MANAGEMENT OF *SCLEROTINIA SCLEROTIORUM* IN SOYBEAN USING THE BIOFUNGICIDES *BACILLUS AMYLOLIQUEFACIENS* AND *CONIOTHYRIUM MINITANS*

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Dedicated to my mother for her unwavering support of my passions and dreams. To my father for reminding me to live each day to fullest with joy and passion because life is too short. To my friends for their encouragement through times of hardship and triumph. And to my mentors who through their guidance helped me to achieve my academic and professional goals.

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ABSTRACT

Sclerotinia sclerotiorum is a soilborne pathogen of soybean that causes Sclerotinia stem rot, alternatively called white mold. Sclerotinia stem rot can cause significant yield losses under cool and wet environmental conditions. Two biofungicides, *Coniothyrium minitans* and *Bacillus amyloliquefaciens*, are currently available and labeled to limit or suppress *S. sclerotiorum* in soybean. These biofungicides can be applied in place of synthetic foliar fungicides to provide an alternative mode of action for the control of Sclerotinia stem rot. However, limited information is available regarding the efficacy of *C. minitans* and *B. amyloliquefaciens* as biocontrol agents of *S. sclerotiorum* in soybean and the sensitivity of the biofungicides biological activity on *S. sclerotiorum* to pesticides commonly used in soybean production systems. This research aims to provide management recommendations for *S. sclerotiorum* in soybean using *C. minitans* and *B. amyloliquefaciens* and to develop guidelines for how to incorporate the biofungicides into an established soybean pest management program. To assess the effectiveness of *C. minitans* and *B. amyloliquefaciens* as biocontrol agents of *S. sclerotiorum* dual culture, amended media, and soil plate assays were conducted along with experiments in the growth chamber and field. The presence of a distinct inhibition zone surrounding the *B. amyloliquefaciens* colony in the dual culture assay and the absence of mycelial growth on the media plates amended with *B. amyloliquefaciens* confirmed that the bacteria can control the mycelial growth of *S. sclerotiorum* through antibiosis. The absence of an inhibition zone surrounding the *C. minitans* isolate in the dual culture assay along with the degradation of sclerotia following treatment with *C. minitans* in the soil plate assay indicates an inability to limit the mycelial growth of *S. sclerotiorum* and confirms that the primary mode of action is mycoparasitism. In the growth chamber, *B. amyloliquefaciens* at 14.03 L/ha applied using the dip method significantly reduced Sclerotinia stem rot lesion length when compared to the non-treated control and resulted in the lowest lesion area under the disease progress curve (lAUDPC). When *B. amyloliquefaciens* and *C. minitans* were applied in the field, no differences were observed between treatments for soybean moisture, test weight, or yield. To evaluate the sensitivity of *B. amyloliquefaciens* and *C. minitans* biological activity on *S. sclerotiorum* to pesticides commonly used in soybean production systems a poison plate assay as well as soil plate, growth chamber, and field experiments were conducted. In the poison plate assay *C. minitans* was most sensitive to the preemergence herbicide flumioxazin and the synthetic

fungicides boscalid and fluazinam, while *B. amyloliquefaciens* was sensitive only to the synthetic fungicide fluazinam. In the soil plate assay the mycoparasitic activity of *C. minitans* on sclerotia of *S. sclerotiorum* was sensitive to flumioxazin, metribuzin, glyphosate, picoxystrobin, and boscalid. In the controlled environment experiments, none of the pesticides tested decreased the efficacy of *B. amyloliquefaciens*. There were no significant interactions between *C. minitans* and *B. amyloliquefaciens* with preemergence herbicides, postemergence herbicides, and synthetic fungicides for soybean moisture, test weight, and yield. This research demonstrates that *B. amyloliquefaciens* and *C. minitans* are effective biocontrol agents of *S. sclerotiorum* in soybean. However, antagonistic relationships exist between the biofungicides and certain preemergence, postemergence, and synthetic fungicides used in soybean production systems.

CHAPTER 1. INTRODUCTION

Soybean (*Glycine max*) (L.) Merrill is an important and valuable crop worldwide. It is estimated that soybean is grown on 6% of available arable land (Hartman et al. 2011). The top soybean producers are Brazil at 121.8 million metric tons, followed by the United States, Argentina, and China at 112.5, 48.7, and 19.6 million metric tons respectively (FAO 2020). Soybean is mainly used as a protein and oil source for human and livestock consumption (Guriqbal 2010). Soybean contains approximately 40% protein, 23% carbohydrates, 20% oil, 5% minerals, 4% fiber, and 8% water (Guriqbal 2010). In the United States 34.9 million hectares of soybean were planted in 2021, with 2.3 million hectares planted in Indiana (NASS 2021). The average yield for soybean in Indiana was 4,001.4 kg/ha in 2021 (NASS 2021). Disease, insects, weeds, and abiotic factors can limit soybean yield and cause economic losses.

Soybean is susceptible to hundreds of different species of pathogens (Hartman et al. 2015). Across the soybean producing region of North America, yield losses due to soybean diseases are estimated to be about 8.74% (Bradley et al. 2021). In 2019, the most recent year for which data is available, *Heterodera glycines* (soybean cyst nematode), *Sclerotinia sclerotiorum* (Sclerotinia stem rot), seedling diseases, *Fusarium virguliforme* (sudden death syndrome), and *Phytophthora sojae* (Phytophthora root and stem rot) caused the most significant yield losses across the soybean producing region of North America (Bradley et al. 2021). *Sclerotinia sclerotiorum* (Lib.) de Bary is a soilborne pathogen of soybean that causes Sclerotinia stem rot, alternatively called white mold. In 2021, 77.4 million kg were lost to white mold in Indiana, up from the 9.5 million kg lost in 2020 (Crop Protection Network 2020; Crop Protection Network 2021).

S. sclerotiorum belongs to the *Sclerotiniaceae* family in the order of Helotiales belonging to the Discomycetes in the Ascomycota phylum. The fungus overwinters in the form of sclerotia that germinate to produce apothecia under cool (15 to 20 °C) and wet (-0.03 to -0.07 MPa) environmental conditions in the spring (Hao et al. 2007; Willetts 1971). The apothecia produce ascospores which are transported to the soybean plant by wind (Adams and Ayers 1979). The ascospores infect the plant through a wound or natural opening. Mycelium grows from the infected plant tissue and produces sclerotia which drop to the soil completing the disease cycle (Adams and Ayers 1979). Early disease symptoms include water-soaked lesions on the main stem (Hartman et al. 2015). As the disease progresses, the lesions appear bleached and encircle the stem. The leaves

will remain on the stem but turn brown, and eventually the entire plant will prematurely senesce. Disease signs include fluffy white mycelium growing from the bleached lesions and sclerotia either on the surface of or within the stem (Hartman et al. 2015).

Control of *S. sclerotiorum* is challenging because the sclerotia can lay dormant in the soil during winter months and remain viable for up to five years (Adams and Ayers 1979; Duncan et al. 2006; Hao et al. 2007; Willetts 1971). Many options are available for the management of white mold in soybean. These options include cultural practices such as selection of a resistant variety, increasing row spacing, decreasing planting populations, and rotating to a non-host crop as well as the application of synthetic foliar fungicides at the beginning reproductive stages (Mueller et al. 2002; Peltier et al. 2012; Smith et al. 2020; Webster et al. in press; Willbur et al. 2019b, 2019a).

The exact degree of genetic diversity in *S. sclerotiorum* populations is still not fully understood. There is evidence to suggest that populations within a state or province are clonal, yet populations among different countries are distinct (Atallah et al. 2004; Attanayake et al. 2014; Cubeta et al. 1997; Dunn et al. 2017; Gambhir et al. 2021; Hemmati et al. 2009; Lehner et al. 2017; Sexton and Howlett 2004; Silva et al. 2021; Yu et al. 2020;). Because of this, it is unclear how quickly the pathogen will develop resistance to synthetic fungicides. Fungicide resistance to dicarboximide has been reported in China and resistance to benomyl has been discovered in Canada (Gossen et al. 2001; Zhou et al. 2014). It has also been demonstrated that repeated fungicide applications, especially low dose applications, can lead to the development of resistance in *S. sclerotiorum* populations (Lehner et al. 2015). It is essential to identify alternative disease management strategies before fungicide resistance becomes widespread in *S. sclerotiorum* populations. Biofungicides, a microbial or biochemical product used to control or limit the activity of a pathogen, can be applied in place of synthetic fungicides to provide additional modes of action. *Coniothyrium minitans* (Contans WG; Sipcam Agro. USA Inc., Durham, NC) and *Bacillus amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Colombia, NC) are two biofungicides labeled to control or suppress white mold in soybean. These biofungicides were selected as the products are readily available and farmers are familiar with the products.

The mycoparasitic activity of *C. minitans* on *S. sclerotiorum* was first discovered in California in 1947 by Campbell but did not gain popularity until the 1970s after an increased interest in using biocontrols (Campbell 1947; Whipps and Gerlagh 1992). In vitro, *C. minitans* can degrade sclerotia through the expression of cell wall degrading enzymes, specifically glucanase

and glycosidase (Muthumeenakshi et al. 2007; Whipps and Gerlagh 1992). Previous work has demonstrated the mycoparasitic activity of *C. minitans* on sclerotia of *Sclerotinia* spp. (Budge et al. 1995; Partridge et al. 2006b; Whipps and Budge 1990; Zeng et al. 2012). Furthermore, it has been shown that *C. minitans* can decrease the inoculum potential of *S. sclerotiorum* in lettuce, celery, cabbage, sunflower, and oilseed rape under field conditions (Budge and Whipps 1991; Chitrampalam et al. 2008, 2010; Eirian Jones et al. 2014; Matheron and Porchas 2019; McLaren et al. 1994; McQuilken et al. 1995; Rabeendran et al. 2006).

B. amyloliquefaciens was first isolated by Fukomoto in Japan in 1943 (Priest et al. 1987). *B. amyloliquefaciens* is known for its ability to prevent pathogen infection by controlling the mycelial growth of *S. sclerotiorum* through the expression of secondary metabolites (Chen et al. 2009). These enzymes include lipopeptides and polyketides which have antifungal, antibacterial, and nematocidal properties (Chen et al. 2009; Farzand et al. 2020; Hou et al. 2006). Previous work has demonstrated that *B. amyloliquefaciens* can provide protection from *S. sclerotiorum* infection in squash, tomato, eggplant, and canola under greenhouse conditions and in snap, dry, and common bean as well as canola under field conditions (Abdullah et al. 2008; Fernando et al. 2007; Pethybridge et al. 2019; Sabaté et al. 2018; Wu et al. 2014).

C. minitans and *B. amyloliquefaciens* are commercially available as the products Contans WG and Double Nickel LC respectively and are labeled to limit or suppress *S. sclerotiorum* in soybean however, to the best of our knowledge no research has been done to confirm the efficacy of these products in soybean. We hypothesize that both *C. minitans* and *B. amyloliquefaciens* are effective biocontrol agents of *S. sclerotiorum* in soybean. By confirming the ability of *C. minitans* and *B. amyloliquefaciens* to limit or suppress *S. sclerotiorum* in soybean a wider variety of options are available for managing the disease.

An integrated pest management strategy to maximize soybean yield includes the application of herbicides, fungicides, and insecticides at various times during the growing season. When including biofungicides into an established soybean integrated management strategy, it is essential to recognize that, because the biofungicides are living organisms, other applied pesticides could negatively impact the effectiveness of the products. Partridge et al. (2006a) found that the radial mycelial growth of *C. minitans* was significantly reduced by the fungicides azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, and tebuconazole as well as the preemergence herbicide flumioxazin. The radial mycelial growth of *C. minitans* was inhibited by the fungicides iprodione,

mancozeb, metalaxyl+thiram, thiram, tolclofos-methyl, and zineb as well as the insecticides malathion and pirimicarb in the experiments conducted by Budge and Whipps (2001). Li et al. (2002) found that the mycelial radial growth of *C. minitans* was greatly reduced by the fungicides benomyl and vinclozolin. No previous research has been done to explore how to appropriately incorporate *C. minitans* and *B. amyloliquefaciens* into season-long soybean pest management practices. We hypothesize that some of the pesticides will negatively impact the ability of *C. minitans* and *B. amyloliquefaciens* to control or limit *S. sclerotiorum* in soybean.

This study assessed the ability of *C. minitans* and *B. amyloliquefaciens* to control the mycelial growth or degrade the sclerotia of *S. sclerotiorum* in vitro. Furthermore, the efficacy of the biofungicides was tested under growth chamber and field conditions. The sensitivity of *C. minitans* and *B. amyloliquefaciens* to the preemergence herbicides flumioxazin, S-metolachlor, and metribuzin; the postemergence herbicides cloransulam-methyl, glyphosate, dicamba, glufosinate, and 2,4-D; and the synthetic fungicides picoxystrobin, boscalid, and fluazinam was evaluated by in vitro assays to establish the sensitivity level of the biofungicides to pesticides commonly used in soybean production systems. The interaction between *C. minitans* and *B. amyloliquefaciens* and the preemergence herbicides, postemergence herbicides, and synthetic fungicides was also investigated in soil plate assays as well as in the growth chamber and field. This research will help farmers make better informed management decisions by providing support for an alternative mode of action and recommendations for how to properly incorporate the biofungicides into an established soybean integrated management strategy.

1.1 References

Abdullah, M. T., Ali, N. Y., and Suleman, P. 2008. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*. Crop Protec. 27:1354–1359.

Adams, P. B. and Ayers, W. A. 1979. Ecology of *Sclerotinia* species. Phytopathology. 69:896–899.

Atallah, Z. K., Larget, B., Chen, X., and Johnson, D. A. 2004. High genetic diversity, phenotypic uniformity, and evidence of outcrossing in *Sclerotinia sclerotiorum* in the Columbia Basin of Washington State. Phytopathology. 94:737–742.

Attanayake, R. N., Tennekoon, V., Johnson, D. A., Porter, L. D., del Río-Mendoza, L., Jiang, D., and Chen, W. 2014. Inferring outcrossing in the homothallic fungus *Sclerotinia sclerotiorum* using linkage disequilibrium decay. Heredity. 113:353–363.

Bradley, C. A., Allen, T. W., Sisson, A. J., Bergstrom, G. C., Bissonnette, K. M., Bond, J., Byamukama E., Chilvers, M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault N. S., Esker, P. D., Faske, T. R., Fiorellino N. M., Geisler, L. J., Hartman, G. L., Hollier, C. A., Isakeit T., Jackson-Ziems, T. A., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koehler, A. M., Kratochvil, R. J., Kurle, J. E., Malvick, D. K., Markell, S. G., Mathew, F. M., Mehl, H. L., Mehl K. M., Mueller, D. S., Mueller, J. D., Nelson, B. D., Overstreet, C., Padgett, G. B., Price, P. P., Sikora, E. J., Small, I., Smith, D. L., Spurlock, T. N., Tande, C. A., Telenko, D. E. P., Tenuta, A. U., Thiessen, L. D., Warner, F., Wiebold, W. J., and Wise, K. A. 2021. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2015 to 2019. Plant Health Prog. 22:483–495.

Budge, S. P., McQuilken, M. P., Fenlon, J. S., and Whipps, J. M. 1995. Use of *Coniothyrium minitans* and *Gliocladium virens* for biological control of *Sclerotinia sclerotiorum* in glasshouse lettuce. Bio. Control. 5:513–522.

Budge, S. P. and Whipps, J. M. 1991. Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. Plant Pathol. 40:59–66.

Budge, S. P. and Whipps, J. M. 2001. Potential for integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. Phytopathology. 91:221–227.

Campbell, W. A. 1947. A new species of *Coniothyrium* parasitic on sclerotia. Mycologia. 39:190–195.

Chen, X. H., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Süssmuth, R., Piel, J., and Borriss, R. 2009. Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. J. Biotech. 140:27–37.

Chitrampalam, P., Figuli, P. J., Matheron, M. E., Subbarao, K. v., and Pryor, B. M. 2008. Biocontrol of lettuce drop caused by *Sclerotinia sclerotiorum* and *Sclerotinia minor* in desert agroecosystems. Plant Dis. 92:1625–1634.

Chitrampalam, P., Turini, T. A., Matheron, M. E., and Pryor, B. M. 2010. Effect of sclerotium density and irrigation on disease incidence and on efficacy of *Coniothyrium minitans* in suppressing lettuce drop caused by *Sclerotinia sclerotiorum*. Plant Dis. 94:1118–1124.

Crop Protection Network. 2020. Estimates of corn, soybean, and wheat yield losses due to diseases and insect pests: an online tool. Https://loss.cropprotectionnetwork.org/. Doi.org/10.31274/cpn-20191121-0.

Crop Protection Network. 2021. Estimates of corn, soybean, and wheat yield losses due to diseases and insect pests: an online tool. Https://loss.cropprotectionnetwork.org/. Doi.org/10.31274/cpn-20191121-0.

Cubeta, M. A., Cody, B. R., Kohli, Y., and Kohn, L. M. 1997. Clonality in *Sclerotinia sclerotiorum* on infected cabbage in eastern North Carolina. Phytopathology. 87:1000–1004.

Duncan, R. W., Dilantha Fernando, W. G., and Rashid, K. Y. 2006. Time and burial depth influencing the viability and bacterial colonization of sclerotia of *Sclerotinia sclerotiorum*. Soil Biol. & Biochem. 38:275–284.

Dunn, A. R., Kikkert, J. R., and Pethybridge, S. J. 2017. Genotypic characteristics in populations of *Sclerotinia sclerotiorum* from New York State, USA. Annals of App. Biol. 170:219–228.

Eirian Jones, E., Rabeendran, N., and Stewart, A. 2014. Biocontrol of *Sclerotinia sclerotiorum* infection of cabbage by *Coniothyrium minitans* and *Trichoderma* spp. Biocontrol Sci. and Tech. 24:1363–1382.

Farzand, A., Moosa, A., Zubair, M., Khan, A. R., Ayaz, M., Massawe, V. C., and Gao, X. 2020. Transcriptional profiling of diffusible lipopeptides and fungal virulence genes during *Bacillus amyloliquefaciens* EZ1509-mediated suppression of *Sclerotinia sclerotiorum*. Phytopathology. 110:317–326.

Fernando, W. G. D., Nakkeeran, S., Zhang, Y., and Savchuk, S. 2007. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. Crop Protec. 26:100–107.

Food and Agriculture Organization of the United Nations (FAO). 2020. Production of soybeans: Top 10 producers. Available at: https://www.fao.org/faostat/en/#data/QCL/visualize.

Gambhir, N., Kamvar, Z. N., Higgins, R., Amaradasa, B. S., and Everhart, S. E. 2021. Spontaneous and fungicide-induced genomic variation in *Sclerotinia sclerotiorum*. Phytopathology. 111:160–169.

Gossen, B. D., Rimmer, S. R., and Holley, J. D. 2001. First report of resistance to benomyl fungicide in *Sclerotinia sclerotiorum*. Plant Dis. 85:1206–1206.

Guriqbal, S. 2010. *The Soybean Botany, Production, and Uses*. Cambridge: CAB International.

Hao, J. J., Subbarao, K. V., and Duniway, J. M. 2007. Germination of *Sclerotinia minor* and *Sclerotinia sclerotiorum* sclerotia under various soil moisture and temperature combinations. Phytopathology. 93:443–45.

Hartman, G. L., Rupe, J. C., Sikora, E. J., Domier, L. L., Davis, J. A., and Steffey, K. L., eds. 2015. *Compendium of Soybean Diseases and Pests*. Fifth Edition. The American Phytopathological Society.

Hartman, G. L., West, E. D., and Herman, T. K. 2011. Soybean-worldwide production, use, and constraints caused by pathogens and pests. Food Security. 3:5–17.

Hemmati, R., Javan-Nikkhah, M., and Linde, C. C. 2009. Population genetic structure of *Sclerotinia sclerotiorum* on canola in Iran. European J. Plant Path. 125:617–628.

Hou, X., Boyetchko, S. M., Brkic, M., Olson, D., Ross, A., and Hegedus, D. 2006. Characterization of the anti-fungal activity of a *Bacillus* spp. associated with sclerotia from *Sclerotinia sclerotiorum*. Appl. Microbiol. and Biotech. 72:644–653.

Lehner, M. S., de Paula Jú nior, T. J., del Ponte, E. M., G Mizubuti, E. S., and Pethybridge, S. J. 2017. Independently founded populations of *Sclerotinia sclerotiorum* from a tropical and a temperate region have similar genetic structure. PLoS ONE. 12:1–14.

Lehner, M. S., Paula Júnior, T. J., Silva, R. A., Vieira, R. F., Carneiro, J. E. S., Schnabel, G., and Mizubuti, E. S. G. 2015. Fungicide Sensitivity of *Sclerotinia sclerotiorum*: A thorough assessment using discriminatory dose, EC50, high-resolution melting analysis, and description of new point mutation associated with thiophanate-methyl resistance. Plant Dis. 99:1537–1543.

Li, G. Q., Huang, H. C., and Acharya, S. N. 2002. Sensitivity of *Ulocladium atrum*, *Coniothyrium minitans*, and *Sclerotinia sclerotiorum* to benomyl and vinclozolin. Canadian J. Bot. 80:892–898.

Matheron, M. E. and Porchas, M. 2019. Optimizing fungicide inputs for management of lettuce drop caused by *Sclerotinia minor* and *Sclerotinia sclerotiorum*. Plant Health Prog. 20:238–243.

McLaren, D. L., Huang, H. C., Kozub, G. C., and Rimmer, S. R. 1994. Biological control of Sclerotinia wilt of sunflower with *Talaromyces flavus* and *Coniothyrium minitans*. Plant Dis. 78:231–235.

McQuilken, M. P., Mitchell, S. J., Budge, S. P., Whipps, J. M., Fenlon, J. S., and Archer, S. A. 1995. Effect of *Coniothyrium minitans* on sclerotial survival and apothecial production of *Sclerotinia selerotiorum* in field-grown oilseed rape. Plant Path. 4: 883-896.

Mueller, D. S., Ozkan, E., Bradley, C. A., and Pedersen, W. L. 2002. Efficacy of fungicides on *Sclerotinia sclerotiorum* and their potential for control of Sclerotinia stem rot on soybean. Plant Dis. 86:26-31.

Muthumeenakshi, S., Sreenivasaprasad, S., Rogers, C. W., Challen, M. P., and Whipps, J. M. 2007. Analysis of cDNA transcripts from *Coniothyrium minitans* reveals a diverse array of genes involved in key processes during sclerotial mycoparasitism. Fungal Gen. and Biol. 44:1262–1284.

National Agricultural Statistics Service (NASS). 2021. Soybean - Area harvested. Available at: https://quickstats.nass.usda.gov/#86FAC1E2-459A-3BF8-B6F4-13D383AD6851.

Partridge, D. E., Sutton, T. B., and Jordan, D. L. 2006a. Effect of environmental factors and pesticides on mycoparasitism of *Sclerotinia minor* by *Coniothyrium minitans*. Plant Dis. 90:1407–1412.

Partridge, D. E., Sutton, T. B., Jordan, D. L., Curtis, V. L., and Bailey, J. E. 2006b. Management of Sclerotinia blight of peanut with the biological control agent *Coniothyrium minitans*. Plant Dis. 90:957–963.

