoes

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Master of Science

Two fungal pathogen species, *Helminthosporium solani* and *Pythium ultimum*, cause significant economic loss to potato (*Solanum tuberosum*) growers throughout the world. These pathogens have substantial differences in cellular makeup, pathogenicity, and modes of infection. We studied the efficacy of 82 isolates within the bacterial genus *Streptomyces* in inhibiting these pathogens under laboratory and greenhouse conditions. Derivatives of *Streptomyces* have significant implications in medicinal use because of their antibiotic and antifungal properties. Under in-vitro conditions, 25% of *Streptomyces* isolates inhibited growth of *P. ultimum*, up to 81%. Ninety-five percent of the *Streptomyces* isolates inhibited growth of *H. solani*, with a maximum of 70%. In storage, these findings lead us to believe substantial differences between *Streptomyces* isolates will allow for some isolates to be effective biological controls at controlling diseases on common pathogens of potatoes.

Keywords: Helminthosporium, Pythium, biocontrol, chitinase, Streptomyces

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INTRODUCTION

Potatoes are the fourth-most economically important crop worldwide (FAOStat 2017). In 2017, the United States produced more than 20 million tons of potatoes, leading to an industry that is estimated to be worth more than $4.5 billion (NASS, 2017). Over the past six decades, damage to potato crops by two fungal species, *Helminthosporium solani* and *Pythium ultimum*, have led to substantial financial losses to producers throughout the country. *Helminthosporium solani* is responsible for what is widely termed “silver scurf”, which are tan to silver blemishes on the surface of potatoes, which render them unsaleable in fresh markets (Errampalli et al., 2001). Silver scurf, which may cause up to 13% tuber yield loss, has become more prevalent in the northwestern United States over the past forty years (Geary et al., 2007). Control of silver scurf has proven difficult given that *H. solani* has been shown to quickly develop resistance to traditional fungicides, especially Thiabendazole, which has been a commonly applied postharvest fungicide since the 1960s (Errampalli et al., 2001). A few salts and other products have been proven to decrease *H. solani* germination, but only when applied hours or days after infection of the tuber, making it ineffective in commercial settings (Hervieux et al., 2002).

Pythium ultimum is an oomycete and the cause of *Pythium* leak disease. Pythium leak liquefies the inside of potato tubers, causing them to turn into a brown or gray watery rot (Kuznetsova et al., 2018). This disease causes annual yield losses between 5-9% in well-managed fields (Kuznetsova et al., 2018), and can be up to 30% yield loss in highly susceptible or poorly managed fields (Triki et al., 2001). Oospores of *P. ultimum* have the capability to stay viable in the soil for 12 years, which impedes control of the fungal pathogen (Hoppe, 1966). As found with *H. solani*, the built-up resistance of *P. ultimum* to common fungicides presents a sizable challenge to growers (Taylor, R. J., Salas, B., Secor, G. A., Rivera, V., & Gudmestad, N.

C., 2002). Metaxyl-based fungicides have been effective in controlling *P. ultimum* in the past, but over-reliance on them has also caused resistance issues (Taylor, R. J., Salas, B., Secor, G. A., Rivera, V., & Gudmestad, N. C., 2002).

As is widely known, *Streptomyces* has the capability for creating a diverse array of antifungal, antibacterial, and other secondary metabolites (Elleuch et al., 2010; Wanner and Kirk, 2015; Rocio Suarez-Moreno et al., 2019). Researchers have been working with Streptomyces to develop it as a potential biological control for agricultural crop diseases. More than 60% of antibiotics in industrial and pharmaceutical use have been estimated to come from secondary metabolites produced by *Streptomyces* (Wanner and Kirk, 2015). The ability of *Streptomyces* to produce commercially and medically useful secondary metabolites makes it appealing for developing biological controls against *P. ultimum*, and *H. solani*. However, in some instances, less than 1% of the time, *Streptomyces* can cause potato scab. In addition, Wanner (2007) reported that non-pathogenic isolates of *Streptomyces* lead to disease suppression and promote potato plant growth.

Given that *Streptomyces* produces secondary metabolites and stimulates plant growth, it has been tested as a biological control in controlling Dutch elm disease (O'Brien et al., 1983); *Botrytis* and *Rhizoctonia* in lettuce (*Lactuca sativa*) (Tahvonen and Lahdenpera, 1988), bacteria in rice (Rocio Suarez-Moreno et al., 2019) and tomato cultivation (O'Brien et al., 1983; Tahvonen and Lahdenpera, 1988; Rocio Suarez-Moreno et al., 2019; Wu et al., 2019). Individual isolates of *Streptomyces* have been tested and can control *P. ultimum* (Sellem et al., 2017a). However, researchers appear to have only focused on only a few isolates (O'Brien et al., 1983; Tahvonen and Lahdenpera, 1988; Wu et al., 2019; Rocio Suarez-Moreno et al., 2019). Hence, they have been unable to make comparisons among *Streptomyces* isolates. To understand the

mechanisms to improve biological control of *Streptomyces* isolates, many isolates need to be tested and compared.

Eighty-two isolates of *Streptomyces* were collected from soil on potatoes from potato-growing regions of North America. These isolates are non-pathogenic to potato tubers, isolates that tested positive for the pathogenic gene were removed from this study. The purpose of this research was to determine if (1) *Streptomyces* isolates were antagonistic to *H. solani* and *P. ultimum* in Petri dish competition bioassays; (2) the top ten *H. solani*-limiting *Streptomyces* isolates would limit disease spread in the greenhouse with potatoes grown in soil; and (3) the top ten *P. ultimum*-limiting *Streptomyces* isolates would limit disease spread in a quick rot assay.

MATERIALS AND METHODS

Plate Bioassay

Petri-dish bioassays were used to quantify percent inhibition of *Streptomyces* isolates to *P. ultimum* and *H. solani*. Healthy colonies were established on oat (*Avena sativa*) agar (Babcock et al., 1993), and harvested from the agar with distilled water and quantified using a hemocytometer (Bright-Line, Hausser Scientific, Horsham PA). A ml of 10^7 colony-forming units (cfu) of *Streptomyces* were pipetted onto a 2.5-cm-dia.circle of autoclaved filter paper and placed in the center of a Petri dish (92 mm x 16 mm) filled with oat agar. Marks were made 8 mm from the edge of each filter paper in each cardinal direction. Fungal cultures were prepared and handled using aseptic techniques in a sterile biosafety cabinet (SterilGard III Advance, The Baker Company, Sanford Maine). Cork borer plugs of 0.8 cm in diameter were taken from the edge of actively growing fungal cultures, which 8-12 days old. The fungal culture was placed face down with the inner edge just touching the marked line. *Helminthosporium solani* samples

were plated the same day as *Streptomyces*. *Pythium ultimum* was plated 48 hours after *Streptomyces*.

Seven days after transferring the fungal plugs, we measured the distance *P. ultimum* had grown towards *Streptomyces* isolates with a digital caliper (01408A Electronic Digital Caliper, Neiko Tools, Grand Rapids, Michigan), and compared it to the distance *P. ultimum* grew away from the *Streptomyces* filter paper disk. Data were collected from 20 samples per isolate, averaged, and compared to determine percent inhibition from the *Streptomyces*. Three weeks after transferring the fungal plugs, *H. solani* underwent the same measuring and percent-inhibition calculations. Both *H. solani* and *P. ultimum* tests were conducted on 82 *Streptomyces* isolates (Figure 1). The statistical measurements were conducted using R (R Core Team 2017). Isolates were analyzed using ANOVA and Beta GLM tests, and Dunnett's multiple comparison test was used to determine which isolates were significantly different by comparing the least squares differences. A conservative Tukey-Kramer grouping was then used to determine the levels of significance and are listed in Tables 1 and 2.

Quick Rot Bioassay

Ten *Streptomyces* isolates (ID05-47C, DE06-01C, TX06-03A, FL07-03A, ID06-12A, ID06-15, MD13-6B, NY06-09D, and NE06-04A), which had the highest mean inhibition against *P. ultimum* in the Petri plate bioassay were taken and shaken in 50 mL of sterile oat broth for 10-15 days at 120 rpm (Innova 2100 platform shaker, New Brunswick Scientific, Edison New Jersey). Concurrent with the *Streptomyces* being shaken, a 2-L flask of *P. ultimum* was also being grown in sterile oat broth and shaken for 10-15 days at 120 rpm (Gyromax 703R Orbital Shaker, Concord California). The number of spores of *Streptomyces* were then quantified using a

hemocytometer and adjusted to 10^7 cfu mL^{-1}. We mixed 1 mL of 107 cfu broth of *Streptomyces* with 15 g of gum arabic and sufficient water to dilute the solution to 10^7 cfu in a 50-mL solution, which was used to coat tubers. The tubers were then dipped into the gum arabic-*Streptomyces* solution for thirty seconds until the tuber was thoroughly coated. The tubers were stored at 22.2°C at an elevated humidity (about 80%) for 48 hours. On opposing sides of each tuber, the periderm was wounded by lightly tapping multiple times with a 4-mm-toothed meat tenderizer.

After wounding, the tubers were placed in a sterile container. *Pythium ultimum* broth was then added until it covered the tubers. Once submerged, tubers were shaken at 120 rpm (Gyromax 703 Orbital Shaker, Concord California) for 5 minutes. After this process, the tubers were stored at 28°C at 80% relative humidity for 1 week. The tubers were sectioned into quarters after storage to be analyzed for damage by *P. ultimum* and other diseases. Data from each potato quarter were then averaged to estimate the amount of disease per potato. Ten tubers were analyzed for each of the 10 *Streptomyces* isolates, as well as a control with no coating, and a control with gum arabic and water coating rather than a gum and *Streptomyces* coating. Before analyzing data from the quick rot experiment the data needed to be transformed into proportions in the interval [0,1] by dividing by 100.To avoid losing observations for the model fit, percentage values were recorded. The Beta distribution assumes values only in the open interval (0,1), i.e. 0 and 1 values are not possible under this distribution. Therefore, to deal with proportion data that includes zeroes in the fit of a Beta GLM, there has to be a transformation that squeezes data that lies in the closed interval [0,1] to be in the open interval (0,1). The following transformation $Z=[Y(N-1)+.5]/N$ as depicted by Smithson and Verkuilen, 2006 was used (Table 3).

Greenhouse Bioassay

The ten *Streptomyces* isolates with the highest mean inhibition against *H. solani* from the Petri-plate assays were used for greenhouse bioassay trials. The greenhouse trials were done by a method finalized by Christopher Clarke from the USDA Beltsville ARS office (personal communication). Streptomyces isolates were initially streaked onto yeast malt extract (YME) plates and stored in an incubator at 27°C for 2 weeks. During the isolate culture period, 50 mg bags of vermiculite were autoclaved twice. The spores were then harvested from the YME plates by filling the plate with 5 ml of sterile water and gently rubbing with a sterile glass rod. The spores were quantified with a hemocytometer and diluted to a concentration of 10^6 spores mL^{-1}. Four mL of solution containing 10^6 spores was added to 50 mL of YME in a sterile flask and shaken at 120 rpm at 28°C for 72 hours. While the *Streptomyces* solution was shaking, the vermiculite bag was autoclaved a third time. After 72 hours of shaking, the *Streptomyces* was centrifuged, decanted the top 36 ml solution, and resuspended the bottom 18 ml solution. Five mL of the resuspension were added to the vermiculite with 50 mL of Say's solution (40g of sucrose, 2.4 g of asparagine, 1.2 g of K_2HPO_4 and 20 g of yeast extract in 1 liter of distilled water). The bag of vermiculite with the added *Streptomyces* was incubated for 14 days at 28°C. During this incubation period, we mixed the bag three times a week. A 50:50 sand-soil mixture was autoclaved, allowed to sit at 27°C for 2 days, and autoclaved again. The sand:soil mixture was autoclaved a third time after sitting for 2 days.

The seed-tubers were then washed and sterilized in 0.5% sodium hypochlorite with 1 drop of dish soap for 2-3 minutes. The seed-tubers were then stored in high-humidity buckets with *H. solani*-infected potatoes. Approximately 1 g of vermiculite was then removed from the bag of vermiculite and placed in 10 mL of water. The dilutions were then plated onto YME plates,

sealed with parafilm and incubated at 28°C for 48 hours. The dilutions on the YME plates were counted using a hemacytometer (Bright-line, Hausser Scientific, Horsham PA) to get an estimate of the concentration of *Streptomyces* in the vermiculite. The 50:50 sand-soil medium was then mixed with the vermiculite solution to obtain 10^7 cfu mL^{-1} Streptomyces. The seed-tubers had 5-10% silver scurf lesions and planted in the pots in the sterile sand-soil mixture. The 15.3-cm-dia. pots and 20.3 cm tall, were filled two-thirds full, the vermiculite was placed on the sand soil mixture, and the seed-tuber was placed on the vermiculite. The rest of the pot was filled with the sand-soil mixture to cover the seed-tuber. The potatoes were grown for 70 days. The progeny tubers were analyzed for *H. solani* growth, mass of progeny tubers, and other quality issues—quality issues included discoloration, russeting, bruising, or tuber cracking— and were held in storage for 30 days before being re-analyzed for *H. solani* growth and quality issues. The statistical analysis conducted was a chi-square test to determine if the amount of *H. solani* or the amount of quality issues was different between the treated and untreated progeny tubers. The significance was examined at $P<0.05$.