Peltier, A. J., Bradley, C. A., Chilvers, M. I., Malvick, D. K., Mueller, D. S., Wise, K. A., and Esker, P. D. 2012. Biology, yield loss and control of Sclerotinia stem rot of soybean. J. Integr. Pest Manag. 3:1–7.

Pethybridge, S. J., Gugino, B. K., and Kikkert, J. R. 2019. Efficacy of Double Nickel LC (*Bacillus amyloliquefaciens* D747 strain) for management of white mold in snap and dry bean. Plant Health Prog. 20:61–66.

Priest, F. G., Goodfellow, M., Shute, L. A., and Berkeley, R. C. W. 1987. *Bacillus amyloliquefaciens* sp. nov. nom. rev. Int. J. Sys. Bacteriol. 37:69–71.

Rabeendran, N., Jones, E. E., Moot, D. J., and Stewart, A. 2006. Biocontrol of Sclerotinia lettuce drop by *Coniothyrium minitans* and *Trichoderma hamatum*. Bio. Control. 39:352–362.

Sabaté, D. C., Brandan, C. P., Petroselli, G., Erra-Balsells, R., and Audisio, M. C. 2018. Biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary on common bean by native lipopeptide-producer *Bacillus* strains. Microbiol. Res. 211:21–30.

Sexton, A. C. and Howlett, B. J. 2004. Microsatellite markers reveal genetic differentiation among populations of *Sclerotinia sclerotiorum* from Australian canola fields. Current Gen. 46:357–365.

Silva, R. A., Ferro, C. G., Lehner, M. da S., Paula, T. J., and Mizubuti, E. S. G. 2021. The population of *Sclerotinia sclerotiorum* in Brazil is structured by mycelial compatibility groups. Plant Dis. 105:3376–3384.

Smith, D., Bradley, C., Chilvers, M., Esker, P., Malvick, D., Mueller, D., Peliter, A., Sisson, A., Wise, K., Tenuta, A., and Faske, T. 2020. Soybean disease management: White mold. Crop Protection Network. CPN 1005. doi.org/10.31274/cpn-20190620-030.

Webster, R. W., Roth, M. G., Mueller, B. D., Mueller, D. S., Willbur, J. F., Mourtzinis, S., Conley, S. P., and Smith, D. L. In press. Integration of row spacing, seeding rates, and fungicide applications for control of Sclerotinia stem rot in *Glycine max*. Plant Dis.

Whipps, J. M. and Budge, S. P. 1990. Screening for sclerotial mycoparasites of *Sclerotinia sclerotiorum*. Mycol. Res. 94:607–612.

Whipps, J. M. and Gerlagh, M. 1992. Biology of *Coniothyrium minitans* and its potential for use in disease biocontrol. Mycol. Res. 96:897–907.

Willbur, J. F., Mitchell, P. D., Fall, M. L., Byrne, A. M., Chapman, S. A., Floyd, C. M., Bradley, C. A., Ames, K. A., Chilvers, M. I., Kleczewski, N. M., Malvick, D., K., Mueller, B. D., Mueller, D. S., Kabbage, M., Conley, S. P., and Smith, D. L. 2019a. Meta-analytic and economic approaches for evaluation of pesticide impact on Sclerotinia stem rot control and soybean yield in the North Central United States. Phytopathology. 109:1157–1170.

Willbur, J., McCaghey, M., Kabbage, M., and Smith, D. L. 2019b. An overview of the *Sclerotinia sclerotiorum* pathosystem in soybean: Impact, fungal biology, and current management strategies. Trop. Plant Path. 44:3–11.

Willetts, H. J. 1971. The survival of fungal sclerotia under adverse environmental conditions. Biol. Rev. 46:387–407.

Wu, Y., Yuan, J., Raza, W., Shen, Q., and Huang, Q. 2014. Biocontrol traits and antagonistic Potential of *Bacillus amyloliquefaciens* strain NJZJSB3 against *Sclerotinia sclerotiorum*, a causal agent of canola stem rot. J. Microbiol. Biotech. 24:1327–1336.

Yu, Y., Cai, J., Ma, L., Huang, Z., Wang, Y., Fang, A.,Yang, Y., Qing, L., and Bi, C. 2020. Population structure and aggressiveness of *Sclerotinia sclerotiorum* from rapeseed (*Brassica napus*) in Chongqing City. Plant Dis. 104:1201–1206.

Zeng, W., Wang, D., Kirk, W., and Hao, J. 2012. Use of *Coniothyrium minitans* and other microorganisms for reducing *Sclerotinia sclerotiorum*. Biol. Control. 60:225–232.

Zhou, F., Zhang, X. L., Li, J. L., and Zhu, F. X. 2014. Dimethachlon resistance in *Sclerotinia sclerotiorum* in China. Plant Dis. 98:1221–1226.

CHAPTER 2. EFFICACY OF BIOCONTROL AGENTS *CONIOTHYRIUM MINITANS* AND *BACILLUS AMYLOLIQUEFACIENS* FOR CONTROLLING *SCLEROTINIA SCLEROTIORUM* IN SOYBEAN

2.1 Abstract

Sclerotinia sclerotiorum is a soilborne pathogen of soybean that causes Sclerotinia stem rot, alternatively called white mold. Sclerotinia stem rot can cause significant yield losses under cool and wet environmental conditions. Two biofungicides, *Coniothyrium minitans* and *Bacillus amyloliquefaciens,* are currently available and labeled to control or suppress *S. sclerotiorum* in soybean. These biofungicides can be applied in place of synthetic foliar fungicides to provide an alternative mode of action for the control of Sclerotinia stem rot. However, limited information is available regarding the efficacy of *C. minitans* and *B. amyloliquefaciens* as biocontrol agents of *S. sclerotiorum* in soybean. To assess the effectiveness of *C. minitans* and *B. amyloliquefaciens* as biocontrol agents of *S. sclerotiorum*, dual culture, amended media, and soil plate assays were conducted along with experiments in the growth chamber and field. The presence of a distinct inhibition zone surrounding the *B. amyloliquefaciens* colony in the dual culture assay and the absence of mycelial growth on the media plates amended with *B. amyloliquefaciens* confirms that the bacteria can control the mycelial growth of *S. sclerotiorum* through antibiosis. The absence of an inhibition zone surrounding the *C. minitans* isolate in the dual culture assay along with the degradation of sclerotia following treatment with *C. minitans* in the soil plate assay indicates an inability to limit the mycelial growth of *S. sclerotiorum* and confirms that the primary mode of action is mycoparasitism. In the growth chamber, *B. amyloliquefaciens* at 14.03 L/ha applied using the dip method significantly reduced Sclerotinia stem rot lesion length over the non-treated control and resulted in the lowest lesion area under the disease progress curve (lAUDPC). When *B. amyloliquefaciens* and *C. minitans* were applied in the field, no differences were observed between treatments for soybean moisture, test weight, or yield.

2.2 Introduction

Soybean (*Glycine max)* is susceptible to hundreds of different species of pathogens (Hartman et al. 2015). Across the soybean producing region of North America, yield losses due to soybean

diseases are estimated to be about 8.74% (Bradley et al. 2021). *Sclerotinia sclerotiorum* (Lib.) de Bary is a soilborne pathogen of soybean that causes Sclerotinia stem rot, alternatively called white mold. Sclerotinia stem rot can cause significant yield losses under cool and wet environmental conditions. In 2021, 77.4 million kg were lost to white mold in Indiana, up from the 9.5 million kg lost in 2020 (Crop Protection Network 2020; Crop Protection Network 2021). Control of *S. sclerotiorum* is challenging because specialized survival structures called sclerotia can lay dormant in the soil during winter months and remain viable for up to five years (Adams and Ayers 1979; Duncan et al. 2006; Hao et al. 2007; Willetts 1971). Many options are available for the management of Sclerotinia stem rot in soybean. These options include cultural practices such as selection of a resistant variety, increasing row spacing, decreasing planting populations, and rotating to a non-host crop as well as the application of synthetic foliar fungicides at the beginning reproductive stages (Mueller et al. 2015; Peltier et al. 2012; Willbur et al. 2019a, 2019b).

Synthetic foliar fungicides can be applied at the beginning bloom growth stage to control Sclerotinia stem rot in soybean (Willbur et al. 2019a). However, due to the variation in the genetic background of *S. sclerotiorum* there is a risk that the pathogen will develop resistance to synthetic fungicides (Gambhir et al. 2021). It has also been demonstrated that repeated fungicide applications, especially low dose applications, can lead to the development of resistance in *S. sclerotiorum* populations (Lehner et al. 2015). It is essential to identify alternative disease management strategies before fungicide resistance becomes widespread in *S. sclerotiorum* populations. Biofungicides, a microbial or biochemical product used to control or limit the activity of a pathogen, can be applied in place of synthetic fungicides to provide an additional mode of action (US EPA - Biopesticides n.d.). *Coniothyrium minitans* (Contans WG; Sipcam Agro. USA Inc., Durham, NC) and *Bacillus amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Colombia, NC) are two biofungicides currently available and labeled to limit or suppress Sclerotinia stem rot in soybean.

The mycoparasitic activity of *C. minitans* on *S. sclerotiorum* was first discovered in California in 1947 by Campbell (Campbell 1947; Whipps and Gerlagh 1992). In vitro *C. minitans* can degrade sclerotia through the expression of cell wall degrading enzymes, specifically glucanase and glycosidase (Muthumeenakshi et al. 2007; Whipps and Gerlagh 1992). *B. amyloliquefaciens* was first isolated by Fukomoto in Japan in 1943 (Priest et al. 1987). *B. amyloliquefaciens* is known for its ability to prevent pathogen infection by controlling mycelial

growth of *S. sclerotiorum* through the expression of secondary metabolites (Chen et al. 2009). These enzymes include lipopeptides and polyketides which have antifungal, antibacterial, and nematocidal properties (Chen et al. 2009; Hou et al. 2006).

Previous work has demonstrated that *B. amyloliquefaciens* can provide protection from *S. sclerotiorum* infection in various crops under greenhouse and field conditions (Abdullah et al. 2008; Fernando et al. 2007; Pethybridge et al. 2019; Wu et al. 2014), and *C. minitans* can decrease the inoculum potential of *S. sclerotiorum* in several crops under field conditions (Budge and Whipps 1991; Chitrampalam et al. 2008, 2010; Matheron and Porchas 2019; McLaren et al. 1994; McQuilken et al. 1995), however limited information is available on the effectiveness of these products in soybean. The objective of this research was to evaluate the effectiveness of *C. minitans* and *B. amyloliquefaciens* in vitro as biocontrol agents of *S. sclerotiorum*. And to assess the efficacy of *C. minitans* and *B. amyloliquefaciens* for management of Sclerotinia stem rot under controlled environmental conditions and in the field.

2.3 Materials and Methods

2.3.1 *Sclerotinia sclerotiorum* isolate information

An isolate of *S. sclerotiorum* originating from an infected soybean plant in Porter County, Indiana was obtained in the fall of 2019. The isolate was confirmed to be *S. sclerotiorum* through observation of the isolate morphology (Hartman et al. 2015). The isolate had fluffy white mycelium and produced black sclerotia after 1-2 weeks of incubation. This isolate was grown on full strength potato dextrose agar (PDA) (BD Difco Dehydrated Culture Media; Fisher Scientific, Waltham, MA) and incubated at 25ºC with 12 h light and 12 h dark for one week before being transferred to a new PDA plate.

2.3.2 Biofungicide isolate information

Commercial formulations of all biopesticides were used in this study (Table 1). For the dual culture assay, an isolate of *C. minitans* was obtained by plating out the commercial formulation Contans WG (Sipcam Agro. USA Inc., Durham, NC). The isolate was grown on full strength PDA amended with 0.05% Rifampicin (v/v) (BioReagents; Fisher Scientific, Waltham, MA) and incubated at 20ºC with 12 h light and 12 h dark for one week. The isolate was transferred

to a new PDA+Rifampicin plate and placed back in the incubator for an additional week before being used for experiments. All other experiments used the recommended field application rates, which are 2.24 kg/ha (2.0 lb/A) for *C. minitans* (Contans WG; Sipcam Agro. USA Inc., Durham, NC) and 4.68 L/ha (2.0 qt/A) or 14.03 L/ha (6.0 qt/A) for *B. amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Colombia, NC).

Table 1: Biofungicide product, manufacturer, active ingredient, Fungicide Resistance Action Committee codes, and application rates for products used in the studies.

Product Name	Manufacturer	Active ingredient (%)	FRAC code[z]	Application rate (Imperial units)	Application rate (SI units)
Contans WG	Sipcam Agro USA Inc. Durham, NC	*Coniothyrium minitans* strain CON/M/91-08 (5.00%)	BM02	1.0 to 4.0 lbs/A	1.12 to 4.48 kg/ha
Double Nickel LC	Certis USA LLC Columbia, NC	*Bacillus amyloliquefaciens* strain D747 (98.85%)	BM02	0.5 to 6.0 qt/A	1.17 to 14.03 L/ha

[z] FRAC = Fungicide resistance action committee. BM02: Biologicals with multiple modes of action, microbial.

2.3.3 Dual culture assay

An 8-mm plug taken from the edge of an actively growing 7-day old culture of *S. sclerotiorum* was placed upside down on full strength PDA 2 cm away from either 10 µL of *B. amyloliquefaciens*, non-diluted formulated product, or an 8-mm plug of *C. minitans* that was taken from an actively growing 7-day old culture. *B. amyloliquefaciens* was then spread into an approximately 1-cm circle with a sterile metal inoculating loop. The plates were incubated at 25°C with 12 h light and 12 h dark for one week. After one week, the presence or absence of an inhibition zone was recorded. An inhibition zone was defined as a clear area surrounding either the *B. amyloliquefaciens* colony or *C. minitans* isolate where *S. sclerotiorum* cannot grow. Four replicates were included in each experiment and the experiment was repeated four times.

2.3.4 Amended media assay

PDA was autoclaved and cooled to at least 65°F. The PDA was then amended with *B. amyloliquefaciens* at a concentration equal to the field application rate of 4.68 L/ha using non-diluted formulated product. Non-amended PDA was used as the control. Eight mm plugs were taken from the edge of an actively growing 7-day old *S. sclerotiorum* culture and placed upside down in the center of either the amended or non-amended plates. The plates were incubated at 25°C with 12 h light and 12 h dark for one week. After one week, the radial growth (mm) along two axes was measured for each plate. The two axes were averaged before analysis. Three replicates were included in each experiment and the experiment was repeated three times.

2.3.5 Soil plate assay

A modified soil plate technique described by Smith et al. (1991) was used. Potting mix (Redi-Earth Propagation Mix; Sungro Horticulture, Agawam, MA) was autoclaved for 35 min, then 6 g of the potting mix was placed into a 9-cm plastic Petri dish. Each Petri dish was then sprayed with 5 mL of deionized water using a hand atomizer. Five sclerotia were surface sterilized in 10% sodium hypochlorite for 30 s. The sclerotia were then rinsed in sterile deionized water, dried on a sterile paper towel, and placed on the soil surface. The application rate for *C. minitans* was converted from rate per hectare to rate per Petri dish using the surface area of the Petri dish. *C. minitans* was applied as the formulated product Contans WG at a field application rate of 2.24

kg/ha using a hand atomizer (Solid USA, Irvine, CA). Each experiment had a non-treated control that did not receive a treatment. After the treatments were applied, the plates were incubated at 20°C with 12 h light and 12 h dark for four weeks.

After four weeks, all five sclerotia were collected from each plate, surface sterilized in 10% sodium hypochlorite, rinsed in sterile deionized water, and dried on a sterile paper towel. Each sclerotia were then individually plated on PDA + 0.05% Rifampicin (v/v) plates. The plates were then placed in an incubator at 25°C with 12 h light and 12 h dark for one week. After one week, the radial growth (mm) along two axes of each plate was measured. Before the data were analyzed, the values for the two axes were averaged and then the five sclerotia per treatment were averaged. Four replicates were included in each experiment and the experiment was repeated twice.

2.3.6 Controlled environment experiments

The growth chamber (Conviron BDW190; Controlled Environments Limited, Winnipeg, Canada) conditions were set to 14 h light and 10 h dark with a light intensity of 500 μmol/m^2s and a constant temperature of 20°C. The plants were watered both on the soil surface and from the base on trays, after treatments were applied the plants were only watered from the base. The soybean variety, P34A79X, was sowed in 15-cm pots at a rate of two seeds per pot. The plants were thinned to one seedling per pot two weeks after planting. The experiment had a randomized complete block design with four replications and was repeated twice. Prior to treatment application, the fourth vegetative (V4) leaf was cut leaving an exposed petiole. *B. amyloliquefaciens* was applied as formulated product at field application rates of 4.68 L/ha and 14.03 L/ha using the dip method or a spray booth. In the dip method, the exposed petiole was dipped in a solution of the treatments. A spray booth (Generation III; DeVries Manufacturing, Hollandale, MN) fitted with an XR8002 nozzle applied the treatments at 140.2 L/ha and 206.84 kPa with a sprayer height of about 50.8 cm. The plants were transferred to a greenhouse with 14 h light and 10 h dark with a minimum temperature of 18°C and a maximum temperature of 29°C one day prior to treatment applications. After the treatments were applied, the plants were allowed to dry on the greenhouse bench overnight. The plants were then transferred back to the growth chamber and inoculated using the pipet tip method (Botha et al. 2009). Plugs were cut from an actively growing 7-day old *S. sclerotiorum* isolate using a 200 μL pipet tip (Labtips Pipette Tips; Fisher Scientific, Waltham, MA). The pipet tip containing the *S. sclerotiorum* plug was then placed on the exposed petiole.

Lesion length along the main stem (mm) was measured 6, 11, and 14 days after inoculation (DAI) using calipers. The lesion area under the disease progress curve (lAUDPC) was calculated using Equation 1 (Simko and Piepho 2012). Where t_i is the current time point and y_i is the corresponding disease rating, t_{i+1} is the next time point in the series and y_{i+1} is the corresponding disease rating.

Equation 1. Lesion area under the disease progress curve (lAUDPC).

$$\text{lAUDPC} = \sum_{i=1}^{N_{i-1}} \left(\frac{y_i + y_{i+1}}{2}\right) * (t_{i+1} - t_i)$$

2.3.7 Field experiments

Trials were established in 2020 and 2021 at the Agronomy Center for Research and Education (ACRE) in West Lafayette, IN and the Pinney Purdue Agricultural Center (PPAC) in Wanatah, IN. Field trial information on variety, planting date, irrigation, biofungicide application date, growth stage at the time of application, and harvest date are found in Table 2. The experiments were a randomized complete block design with four replications. Plots were either 1.5-m or 2.04-m wide and 9.14-m long and consisted of four rows. In 2020 the previous crop was corn, in 2021 the previous crop was sunflower. Standard practices for weed management in soybean production in Indiana were followed. All plots were inoculated with *S. sclerotiorum* at 0.04 g/cm within the seedbed at planting and in 2021 sclerotia at 5.0 g/plot were also spread between the middle two rows prior to emergence. At PPAC, overhead irrigation was applied weekly at approximately 25 mm unless weekly rainfall was 25 mm or higher to encourage disease. In 2020, a Lee self-propelled sprayer equipped with a 3-m boom, fitted with six TJ-VS 8002 nozzles spaced 50.8-cm apart was used to make the treatment applications and in 2021, a CO_2 backpack sprayer equipped with a 3-m boom, fitted with six TJ-VS 8002 nozzles spaced 50.8-cm apart was used. All treatments were applied at 140.2 L/ha and 206.84 kPa. The two center rows of each plot were harvested with a Kincaid XP8 combine and yields were adjusted to 13% moisture.

Table 2. Trial details for field experiments used to assess the ability of *Coniothyrium minitans* and *Bacillus amyloliquefaciens* to control Sclerotinia stem rot in soybean.

Year	Location[z]	Variety	Planting Date	Irrigation (Y/N)[y]	Treatment, rate/ha, and timing[x]	Application Date	Harvest Date
2020	ACRE	P34A79X	6/1/20	N	*C. minitans*, 2.24 kg, Pre *B. amyloliquefaciens*, 4.68 L, R1	6/2/2020 7/15/2020	10/14/2020
	PPAC	P34A79X	6/6/20	Y	*C. minitans*, 2.24 kg, Pre *B. amyloliquefaciens*, 4.68 L, R1	6/7/2020 7/21/2020	11/2/2020
2021	ACRE	P34A79X	5/15/21	N	*C. minitans*, 2.24 kg, Pre *B. amyloliquefaciens*, 4.68 L, R2	5/15/2021 7/13/2021	10/18/2021
	PPAC	P34A79X	5/24/21	Y	*C. minitans*, 2.24 kg, Pre *B. amyloliquefaciens*, 4.68 L, R2	5/26/2021 7/19/2021	10/1/2021

[z] ACRE = Agronomy Center for Research and Education, West Lafayette, IN. PPAC = Pinney Purdue Agricultural Center, Wanatah, IN.
[y] Irrigation applied weekly at approximately 25 mm unless weekly rainfall was 25 mm or higher to encourage disease.
[x] *Coniothyrium minitans* applied as formulated product Contans WG (Sipcam Agro USA Inc., Durham, NC), *Bacillus amyloliquefaciens* applied as formulated product Double Nickel LC (Certis USA LLC, Columbia, NC). Timing: Pre = Preemergence, R1 = Beginning bloom, R2 = Full bloom.

2.3.8 Data analyses

All data were analyzed in SAS 9.4 (SAS Institute Inc., Cary, NC). A Student's t-test was used to separate treatment effect on radial mycelial growth of *S. sclerotiorum* in the amended media assay and sclerotia viability in the soil plate assay. The assumption of equal variances was met in each experiment, therefore significant differences between treatments were assessed using the pooled variance procedure at $p \leq 0.05$.

Outliers were removed from the controlled environment and field experiment datasets if the absolute value of the studentized residual was greater than 3. Typically, a value of 2.5 for the absolute value of the studentized residual is used to identify outliers, however to allow for variation in the dataset a value of 3 was selected instead. Plots of the residuals were used to select the best distribution for each dataset respectively. The data were combined across repetition in the controlled environment dataset, and year and location in the field experiment dataset prior to analysis. A generalized linear mixed model with a normal distribution utilizing PROC GLIMMIX was used to determine the effect of treatment on Sclerotinia stem rot lesion length (mm) in the controlled environment experiment, as well as to determine the effect of treatment on moisture, test weight, and yield in the field experiment. In the controlled environment and field experiments, treatment was the main effect in the model, and the random effect was experiment. Significant differences between treatments in each experiment were assessed using Fisher's least significant difference at $\alpha = 0.05$.

2.4 Results

2.4.1 Dual culture assay

A dual culture experiment was conducted to observe if *C. minitans* and *B. amyloliquefaciens* can limit the mycelial growth of *S. sclerotiorum* in vitro. A distinct inhibition zone was recorded surrounding the *B. amyloliquefaciens* colony, while no inhibition zone was recorded between *C. minitans* and *S. sclerotiorum* on all 16 plates (Figure 1 and Table 3).

Table 3. *Sclerotinia sclerotiorum* plated in dual culture with either *Bacillus amyloliquefaciens* or *Coniothyrium minitans*.

Colony or Isolate	Inhibition Zone #[z]	No Inhibition Zone #[z]
B. amyloliquefaciens	16	0
C. minitans	0	16

[z] Data represent the number of plates where a distinct inhibition zone was recorded surrounding the *S. sclerotiorum* isolate after one week of incubation at 25°C. Data represent the total number of plates from four experiments with four replicates.