Linear Model

Using the chitinase data (Christopher Clarke, data not shown) and our inhibition data (Figures 2D and 3G). Two linear models were created to determine which factors—of *H. solani* inhibition, *P. ultimum* inhibition, Chitobiosidase (CB), Triacetylchitotriosidase (CT), and beta-N-acetylglucosaminidase (GA)—could most accurately predict the inhibition of whichever pathogen was not included in the model. Every combination of the three chitinases (CB, CT, and GA) and the *H. solani* inhibition or *P. ultimum* inhibition were created as general linear models

in R (R Core Team 2017). The model selection was based on Akaike's information criteria (AIC), with a normally distributed Gaussisn error structure (Tables 4, 5).

RESULTS

Plate Bioassay

Out of the 82 assayed *Streptomyces* isolates, 24 of them inhibited *P. ultimum* growth. Nine *Streptomyces* isolates (ID05-47C, DE06-01C, TX06-03A, FL07-03A, ID06-12A, ID06-15, MD13-6B, NY06-09D, and NE06-04A) had greater than 30% reduction in growth. The isolates reduced *P. ultimum* by 82%, 76%, 70%, 49%, 45%, 34%, 32%, 31%, and 30% respectively (Figure 2B). Growth comparisons were made by using the growth away from the *Streptomyces* minus the growth towards the *Streptomyces*. The two *P. ultimum* isolates, which were the most inhibitive, ID05-47C and DE06-01C, had much more consistent inhibition as indicated by the small variance boxes (Figure 2C). The other seven isolates, which had greater than 30% inhibition, had larger variance boxes.

Isolate TX06-03A median was above 90% inhibition. However, the mean equaled 64% inhibition, and the lowest quartile of data points ranged between 0-23%. The 18 isolates, which had less than 30% inhibition, had a limited number of data points (typically 4-6), that inhibited *P. ultimum* which had increased amounts of inhibition, but the median of the isolate data points was zero (Figure 2C). Significant differences were determined at the $P<0.05$ level. Only three of the isolates listed above were significantly different from the isolates that showed no inhibition, isolates ID05-47C, DE06-01C, and TX06-03A. Isolates ID05-47C and TX06-03A, were not significantly different between themselves or DE06-01C but they were significantly different than every other isolate (Figure 2B). DE06-01C was not significantly different from isolates

ID05-47C, TX06-03A, ID06-12A, FL07-03A, NY06-09D, ID06-15, MD13-6B, NE06-04A, or ID06-19 but was significantly different than all other isolates. FL07-03A was not significantly different from TX06-03A, ID06-12A, ID06-15, MD13-6B, ID06-19, NY06-09D, or NE06-04A, but was significantly different than all other isolates. Significant differences among isolates continued in a descending order and can be determined in Table 1.

Eighty of the 82 *Streptomyces* isolates inhibited *H. solani* growth, only WI06-36G and ID06-18 did not inhibit *H solani* growth (Figure 3G). Several isolates (ON07-05A, NE06-04A, NY06-04A, ID06-12A, ID05-47C, MI06-06C, ME02-AFT2, ID05-37A, ME02-7008A) had a mean greater than 60% *H. solani* inhibition (Figure 3G). Of these nine isolates, five of them (ON07-05A, NE06-04A, ID06-12A, ID05-47C, and ID05-37A) had medians and means above 65% inhibition. However even the top performing strains, listed above, were not significantly different from the top 55 isolates (Table 2).

Quick Rot Bioassay

The control without the gum arabic coating displayed minimal symptoms of *P. ultimum* infection, mean of 1% total tuber rot. The controls had no statistical differences from any *Streptomyces* isolate except TX06-03A. TX06-03A actually had more *P. ultimum* than the controls or any other isolate (Table 3).

Greenhouse Bioassay

None of the progeny tubers had any *H. solani* present, either at the post-harvest or post-storage stages. Nearly 40% of the progeny tubers of seed tubers treated with TX06-03A exhibited quality issues. While the progeny of the control tubers had quality issues in 19% of the

tubers. We found through the greenhouse bioassay that quality issues of potatoes, which were treated with the TX06-03A isolate of *Streptomyces*, increased compared to potatoes without *Streptomyces* ($P = 2.688e^{-19}$).

Linear Models

The linear models indicated which models were the best among the chitinase variables CB, CT, GA productions, and *H. solani* or *P. ultimum* inhibition. The model with the best prediction of *P. ultimum* control was only *H. solani* inhibition with a weight of .332 or a 33.2% chance of being the best model. The best eight models in predicting *P. ultimum* all include *H. solani* inhibition (Table 5). The model that most likely is the best model in predicting *H. solani* includes only the *P. ultimum* inhibition with a weight of .317 or 31.7% chance of being the best model. The best eight models in predicting *H. solani* all include *P. ultimum* inhibition (Table 4).

DISCUSSION

The results of our competition assays in Petri dishes supports the use of some *Streptomyces* isolates as effective biological-control agents against the fungal pathogens *H. solani* and *P. ultimum*. However, differences among the isolates complicates their potential use as biological controls. Levels of control differ drastically between the inhibition of *H. solani* compared to *P. ultimum*. Data in the *P. ultimum* inhibition assay varied widely (Figure 2E). Of the 82 *Streptomyces* isolates tested, approximately 25% inhibited *P. ultimum*. Only one isolate, ID05-47C, consistently inhibited *P. ultimum* with no outliers, but outliers are prominent throughout the remainder of the isolates. In contrast, 95% of *Streptomyces* isolates inhibited *H.* solani (Figure 3F). The variance is smaller because *H. solani* bioassays were quite consistent, the only isolate

with more than two outliers was MI02-7B (Figure 3G). Percent inhibition was consistently higher in *H. solani* and was found in nearly all *Streptomyces* isolates (Figures 2D, 2E, 3F, 3G). The two most inhibitive isolates of *P. ultimum* consistently limited growth, but almost every *Streptomyces* isolate inhibited *H. solani* consistently (Figure 3G).

Differences in results between *H. solani* and *P. ultimum* inhibition are likely due to unique morphological characteristics between the fungi. In terms of taxonomic classification, these organisms differ at the kingdom level. *Pythium ultimum* is found within the Chromista (or Stramenopile) kingdom, while *H. solani* is part of the Fungi kingdom.

Biologically, these two genera have stark differences. For example, *P. ultimum* has motile spores, which include tripartite mastigonemes (Andersen, 2004), whereas *H. solani* spores lack flagella (Roncero, 2002). Cell wall composition also differs between the two genera (Cherif et al., 1993), which likely has some bearing on the degree of inhibition by *Streptomyces* (Mizuhara et al., 2011). Cell walls of taxa the Fungi kingdom, consist primarily of chitin (Roncero, 2002), and that of the kingdom Chromista primarily contain cellulose (Ruiz-Herrera and Ortiz-Castellanos, 2010). However, there is additional information to consider regarding these differences in cell structure.

The prevailing hypothesis is that Chromista cell walls evolved from chitin-containing cell walls in order to utilize beta-1,3-glucan and beta-1,6-glucan enzymes, which led to cell-wall cellulose synthesis (Ruiz-Herrera and Ortiz-Castellanos, 2010). This evolution has created taxa with varying degrees of combinations of chitin and cellulose in their walls (Cherif et al., 1993). However, considerable ambiguity exists related to determining which cell wall components inhibit *P. ultimum* (Cherif et al., 1993; Benhamou and Chet, 1997; Dunne et al., 1997). Some researchers suggest that beta-1,3-glucanase acts as a primary factor in *P. ultimum* inhibition

(Benhamou and Chet, 1997), while others believe beta-1,4-glucanase (Kitamura et al., 2002), or the culmination of various enzymes (Dunne et al., 1997), play a pivotal role in controlling *P. ultimum*. What emerges from the published literature (Yan et al., 2008) is that chitinase plays a minor role in inhibiting *P. ultimum*. However, chitinase appears to effective in inhibiting *H. solani*—or other diverse taxa in the Fungi kingdom (Gupta et al., 1995; Liu et al., 2019). Our results are similar to these findings. Our most inhibitory isolates of *P. ultimum*, ID05-47C, DE06-01C, and TX06-03A, did not produce the most chitinase relative to our other *Streptomyces* isolates (Christopher Clarke, data not shown), but were the most inhibitory of our isolates in the plate bioassay. Clearly, some other factor such as: antibiotic production or antagonistic competition are primarily responsible for *P. ultimum* inhibition (Tables 4, 5). However, our linear model has limited scope. Other variables may be able to produce much better models, and the consistency of the plate bioassays were poor at showing significant differences which may be forcing our linear model to extrapolate insignificant differences.

We expected chitinase to be a driving factor inhibiting *H. solani* growth (Kunz et al., 1992; Vierheilig et al., 2001; Reyes-Ramirez et al., 2004). Our data suggests that chitinase production is not likely the primary driver for inhibition (Tables 4, 5). Chitinase may be an underlying reason that 95% of the *Streptomyces* isolates inhibited the growth of *H. solani* (Figure 3F), but the highest chitinase-producing *Streptomyces* isolates are average at inhibiting *H. solani* (ME14-692C, NB05-1C, ME16-692B) (Christopher Clarke, data not shown). However, other low-producing chitinase isolates (ID05-47C, ME14-692B, ID05-17A) were ranked first, second, and third respectively, in inhibition Petri plate bioassays.

There are a few isolates that exhibited unique properties. For example, ID05-47C outperformed the other isolates in inhibiting *P. ultimum* (Figures 2D, 2E). In addition, this

particular isolate ranked high among those that inhibited *H. solani.* (Figures 3F, 3G). This taxon should be studied more regarding its secondary-metabolite production. Its chitinase output is below average, but it does produce both chitobiosidase (CB) and triacetylchitotriosidase (CT) chitinases (Christopher Clarke, data not shown).

Potential exists that producing both of those chitinases may be important to inhibiting growth of *H. solani*. Nonetheless, other isolates, which did not produce much CB or CT chitinase (ON07-05A, ID05-17A), still inhibited *H. solani* growth. We do not know if secondary metabolite production was involved (Wanner, 2007; Sellem et al., 2017b) or if the *Streptomyces* isolates are able to outcompete the *H. solani* for resource acquisition. One possibility is that they may be a combination of both.

Isolate TX06-03A also complicates broad implications of our research regarding *Streptomyces*. The *Streptomyces* range of inhibition of *P. ultimum* had a lot of variation, but there is a distinct pattern among those that cause inhibition. Two isolates, ID05-47C and DE06-01C, consistently inhibited *P. ultimum* growth, while the other evaluated isolates only occasionally inhibited. The median value of *P. ultimum* growth inhibition of isolate TX06-03A was 90%, but 25% of the samples had no inhibition (Figure 2E). Combined with the quick-rot results where disease incidence was higher, these results suggest there may be some environmental factor driving inhibition (Figure 4H). Another possibility is that TX06-03A encourages other microbes to infect tubers. The other reason this may be the case is the quick rot study. In our experiment, the only isolate that showed an increase of total disease incidence from the control with gum arabic was TX06-03A (Figure 4H). Whatever allowed the TX06-03A isolate to be inhibitive in the Petri dish clearly was overridden or never activated during the quick-rot study (Figures 2D,

4H). An increase in quality issues in the greenhouse study also calls into question the effectiveness of TX06-03A in inhibiting *H. solani* growth.

Our quick rot study showed some isolates reduced disease levels of *Pythium* leak (Figure 4H). Three *Streptomyces* isolates (ID06-12A, ID06-15, NE06-04A) displayed lower levels of *P. ultimum* than the controls with the gum arabic coating (Figure 4H). These isolates indicate potential in controlling *P. ultimum* in field or storage applications.

Since some *Streptomyces* isolates could be effective in controlling *H. solani* and *P. ultimum* (Figures 2D, 3F) there is further research that should be performed prior to commercial application. The effectiveness of these isolates in commercial fields should be determined. When in field soil, they will be under tremendous competitive pressures. Our experiments did not look at using *Streptomyces* in conjunction with more microorganisms than the disease agents. Further research is needed to determine how long the *Streptomyces* isolates of interest last in soil, how treatments would be applied to seed potatoes, determine which mechanisms inhibit the pathogens, attempt tests using combinations of *Streptomyces*, and scale up the production of *Streptomyces* cultivation.

Our study utilized 82 different isolates, which allowed for a high degree of comparative evaluations, whereas most studies with *Streptomyces* include less than five isolates (Elleuch et al., 2010; Chen, X. et al., 2016; Chen, Xiaoyulong et al., 2017; Jeon et al., 2019). Continuing research with large numbers of isolates of *Streptomyces* will enable impactful discoveries, which could lead to determining mechanisms of inhibition employed by *Streptomyces*. Sellem et al. (2017a) hypothesized that several secondary metabolites inhibit Fungi and Chromista taxa growth (Sellem et al., 2017a). Determining what these substances are could lead to other methods of control for potato pathogens.

Analyzing the genetic differences between inhibitory and non-inhibitory isolates of *Streptomyces* could also determine which genes synthesize secondary metabolites. If we knew the genes that made the secondary metabolites, they could potentially be put in the genome of potato plants to allow them to protect themselves. There is a need to expand research of secondary metabolites and field trials to continue this research. This knowledge and technology could potentially be used across multiple crops as well. Understanding *Streptomyces,* their mechanisms for inhibition, and the genes that encode for it could lead to a more sustainable agricultural system to help feed a growing population through biological systems.