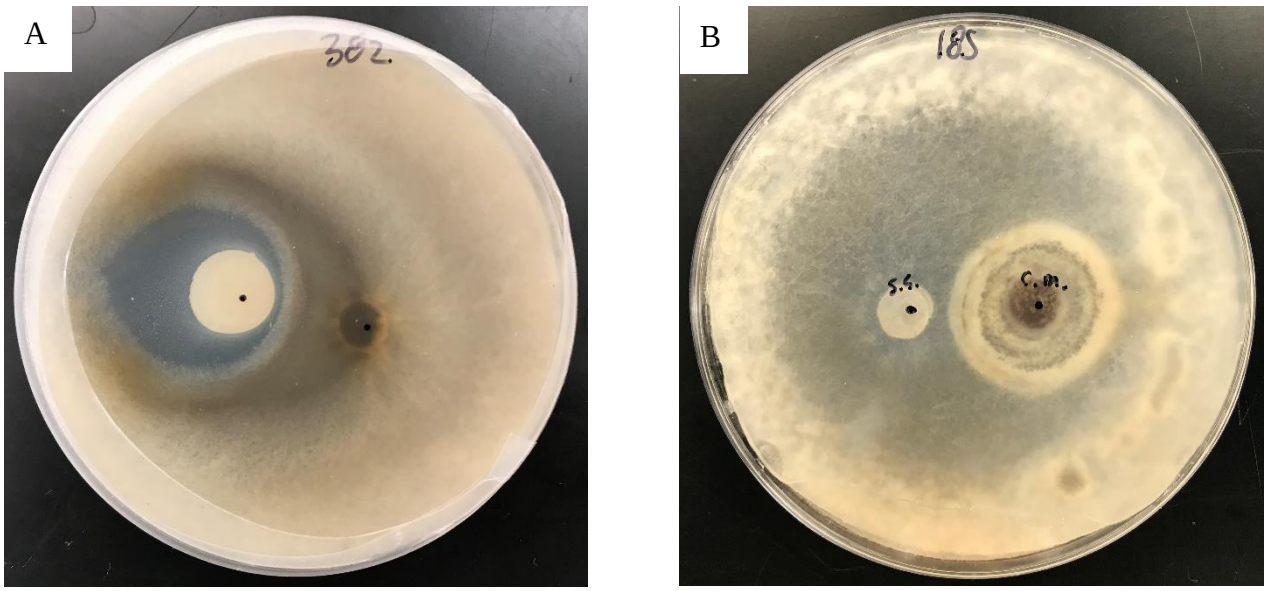

Figure 1. *Sclerotinia sclerotiorum* plated in dual culture with *Bacillus amyloliquefaciens* and *Coniothyrium minitans*. A) A clear inhibition zone was observed surrounding the *B. amyloliquefaciens* colony (left) where mycelium of *S. sclerotiorum* (right) cannot grow. B) No inhibition zone was observed between *S. sclerotiorum* (left) and *C. minitans* (right).

2.4.2 Amended media assay

PDA media amended with *B. amyloliquefaciens* was used to quantify the level of *S. sclerotiorum* mycelial growth control provided by the biofungicide. *B. amyloliquefaciens* significantly reduced the radial mycelial growth of *S. sclerotiorum*. The average radial growth for the non-amended control was 85.0 mm, while the average radial growth for the PDA amended with *B. amyloliquefaciens* was 0.0 mm (Figure 2 and Table 4).

Table 4. Control of radial mycelial growth of *Sclerotinia sclerotiorum* with *Bacillus amyloliquefaciens.*

Treatment	Average radial growth (mm)[z]
Non-amended control	85.0 a
B. amyloliquefaciens	0.0 b
***P*-value[y]**	<0.0001

[z] Data represent the average radial mycelial growth of *S. sclerotiorum* after one week of incubation at 25°C. Data represent the mean of three replicates from three experiments. Data were pooled over three experiments prior to analysis.
[y] Means separated using Fisher's least significant difference ($\alpha = 0.05$).

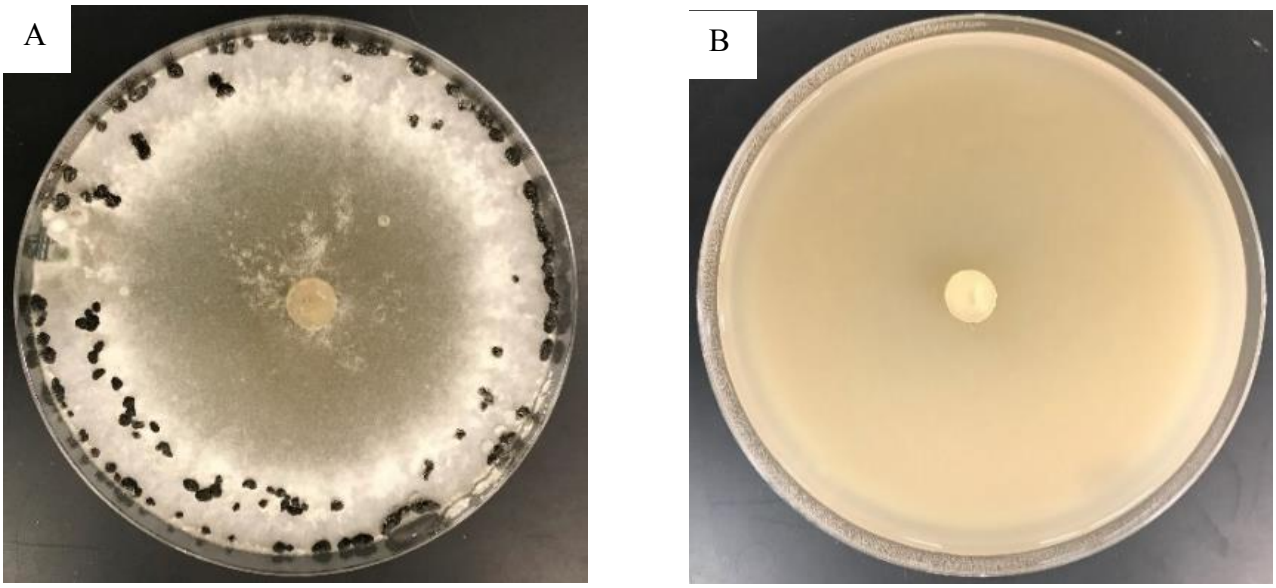

Figure 2. Radial mycelial growth of *S. sclerotiorum* for the A) Non-amended media or B) Media amended with *B. amyloliquefaciens*.

2.4.3 Soil plate assay

A modified soil plate technique was used to assess the ability of *C. minitans* to degrade the sclerotia of *S. sclerotiorum*. Four weeks after being treated with *C. minitans*, the viability of *S. sclerotiorum* sclerotia was significantly reduced following treatment with *C. minitans* leading to a decrease in radial mycelial growth. The average radial growth of the sclerotia from the non-treated

control was 83.78 mm, while the radial growth of the sclerotia treated with C. *minitans* was 38.18 mm (Figure 3 and Table 5).

Table 5. Degradation of sclerotia of *Sclerotinia sclerotiorum* by *Coniothyrium minitans* leading to a decrease in radial mycelial growth.

Treatment	**Average radial growth (mm)[z]**
Non-treated control	83.78 a
C. minitans	38.18 b
***P*-value[y]**	<0.0001

[z] Data represent the average radial mycelial growth of sclerotia of *S. sclerotiorum* after four weeks of incubation at 20°C. Data represent the mean of four replicates from two experiments. Data were pooled over two experiments prior to analysis.

[y] Means separated using Fisher's least significant difference ($\alpha = 0.05$).

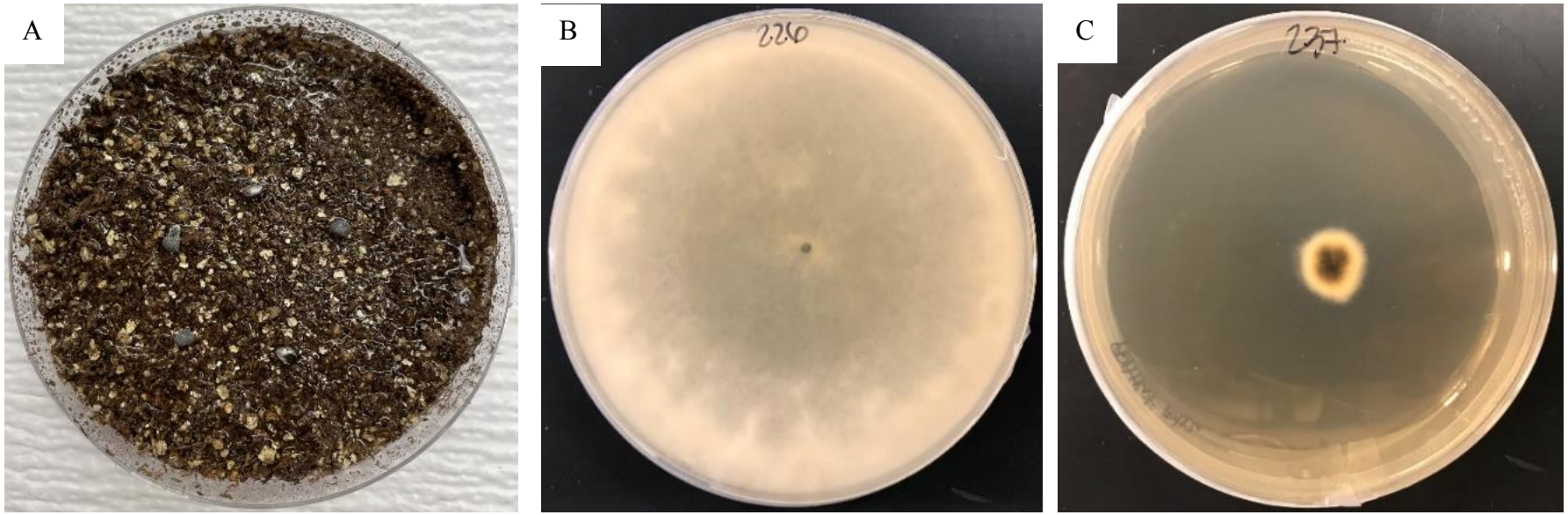

Figure 3. A) Sclerotia plated on soil surface in the soil plate assay. Radial mycelial growth of *S. sclerotiorum* for the B) Non-treated control or C) *C. minitans* treatment.

2.4.4 Controlled environment experiments

The efficacy of *B. amyloliquefaciens*, applied using both the dip and spray methods, was evaluated in a controlled environment. Significant differences were found between treatments at 6, 11, and 14 DAI (Figure 4). *B. amyloliquefaciens* applied at 4.68 and 14.03 L/ha using the spray method did not reduce Sclerotinia stem rot lesion length when compared to the non-treated control for all dates. At both 6 DAI and 11 DAI *B. amyloliquefaciens* applied using the dip method at 14.03 L/A was not statistically different from *B. amyloliquefaciens* applied at 4.68 L/ha. However, by 14 DAI *B. amyloliquefaciens* applied at 14.03 L/A was significantly different from all other treatments including *B. amyloliquefaciens* applied 4.68 L/ha and the non-treated control.

B. amyloliquefaciens applied at 4.68 and 14.03 L/ha using the spray method were not statistically different from the non-treated control for Sclerotinia stem rot lAUDPC (Figure 5). *B. amyloliquefaciens* at 4.68 L/ha applied using the dip method significantly reduced lAUDPC over the non-treated control. *B. amyloliquefaciens* at 14.03 L/ha applied using the dip method resulted in the lowest lAUDPC.

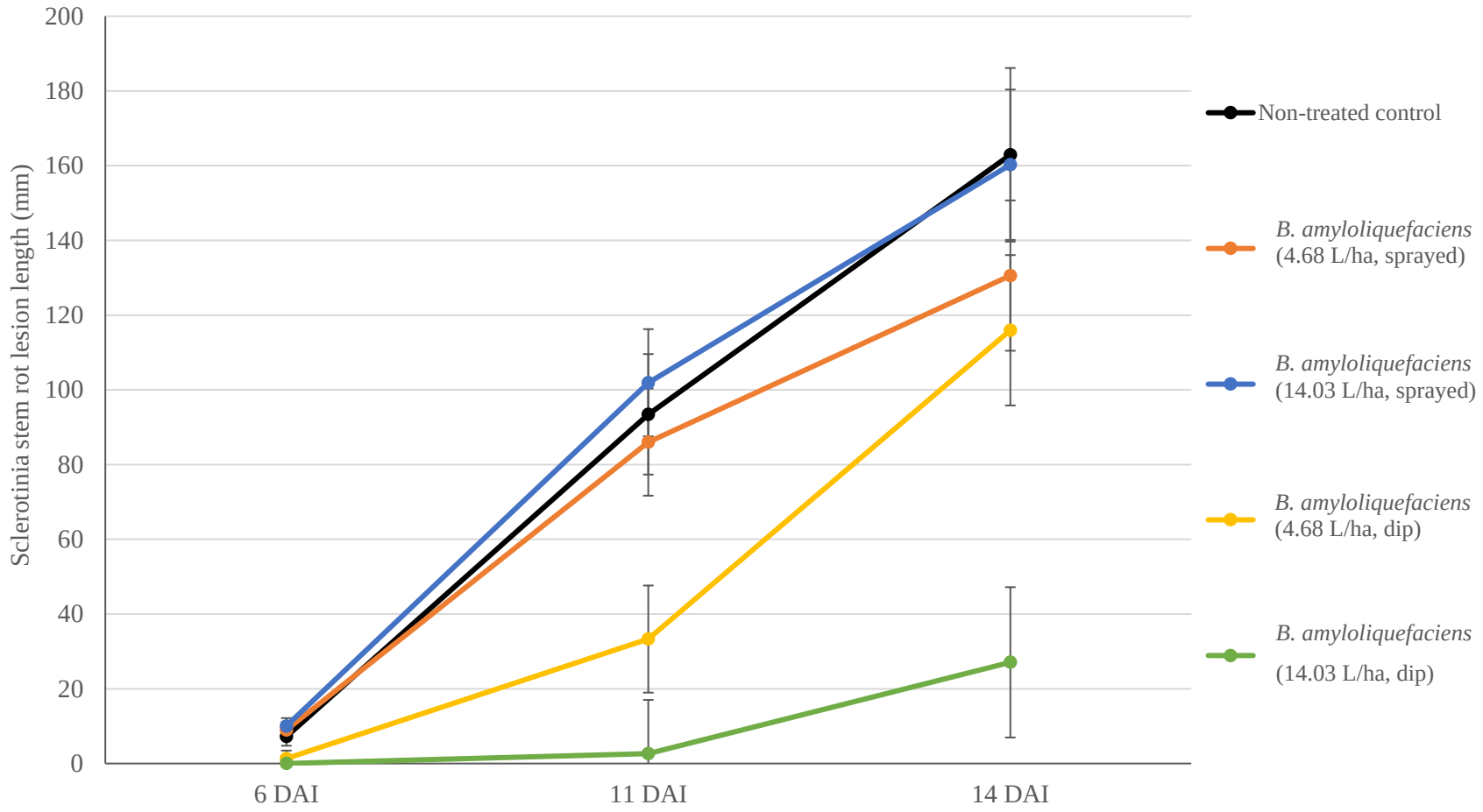

Figure 4. Efficacy of *Bacillus amyloliquefaciens* at 4.68 L/ha or 14.03 L/ha applied using a dip or spray method for reducing Sclerotinia stem rot lesion length (mm) at 6, 11 and 14 days after inoculation (DAI) in the growth chamber. Four replicates were included in each experiment and the experiment was repeated twice. Data pooled over two experiments prior to analysis. Least squares means separated using Fisher's least significant difference ($\alpha = 0.05$). Error bars represent standard error of the mean. 6 DAI: $F = 4.80$, $p = 0.0038$. 11 DAI: $F = 12.13$, $p = 0.0001$. 14 DAI: $F = 7.24$, $p = 0.0022$.

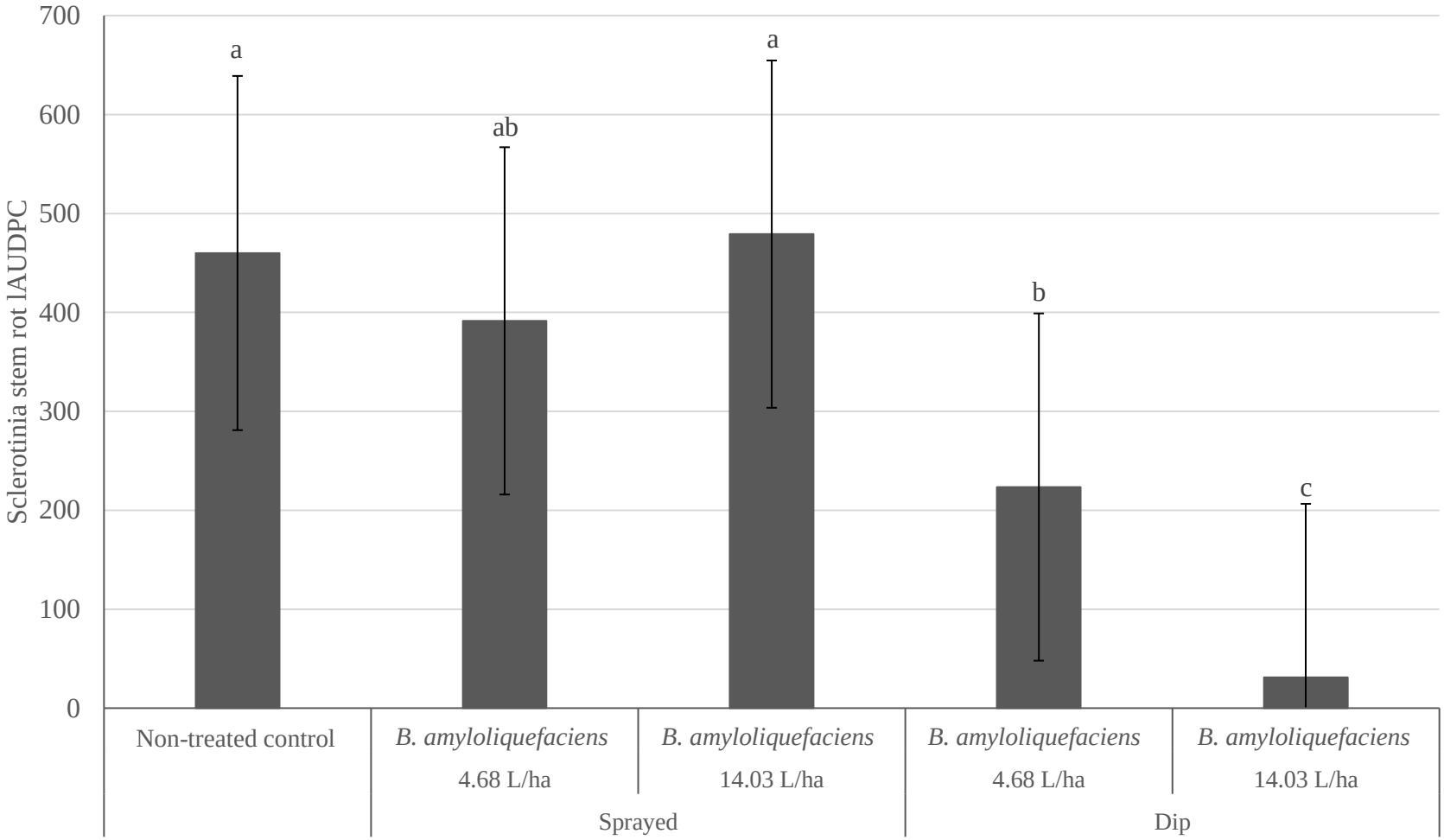

Figure 5. Efficacy of *Bacillus amyloliquefaciens* at 4.68 L/ha or 14.03 L/ha applied using a dip or spray method to decrease Sclerotinia stem rot lesion area under the disease progress curve (lAUDPC) in the growth chamber. Four replicates were included in each experiment and the experiment was repeated twice. Data pooled over two experiments prior to analysis. Least squares means separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not statistically different. Error bars represent standard error of the mean. $F = 9.19$, $p = 0.0001$.

2.4.5 Field experiments

Field experiments were conducted to determine the effect of applying *C. minitans* and *B. amyloliquefaciens* on soybean moisture, test weight, and yield. Weather conditions were not conducive for the development of Sclerotinia stem rot in 2020 and 2021 at either ACRE or PPAC. No differences were observed between treatments and the non-treated control for soybean moisture, test weight, or yield (Table 6). The average soybean yield was 5500.4 kg/ha for the *C. minitans* treatment and 5467.5 kg/ha for the *B. amyloliquefaciens* treatment when compared to 5487.5 for the non-treated control.

Table 6. Effect of *Coniothyrium minitans* and *Bacillus amyloliquefaciens* on soybean moisture, test weight, and yield in Indiana field experiments.

Treatment and rate/ha[z]	Moisture (%)	Test Weight kg/hL (lb/bu)	Yield kg/ha (bu/A)[y]
Non-treated control	12.0	71.3 (55.4)	5487.5 (81.6)
C. minitans 2.24 kg	11.9	71.1 (55.2)	5500.4 (81.8)
B. amyloliquefaciens 4.68 L	11.9	71.3 (55.4)	5467.5 (81.3)
***P*-value[x]**	0.6659	0.3295	0.9728

[z] *Coniothyrium minitans* applied as formulated product Contans WG (Sipcam Agro USA Inc., Durham, NC) and *Bacillus amyloliquefaciens* applied as formulated product Double Nickel LC (Certis USA LLC, Columbia, NC).
[y] Yields were adjusted to 13% moisture.
[x] Experiment was conducted at the Agronomy Center for Research and Education (ACRE) and Pinney Purdue Agricultural Center (PPAC) in 2020 and 2021. Data pooled across experiments prior to analysis. Least squares means separated using Fisher's least significant difference ($\alpha = 0.05$).

2.5 Discussion

The results reported here are consistent with previous research that found that *B. amyloliquefaciens* can control the mycelial growth of *S. sclerotiorum* (Abdullah et al. 2008; Wu et al. 2014). The presence of a distinct inhibition zone surrounding the *B. amyloliquefaciens* colony in the dual culture assay and the absence of mycelial growth on the media plates amended with *B. amyloliquefaciens* confirms that the bacteria can control the mycelial growth of *S. sclerotiorum*. This is likely to occur through antibiosis where the lipopeptides produced by *B. amyloliquefaciens*,

which include surfactin, bacillomycin, fengycin, and iturin limit the mycelium of *S. sclerotiorum* (Chen et al. 2009; Hou et al. 2006; Stein 2005).

The absence of an inhibition zone surrounding the *C. minitans* isolate in the dual culture assay indicates an inability to limit the mycelial growth of *S. sclerotiorum*. The results of the soil plate assay confirmed that the primary mode of action of *C. minitans* is mycoparasitism of sclerotia (Muthumeenakshi et al. 2007; Whipps and Gerlagh 1992). Consistent with previous research, *C. minitans* degraded the sclerotia of *Sclerotinia* spp., leading to a decrease in radial mycelial growth (Partridge et al. 2006; Whipps and Budge 1990). Successful control of *S. sclerotiorum* by *C. minitans* and *B. amyloliquefaciens* is observed in the laboratory because the biofungicides are in direct contact with the sclerotia and mycelium of *S. sclerotiorum* without confounding environmental factors.