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FIGURES

Figure 1A. Control Plate of *Pythium. ultimum*, has no inhibition and is taken over by *P. ultimum*.

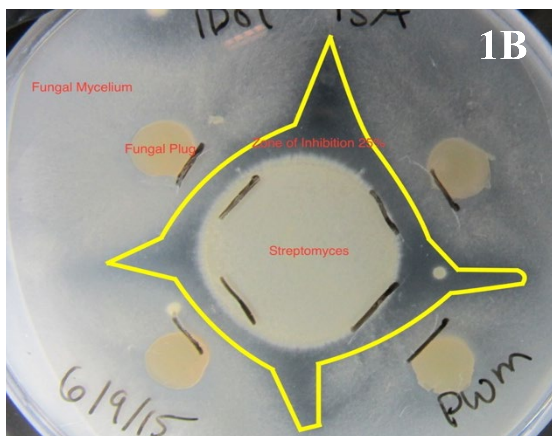

Figure 1B. Mild inhibition, ~25% of Pythium ultimum.

Figure 1C. Complete, 100% Inhibition of *Pythium ultimum*

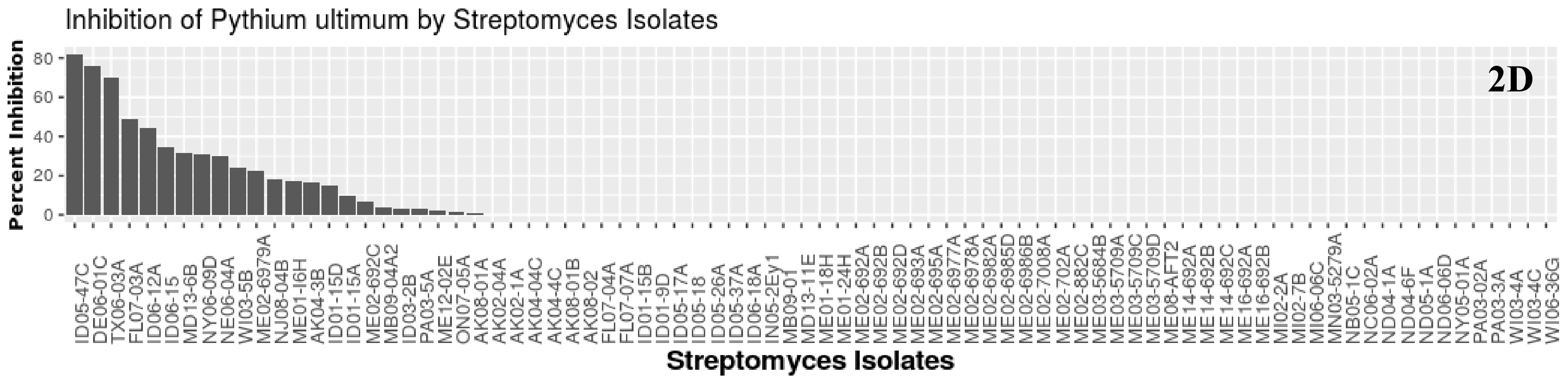

Figure 2D. Eighty-two Streptomyces isolates were collected from potato growing regions in North America and inhibition assays were performed in Petri dishes to determine which isolates inhibited the growth of *Pythium ultimum*. Twenty-seven isolates inhibited *Pythium ultimum*

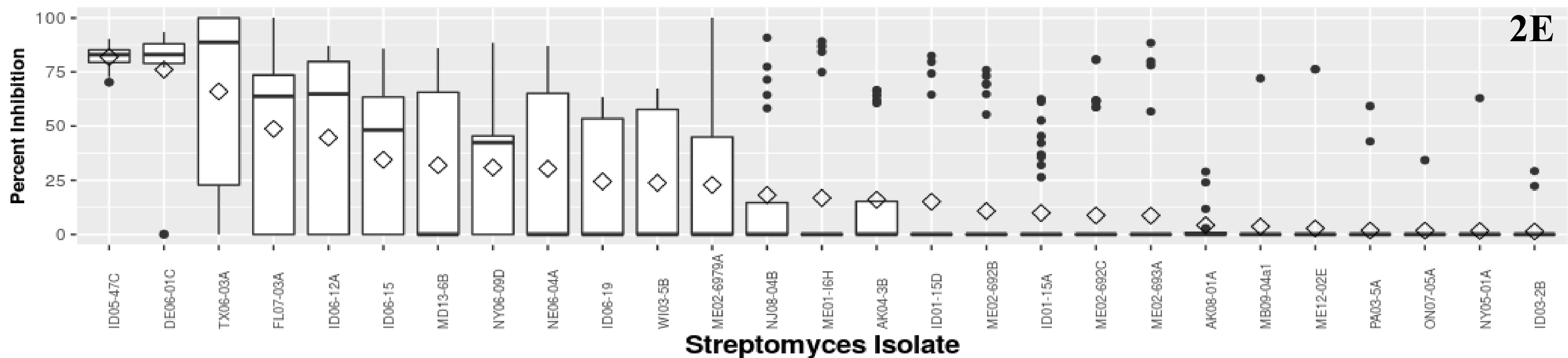

Figure 2E. Twenty-seven *Streptomyces* isolates that inhibited *Pythium ultimum* and their variance between each inhibitory *Streptomyces* isolate of *Pythium ultimum*, listed from most inhibitory to the least. Note the diamond is the mean. The horizontal line in the box is the median. The four categories (bottom line, bottom box, top box, and upper line) represent quartiles. Black dots represent outliers.

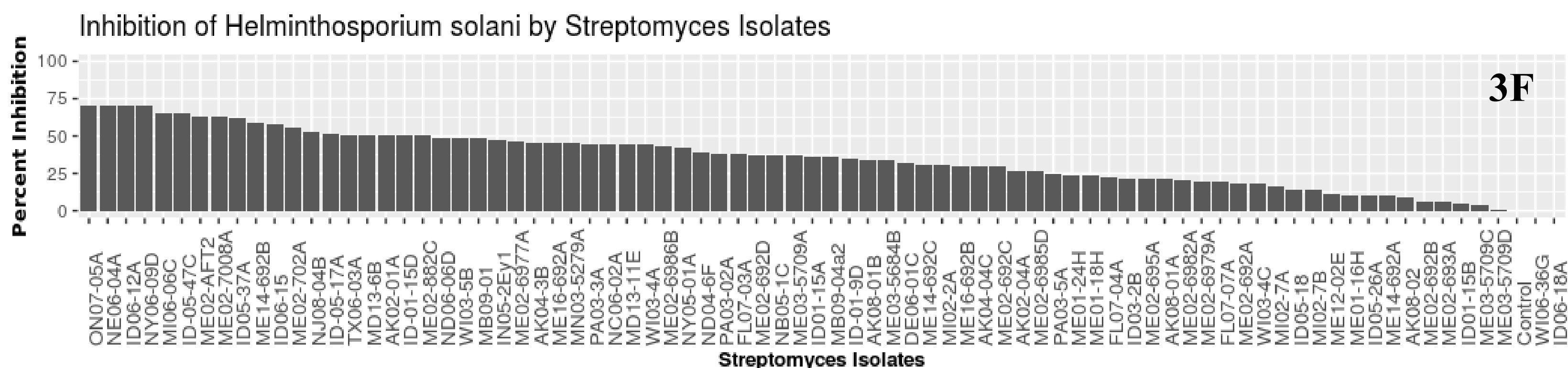

Figure 3F. Eighty-two *Streptomyces* isolates were collected from potato growing regions in North America and inhibition assays were performed in Petri dishes to determine which isolates inhibited the growth of *H. solani*. Fifty-three isolates inhibited *Helminthosporium solani* by 40%.

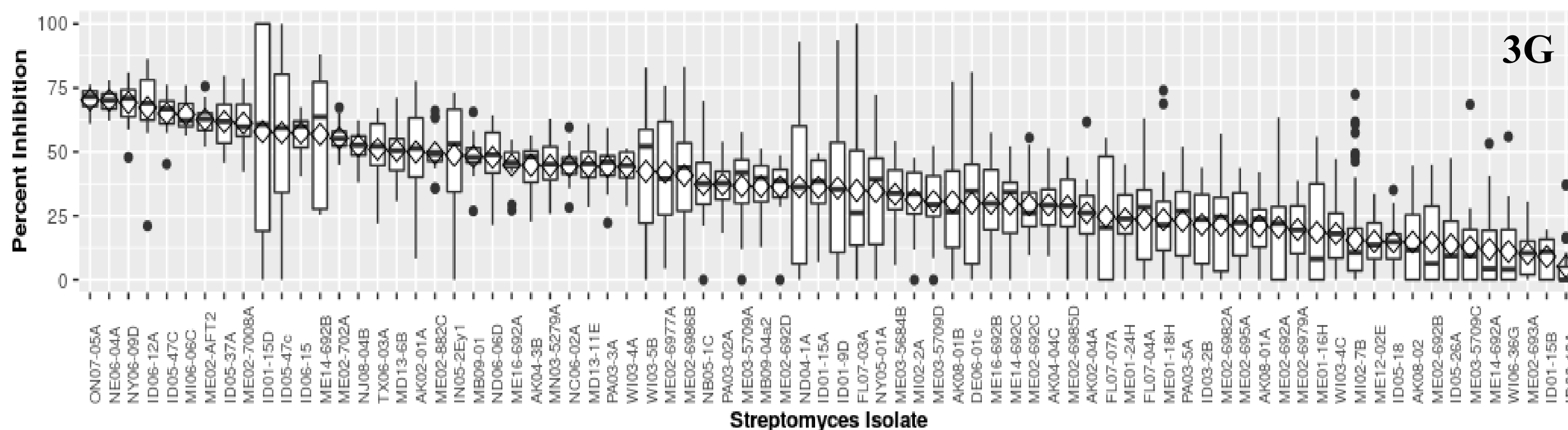

Figure 3G. Variance between each inhibitory *Streptomyces* isolate of *H. solani*, listed from most inhibitory to the least. Note the diamond is the mean. The horizontal line in the box is the median. The four categories (bottom line, bottom box, top box, and upper line) represent quartiles. Black dots represent outliers.

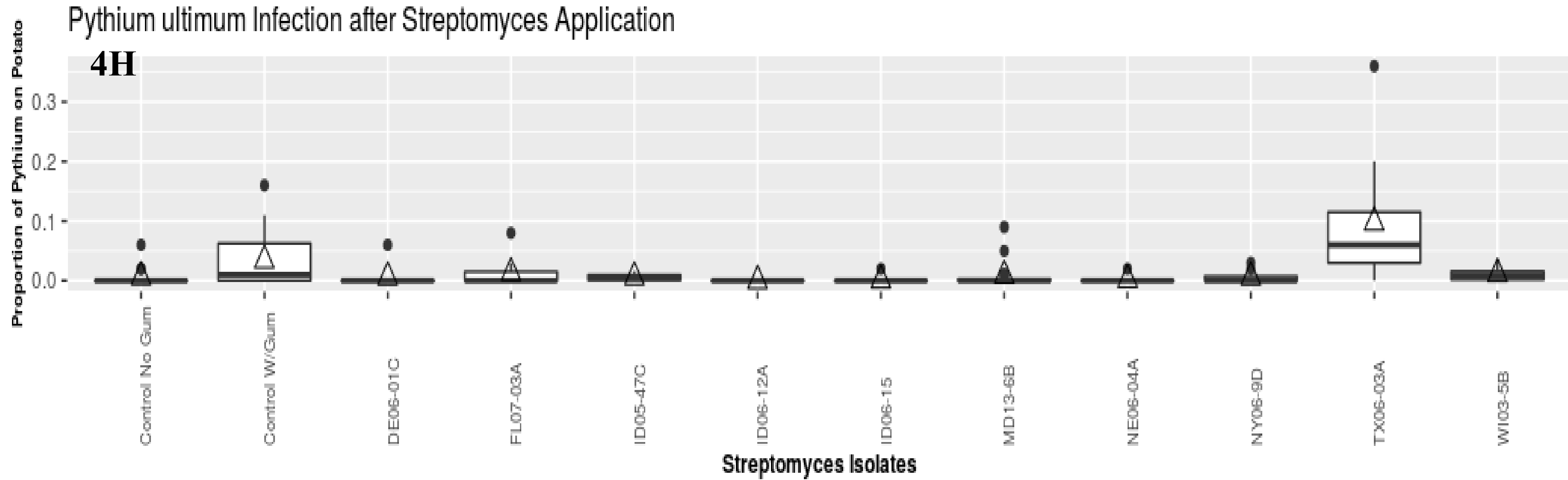

Figure 4H. Ten isolates of *Streptomyces* and the variance of how much *Pythium ultimum* was found on potato tubers when inoculated. Note the diamond is the mean. The horizontal line in the box is the median. The four categories (bottom line, bottom box, top box, and upper line) represent quartiles. Black dots represent outliers.

TABLES

Table 1. Significantly different isolates inhibiting of *P. ultimum* plate bioassay conservative Tukey-Kramer grouping for least square means P<0.05. All isolates were analyzed. Differing letters denote a different significance level.