B. amyloliquefaciens can be applied at 1.17 up to 14.03 L/ha (0.5 qt/A to 6 qt/A). Two application rates were tested in the controlled environment experiments, 4.68 and 14.03 L/ha, corresponding to the recommended application rate and the maximum labeled application rate. *B. amyloliquefaciens* at 14.03 L/ha applied using the dip method limited Sclerotinia stem rot lesion length development in soybean and resulted in the lowest lAUDPC. Furthermore, *B. amyloliquefaciens* applied using the dip method was still able to reduce lAUDPC over the non-treated control when applied at 4.68 L/ha. These results are consistent with previous literature which found that *B. amyloliquefaciens* was able to control *S. sclerotiorum* in squash, tomato, eggplant, canola, snap bean, and dry bean (Abdullah et al. 2008; Fernando et al. 2007; Pethybridge et al. 2019).

It is very challenging to achieve uniform Sclerotinia stem rot disease pressure in a controlled environment such as a growth chamber. The pipet tip inoculation method was selected as it produced the most consistent Sclerotinia stem rot disease pressure, however it also bypasses the plant's natural defense mechanisms. Through antibiosis, *B. amyloliquefaciens* acts as a plant protectant which is why disease control was observed for the dip method and not the spray method. Using the dip method to apply *B. amyloliquefaciens*, the biofungicide was in direct contact with *S. sclerotiorum* leading to a decrease in lesion length. Other inoculation methods in the controlled environment that do not bypass the plant's natural defense mechanisms should be explored in the future. After consistent Sclerotinia stem rot disease pressure is achieved using alternative

inoculation methods, the efficacy of *B. amyloliquefaciens* for controlling Sclerotinia stem rot in soybean should be evaluated again in the growth chamber using the spray method.

When *B. amyloliquefaciens* and *C. minitans* were applied in the field, no differences were observed between treatments for soybean moisture, test weight, or yield. Weather conditions were not conducive to Sclerotinia stem rot development in the field trials during the 2020 and 2021 growing seasons and disease was not observed in the plots. Therefore, in a year with low disease pressure, if applications of C. *minitans* are made to reduce the amount of inoculum in the soil or *B. amyloliquefaciens* is applied proactively to prevent the development of *S. sclerotiorum*, these results indicate that there will not be a negative impact on the yield of soybean.

Future work should focus primarily on evaluating the efficacy of *B. amyloliquefaciens* and *C. minitans* for controlling *S. sclerotiorum* in soybean under controlled environmental conditions using other plant inoculation techniques and in the field under high disease pressure. The application timing of the biofungicides in soybean and the interaction between the biofungicides and other applied pesticides should also be explored.

2.6 References

Abdullah, M. T., Ali, N. Y., and Suleman, P. 2008. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*. Crop Prot. 27:1354–1359.

Adams, P. B. and Ayers, W. A. 1979. Ecology of *Sclerotinia* species. Phytopathology. 69:896–899.

Botha, C., McLaren, N. W., and Swart, W. J. 2009. Evaluation of greenhouse inoculation techniques used to screen for Sclerotinia stem rot resistance in soybeans. South African J. Plant and Soil. 26:48–50.

Bradley, C. A., Allen, T. W., Sisson, A. J., Bergstrom, G. C., Bissonnette, K. M., Bond, J., Byamukama E., Chilvers, M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault N. S., Esker, P. D., Faske, T. R., Fiorellino N. M., Geisler, L. J., Hartman, G. L., Hollier, C. A., Isakeit T., Jackson-Ziems, T. A., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koehler, A. M., Kratochvil, R. J., Kurle, J. E., Malvick, D. K., Markell, S. G., Mathew, F. M., Mehl, H. L., Mehl K. M., Mueller, D. S., Mueller, J. D., Nelson, B. D., Overstreet, C., Padgett, G. B., Price, P. P., Sikora, E. J., Small, I., Smith, D. L., Spurlock, T. N., Tande, C. A., Telenko, D. E. P., Tenuta, A. U., Thiessen, L. D., Warner, F., Wiebold, W. J., and Wise, K. A. 2021. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2015 to 2019. Plant Health Prog. 22:483–495.

Budge, S. P. and Whipps, J. M. 1991. Glasshouse trials of *Coniothyrium minitans* and *Trichoderma* species for the biological control of *Sclerotinia sclerotiorum* in celery and lettuce. Plant Pathol. 40:59–66.

Campbell, W. A. 1947. A new species of *Coniothyrium* parasitic on sclerotia. Mycologia. 39:190–195.

Chen, X. H., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Süssmuth, R., Piel, J., and Borriss, R. 2009. Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens. J. of Biotechn. 140:27–37.

Chitrampalam, P., Figuli, P. J., Matheron, M. E., Subbarao, K. V., and Pryor, B. M. 2008. Biocontrol of lettuce drop caused by *Sclerotinia sclerotiorum* and *Sclerotinia minor* in desert agroecosystems. Plant Dis. 92:1625–1634.

Chitrampalam, P., Turini, T. A., Matheron, M. E., and Pryor, B. M. 2010. Effect of sclerotium density and irrigation on disease incidence and on efficacy of *Coniothyrium minitans* in suppressing lettuce drop caused by *Sclerotinia sclerotiorum*. Plant Dis. 94:1118–1124.

Crop Protection Network. 2020. Estimates of corn, soybean, and wheat yield losses due to diseases and insect pests: an online tool. Https://loss.cropprotectionnetwork.org/. Doi.org/10.31274/cpn-20191121-0.

Crop Protection Network. 2021. Estimates of corn, soybean, and wheat yield losses due to diseases and insect pests: an online tool. Https://loss.cropprotectionnetwork.org/. Doi.org/10.31274/cpn-20191121-0.

Duncan, R. W., Dilantha Fernando, W. G., and Rashid, K. Y. 2006. Time and burial depth influencing the viability and bacterial colonization of sclerotia of *Sclerotinia sclerotiorum*. Soil Biol. & Biochem. 38:275–284.

Fernando, W. G. D., Nakkeeran, S., Zhang, Y., and Savchuk, S. 2007. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. Crop Prot. 26:100–107.

Gambhir, N., Kamvar, Z. N., Higgins, R., Amaradasa, B. S., and Everhart, S. E. 2021. Spontaneous and fungicide-induced genomic variation in *Sclerotinia sclerotiorum*. Phytopathology. 111:160–169.

Hao, J. J., Subbarao, K. V., and Duniway, J. M. 2007. Germination of *Sclerotinia minor* and *Sclerotinia sclerotiorum* sclerotia under various soil moisture and temperature combinations. Phytopathology. 93:443–450.

Hartman, G. L., Rupe, J. C., Sikora, E. J., Domier, L. L., Davis, J. A., and Steffey, K. L., eds. 2015. *Compendium of Soybean Diseases and Pests*. Fifth Edition. The American Phytopathological Society.

Hou, X., Boyetchko, S. M., Brkic, M., Olson, D., Ross, A., and Hegedus, D. 2006. Characterization of the anti-fungal activity of a *Bacillus* spp. associated with sclerotia from *Sclerotinia sclerotiorum*. Appl. Microbiol. and Biotech. 72:644–653.

Lehner, M. S., Paula Júnior, T. J., Silva, R. A., Vieira, R. F., Carneiro, J. E. S., Schnabel, G., and Mizubuti, E. S. G. 2015. Fungicide sensitivity of *Sclerotinia sclerotiorum*: A thorough assessment using discriminatory dose, EC50, high-resolution melting analysis, and description of new point mutation associated with thiophanate-methyl resistance. Plant Dis. 99:1537–1543.

Matheron, M. E. and Porchas, M. 2019. Optimizing fungicide inputs for management of lettuce drop caused by *Sclerotinia minor* and *Sclerotinia sclerotiorum*. Plant Health Prog. 20:238–243.

McLaren, D. L., Huang, H. C., Kozub, G. C., and Rimmer, S. R. 1994. Biological control of Sclerotinia wilt of sunflower with *Talaromyces flavus* and *Coniothyrium minitans*. Plant Dis. 78:231–235.

McQuilken, M. P., Mitchell, S. J., Budge, S. P., Whipps, J. M., Fenlon, J. S., and Archer, S. A. 1995. Effect of *Coniothyrium minitans* on sclerotial survival and apothecial production of *Sclerotinia selerotiorum* in field-grown oilseed rape. Plant Pathol. 44:883-896.

Mueller, D., Bradley, C., Chilvers, M., Esker, P., Malvick, D., Peltier, A., Sisson, A., Wise, K., Tenuta, A., and Faske, T. 2015. Soybean disease management: White mold. Crop Protection Network. CPN 1005. doi.org/10.31274/cpn-20190620-030.

Muthumeenakshi, S., Sreenivasaprasad, S., Rogers, C. W., Challen, M. P., and Whipps, J. M. 2007. Analysis of cDNA transcripts from *Coniothyrium minitans* reveals a diverse array of genes involved in key processes during sclerotial mycoparasitism. Fungal Gen. and Biol. 44:1262–1284.

Partridge, D. E., Sutton, T. B., and Jordan, D. L. 2006. Effect of environmental factors and pesticides on mycoparasitism of *Sclerotinia minor* by *Coniothyrium minitans*. Plant Dis. 90:1407–1412.

Peltier, A. J., Bradley, C. A., Chilvers, M. I., Malvick, D. K., Mueller, D. S., Wise, K. A., and Esker, P. D. 2012. Biology, yield loss and control of Sclerotinia stem rot of soybean. J. Int. Pest Manag. 3:1–7.

Pethybridge, S. J., Gugino, B. K., and Kikkert, J. R. 2019. Efficacy of Double Nickel LC (*Bacillus amyloliquefaciens* D747 strain) for management of white mold in snap and dry bean. Plant Health Prog. 20:61–66.

Priest, F. G., Goodfellow, M., Shute, L. A., and Berkeley, R. C. W. 1987. *Bacillus amyloliquefaciens* sp. nov. nom. rev. Int. J. Syst. Bacteriol. 37:69–71.

Simko, I. and Piepho, H.-P. 2012. The area under the disease progress stairs: Calculation, advantage, and application. Phytopathology. 102:381-389.

Smith, F. D., Phipps, P. M., and Stipes, R. J. 1991. Agar plate, soil plate, and field evaluation of fluazinam and other fungicides for control of *Sclerotinia minor* on peanut. Plant Dis. 75:1138-1143.

Stein, T. 2005. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. Molec. Microbiol. 56:845–857.

United States Environmental Protection Agency - Biopesticides. Available at: https://www.epa.gov/pesticides/biopesticides.

Whipps, J. M. and Budge, S. P. 1990. Screening for sclerotial mycoparasites of *Sclerotinia sclerotiorum*. Mycol. Res. 94:607–612.

Whipps, J. M. and Gerlagh, M. 1992. Biology of *Coniothyrium minitans* and its potential for use in disease biocontrol. Mycol. Res. 96:897–907.

Willbur, J. F., Mitchell, P. D., Fall, M. L., Byrne, A. M., Chapman, S. A., Floyd, C. M., Bradley, C. A., Ames, K. A., Chilvers, M. I., Kleczewski, N. M., Malvick, D. K., Mueller, B. D., Mueller, D. S., Kabbage, M., Conley, S. P., and Smith, D. L. 2019a. Meta-analytic and economic approaches for evaluation of pesticide impact on Sclerotinia stem rot control and soybean yield in the North Central United States. Phytopathology. 109:1157–1170.

Willbur, J., McCaghey, M., Kabbage, M., and Smith, D. L. 2019b. An overview of the *Sclerotinia sclerotiorum* pathosystem in soybean: Impact, fungal biology, and current management strategies. Trop. Plant Path. 44:3–11.

Willetts, H. J. 1971. The survival of fungal sclerotia under adverse environmental conditions. Biol. Rev. 46:387–407.

Wu, Y., Yuan, J., Raza, W., Shen, Q., and Huang, Q. 2014. Biocontrol traits and antagonistic potential of *Bacillus amyloliquefaciens* strain NJZJSB3 against *Sclerotinia sclerotiorum*, a causal agent of canola stem rot. J. Microbiol. Biotech. 24:1327–1336.

CHAPTER 3. INTEGRATION OF *SCLEROTINIA SCLEROTIORUM* TARGETED BIOFUNGICIDES *CONIOTHYRIUM MINITANS* AND *BACILLUS AMYLOLIQUEFACIENS* INTO SEASON LONG SOYBEAN PEST MANAGEMENT PRACTICES

3.1 Abstract

Two biofungicides, *Coniothyrium minitans* and *Bacillus amyloliquefaciens,* are commercially available and have been shown to limit or suppress *Sclerotinia sclerotiorum* in soybean. *S. sclerotiorum* is a soilborne pathogen of soybean that can cause significant yield losses under cool and wet environmental conditions. Integrated soybean pest management practices include the application of herbicides, fungicides, and insecticides at various times during the growing season in order to minimize yield losses. However, limited information is available regarding how biofungicides can be successfully incorporated into a soybean integrated pest management program. To assess the sensitivity of *C. minitans* and *B. amyloliquefaciens* biological activity on *S. sclerotiorum* to pesticides commonly used in soybean production systems a poison plate assay as well as soil plate, growth chamber, and field experiments were conducted. In the poison plate assay *C. minitans* was most sensitive to the preemergence herbicide flumioxazin and the synthetic fungicides boscalid and fluazinam while *B. amyloliquefaciens* was sensitive only to the synthetic fungicide fluazinam. In the soil plate assay, the mycoparasitic activity of *C. minitans* on *S. sclerotiorum* was sensitive to flumioxazin, metribuzin, glyphosate, picoxystrobin, and boscalid. In the controlled environment experiments, none of the pesticides tested decreased the efficacy of *B. amyloliquefaciens.* In the field no significant interactions were observed between *C. minitans* and *B. amyloliquefaciens* with preemergence herbicides, postemergence herbicides, and synthetic fungicides for soybean moisture, test weight, and yield.

3.2 Introduction

Soybean yield can be reduced by both biotic and abiotic factors. Biotic factors include weeds, disease, and insects (Hartman et al. 2015). Worldwide between 2001 and 2003, weeds caused an average yield loss of 7.5% in soybean (Oerke 2006). In Indiana, from 2015 to 2019, 7.69% of yield was lost annually to soybean diseases (Bradley et al. 2021). Across the United States in 2020, 2.6% of yield was lost to insects (Musser et al. 2021). Pesticides are applied at various times during the

growing season to minimize these yield losses. In soybean, herbicides are applied at least twice throughout the growing season, once immediately following planting with a second application sometime during the early vegetative growth stages (Loux et al. 2020). Insecticides and fungicides are typically applied at the beginning reproductive stages (Mueller et al. 2016; Myers et al. 2005).

Sclerotinia stem rot in soybean which is caused by the soilborne pathogen *Sclerotinia sclerotiorum*, can cause significant yield losses under cool and wet environmental conditions and is challenging to control as specialized survival structures called sclerotia can lay dormant in the soil during winter months (Duncan et al. 2006; Hao et al. 2007; Adams and Ayers 1979; Willetts 1971). Two biofungicides, *Coniothyrium minitans* (Contans WG; Sipcam Agro. USA Inc., Durham, NC) and *Bacillus amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Colombia, NC), are labeled to limit or suppress Sclerotinia stem rot. Biofungicides are a microbial or biochemical product used to control or limit the activity of a pathogen (US EPA - Biopesticides n.d.). Biofungicides play an important role in in the management of *S. sclerotiorum* serving as either an alternative mode of action for foliar applications or deceasing the amount of inoculum in the soil. However, in order to develop a management plan for Sclerotinia stem rot in soybean using biofungicides it is vital to understand how the products can be successfully incorporated into an established integrated pest management program.

In previous studies under laboratory and growth chamber conditions, the effectiveness of *C. minitans* and *B. amyloliquefaciens* as biocontrol agents of *S. sclerotiorum* in soybean was confirmed (Conrad, unpublished). However, previous research also demonstrated that antagonistic relationships exist between *C. minitans* and other pesticides (Budge and Whipps 2001; Partridge et al. 2006; Li et al. 2002). Therefore, before making the recommendation to apply these products in soybean, it is essential to understand how other pesticides applied in soybean production systems will impact the efficacy of the biofungicides. The objective of this research was to explore the sensitivity of *B. amyloliquefaciens* and *C. minitans* biological activity on *S. sclerotiorum* to preemergence herbicides, postemergence herbicides, and synthetic fungicides commonly used in soybean production systems.

3.3 Materials and Methods

3.3.1 *Sclerotinia sclerotiorum* isolate information

An isolate of *S. sclerotiorum* originating from an infected soybean plant in Porter County, Indiana was obtained in the fall of 2019. The isolate was confirmed to be *S. sclerotiorum* through observation of the isolate morphology (Hartman et al. 2015). The isolate had fluffy white mycelium and produced black sclerotia after 1-2 weeks of incubation. The isolate was plated on full strength potato dextrose agar (PDA) (BD Difco Dehydrated Culture Media, Fisher Scientific, Waltham, MA) and incubated at 25ºC with 12 h light and 12 h dark for one week before being transferred to a new PDA plate.

3.3.2 Biofungicide isolate information

Commercial formulations of all biofungicides were used in this study (Table 7). For the poison plate assay, an isolate of *C. minitans* was obtained by plating out the commercial formulation Contans WG (Sipcam Agro. USA Inc., Durham, NC). The isolate was plated on full strength PDA amended with 0.05% Rifampicin (v/v) (BioReagents; Fisher Scientific, Waltham, MA) and incubated at 20ºC with 12 h light and 12 h dark for one week. The isolate was transferred to a new PDA+Rifampicin plate and placed back in the incubator for an additional week before being used for experiments. In the soil plate assay, growth chamber experiments, and field experiments the recommended field application rate of the biofungicides for soybean were used which are 2.24 kg/ha (2.0 lb/A) for *C. minitans* (Contans WG; Sipcam Agro. USA Inc., Durham, NC) and 4.68 L/ha (2.0 qt/A) for *B. amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Colombia, NC).

3.3.3 Pesticides used

Commercial formulations of all pesticides were applied at the recommended application rate for soybean in the soil plate assay, controlled environment, and field experiments (Table 7). The synthetic fungicides included: picoxystrobin 0.88 L/ha (12 fl oz/A) (Aproach SC; Corteva Agriscience, Johnston, IA), boscalid 0.56 kg/ha (8 oz/A) (Endura WDG; BASF, Research Triangle Park, NC), and fluazinam 0.88 L/ha (12 fl oz/A) (Omega 500F; Syngenta, Greensboro, NC). The

preemergence and postemergence herbicides included: flumioxazin 0.21 kg/ha (3 oz/A) (Valor SX WDG; Valent USA LLC, Walnut Creek, CA), S-metolachlor 3.04 L/ha (2.6 pt/A) (Dual Magnum EC; Syngenta, Greensboro, NC), metribuzin 0.67 kg/ha (0.6 lb/A) (Tricor DF; Corteva Agriscience, Johnston, IA), cloransulam-methyl 0.04 kg/ha (0.6 oz/A) (First Rate WDG; Corteva Agriscience, Johnston, IA), glyphosate 1.60 L/ha (22 fl oz/A) (RoundUp PowerMax EC; Bayer Crop Science, Research Triangle Park, NC), dicamba 1.60 L/ha (22 fl oz/A) (XtendiMax EC; Bayer Crop Science, Research Triangle Park, NC), glufosinate 3.14 L/ha (43 fl oz /A) (Liberty EC; BASF, Research Triangle Park, NC), and 2,4-D 2.34 L/ha (2 pt/A) (Enlist One EC; Corteva Agriscience, Johnston, IA). In the soil plate assay, growth chamber, and field experiments dicamba treatments included the required volatility reducing agent potassium hydroxide at 1.46 L/ha (20 fl oz/A) (Voliminate; Precision Laboratories, Waukegan, IL).

Table 7. Biofungicide, fungicide, and herbicide products used in study, manufacturer, active ingredient, Fungicide Resistance Action Committee or Herbicide Resistance Action Committee codes, and application rates.

Product Name	Manufacturer	Active ingredient (%)	FRAC code or HRAC code[z]	Application rate (Imperial units)	Application rate (SI units)
Contans WG	Sipcam Agro USA Inc. Durham, NC	*Coniothyrium minitans* strain CON/M/91-08 (5.0%)	BM02	1.0 to 4.0 lbs/A	1.12 to 4.48 kg/ha
Double Nickel LC	Certis USA LLC Columbia, NC	*Bacillus amyloliquefaciens* strain D747 (98.85%)	BM02	0.5 to 6.0 qt/A	1.17 to 14.03 L/ha
Aproach SC	Corteva Agriscience Johnston, IA	Picoxystrobin (22.5%)	11	8.0 to 12.0 fl oz/A	0.58 to 0.88 L/ha
Endura WDG	BASF Research Triangle Park, NC	Boscalid (70.0%)	7	5.5 to 11.0 oz/A	0.39 to 0.77 kg/ha
Omega 500F	Syngenta Greensboro, NC	Fluazinam (40.0%)	29	12.0 to 16.0 fl oz/A	0.88 to 1.17 L/ha
Valor SX WDG	Valent USA LLC Walnut Creek, CA	Flumioxazin (51.0%)	14	2.0 to 12.0 oz/A	0.14 to 0.84 kg/ha
Dual Magnum EC	Syngenta Greensboro, NC	S-metolachlor (83.7%)	15	1.0 to 2.6 pt/A	1.17 to 3.04 L/ha
Tricor DF	Corteva Agriscience Johnston, IA	Metribuzin (75%)	5	0.5 to 1.3 lb/A	0.56 to 1.49 kg/ha
First Rate WDG	Corteva Agriscience Johnston, IA	Cloransulam-methyl (84%)	2	0.6 to 0.75 oz/A	0.04 to 0.05 kg/ha

Table 7 continued

RoundUp PowerMax EC	Bayer Crop Science Research Triangle Park, NC	Glyphosate (48.7%)	9	22.0 fl oz/A	1.60 L/ha
XtendiMax EC	Bayer Crop Science Research Triangle Park, NC	Dicamba (42.8%)	4	22.0 fl oz/A	1.60 L/ha
Liberty EC	BASF Research Triangle Park, NC	Glufosinate (24.5%)	10	32.0 to 43.0 fl oz/A	2.34 to 3.14 L/ha
Enlist One EC	Corteva Agriscience Johnston, IA	2,4-Dichlorophenoxyacetic acid (55.7%)	4	1.5 to 2.0 pt/A	1.75 to 2.33 L/ha
Voliminate	Precision Laboratories Waukegan, IL	Potassium Hydroxide (50%)		20.0 fl oz/A	1.46 L/ha

[z] FRAC = Fungicide resistance action committee. BM02: Biologicals with multiple modes of action, microbial. 11: Quinone outside Inhibitors (QoI). 7: Succinate-dehydrogenase inhibitors (SDHI). 29: Uncouplers of oxidative phosphorylation.
HRAC = Herbicide resistance action committee. 14: Inhibition of protoporphyrinogen oxidase. 15: Inhibition of very long chain fatty acids. 5: Inhibition of photosynthesis at PS ll. 2: Inhibition of acetolactate synthase. 9: Inhibition of enolpyruvyl shikimate phosphate synthase. 4: Auxin mimics. 10: Inhibition of glutamine synthetase.