Strain	Estimate			
TX06-03A	0.7454	A		
ID05-47C	0.6526	A		
DE06-01C	0.372	B	A	
FL07-03A	-0.676	B	C	
ID06-12A	-0.9369	B	C	D
NY06-09D	-1.118	B	C	D
ID06-15	-1.15	B	C	D
MD13-6B	-1.2792	B	C	D
NE06-04A	-1.3398	B	C	D
ID06-19	-1.3993	B	C	D
WI03-5B	-1.4609	C	D	
ME02-6979A	-1.4763	C	D	
NJ08-04B	-1.6688	C	D	
AK04-3B	-1.693	C	D	
ME01-I6H	-1.725	C	D	
ID01-15D	-1.7452	C	D	
ID01-15A	-1.7641	C	D	
AK08-01A	-1.8052	C	D	
ME02-692B	-1.8186	D		
ME02-693A	-1.8687	D		
MB09-04a1	-1.9575	D		

ON07-05A	-1.9695	D		
ID03-2B	-1.9714	D		
ME12-02E	-1.974	D		
PA03-5A	-1.9813	D		
NY05-01A	-1.9898	D		
Control	-2.0183	D		

Table 2. Significantly different isolates inhibiting of *Helminthosporium solani* plate bioassay. conservative Tukey-Kramer grouping for least square means P<0.05. All isolates were analyzed. Differing letters denote a different significance level.

Strain	Estimate								
ON07-05A	70.3088	A							
NE06-04A	70.1219	A							
NY06-09D	69.2213	A							
ID06-12A	67.2486	A							
ID05-47C	65.0148	B	A						
MI06-06C	64.6698	B	A	C					
ME02-AFT2	62.5505	B	A	C					
ID05-37A	61.803	B	A	C					
ME02-7008A	61.2398	B	A	C					
ID06-15	57.3637	B	D	A	C				
ME14-692B	56.8286	E	B	D	A	C			
ME02-702A	55.4107	E	B	D	A	C			
ID05-47C	53.8843	E	B	D	A	C			
NJ08-04B	52.3501	E	B	D	A	C			
TX06-03A	50.9834	E	B	D	A	C			
MD13-6B	50.4567	E	B	D	A	C			
AK02-01A	49.9096	E	B	D	A	C			
ME02-882C	49.5656	E	B	D	A	C			
ID01-15D	48.8067	E	B	D	A	C			
MB09-01	48.15	E	B	D	A	C			
ND06-06D	47.8619	E	B	D	A	C			
IN05-2Ey1	46.6516	E	B	D	A	C			
ME16-692A	44.855	E	B	D	A	C	F		
AK04-3B	44.7623	E	B	D	A	C	F		
MN03-5279A	44.5839	E	B	D	A	C	F		

NC06-02A	44.5387	E	B	D	A	C	F		
MD13-11E	44.3278	E	B	D	A	C	F		
PA03-3A	44.2767	E	B	D	A	C	F		
WI03-4A	44.1076	E	B	D	A	C	F		
ME02-6977A	42.1442	E	B	D	A	C	F		
WI03-5B	38.9126	E	B	D	A	G	C	F	
ME02-6986B	37.9703	E	B	D	A	G	C	F	
PA03-02A	37.2788	E	B	D	A	G	C	F	
MB09-04a2	36.512	E	B	D	A	G	C	F	
ME03-5709A	36.2212	E	B	D	A	G	C	F	
ID01-15A	36.1437	E	B	D	A	G	C	F	
ME02-692D	34.5457	E	B	D	A	G	C	F	
ME03-5684B	33.4158	E	B	D	A	G	C	F	
NY05-01A	32.3608	E	B	D	H	A	G	C	F
ND04-1A	31.5489	E	B	D	H	A	G	C	F
FL07-03A	31.3486	E	B	D	H	A	G	C	F
ME03-5709D	30.5804	E	B	D	H	A	G	C	F
MI02-2A	30.3248	E	B	D	H	A	G	C	F
ME16-692B	29.4662	E	B	D	H	A	G	C	F
ME02-692C	29.2803	E	B	D	H	A	G	C	F
AK04-04C	29.2422	E	B	D	H	A	G	C	F
DE06-01C	28.6973	E	B	D	H	A	G	C	F
ME14-692C	28.5888	E	B	D	H	A	G	C	F
AK08-01B	27.7408	E	B	D	H	A	G	C	F
AK02-04A	25.5508	E	B	D	H	A	G	C	F
ID01-9D	24.0076	E	B	D	H	A	G	C	F
ME02-6985D	23.0986	E	B	D	H	A	G	C	F
ME01-24H	23.0126	E	B	D	H	A	G	C	F

PA03-5A	22.6758	E	B	D	H	A	G	C	F
AK08-01A	21.1075	E	B	D	H	G	C	F	
ID03-2B	20.9847	E	B	D	H	G	C	F	
ME02-695A	20.9642	E	B	D	H	G	C	F	
ME02-6982A	20.4672	E	B	D	H	G	C	F	
ME02-6979A	19.4861	E	D	H	G	C	F		
ME01-18H	19.2732	E	D	H	G	C	F		
FL07-07A	18.5389	E	D	H	G	C	F		
WI03-4C	17.6403	E	D	H	G	C	F		
FL07-04A	15.4495	E	D	H	G	F			
ME02-692A	15.0012	E	D	H	G	F			
ID05-18	14.1836	E	D	H	G	F			
MI02-7B	10.6079	E	H	G	F				
ID05-26A	7.8614	E	H	G	F				
AK08-02	6.5243	E	H	G	F				
ME03-5709C	0.9708	H	G	F					
ME02-692B	0.3675	H	G	F					
Control	0.003195	H							

Table 3. Significance of *Pythium ultimum* quick rot bioassay GLM with P<0.05.

Strain	Strain	Estimate	Standard Error	DF	T value	Pr>t	Adj P
Control No Gum	Control W/Gum	-0.5743	0.2939	123	-1.95	0.053	0.6324
Control No Gum	DE06-01C	0.05748	0.3563	123	0.16	0.8721	1
Control No Gum	FL07-03A	-0.1549	0.3482	123	-0.44	0.6572	1
Control No Gum	ID06-12A	0.1834	0.3607	123	0.51	0.612	1
Control No Gum	ID06-15	0.1459	0.2947	123	0.5	0.6213	1
Control No Gum	MD13-6B	-0.03975	0.2938	123	-0.14	0.8926	1
Control No Gum	NE06-04A	0.1036	0.358	123	0.29	0.7728	1
Control No Gum	NY06-9D	-0.06026	0.3519	123	-0.17	0.8643	1
Control No Gum	TX06-03A	-1.6081	0.2873	123	-5.6	<.0001	<.0001
Control W/Gum	DE06-01C	0.6318	0.342	123	1.85	0.0671	0.7038
Control W/Gum	FL07-03A	0.4195	0.3328	123	1.26	0.21	0.9604
Control W/Gum	ID06-12A	0.7578	0.347	123	2.18	0.0309	0.4733
Control W/Gum	ID06-15	0.7203	0.2775	123	2.6	0.0106	0.2311
Control W/Gum	MD13-6B	0.5346	0.2759	123	1.94	0.055	0.6437
Control W/Gum	NE06-04A	0.6779	0.3439	123	1.97	0.0509	0.6205
Control W/Gum	NY06-9D	0.5141	0.337	123	1.53	0.1298	0.8795
Control W/Gum	TX06-03A	-1.0338	0.2641	123	-3.91	0.0001	0.0056
DE06-01C	FL07-03A	-0.2123	0.3895	123	-0.55	0.5866	0.9999
DE06-01C	ID06-12A	0.126	0.4007	123	0.31	0.7538	1
DE06-01C	ID06-15	0.08846	0.3424	123	0.26	0.7966	1
DE06-01C	MD13-6B	-0.09723	0.3417	123	-0.28	0.7765	1
DE06-01C	NE06-04A	0.04609	0.3982	123	0.12	0.908	1
DE06-01C	NY06-9D	-0.1177	0.3928	123	-0.3	0.7649	1
DE06-01C	TX06-03A	-1.6656	0.3365	123	-4.95	<.0001	0.0001
FL07-03A	ID06-12A	0.3383	0.3936	123	0.86	0.3917	0.9973