3.3.4 Poison plate assay

Stock solutions with a concentration of 100 mg/mL of each pesticide were created by dissolving 20 mg of analytical grade pesticide in 200 µL of solvent (Sigma-Aldrich, St. Louis, MO). The purity of all analytical grade pesticides was greater than 95%. The stock solutions for flumioxazin, S-metolachlor, metribuzin, cloransulam-methyl, dicamba, and 2,4-D were created using dimethylformamide (DMF). The stock solution for glufosinate was created using sterile deionized water. The stock solution for glyphosate was created using sterile deionized water plus 50 µL of a 10% sodium hydroxide solution. Picoxystrobin, boscalid, and fluazinam stock solutions were created using dimethyl sulfoxide (DMSO). The concentration of each stock solution was then adjusted to achieve final concentrations of 0.01, 0.1, 1, and 10 µg/mL when added to PDA media. PDA was autoclaved and cooled to at least 18°C before amending with each pesticide. The non-treated controls were PDA where no pesticides were added and PDA with the respective solvents.

A 6-mm plug taken from an actively growing 7-day old *C. minitans* isolate was placed upside down in the center of each Petri dish. The Petri dishes were incubated at 20°C with 12 h light and 12 h dark. Radial growth (mm) along two axes was measured after 5, 10, and 14 days. The two axes were averaged before analysis. The preemergence herbicides, postemergence herbicides, and synthetic fungicides were separated into their own experiments with their respective controls. Each experiment had a randomized complete block design with four replications and were repeated twice. The percentage of mycelial growth inhibition (PMGI) was calculated according to Equation 2. Where D_{PDA} is the average isolate diameter of the control and D_F is the average isolate diameter of the plates amended with each pesticide respectively after 14 days of growth.

Equation 2. Percentage of mycelial growth inhibition (PMGI).

$$\text{PMGI} = 100 * \left[\frac{D_{PDA} - D_F}{D_{PDA}}\right]$$

A 10^6 serial dilution of *B. amyloliquefaciens* was created using sterile deionized water. Ten µL of the 10^6 serial dilution was placed on the center of each Petri dish. The solution was then spread into an approximately 1-cm circle with a sterile metal inoculating loop. The Petri dishes were incubated at 25°C with 12 h light and 12 h dark. After two days, the plates were evaluated

for colony growth, where yes meant that colony growth occurred and no meant there was no growth.

3.3.5 Soil plate assay

A modified soil plate technique described by Smith et al. (1991) was used. Potting mix (Redi-Earth Propagation Mix; Sungro Horticulture, Agawam, MA) was autoclaved for 35 min, then 6 g of potting mix was placed into a 9-cm plastic Petri dish. Each Petri dish was then sprayed with 5 mL of deionized water using a hand atomizer. Five sclerotia were surface sterilized in 10% sodium hypochlorite for 30 s. The sclerotia were then rinsed in sterile deionized water, dried on a sterile paper towel, and placed on the soil surface. The application rate for all pesticides was converted from rate per hectare to rate per Petri dish using the surface area of the Petri dish (57 cm^2). *C. minitans* was applied first as the formulated product Contans WG at a field application rate of 2.24 kg/ha using a hand atomizer (Solid USA, Irvine, CA). The hand atomizer was then used to make a subsequent application of each pesticide at the recommended field application rate. Each experiment had a non-treated control that did not receive a treatment. After the treatments were applied, the plates were incubated at 20°C with 12 h light and 12 h dark for four weeks.

After four weeks, all five sclerotia were collected from each plate, surface sterilized in 10% sodium hypochlorite, rinsed in sterile deionized water, and dried on a sterile paper towel. Each sclerotia were then individually plated on PDA + 0.05% Rifampicin (v/v) plates. The plates were then placed in an incubator at 25°C with 12 h light and 12 h dark for one week. After one week, the radial growth (mm) along two axes of each plate was measured. Before the data were analyzed, the values for the two axes were averaged and then the five sclerotia per treatment were averaged. The interaction between *C. minitans* and preemergence herbicides, postemergence herbicides, and synthetic fungicides were separated into their own respective experiments. Four replicates were included in each experiment and the experiment was repeated twice. The PMGI was calculated according to Equation 2 and used as the observed values for the Colby's method analysis. The expected values for the Colby's method analysis were calculated according to Equation 3 (Colby 1967). Where A is the PMGI for the *C. minitans* treatment and B is the PMGI for the other applied pesticides.

Equation 3. Colby's method equation.

$$E = A + B - \frac{A*B}{100}$$

3.3.6 Controlled environment experiments

The growth chamber (Conviron BDW190; Controlled Environments Limited, Winnipeg, Canada) conditions were set to 14 h light and 10 h dark with a light intensity of 500 μmol/m^2s and a constant temperature of 20°C. The plants were watered both on the soil surface and from the base on trays, after treatments were applied the plants were only watered from the base. The soybean varieties, P34A79X and P32A87L, were sowed in 15-cm pots at a rate of two seeds per pot. The variety P34A79X was selected as it has the RoundUp Ready 2 Xtend herbicide tolerance trait which allows for the application of glyphosate and dicamba, and P32A87L was selected as it has the Liberty Link herbicide tolerance trait which allows for the application of glufosinate. The plants were thinned to one seedling per pot two weeks after planting. Each experiment had a randomized complete block design with four replications and were repeated twice. Prior to treatment application the fourth vegetative leaf (V4) was cut leaving an exposed petiole. *B. amyloliquefaciens* was applied as formulated product using the dip method where the exposed petiole was dipped in a solution of the treatment. All other treatments were applied using a spray booth (Generation III; DeVries Manufacturing, Hollendale, MN) fitted with an XR8002 nozzle that applied the treatments at 206.84 kPa with a sprayer height of about 50.8 cm. The herbicides were applied at 187.1 L/ha and the synthetic fungicides were applied at 140.2 L/ha. The plants were transferred to a greenhouse with 14 h light and 10 h dark with a minimum temperature of 18°C and a maximum temperature of 29°C one day prior to treatment applications.

For the experiment looking at the interaction between *B. amyloliquefaciens* and postemergence herbicides, the herbicide treatments were applied on the first day, allowed to dry on the bench in the greenhouse overnight, and *B. amyloliquefaciens* was applied the following day. For the experiment looking at the interaction between *B. amyloliquefaciens* and synthetic fungicides, *B. amyloliquefaciens* was applied on the first day, allowed to dry on the bench in the greenhouse overnight, and the following day the synthetic fungicides were applied. All plants were transferred back to the growth chamber on the third day and inoculated using the pipet tip method (Botha et al. 2009). Plugs were cut from an actively growing 7-day old *S. sclerotiorum* isolate

using a 200 µL pipet tip (Labtips Pipette Tips; Fisher Scientific, Waltham, MA). The pipet tip containing the *S. sclerotiorum* plug was then placed on the exposed petiole. Lesion length along the main stem (mm) was measured 6, 11, and 14 days after inoculation (DAI) using calipers. The reduction in lesion length was calculated according to Equation 2 and used as the observed values for the Colby's method analysis. The expected values for the Colby's method analysis were calculated according to Equation 3. Lesion area under the disease progress curve (lAUDPC) was calculated using Equation 4 (Simko and Piepho 2012). Where t_i is the current time point and y_i is the corresponding disease rating, t_{i+1} is the next time point in the series and y_{i+1} is the corresponding disease rating.

Equation 4. Lesion area under the disease progress curve (lAUDPC).

$$\text{lAUDPC} = \sum_{i=1}^{N_{i-1}} \left(\frac{y_i + y_{i+1}}{2}\right) * (t_{i+1} - t_i)$$

3.3.7 Field experiments

Three separate experiments were conducted to examine the interaction between the biofungicides and preemergence herbicides, postemergence herbicides, and synthetic fungicides respectively. The three experiments were established at the Agronomy Center for Research and Education (ACRE) in West Lafayette, IN and the Pinney Purdue Agricultural Center (PPAC) in Wanatah, IN in 2020 and 2021. Field trial information on variety, planting date, irrigation, pesticide application date, growth stage at the time of application, and harvest date are found in Tables 8 and 9. The experiments were a randomized complete block design with four replications. Plots were either 1.5-m or 2.04-m wide and 9.14-m long and consisted of four rows. In 2020 the previous crop was corn, in 2021 the previous crop was either sunflower or soybean. Standard practices for weed management in soybean production in Indiana were followed. All plots were inoculated with *S. sclerotiorum* at 0.04 g/cm within the seedbed at planting and in 2021 sclerotia at 5 g/plot were also spread between the middle two rows prior to emergence in the experiment looking at the interaction between the biofungicides and synthetic fungicides. At PPAC, overhead irrigation was applied weekly at approximately 25 mm unless weekly rainfall was 25 mm or higher to encourage disease. In 2020, a Lee self-propelled sprayer equipped with a 3-m boom, fitted with six TJ-VS 8002 nozzles spaced 50.8-cm apart was used to make the treatment applications and in

2021, a CO_2 backpack sprayer equipped with a 3-m boom, fitted with six TJ-VS 8002 nozzles spaced 50.8-cm apart was used. The biofungicides and synthetic fungicides were applied at 140.2 L/ha and the herbicides were applied at 187.08 L/ha. All treatments were applied at 206.84 kPa. The two center rows of each plot were harvested with a Kincaid XP8 combine and yields were adjusted to 13% moisture.

Table 8. Trial details for field experiments used to explore the interaction between *Coniothyrium minitans* and *Bacillus amyloliquefaciens* and preemergence herbicides, postemergence herbicides, and synthetic fungicides.

Year	Location[z]	Experiment	Variety	Planting Date	Irrigation (Y/N)[y]	Harvest Date
2020	ACRE	Preemergence herbicides	P34A79X	6/1/2020	N	10/14/2020
		Postemergence herbicides	P34A79X	6/1/2021	N	10/14/2020
		Synthetic fungicides	P34A79X	6/1/2020	N	10/14/2020
	PPAC	Preemergence herbicides	P34A79X	6/6/2020	Y	11/2/2020
		Postemergence herbicides	P34A79X	6/6/2020	Y	11/2/2020
		Synthetic fungicides	P34A79X	6/6/2020	Y	11/2/2020
2021	ACRE	Preemergence herbicides	P34A79X	5/15/2021	N	10/10/2021
		Postemergence herbicides	P34A79X	5/15/2021	N	10/18/2021
		Synthetic fungicides	P34A79X	5/15/2021	N	10/18/2021
	PPAC	Preemergence herbicides	P34A79X	5/25/2021	Y	9/29/2021
		Postemergence herbicides	P34A79X	5/25/2021	N	9/29/2021
		Synthetic fungicides	P34A79X	5/24/2021	Y	10/1/2021

[z] ACRE = Agronomy Center for Research and Education, West Lafayette, IN. PPAC = Pinney Purdue Agricultural Center, Wanatah, IN.
[y] Irrigation was applied weekly at approximately 25 mm unless weekly rainfall was 25 mm or higher to encourage disease.

Table 9. Treatment details for field experiments used to explore the interaction between *Coniothyrium minitans* and *Bacillus amyloliquefaciens* and preemergence herbicides, postemergence herbicides, and synthetic fungicides.

		Application date and timing[y]			
Experiment	**Treatment and rate/ha[z]**	**ACRE 2020[x]**	**PPAC 2020**	**ACRE 2021**	**PPAC 2021**
Preemergence herbicides	*C. minitans* 2.24 kg	6/2/2020 Pre	6/7/2020 Pre	5/15/2021 Pre	5/26/2021 Pre
	B. amyloliquefaciens 4.68 L	7/15/2020 R1	7/21/2020 R1	7/13/2021 R2	7/30/2021 R3
	Flumioxazin 0.21 kg	6/2/2020 Pre	6/7/2020 Pre	5/15/2021 Pre	5/26/2021 Pre
	S-metolachlor 3.04 L	6/2/2020 Pre	6/7/2020 Pre	5/15/2021 Pre	5/26/2021 Pre
	Metribuzin 0.67 kg	6/2/2020 Pre	6/7/2020 Pre	5/15/2021 Pre	5/26/2021 Pre
Postemergence herbicides	*C. minitans* 2.24 kg	6/2/2020 Pre	6/7/2020 Pre	5/15/2021 Pre	5/26/2021 Pre
	B. amyloliquefaciens 4.68 L	7/15/2020 R1	7/21/2020 R1	7/13/2021 R2	7/30/2021 R3
	Cloransulam-methyl 0.04 kg	6/18/2020 V1	6/19/2020 VC	6/16/2021 V2	6/20/2021 V2/V3
	Glyphosate 1.60 L	6/18/2020 V1	6/19/2020 VC	6/16/2021 V2	6/20/2021 V2/V3
	Dicamba 1.60 L	6/18/2020 V1	6/18/2020 VC	6/16/2021 V2	6/20/2021 V2/V3
Synthetic fungicide	*C. minitans* 2.24 kg	6/2/2020 Pre	6/7/2020 Pre	5/15/2021 Pre	5/26/2021 Pre
	B. amyloliquefaciens 4.68 L	7/15/2020 R1	7/21/2020 R1	7/13/2021 R2	7/19/2021 R2
	Picoxystrobin 0.88 L	7/17/2020 R1	7/23/2020 R1	7/14/2021 R2	7/19/2021 R2
	Boscalid 0.56 kg	7/17/2020 R1	7/23/2020 R1	7/14/2021 R2	7/19/2021 R2
	Fluazinam 0.88 L	7/17/2020 R1	7/23/2020 R1	7/14/2021 R2	7/19/2021 R2

[z] *Coniothyrium minitans* (Contans WG; Sipcam Agro USA Inc., Durham, NC), *Bacillus amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Columbia, NC), flumioxazin (Valor SX WDG; Valent USA LLC, Walnut Creek, CA), S-metolachlor (Dual Magnum EC; Syngenta, Greensboro, NC), metribuzin (Tricor DF; Corteva Agriscience, Johnston, IA), cloransulam-methyl (First Rate WDG, Corteva Agriscience, Johnston, IA), glyphosate (RoundUp PowerMax EC, Bayer Crop Science, Research Triangle Park, NC), dicamba (XtendiMax EC, Bayer Crop Science, Research Triangle Park, NC), picoxystrobin (Aproach SC, Corteva Agriscience, Johnston, IA), boscalid (Endura WDG, BASF, Research Triangle Park, NC), and fluazinam (Omega 500F, Syngenta, Greensboro, NC).

[y] Timing: Pre = Preemergence, VC = Cotyledon, V1 = First vegetative, V2 = Second vegetative, V3 = Third vegetative, R1 = Beginning bloom, R2 = Full bloom, R3 = Beginning pod. In 2020 treatments were applied using a Lee self-propelled sprayer, and in 2021 a CO_2 backpack sprayer was used to make applications.

[x] ACRE = Agronomy Center for Research and Education, West Lafayette, IN. PPAC = Pinney Purdue Agricultural Center, Wanatah, IN.

3.3.8 Data analyses

Outliers were first removed from all datasets if the absolute value of the studentized residual was greater than 3. Typically, a value of 2.5 for the absolute value of the studentized residual is used to identify outliers, however to allow for variation in the dataset a value of 3 was selected instead. Plots of the residuals were used to select the best distribution for each dataset. The data were combined across repetition for the poison plate assay, soil plate, and growth chamber datasets, and the data for the field experiments were combined across year and location prior to analysis.

To determine the effect of treatment on the radial growth (mm) of *C. minitans* in the poison plate assay and to determine the effect of treatment on the radial growth (mm) of *S. sclerotiorum* in the soil plate assay the data were analyzed using a generalized linear mixed model with a lognormal distribution utilizing PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC). Treatment was the main effect in the model, and the random effect was experiment. To determine the effect of treatment on the colony formation (yes/no) of *B. amyloliquefaciens* in the poison plate assay the data were analyzed using a generalized linear model with a binary distribution utilizing the "brglm" package in RStudio 3.6.2 (R Code Team, Vienna, Austria). Significant differences between treatments in each experiment were assessed using Fisher's least significant difference at $\alpha = 0.05$. The pesticide dose which inhibited the mycelial growth of *C. minitans* by 50% (EC_{50}) in the poison plate assay was calculated using the "drc" package in RStudio 3.6.2.

To determine the effect of treatment on Sclerotinia stem rot lesion length (mm) in the controlled environment experiment as well as to determine the effect of treatment on soybean moisture, test weight, and yield in the field experiments the data were analyzed using a generalized linear mixed model with a normal distribution utilizing PROC GLIMMIX in SAS 9.4. Treatment was the main effect in the model, and the random effect was experiment. Significant differences between treatments in each experiment were assessed using Fisher's least significant difference at $\alpha = 0.05$.

Colby's method was used to classify the relationship between the biofungicides and the other applied chemicals in the soil plate assay and controlled environment experiments. The observed and expected values were compared using a paired T-test in SAS 9.4. The equality of variances was first tested using a Folded F-test ($\alpha = 0.05$). Difference between the means were assessed using a Satterthwaite T-test ($\alpha = 0.05$). The relationship was classified as synergistic if the difference

between the means was significant and the observed value was greater than the expected value. The relationship was antagonistic if the difference between the means was significant and the observed values was less than the expected value. If the difference between the means was not significant the relationship was additive (Colby 1967; Kandel et al. 2018).

3.4 Results

3.4.1 Poison plate assay

Significant differences were observed between treatments for the radial growth (mm) of *C. minitans* in the poison plate assay (Figure 6 and Table 10). Flumioxazin at 0.01 µg/mL did not significantly reduce the radial growth of *C. minitans*, while flumioxazin at 10 µg/mL reduced the radial growth by 83.1%. S-metolachlor and metribuzin at 0.01 µg/mL reduced radial growth of *C. minitans* by 21.3% and 22.8% respectively. At 10 µg/mL S-metolachlor reduced the radial growth of *C. minitans* by 14.6% and metribuzin reduced the radial growth by 14.4%. Cloransulam-methyl at 10 µg/mL reduced the radial growth of *C. minitans* by 13.1%. Cloransulam-methyl at 1, 0.1, and 0.01 µg/mL as well as dicamba, glufosinate, and 2,4-D at all concentrations did not significantly reduce the radial growth of *C. minitans*. Glyphosate at 0.01 µg/mL decreased the radial growth of *C. minitans* by 4.2% while 10 µg/mL increased the radial growth by 4.7%. Picoxystrobin, boscalid, and fluazinam at 0.01 µg/mL did not significantly reduce the radial growth of *C. minitans*. Picoxystrobin at 10 µg/mL reduced the radial growth by 36.7%. Boscalid and fluazinam at 10 µg/mL reduced the radial growth of *C. minitans* by 77.2% and 73.9% respectively.

Only three of the pesticides reduced the radial growth of *C. minitans* by more than 50% (Table 11). The preemergence herbicide flumioxazin reduced the radial growth of *C. minitans* by up to 83.1%. The synthetic fungicides boscalid and fluazinam reduced the radial growth of *C. minitans* by up to 77.2% and 73.9%, respectively. The concentration which reduced the radial growth of *C. minitans* by 50% (EC_{50}) was 0.80 µg/mL for flumioxazin, 0.69 µg/mL for boscalid, and 0.13 µg/mL for fluazinam.

Fluazinam was the only treatment where *B. amyloliquefaciens* growth was affected (Table 12). The probability of *B. amyloliquefaciens* growth on the fluazinam plates was 0.05 regardless of concentration. While the probability of *B. amyloliquefaciens* growth was 0.94 on all other treatments at all concentrations.

Table 10. Effect of preemergence herbicides, postemergence herbicides, and synthetic fungicides on radial mycelial growth (mm) of *Coniothyrium minitans*.

	Pesticide concentration (µg/mL)				
	0	**0.01**	**0.1**	**1**	**10**
Pesticide[z]	***C. minitans* radial growth mm (%)[y]**				
Preemergence herbicides					
Flumioxazin	61.2 a	57.0 (6.8) a	39.8 (35.0) c	31.3 (48.9) d	10.4 (83.1) e
S-metolachlor	56.0 a	44.0 (21.3) c	55.4 (1.0) ab	48.4 (13.4) abc	47.8 (14.6) bc
Metribuzin	61.2 a	47.1 (22.8) bc	53.4 (26.6) ab	44.8 (26.6) c	52.3 (14.4) b
Postemergence herbicides					
Cloransulam-methyl	65.7 a	64.2 (2.2) a	64.4 (1.9) a	65.0 (1.0) a	57.1 (13.1) b
Glyphosate	65.5 b	62.8 (4.2) c	64.9 (1.0) bc	69.4 (-5.9) a	68.6 (-4.7) a
Dicamba	65.7	66.9 (-1.9)	65.4 (0.5)	65.7 (0.0)	66.7 (-1.5)
Glufosinate	68.1 ab	68.1 (-3.8) a	68.1 (-3.7) a	68.7 (-4.6) a	63.6 (3.2) b
2,4-D	65.6	62.6 (4.6)	63.9 (2.7)	64.2 (2.2)	65.9 (-0.2)
Synthetic fungicides					
Picoxystrobin	45.7 a	43.9 (4.3) ab	37.4 (18.4) bc	33.9 (26.1) dc	29.0 (36.7) d
Boscalid	45.9 a	51.2 (-11.5) a	44.5 (3.0) a	17.8 (61.3) b	10.4 (77.2) c
Fluazinam	45.9 b	56.3 (-22.7) a	32.5 (29.2) c	16.0 (65.1) d	12.0 (73.9) e

[z] Analytical grade pesticide with purity greater than 95%.

[y] Data are given as radial growth (mm) of *C. minitans* after 14 days of incubation at 20°C followed by percent mycelial growth inhibition (PMGI) of the non-amended control. Data represent the mean of four replicates from two experiments. Data were pooled over two experiments prior to analysis. Least squares means were separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not significantly different across each row.