FL07-03A	ID06-15	0.3008	0.3341	123	0.9	0.3697	0.9962
FL07-03A	MD13-6B	0.1151	0.3332	123	0.35	0.7303	1
FL07-03A	NE06-04A	0.2584	0.391	123	0.66	0.5099	0.9997
FL07-03A	NY06-9D	0.0946	0.3854	123	0.25	0.8065	1
FL07-03A	TX06-03A	-1.4533	0.3263	123	-4.45	<.0001	0.0008
ID06-12A	ID06-15	-0.03749	0.3469	123	-0.11	0.9141	1
ID06-12A	MD13-6B	-0.2232	0.3463	123	-0.64	0.5205	0.9997
ID06-12A	NE06-04A	-0.07986	0.4021	123	-0.2	0.8429	1
ID06-12A	NY06-9D	-0.2437	0.3968	123	-0.61	0.5403	0.9998
ID06-12A	TX06-03A	-1.7916	0.342	123	-5.24	<.0001	<.0001
ID06-15	MD13-6B	-0.1857	0.2769	123	-0.67	0.5037	0.9996
ID06-15	NE06-04A	-0.04237	0.3441	123	-0.12	0.9022	1
ID06-15	NY06-9D	-0.2062	0.3379	123	-0.61	0.5428	0.9998
ID06-15	TX06-03A	-1.7541	0.2711	123	-6.47	<.0001	<.0001
MD13-6B	NE06-04A	0.1433	0.3435	123	0.42	0.6772	1
MD13-6B	NY06-9D	-0.02052	0.3371	123	-0.06	0.9516	1
MD13-6B	TX06-03A	-1.5684	0.2686	123	-5.84	<.0001	<.0001
NE06-04A	NY06-9D	-0.1638	0.3943	123	-0.42	0.6785	1
NE06-04A	TX06-03A	-1.7117	0.3386	123	-5.06	<.0001	<.0001
NY06-9D	TX06-03A	-1.5479	0.331	123	-4.68	<.0001	0.0003

Table 4. Linear model selection table predicting *H. solani* inhibition; variables used to predict *H. solani* inhibition include *P. ultimum* inhibition, GA chitinase, CT chitinase, and CB chitinase.

Model	(Intrc)	Pythm	GA	CT	CB	family	df	logLik	AICc	delta	weight
m1	33.28	0.344				gaussian	3	-331.995	670.3	0	0.317
m7	35.07	0.329			-0.988	gaussian	4	-331.506	671.6	1.25	0.169
m6	34.55	0.328		-0.736		gaussian	4	-331.538	671.6	1.31	0.164
m5	33.14	0.346	0.116			gaussian	4	-331.975	672.5	2.19	0.106
m12	35.02	0.331	0.361		-1.206	gaussian	5	-331.335	673.5	3.2	0.064
m13	35.14	0.3257		-0.414	-0.632	gaussian	5	-331.424	673.7	3.37	0.059
m11	34.38	0.33	0.169	-0.757		gaussian	5	-331.495	673.8	3.52	0.055
m15	35.07	0.328	0.324	-0.309	-0.919	gaussian	6	-331.291	675.8	5.46	0.021
m3	37.56			-1.043		gaussian	3	-335.223	676.8	6.46	0.013
m4	38.22				-1.348	gaussian	3	-335.236	676.8	6.48	0.012
m2	35.93		0.008			gaussian	3	-336.07	678.5	8.15	0.005
m10	38.27			-0.635	-0.796	gaussian	4	-335.059	678.7	8.36	0.005
m9	38.18		0.328		-1.547	gaussian	4	-335.107	678.8	8.45	0.005
m8	37.47		0.089	-1.055		gaussian	4	-335.212	679	8.66	0.004
m14	38.23		0.263	-0.551	-1.03	gaussian	5	-334.979	680.8	10.48	0.002

Table 5. Linear model selection table predicting *Pythium ultimum* inhibition; variables used to predict *P. ultimum* inhibition include *Helminthosporium solani* inhibition, GA chitinase, CT chitinase, CB chitinase.

Model	(Int)	H_sln	CB	CT	GA	family	df	logLik	AICc	delta	weight
m16	-2.78	0.291				gaussian	3	-325.581	657.5	0	0.332
m21	-1.294	0.278		-0.644		gaussian	4	-325.167	658.9	1.4	0.165
m22	-1.164	0.280	-0.713			gaussian	4	-325.282	659.1	1.63	0.147
m20	-2.42	0.291			-0.314	gaussian	4	-325.407	659.4	1.88	0.13
m26	-1.072	0.279		-0.608	-0.268	gaussian	5	-325.041	660.9	3.44	0.06
m28	-0.9837	0.276	-0.283	-0.501		gaussian	5	-325.141	661.1	3.64	0.054
m27	-1.215	0.282	-0.594		-0.19	gaussian	5	-325.226	661.3	3.81	0.05
m30	-1.022	0.278	-0.051	-0.583	-0.259	gaussian	6	-325.04	663.3	5.79	0.018
m18	9.152			-0.934		gaussian	3	-328.853	664	6.54	0.013
m19	9.546		-1.091			gaussian	3	-329.012	664.4	6.86	0.011
m17	8.059				-0.312	gaussian	3	-329.502	665.3	7.84	0.007
m23	9.383			-0.902	-0.243	gaussian	4	-328.759	666.1	8.58	0.005
m25	9.604		-0.503	-0.676		gaussian	4	-328.776	666.1	8.62	0.004
m24	9.559		-1.031		-0.099	gaussian	4	-328.998	666.6	9.06	0.004
m29	9.632		-0.338	-0.736	-0.186	gaussian	5	-328.729	668.3	10.81	0.001