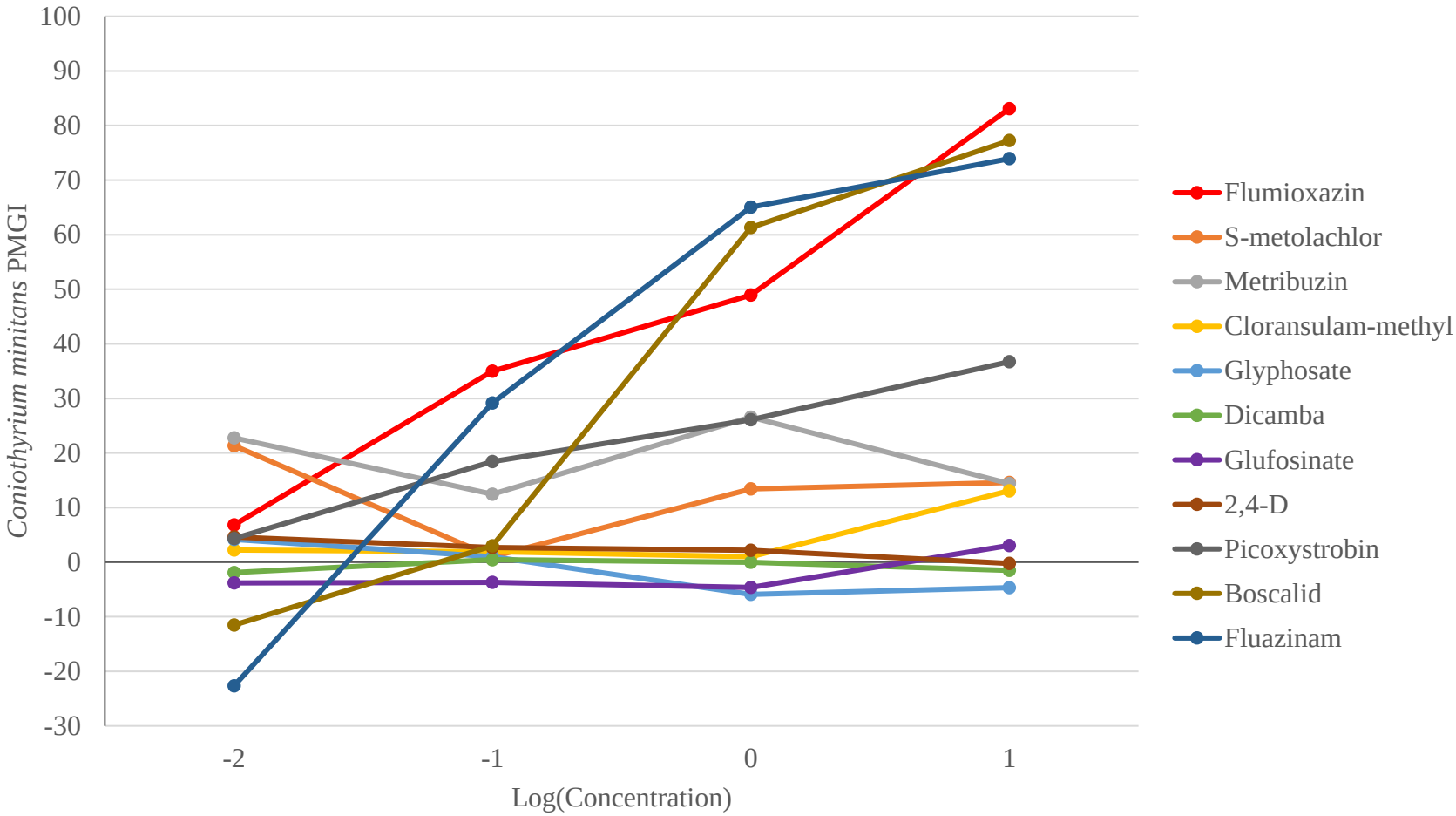

Figure 6. Effect of preemergence herbicides, postemergence herbicides, and synthetic fungicides at concentrations of 0.01, 0.1, 1, and 10 µg/mL on percent mycelial growth inhibition (PMGI) over the non-amended control of *Coniothyrium minitans* after 14 days of incubation at 20°C. Data represent the mean of four replicates from two experiments. Data pooled over two experiments prior to analysis.

Table 11. Concentration of preemergence herbicides, postemergence herbicides, and synthetic fungicides that inhibited the radial mycelial growth of *Coniothyrium minitans* by 50% (EC_{50}).

Pesticide[z]	Recommended application rate (g a.i./ha)[y]	Pesticide concentration (µg/mL)[x]	Mycelial growth EC_{50} (µg/mL)[w]
Preemergence herbicides			
Flumioxazin	108.25	578.57	0.80
S-metolachlor	2160.22	11545.80	>10*
Metribuzin	510.29	2727.37	>10*
Postemergence herbicides			
Cloransulam-methyl	35.72	190.91	>10*
Glyphosate	1071.96	5729.34	>10*
Dicamba	565.23	3021.01	>10*
Glufosinate	891.41	4764.35	>10*
2,4-D	1077.28	5757.78	>10*
Synthetic fungicides			
Picoxystrobin	221.13	1577.21	>10*
Boscalid	399.16	2847.07	0.69
Fluazinam	443.31	3161.38	0.13

[z] Analytical grade pesticide with purity greater than 95%.
[y] Recommended application rate for the commercially formulated product of each pesticide.
[x] Pesticide concentration for the commercially formulated product was calculated using the recommended application rate for each pesticide and a rate of 187.1 L/ha for the preemergence and postemergence herbicides and 140.2 L/ha for the synthetic fungicides.
[w] Data are given as the pesticide dose which inhibits the mycelial growth of *C. minitans* by 50% (EC_{50}). EC_{50} values were calculated using the "drc" package in RStudio 3.6.2. * = No effect at concentrations used in this experiment.

Table 12. Effect of preemergence herbicides, postemergence herbicides, and synthetic fungicides on probability of *Bacillus amyloliquefaciens* colony formation.

	Pesticide concentration (µg/mL)				
	0	**0.01**	**0.1**	**1**	**10**
Pesticide[z]	**Probability of *B. amyloliquefaciens* growth[y]**				
Preemergence herbicides					
Flumioxazin	0.94	0.94	0.94	0.94	0.94
S-metolachlor	0.94	0.94	0.94	0.94	0.94
Metribuzin	0.94	0.94	0.94	0.94	0.94
Postemergence herbicides					
Cloransulam-methyl	0.94	0.94	0.94	0.94	0.94
Glyphosate	0.94	0.94	0.94	0.94	0.94
Dicamba	0.94	0.94	0.94	0.94	0.94
Glufosinate	0.94	0.94	0.94	0.94	0.94
2,4-D	0.94	0.94	0.94	0.94	0.94
Synthetic fungicides					
Picoxystrobin	0.94	0.94	0.94	0.94	0.94
Boscalid	0.94	0.94	0.94	0.94	0.94
Fluazinam	0.94 a	0.05 b	0.05 b	0.05 b	0.05 b

[z] Analytical grade pesticide with purity greater than 95%.

[y] Data represent the probability of *B. amyloliquefaciens* colony growth after 2 days of incubation at 25°C. Four replicates were included in each experiment and the experiment was repeated twice. Data were pooled over two experiments prior to analysis. Least squares means of the probability were separated using Fisher's least significant difference ($\alpha = 0.05$). Probability followed by the same letter are not significantly different across each row.

3.4.2 Soil plate assay

In the soil plate assay significant differences were observed between pesticide treatments for the radial growth (mm) of *S. sclerotiorum*. In the experiment looking at the interaction between *C. minitans* and preemergence herbicides, *C. minitans* reduced the radial growth of *S. sclerotiorum* by 63.1% when compared to the non-treated control (Figure 7 and Table 13). Flumioxazin, S-metolachlor, and metribuzin applied alone were not significantly different from the non-treated control for the radial growth of *S. sclerotiorum*. *C. minitans* followed by S-metolachlor was not significantly different from *C. minitans* applied alone. However, the radial growth of *S. sclerotiorum* for *C. minitans* followed by metribuzin or flumioxazin were significantly different from *C. minitans* applied alone. Using Colby's method, the relationship between *C. minitans* and flumioxazin was classified as antagonism. The relationship between *C. minitans* and both S-metolachlor and metribuzin was classified as additive.

C. minitans reduced the radial growth of *S. sclerotiorum* by 60.9% when compared to the non-treated control in the experiment looking at the interaction between *C. minitans* and postemergence herbicides (Figure 8 and Table 14). Cloransulam-methyl, glyphosate, dicamba, and 2,4-D applied alone were not significantly different from the non-treated control. Glufosinate applied alone significantly reduced the radial growth of *S. sclerotiorum* when compared to the non-treated control. *C. minitans* followed by cloransulam-methyl, dicamba, glufosinate, or 2,4-D were not significantly different from *C. minitans* applied alone. *C. minitans* followed by glyphosate was significantly different from *C. minitans* applied alone for the radial growth of *S. sclerotiorum*. The relationship between *C. minitans* and glyphosate was classified as antagonism using Colby's method. The relationship between *C. minitans* and cloransulam-methyl, dicamba, glufosinate, and 2,4-D was classified as additive.

In the experiment looking at the interaction between *C. minitans* and synthetic fungicides, *C. minitans* reduced the radial mycelial growth of *S. sclerotiorum* by 54.9% when compared to the non-treated control (Figure 9 and Table 15). Picoxystrobin and boscalid applied alone were not significantly different from the non-treated control. Fluazinam applied alone was significantly different from the non-treated control for the radial growth of *S. sclerotiorum*. *C. minitans* followed by fluazinam was not significantly different from *C. minitans* applied alone. *C. minitans* followed by picoxystrobin or boscalid were significantly different from *C. minitans* applied alone for the

radial growth of *S. sclerotiorum*. The relationship between *C. minitans* and picoxystrobin, boscalid, and fluazinam were all classified as antagonism using Colby's method.

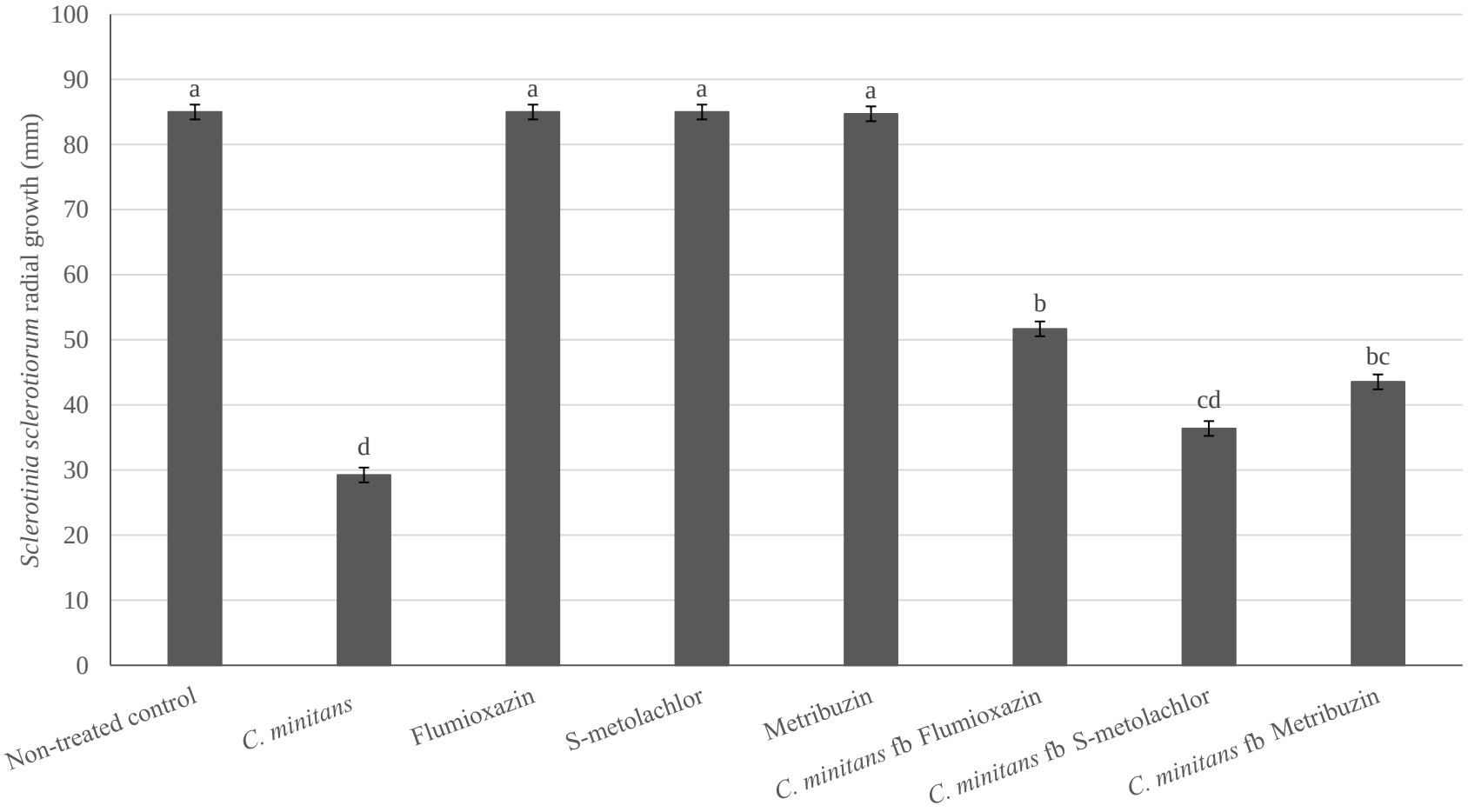

Figure 7. Influence of preemergence herbicides on ability of *Coniothyrium minitans* to degrade sclerotia of *Sclerotinia sclerotiorum*. Data are given as radial growth (mm) of *S. sclerotiorum* after 4 weeks of incubation at 20°C. Data represent the mean of four replicates from two experiments. Data pooled over two experiments prior to analysis. Least squares means were separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not significantly different. Error bars represent standard error of the mean. fb = followed by. $F = 22.89$, $p = 0.0001$.

Table 13. Influence of preemergence herbicides on ability of *Coniothyrium minitans* to degrade sclerotia of *Sclerotinia sclerotiorum*.

Treatment and rate/ha[z]	*S. sclerotiorum* radial growth (mm)[y]	PGMI observed[x]	PMGI expected[w]	*P*-value[v]	Relationship[u]
Non-treated control	85.0 a	.	.	.	.
C. minitans 2.24 kg	29.2 d	63.1	.	.	.
Flumioxazin 0.21 kg	85.0 a	0.0	.	.	.
S-metolachlor 3.04 L	85.0 a	0.0	.	.	.
Metribuzin 0.67 kg	84.7 a	0.3	.	.	.
C. minitans 2.24 kg fb Flumioxazin 0.21 kg	51.7 b	34.5	63.1	0.0053	Antagonism
C. minitans 2.24 kg fb S-metolachlor 3.04 L	36.4 cd	54.6	63.1	0.1946	Additive
C. minitans 2.24 kg fb Metribuzin 0.67 kg	43.5 bc	49.9	63.2	0.2023	Additive

[z] *C. minitans* (Contans WG; Sipcam Agro USA Inc., Durham, NC), flumioxazin (Valor SX WDG; Valent USA LLC, Walnut Creek, CA), S-metolachlor (Dual Magnum EC; Syngenta, Greensboro, NC), and metribuzin (Tricor DF; Corteva Agriscience, Johnston, IA). fb = followed by.

[y] Data are given as radial growth (mm) of *S. sclerotiorum* after 4 weeks of incubation at 20°C. Data represent the mean of four replicates from two experiments. Data were polled over two experiments prior to analysis. Least squares means were separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not significantly different.

[x] PMGI = Percent mycelial growth inhibition.

[w] PMGI expected values were calculated using the Colby's method equation.

[v] Differences between the PMGI observed and expected means were assessed using a Satterthwaite T-test ($\alpha = 0.05$).

[u] The relationship between *C. minitans* and preemergence herbicides was classified using Colby's method. The relationship was classified as synergistic if the difference between the means was significant and the observed value was greater than the expected value. The relationship is antagonistic if the difference between the means was significant and the observed values is less than the expected value. If the difference between the means is not significant the relationship is additive.

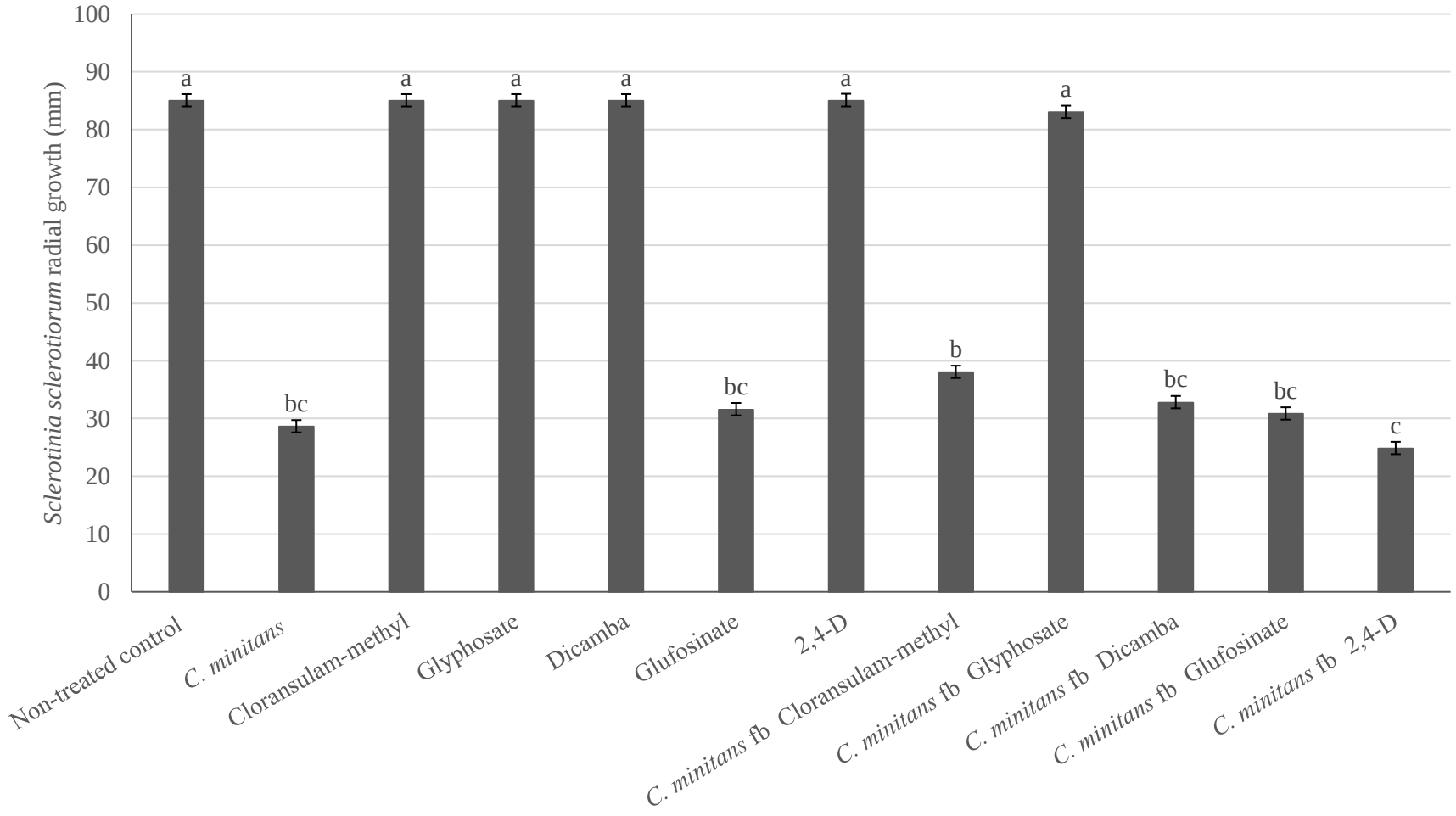

Figure 8. Influence of postemergence herbicides on ability of *Coniothyrium minitans* to degrade sclerotia of *Sclerotinia sclerotiorum*. Data are given as radial growth (mm) of *S. sclerotiorum* after 4 weeks of incubation at 20°C. Data represent the mean of four replicates from two experiments. Data pooled over two experiments prior to analysis. Least squares means were separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not significantly different. Error bars represent standard error of the mean. fb = followed by. $F = 16.16$, $p = 0.0001$.

Table 14. Influence of postemergence herbicides on ability of *Coniothyrium minitans* to degrade sclerotia of *Sclerotinia sclerotiorum*.

Treatment and rate/ha[z]	*S. sclerotiorum* radial growth (mm)[y]	PGMI observed[x]	PMGI expected[w]	*P*-value[v]	Relationship[u]
Non-treated	85.0 a	.	.	.	.
C. minitans 2.24 kg	28.6 bc	60.9	.	.	.
Cloransulam-methyl 0.04 kg	85.0 a	0.0	.	.	.
Glyphosate 1.60 L	85.0 a	0.0	.	.	.
Dicamba 1.60 L	85.0 a	0.0	.	.	.
Glufosinate 3.14 L	31.5 bc	67.3	.	.	.
2,4-D 2.34 L	85.0 a	0.0	.	.	.
C. minitans 2.24 kg fb Cloransulam-methyl 0.04 kg	38.0 b	51.2	60.9	0.4454	Additive
C. minitans 2.24 kg fb Glyphosate 1.60 L	83.0 a	2.2	60.9	0.0001	Antagonism
C. minitans 2.24 kg fb Dicamba 1.60 L	32.8 bc	58.0	60.9	0.7636	Additive
C. minitans 2.24 kg fb Glufosinate 3.14 L	30.8 bc	57.6	85.2	0.0573	Additive
C. minitans 2.24 kg fb 2,4-D 2.34 L	24.8 c	67.4	61.3	0.9096	Additive

[z] *C. minitans* (Contans WG; Sipcam Agro USA Inc., Durham, NC), cloransulam-methyl (First Rate WDG, Corteva Agriscience, Johnston, IA), glyphosate (RoundUp PowerMax EC, Bayer Crop Science, Research Triangle Park, NC), dicamba (XtendiMax EC, Bayer Crop Science, Research Triangle Park, NC), glufosinate (Liberty EC, BASF, Research Triangle Park, NC), and 2,4-D (EnlistOne EC, Corteva Agriscience, Johnston, IA). fb = followed by.

[y] Data are given as radial growth (mm) of *S. sclerotiorum* after 4 weeks of incubation at 20 °C. Data represent the mean of four replicates from two experiments. Data were polled over two experiments prior to analysis. Least squares means were separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not significantly different.

[x] PMGI = Percent mycelial growth inhibition.

[w] PMGI expected values were calculated using the Colby's method equation.

[v] Differences between the PMGI observed and expected means were assessed using a Satterthwaite T-test ($\alpha=0.05$).

[u] The relationship between *C. minitans* and preemergence herbicides was classified using Colby's method. The relationship was classified as synergistic if the difference between the means was significant and the observed value was greater than the expected value. The relationship is antagonistic if the difference between the means was significant and the observed values is less than the expected value. If the difference between the means is not significant the relationship is additive.

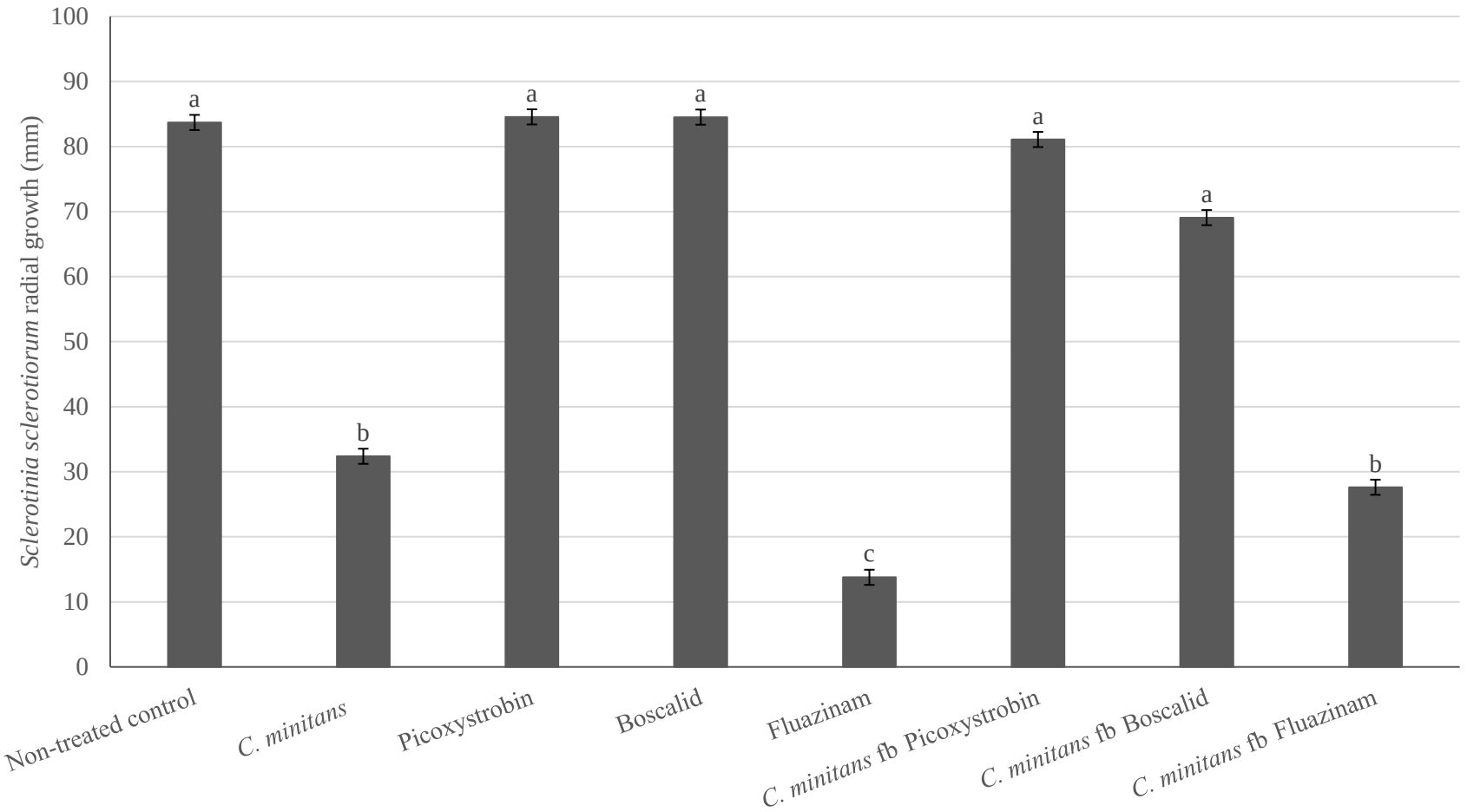

Figure 9. Influence of synthetic fungicides on ability of *Coniothyrium minitans* to degrade sclerotia of *Sclerotinia sclerotiorum*. Data are given as radial growth (mm) of *S. sclerotiorum* after 4 weeks of incubation at 20°C. Data represent the mean of four replicates from two experiments. Data pooled over two experiments prior to analysis. Means were separated using Fisher's least significant difference ($\alpha = 0.05$). Least squares means followed by the same letter are not significantly different. Error bars represent standard error of the mean. fb = followed by. $F = 29.41$, $p = 0.0001$.

Table 15. Influence of synthetic fungicides on ability of *Coniothyrium minitans* to degrade sclerotia of *Sclerotinia sclerotiorum*.

Treatment and rate/ha[z]	*S. sclerotiorum* radial growth (mm)[y]	PGMI observed[x]	PMGI expected[w]	*P*-value[v]	Relationship[u]
Non-treated control	83.7 a	.	.	.	.
C. minitans 2.24 kg	32.4 b	54.9	.	.	.
Picoxystrobin 0.88 L	84.6 a	-1.1	.	.	.
Boscalid 0.56 kg	84.5 a	-1.0	.	.	.
Fluazinam 0.88 L	13.8 c	76.1	.	.	.
C. minitans 2.24 kg fb Picoxystrobin 0.88 L	81.1 a	2.9	54.8	0.0007	Antagonism
C. minitans 2.24 kg fb Boscalid 0.56 kg	69.1 a	14.4	54.7	0.0111	Antagonism
C. minitans 2.24 kg fb Fluazinam 0.88 L	27.6 b	64.3	91.2	0.0005	Antagonism

[z] *C. minitans* (Contans WG; Sipcam Agro USA Inc., Durham, NC), picoxystrobin (Aproach SC, Corteva Agriscience, Johnston, IA), boscalid (Endura WDG, BASF, Research Triangle Park, NC), and fluazinam (Omega 500F, Syngenta, Greensboro, NC). fb = followed by.

[y] Data are given as radial growth (mm) of *S. sclerotiorum* after 4 weeks of incubation at 20°C. Data represent the mean of four replicates from two experiments. Data were polled over two experiments prior to analysis. Least squares means were separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not significantly different.

[x] PMGI = Percent mycelial growth inhibition.

[w] PMGI expected values were calculated using the Colby's method equation.

[v] Differences between the PMGI observed and expected means were assessed using a Satterthwaite T-test (α=0.05).

[u] The relationship between *C. minitans* and preemergence herbicides was classified using Colby's method. The relationship was classified as synergistic if the difference between the means was significant and the observed value was greater than the expected value. The relationship is antagonistic if the difference between the means was significant and the observed values is less than the expected value. If the difference between the means is not significant the relationship is additive.

3.4.3 Controlled environment experiments

Significant differences were observed between treatments in the growth chamber experiments looking at the interaction between *B. amyloliquefaciens* and postemergence herbicides and synthetic fungicides. In the experiment looking at the interaction between *B. amyloliquefaciens* and postemergence herbicides, at 6 days after inoculation (DAI) for the variety P34A79X all treatments were not significantly different from the non-treated control (Figure 10 and Table 16). Also, no significant differences were observed for the variety P32A87L at 6 DAI. At 11 DAI for the variety P34A79X, *B. amyloliquefaciens* significantly decreased Sclerotinia stem rot lesion length over the non-treated control. The Sclerotinia stem rot lesion length for *B. amyloliquefaciens* applied alone was 40.6 mm. Cloransulam-methyl and glyphosate applied alone were not significantly different from the non-treated control. Dicamba applied alone significantly decreased Sclerotinia stem rot lesion length over the non-treated control. The Sclerotinia stem rot lesion length for cloransulam-methyl, glyphosate, or dicamba followed by *B. amyloliquefaciens* were not significantly different from *B. amyloliquefaciens* applied alone. However, cloransulam-methyl followed by *B. amyloliquefaciens* was also not significantly different from the non-treated control. For the variety P32A87L at 11 DAI, *B. amyloliquefaciens* significantly decreased Sclerotinia stem rot lesion length over the non-treated control. The Sclerotinia stem rot lesion length for *B. amyloliquefaciens* applied alone was 51.4 mm. Glufosinate applied alone was also able to significantly decrease Sclerotinia stem rot lesion length over the non-treated control. *B. amyloliquefaciens* applied alone was not significantly different from glufosinate followed by *B. amyloliquefaciens* for Sclerotinia stem rot lesion length. Using Colby's method the relationship between *B. amyloliquefaciens* and cloransulam-methyl, glyphosate, dicamba, and glufosinate were classified as additive.

In the experiment looking at the interaction between *B. amyloliquefaciens* and synthetic fungicides at 6 DAI and 11 DAI all treatments were able to significantly decrease Sclerotinia stem rot lesion length over the non-treated control except boscalid applied alone at 11 DAI (Figure 11 and Table 17). At 14 DAI *B. amyloliquefaciens* significantly decreased Sclerotinia stem rot lesion length over the non-treated control. The Sclerotinia stem rot lesion length for *B. amyloliquefaciens* was 18.4 mm. Picoxystrobin and fluazinam applied alone were also able to significantly decrease Sclerotinia stem rot lesion length. Bosalid applied alone was not significantly different from the non-treated control. The Sclerotinia stem rot lesion length for *B. amyloliquefaciens* followed by

picoxystrobin, boscalid, or fluazinam were not statistically different from *B. amyloliquefaciens* applied alone.

B. amyloliquefaciens applied alone significantly decreased lesion area under the disease progress curve (lAUDPC) when compared to the non-treated control. Picoxystrobin and fluazinam applied alone were also able to significantly decrease lAUDPC when compared to the non-treated control. Boscalid applied alone was not significantly different from the non-treated control for lAUDPC. *B. amyloliquefaciens* followed by picoxystrobin, boscalid, or fluazinam were not significantly different from *B. amyloliquefaciens* applied alone for lAUDPC (Figure 12). The relationship between *B. amyloliquefaciens* and picoxystrobin, boscalid, and fluazinam were classified as additive based on Colby's method.

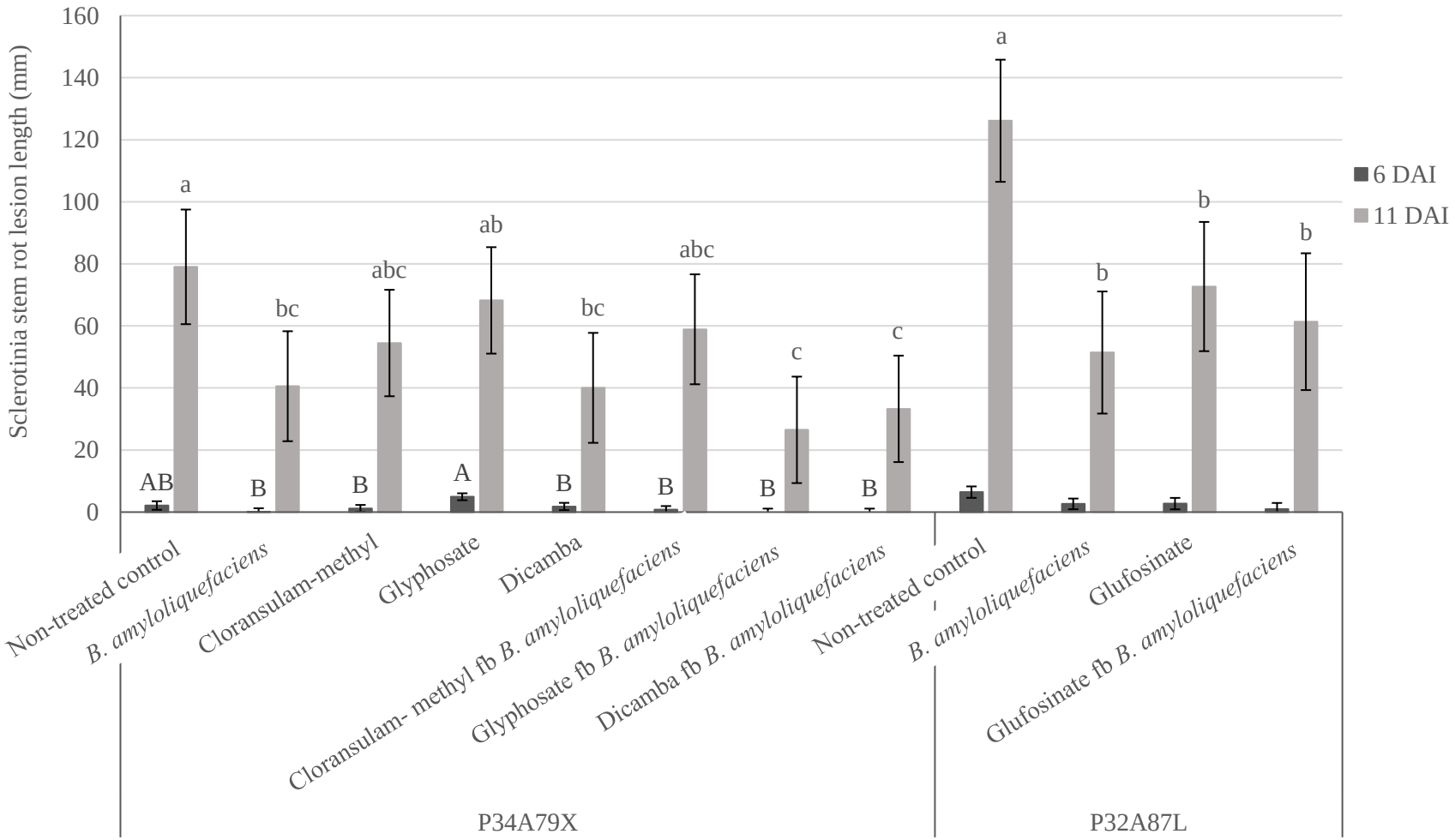

Figure 10. Influence of postemergence herbicides on the ability of *Bacillus amyloliquefaciens* to reduce Sclerotinia stem rot lesion length (mm) at 6 and 11 days after inoculation (DAI). Data represent the mean of four replicates from two experiments. Data pooled over two experiments prior to analysis. Means were separated using Fisher's least significant difference ($\alpha = 0.05$). Least squares means followed by the same letter are not significantly different. Error bars represent standard error of the mean. fb = followed by. Variety P34A79X: 6 DAI: $F = 2.51$, $p = 0.0273$. 11 DAI: $F = 2.31$, $p = 0.0415$. Variety P32A87L: 6 DAI: $F = 2.45$, $p = 0.0976$. 11 DAI: $F = 3.65$, $p = 0.0268$.

Table 16. Influence of postemergence herbicides on the ability of *Bacillus amyloliquefaciens* to reduce Sclerotinia stem rot lesion length.

Variety	Treatment and rate/ha[z]	Sclerotinia stem rot lesion length (mm)[y]	Percent reduction observed	Percent reduction expected[x]	*P*-value[w]	Relationship[v]
P34A79X	Non-treated control	79.0 a	.	.	.	.
	B. amyloliquefaciens 4.68 L	40.6 bc	10.7	.	.	.
	Cloransulam-methyl 0.04 kg	54.5 abc	4.5	.	.	.
	Glyphosate 1.60 L	68.2 ab	-19.0	.	.	.
	Dicamba 1.60 L	40.0 bc	13.9	.	.	.
	Cloransulam-methyl 0.04 kg fb *B. amyloliquefaciens* 4.68 L	58.9 abc	-14.3	-103.4	0.5430	Additive
	Glyphosate 1.60 L fb *B. amyloliquefaciens* 4.68 L	26.5 c	40.1	-112.9	0.3219	Additive
	Dicamba 1.60 L fb *B. amyloliquefaciens* 4.68 L	33.3 c	25.3	16.1	0.4253	Additive
P32A87L	Non-treated control	126.1 a		.	.	.
	B. amyloliquefaciens 4.68 L	51.4 b	60.9	.	.	.
	Glufosinate 3.14 L	72.7 b	38.2	.	.	.
	Glufosinate 3.14 L fb *B. amyloliquefaciens* 4.68 L	61.4 b	46.2	75.8	0.4137	Additive

[z] *Bacillus amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Columbia, NC), cloransulam-methyl (First Rate WDG, Corteva Agriscience, Johnston, IA), glyphosate (RoundUp PowerMax EC, Bayer Crop Science, Research Triangle Park, NC), dicamba (XtendiMax EC, Bayer Crop Science, Research Triangle Park, NC), and glufosinate (Liberty EC, BASF, Research Triangle Park, NC). fb = followed by.

[y] Data are given as Sclerotinia stem rot lesion length (mm) 11 days after inoculation (DAI). Data represent the mean of four replicates from two experiments. Data were polled over two experiments prior to analysis. Least squares means were separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not significantly different.

[x] Percent reduction expected values were calculated using the Colby's method equation.

[w] Differences between the Sclerotinia stem rot lesion length observed and expected means were assessed using a Satterthwaite T-test ($\alpha=0.05$).

[v] The relationship between *C. minitans* and preemergence herbicides was classified using Colby's method. The relationship was classified as synergistic if the difference between the means was significant and the observed value was greater than the expected value. The relationship is antagonistic if the difference between the means was significant and the observed values is less than the expected value. If the difference between the means is not significant the relationship is additive.

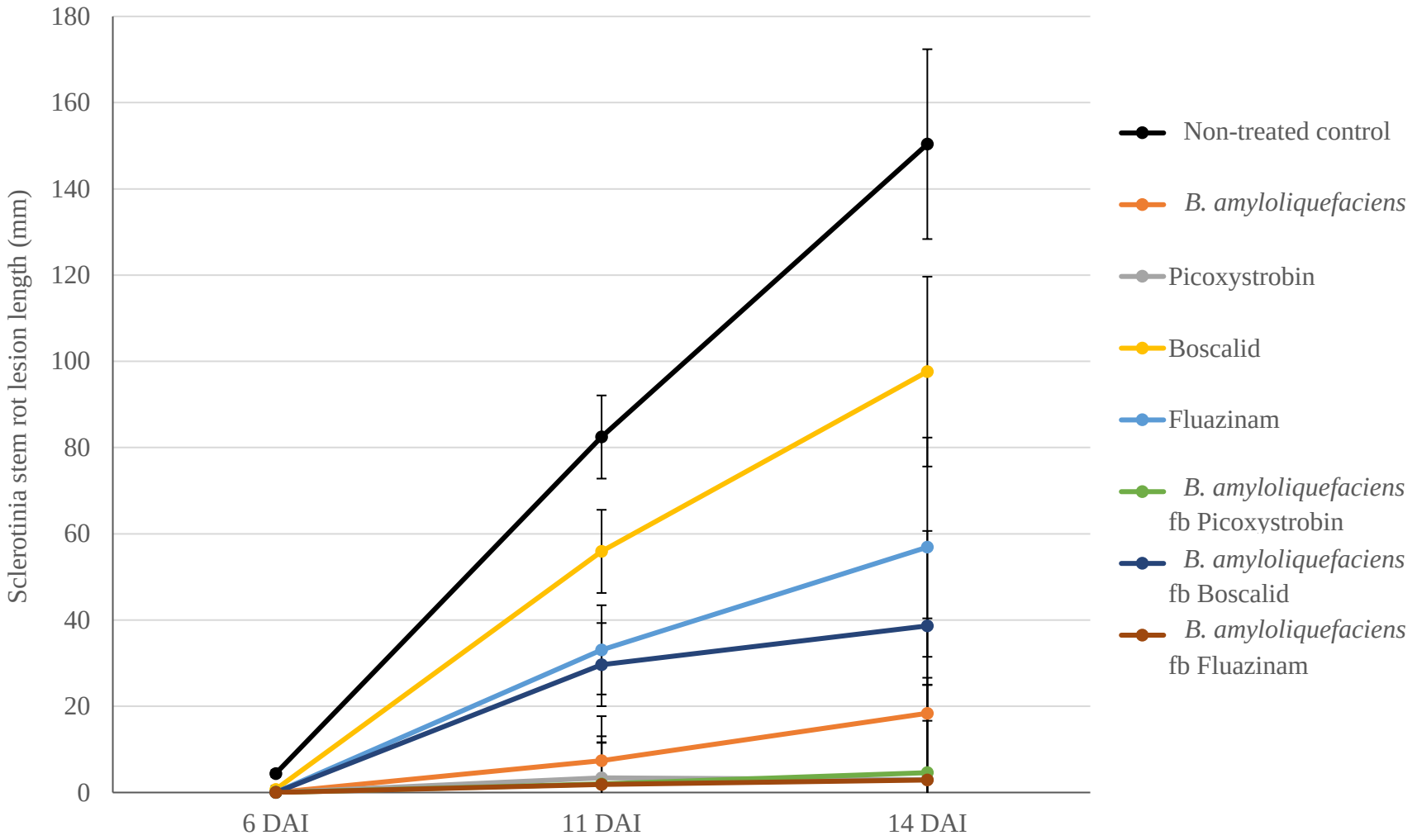

Figure 11. Influence of synthetic fungicides on the ability of *Bacillus amyloliquefaciens* to reduce Sclerotinia stem rot lesion length (mm) at 6, 11, and 14 days after inoculation (DAI). Data represent the mean of four replicates from two experiments. Data pooled over two experiments prior to analysis. Means were separated using Fisher's least significant difference ($\alpha = 0.05$). Error bars represent standard error of the mean. fb =followed by. 6 DAI: F =6.30, p =0.0001, 11 DAI: F =9.46, p =0.0001, 14 DAI: F =5.86, p =0.0005.

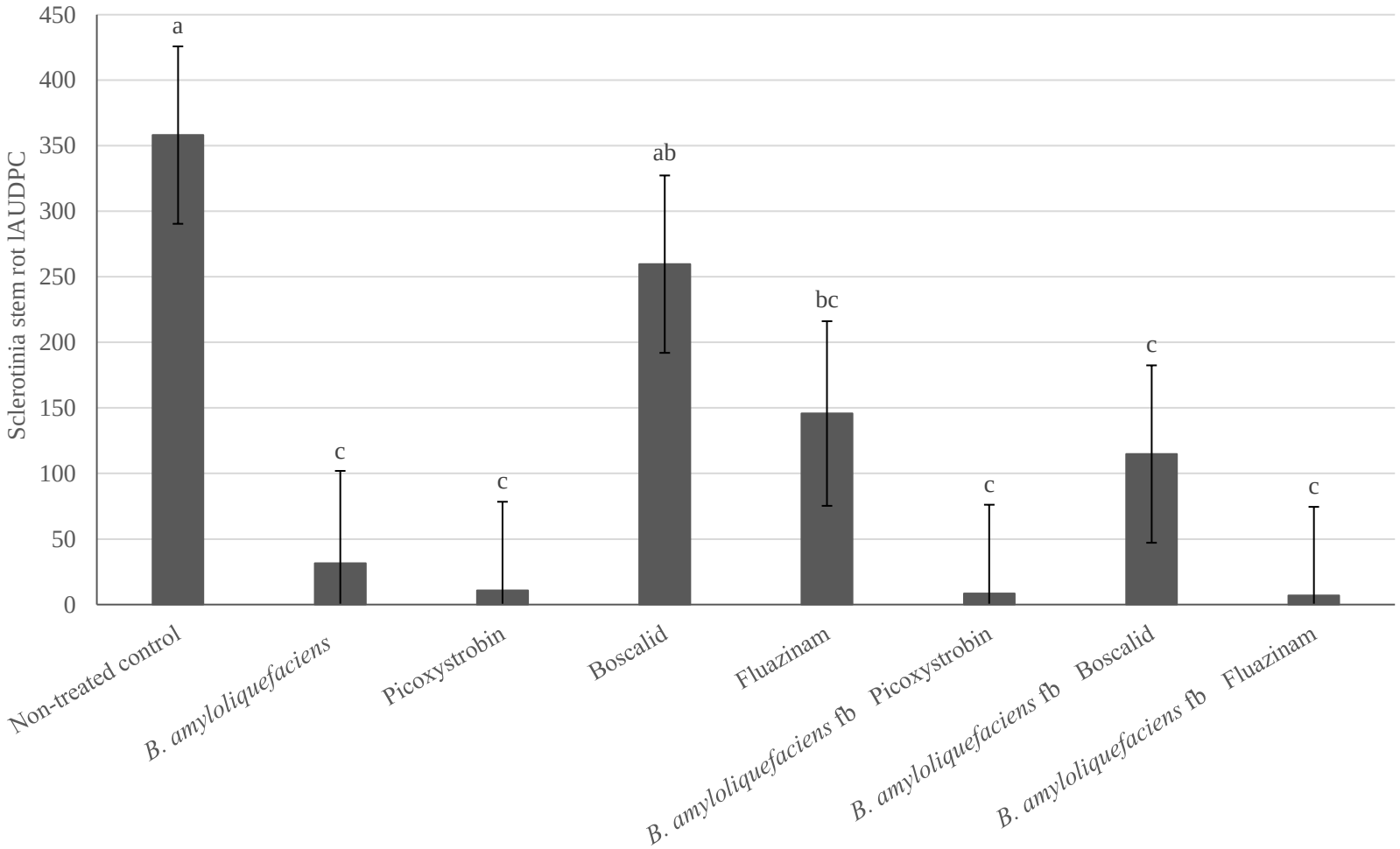

Figure 12. Influence of synthetic fungicides on the ability of *B. amyloliquefaciens* to reduce Sclerotinia stem rot lesion area under the disease progress curve (lAUDPC). Data represent the mean of four replicates from two experiments. Data pooled over two experiments prior to analysis. Means were separated using Fisher's least significant difference ($\alpha = 0.05$). Least squares means followed by the same letter are not significantly different. Error bars represent standard error of the mean. fb = followed by. $F = 6.67$, $p = 0.0001$

Table 17. Influence of synthetic fungicides on the ability of *Bacillus amyloliquefaciens* to reduce Sclerotinia stem rot lesion length.

Treatment and rate/ha[z]	Sclerotinia stem rot lesion length (mm)[y]	Percent reduction observed	Percent reduction expected[x]	*P*-value[w]	Relationship[v]
Non-treated	150.4 a	.	.	.	.
B. amyloliquefaciens 4.68 L	18.4 c	80.1	.	.	.
Picoxystrobin 0.88 L	3.0 c	98.5	.	.	.
Boscalid 0.56 kg	97.6 ab	22.8	.	.	.
Fluazinam 0.88 L	56.9 bc	42.1	.	.	.
Picoxystrobin 0.88 L fb *B. amyloliquefaciens* 4.68 L	4.6 c	96.7	100.0	0.3910	Additive
Boscalid 0.56 kg fb *B. amyloliquefaciens* 4.68 L	38.7 c	79.7	71.7	0.8443	Additive
Fluazinam 0.88 L fb *B. amyloliquefaciens* 4.68 L	2.9 c	98.3	59.7	0.4226	Additive

[z] *Bacillus amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Columbia, NC), picoxystrobin (Aproach SC, Corteva Agriscience, Johnston, IA), boscalid (Endura WDG, BASF, Research Triangle Park, NC), and fluazinam (Omega 500F, Syngenta, Greensboro, NC). fb = followed by.

[y] Data are given as Sclerotinia stem rot lesion length (mm) 14 days after inoculation (DAI). Data represent the mean of four replicates from two experiments. Data were polled over two experiments prior to analysis. Least squares means were separated using Fisher's least significant difference ($\alpha = 0.05$). Means followed by the same letter are not significantly different.

[x] Percent reduction expected values were calculated using the Colby's method equation.

[w] Differences between the Sclerotinia stem rot lesion length observed and expected means were assessed using a Satterthwaite T-test ($\alpha=0.05$).

[v] The relationship between *C. minitans* and preemergence herbicides was classified using Colby's method. The relationship was classified as synergistic if the difference between the means was significant and the observed value was greater than the expected value. The relationship is antagonistic if the difference between the means was significant and the observed values is less than the expected value. If the difference between the means is not significant the relationship is additive.

3.4.4 Field experiments

Three respective experiments were conducted in the field looking at the interaction between *B. amyloliquefaciens* and *C. minitans* with preemergence herbicides, postemergence herbicides, and synthetic fungicides. Weather conditions were not conducive for the development of Sclerotinia stem rot in 2020 and 2021 at either ACRE or PPAC. No significant differences were observed between treatments and the non-treated control for soybean moisture, test weight, and yield (Tables 18-20). Specifically, differences were not observed between the biofungicides applied alone and the biofungicides applied in combination with the preemergence herbicides, postemergence herbicides, synthetic fungicides.

Table 18. Evaluation of the interaction between *Coniothyrium minitans* and *Bacillus amyloliquefaciens* and preemergence herbicides on soybean moisture, test weight, and soybean in Indiana field experiments.

Treatment and rate/ha[z]	Moisture (%)	Test weight kg/hL (lb/bu)	Yield kg/ha (bu/A)[y]
Non-treated control	12.0	71.6 (55.6)	5251.9 (78.1)
C. minitans 2.24 kg	11.9	71.4 (55.5)	5344.6 (79.5)
B. amyloliquefaciens 4.68 L	11.8	71.6 (55.6)	5202.7 (77.4)
Flumioxazin 0.21 kg	11.9	71.4 (55.5)	5093.6 (75.7)
S-metolachlor 3.04 L	11.9	71.4 (55.5)	5147.6 (76.5)
Metribuzin 0.67 kg	11.9	71.6 (55.6)	5341.6 (79.4)
C. minitans 2.24 kg fb Flumioxazin 0.21 kg	12.0	71.6 (55.6)	5160.1 (76.7)
C. minitans 2.24 kg fb S-metolachlor 3.04 L	12.0	71.6 (55.6)	5066.0 (75.3)
C. minitans 2.24 kg fb Metribuzin 0.67 kg	11.9	71.6 (55.6)	4930.4 (73.3)
Flumioxazin 0.21 kg fb *B. amyloliquefaciens* 4.68 L	11.9	71.6 (55.6)	4972.6 (73.9)
S-metolachlor 3.04 L fb *B. amyloliquefaciens* 4.68 L	11.9	71.6 (55.6)	5051.3 (75.1)
Metribuzin 0.67 kg fb *B. amyloliquefaciens* 4.68 L	11.9	71.4 (55.5)	5193.6 (77.2)
***P*-value[x]**	0.6548	0.9597	0.2405

[z] *C. minitans* (Contans WG; Sipcam Agro USA Inc., Durham, NC), *B. amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Columbia, NC), flumioxazin (Valor SX WDG; Valent USA LLC, Walnut Creek, CA), S-metolachlor (Dual Magnum EC; Syngenta, Greensboro, NC), and metribuzin (Tricor DF; Corteva Agriscience, Johnston, IA). fb = followed by.

[y] Yields were adjusted to 13% moisture.

[x] Experiment was repeated at both the Agronomy Center for Research and Education (ACRE) and Pinney Purdue Agricultural Center (PPAC) in 2020 and 2021. Data pooled across experiments prior to analysis. Least squares means separated using Fisher's least significant difference ($\alpha = 0.05$).

Table 19. Evaluation of the interaction between *Coniothyrium minitans* and *Bacillus amyloliquefaciens* and postemergence herbicides on soybean moisture, test weight, and soybean in Indiana field experiments.

Treatment and rate/ha[z]	Moisture (%)	Test Weight kg/hL (lb/bu)	Yield kg/ha (bu/A)[y]
Non-treated control	12.2	71.1 (55.2)	5400.2 (80.3)
C. minitans 2.24 kg	12.3	71.3 (55.4)	5547.7 (82.5)
B. amyloliquefaciens 4.68 L	12.4	71.3 (55.4)	5347.1 (79.5)
Cloransulam-methyl 0.04 kg	12.3	71.3 (55.4)	5621.2 (83.6)
Glyphosate 1.60 L	12.4	71.3 (55.4)	5576.4 (82.9)
Dicamba 1.60 L	12.4	71.2 (55.3)	5586.3 (83.1)
C. minitans 2.24 kg fb Cloransulam-methyl 0.04 kg	12.3	71.1 (55.2)	5457.4 (81.2)
C. minitans 2.24 kg fb Glyphosate 1.60 L	12.3	71.6 (55.6)	5693.8 (84.7)
C. minitans 2.24 kg fb Dicamba 1.60 L	12.3	71.4 (55.5)	5485.2 (81.6)
Cloransulam-methyl 0.04 kg fb *B. amyloliquefaciens* 4.68 L	12.2	71.3 (55.4)	5650.4 (84.0)
Glyphosate 1.60 L fb *B. amyloliquefaciens* 4.68 L	12.1	71.3 (55.4)	5664.2 (84.2)
Dicamba 1.60 L fb *B. amyloliquefaciens* 4.68 L	12.3	71.3 (55.4)	5585.9 (83.1)
***P*-value[x]**	0.4863	0.7196	0.5539

[z] *C. minitans* (Contans WG; Sipcam Agro USA Inc., Durham, NC), *B. amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Columbia, NC), cloransulam-methyl (First Rate WDG, Corteva Agriscience, Johnston, IA), glyphosate (RoundUp PowerMax EC, Bayer Crop Science, Research Triangle Park, NC), and dicamba (XtendiMax EC, Bayer Crop Science, Research Triangle Park, NC). fb = followed by.

[y] Yields were adjusted to 13% moisture.

[x] Experiment was repeated at both the Agronomy Center for Research and Education (ACRE) and Pinney Purdue Agricultural Center (PPAC) in 2020 and 2021. Data pooled across experiments prior to analysis. Least squares means separated using Fisher's least significant difference ($\alpha = 0.05$).

Table 20. Evaluation of the interaction between *Coniothyrium minitans* and *Bacillus amyloliquefaciens* and synthetic fungicides on soybean moisture, test weight, and soybean in Indiana field experiments.

Treatment and rate/ha[z]	**Moisture (%)**	**Test weight kg/hL (lb/bu)**	**Yield kg/ha (bu/A)**[y]
Non-treated control	12.0	71.3 (55.4)	5485.6 (81.6)
C. minitans 2.24 kg	11.9	71.1 (55.2)	5500.4 (81.8)
B. amyloliquefaciens 4.68 L	11.9	71.3 (55.4)	5467.5 (81.3)
Picoxystrobin 0.88 L	11.9	71.3 (55.4)	5483.3 (81.5)
Boscalid 0.56 kg	12.0	71.3 (55.4)	5459.3 (81.2)
Fluazinam 0.88 L	11.9	71.4 (55.5)	5532.6 (82.3)
C. minitans 2.24 kg fb Picoxystrobin 0.88 L	11.8	71.3 (55.4)	5504.4 (81.9)
C. minitans 2.24 kg fb Boscalid 0.56 kg	11.9	71.1 (55.2)	5513.2 (82.0)
C. minitans 2.24 kg fb Fluazinam 0.88 L	11.9	71.6 (55.6)	5555.5 (82.6)
B. amyloliquefaciens 4.68 L fb Picoxystrobin 0.88 L	12.0	71.3 (55.4)	5589.5 (83.1)
B. amyloliquefaciens 4.68 L fb Boscalid 0.56 kg	11.9	71.4 (55.5)	5260.3 (78.2)
B. amyloliquefaciens 4.68 L fb Fluazinam 0.88 L	12.0	71.4 (55.5)	5571.5 (82.8)
C. minitans 2.24 kg fb *B. amyloliquefaciens* 4.68 L	11.9	71.3 (55.4)	5535.8 (82.3)
***P*-value**[x]	0.6328	0.6079	0.8716

[z] *C. minitans* (Contans WG; Sipcam Agro USA Inc., Durham, NC), *B. amyloliquefaciens* (Double Nickel LC; Certis USA LLC, Columbia, NC), picoxystrobin (Aproach SC, Corteva Agriscience, Johnston, IA), boscalid (Endura WDG, BASF, Research Triangle Park, NC), and fluazinam (Omega 500F, Syngenta, Greensboro, NC). fb = followed by.

[y] Yields were adjusted to 13% moisture.

[x] Experiment was repeated at both the Agronomy Center for Research and Education (ACRE) and Pinney Purdue Agricultural Center (PPAC) in 2020 and 2021. Data pooled across experiments prior to analysis. Least squares means separated using Fisher's least significant difference ($\alpha = 0.05$).

3.5 Discussion

The poison plate assay demonstrated that the mycelial growth of *C. minitans* and the colony formation of *B. amyloliquefaciens* could be affected by certain preemergence herbicides, postemergence herbicides, and synthetic fungicides. *C. minitans* was most sensitive to the preemergence herbicide flumioxazin and the synthetic fungicides boscalid and fluazinam. Furthermore, *C. minitans* was slightly sensitive to the preemergence herbicides S-metolachlor and metribuzin and the synthetic fungicide picoxystrobin. *B. amyloliquefaciens* was sensitive only to the synthetic fungicide fluazinam. These results are consistent with previous literature (Partridge et al. 2006; Budge and Whipps 2001; Li et al. 2002). Partridge et al. (2006) found that the radial mycelial growth of *C. minitans* was significantly reduced by the fungicides azoxystrobin, chlorothalonil, fluazinam, pyraclostrobin, and tebuconazole as well as the preemergence herbicide flumioxazin. The radial mycelial growth of *C. minitans* was inhibited by the fungicides iprodione, mancozeb, metalaxyl+thiram, thiram, tolclofos-methyl, and zineb as well as the insecticides malathion and pirimicarb in the experiments conducted by Budge and Whipps (2001). Li et al. (2002) found that the mycelial radial growth of *C. minitans* was greatly reduced by the fungicides benomyl and vinclozolin. Flumioxazin is a group 14 herbicide which inhibits protoporphyrinogen oxidase (PPO). In plants, PPO oxidizes protoporphyrinogen IX to produce protoporphyrin IX which is a precursor molecule for both chlorophyll and heme. However, PPO is also conserved across all eukaryotes and some proteobacteria for the production of heme (Franken et al. 2011). Another herbicide in the group 14 family is lactofen. Previous studies have demonstrated that lactofen can also act as a fungicide to control *S. sclerotiorum* (Dann et al. 1999). Taken together, this evidence suggests that flumioxazin might also have the ability to limit the mycelial growth of other fungi such as *C. minitans*. *B. amyloliquefaciens* belongs to the phylum firmicutes which does not use PPO for the production of heme and is why the bacteria was not affected by flumioxazin (Dailey and Gerdes 2015).

Picoxystrobin and boscalid have broad spectrum activity against various ascomycetes in several crops. Picoxystrobin is a group 11 fungicide that blocks the transfer of electrons at the quinone outside site of cytochrome bc1 in complex III of the electron transport chain (FRAC 2021). The group 7 fungicides, which include boscalid, inhibit succinate dehydrogenase at complex II of the electron transport chain (FRAC 2021). The intended target for picoxystrobin and boscalid in soybean is the ascomycete *S. sclerotiorum*, C. *minitans*, also being an ascomycete, requires the

proper functioning of both these processes in order to produce energy and suggests why *C. minitans* mycelial growth was limited on the plates containing these pesticides. It is thought that the bacteria belonging to the genus *Bacillus* have a slight modification to their cytochrome bc1 complex which is why *B. amyloliquefaciens* was still able to grow on the plates amended with picoxystrobin (Yu et al. 1995; Yu and le Brun 1998). In the mitochondria of eukaryotes and gram-negative bacteria ubiquinone is used as the electron acceptor at complex II, however in gram-positive bacteria like *B. amyloliquefaciens* menaquinone is used instead (Schirawski and Unden 1998). This difference in the electron acceptor at complex II is why the growth of *B. amyloliquefaciens* was not affected by boscalid. Fluazinam is a group 29 fungicide that uncouples oxidative phosphorylation (FRAC 2021). Oxidative phosphorylation is conserved across prokaryotes, eukaryotes, and plants (Nath and Villadsen 2015). Therefore, it is logical to conclude that both *C. minitans* and *B. amyloliquefaciens* might be sensitive to fluazinam.

Similar results were observed in the soil plate assay and controlled environment experiments. The ability of *C. minitans* to degrade the sclerotia of *S. sclerotiorum* was reduced by flumioxazin, picoxystrobin, and boscalid. Interestingly, *C. minitans* was only somewhat sensitive to metribuzin in the poison plate assay, but significantly reduced the mycoparasitic activity of *C. minitans* in the soil plate assay. Glyphosate had no effect on the radial growth of *C. minitans* in the poison plate assay, but significantly decreased the mycoparasitic activity of *C. minitans* in the soil plate assay. The radial growth of *C. minitans* was reduced by fluazinam and S-metolachlor in the poison plate assay, but had no effect on the mycoparasitic activity of *C. minitans* in the soil plate assay. Furthermore, none of the postemergence herbicides or synthetic fungicides, including fluazinam, decreased the efficacy of *B. amyloliquefaciens* in the controlled environment experiments. The differences observed between the results of the poison plate assay and the soil plate assay or controlled environment experiments could be explained by how closely the biofungicides and other pesticides interacted. In the poison plate assay, the biofungicides were in direct contact with the pesticides, while in the soil plate assay and controlled environment experiments, there was more variability during product application; therefore, it is possible that the biofungicides did not interact as closely with the other pesticides.

There were no significant interactions between *C. minitans* and *B. amyloliquefaciens* with preemergence herbicides, postemergence herbicides, and synthetic fungicides for soybean moisture, test weight, and yield. Weather conditions were not conducive for the development of

Sclerotinia stem rot in field trials during the 2020 and 2021 growing seasons, and the disease was not observed in the plots. Therefore, in a year with low disease pressure, yield will not be negatively impacted if the biofungicides are incorporated into season long soybean management practices.

These results demonstrate that antagonistic relationships exist between the biofungicides *C. minitans* and *B. amyloliquefaciens* and certain preemergence herbicides, postemergence herbicides, and synthetic fungicides. Particularly the preemergence herbicides flumioxazin and metribuzin, the postemergence herbicide glyphosate, and the synthetic fungicides picoxystrobin, boscalid, and fluazinam. Caution should be used when timing the application of either biofungicide to avoid direct contact with these pesticides. The list of pesticides tested here is by no means a comprehensive list of all pesticides that could be applied to soybean throughout the growing season. But instead highlights the potential impact of some of the most commonly used products. Future work should include exploring the interaction between *C. minitans* and *B. amyloliquefaciens* with other pesticides commonly used in soybean. The interaction should also be explored in the field under high Sclerotinia stem rot disease pressure to classify the relationship between the biofungicides and the other applied pesticides under conditions encountered in soybean production systems.

3.6 References

Adams, P. B. and Ayers, W. A. 1979. Ecology of *Sclerotinia* species. Phytopathology. 69:896–899.

Botha, C., McLaren, N. W., and Swart, W. J. 2009. Evaluation of greenhouse inoculation techniques used to screen for Sclerotinia stem rot resistance in soybeans. South African J. Plant and Soil. 26:48–50.

Bradley, C. A., Allen, T. W., Sisson, A. J., Bergstrom, G. C., Bissonnette, K. M., Bond, J., Byamukama E., Chilvers, M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault N. S., Esker, P. D., Faske, T. R., Fiorellino N. M., Geisler, L. J., Hartman, G. L., Hollier, C. A., Isakeit T., Jackson-Ziems, T. A., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koehler, A. M., Kratochvil, R. J., Kurle, J. E., Malvick, D. K., Markell, S. G., Mathew, F. M., Mehl, H. L., Mehl K. M., Mueller, D. S., Mueller, J. D., Nelson, B. D., Overstreet, C., Padgett, G. B., Price, P. P., Sikora, E. J., Small, I., Smith, D. L., Spurlock, T. N., Tande, C. A., Telenko, D. E. P., Tenuta, A. U., Thiessen, L. D., Warner, F., Wiebold, W. J., and Wise, K. A. 2021. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2015 to 2019. Plant Health Prog. 22:483–495.

Budge, S. P. and Whipps, J. M. 2001. Potential for integrated control of *Sclerotinia sclerotiorum* in glasshouse lettuce using *Coniothyrium minitans* and reduced fungicide application. Phytopathology. 91:221–227.

Colby, S. R. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds. 15:20.

Dailey, H. A. and Gerdes, S. 2015. HemQ: An iron-coproporphyrin oxidative decarboxylase for protoheme synthesis in Firmicutes and Actinobacteria. Archives of Biochem. and Biophys. 574:27-35.

Dann, E. K., Diers, B. W., and Hammerschmidt, R. 1999. Suppression of Sclerotinia stem rot of soybean by lactofen herbicide treatment. Phytopathology. 89:598–602.

Duncan, R. W., Dilantha Fernando, W. G., and Rashid, K. Y. 2006. Time and burial depth influencing the viability and bacterial colonization of sclerotia of *Sclerotinia sclerotiorum*. Soil Biol. & Biochem. 38:275–284.

FRAC Code List 2021. Fungal control agents sorted by cross resistance pattern and mode of action (Including coding for FRAC groups on product labels). Available at: https://www.frac.info/docs/default-source/publications/frac-code-list/frac-code-list-2021--final.pdf?sfvrsn=f7ec499a_2

Franken, A. C. W., Lokman, B. C., Ram, A. F. J., Punt, P. J., van den Hondel, C. A. M. J. J., and de Weert, S. 2011. Heme biosynthesis and its regulation: Towards understanding and improvement of heme biosynthesis in filamentous fungi. Applied Microbiol. and Biotech. 91:447–460.

Fungicide Resistance Action Committee (FRAC) 2021. Quinone outside Inhibitor (QoI): Introduction and general information. Available at: https://www.frac.info/frac-teams/working-groups/qol-fungicides/information.

Fungicide Resistance Action Commottee (FRAC) 2021. Succinate Dehydrogenase Inhibitor (SDHI): Introduction and general information. Available at: https://www.frac.info/frac-teams/working-groups/sdhi-fungicides/information.

Hao, J. J., Subbarao, K. V., and Duniway, J. M. 2007. Germination of *Sclerotinia minor* and *Sclerotinia sclerotiorum* sclerotia under various soil moisture and temperature combinations. Phytopathology. 93:443–450.

Hartman, G. L., Rupe, J. C., Sikora, E. J., Domier, L. L., Davis, J. A., and Steffey, K. L., *eds.* 2015. *Compendium of Soybean Diseases and Pests*. Fifth Edition. The American Phytopathological Society.

Kandel, Y. R., Mueller, D. S., Legleiter, T., Johnson, W. G., Young, B. G., and Wise, K. A. 2018. Impact of fluopyram fungicide and preemergence herbicides on soybean injury, population, sudden death syndrome, and yield. Crop Protec. 106:103–109.

Li, G. Q., Huang, H. C., and Acharya, S. N. 2002. Sensitivity of *Ulocladium atrum*, *Coniothyrium minitans*, and *Sclerotinia sclerotiorum* to benomyl and vinclozolin. Canadian J. of Bot. 80:892–898.

Loux, M. M., Doohan, D., Dobbels, A. F., Johnson, W. G., Young, B. G., Zimmer, M., and Hager, A. 2020. *Weed Control Guide: 2020 Ohio, Indiana, and Illinois*. WS-16.

Mueller, D., Wise, K., Sisson, A., Smith, D., Sikora, E., Bradley, C., and Robertson, A. 2016. *A Farmer's Guide to Soybean Diseases*.

Musser, F., Conley, S. P., and Davis, J. A. 2021. 2020 Soybean insect losses in the United States. Midsouth Entom. 13:1–15.

Myers, S. W., Hogg, D. B., and Wedberg, J. L. 2005. Determining the optimal timing of foliar insecticide applications for control of soybean aphid (Hemiptera: *Aphididae*) on soybean. Journal of Econ. Entom. 98:2006–2012.

Nath, S. and Villadsen, J. 2015. Oxidative phosphorylation revisited. Biotech. and Bioeng. 112:429–437.

Oerke, E. C. 2006. Crop losses to pests. J. of Ag. Science. 144:31–43.

Partridge, D. E., Sutton, T. B., and Jordan, D. L. 2006. Effect of environmental factors and pesticides on mycoparasitism of *Sclerotinia minor* by *Coniothyrium minitans*. Plant Dis. 90:1407–1412.

Schirawski, J. and Unden, G. 1998. Menaquinone-dependent succinate dehydrogenase of bacteria catalyzes reversed electron transport driven by the proton potential. European J. Biochem. 257:210–215.

Simko, I. and Piepho, H.-P. 2012. The area under the disease progress stairs: Calculation, advantage, and application. Phytopathology. 102:381.

Smith, F. D., Phipps, P. M., and Stipes, R. J. 1991. Agar plate, soil plate, and field evaluation of fluazinam and other fungicides for control of *Sclerotinia minor* on Peanut. Plant Dis. 75:1138–1143.

United States Environmental Protection Agency - Biopesticides. Available at: https://www.epa.gov/pesticides/biopesticides.

Willetts, H. J. 1971. The survival of fungal sclerotia under adverse environmental conditions. Biol. Rev. 46:387–407.

Yu, J. and le Brun, N. E. 1998. Studies of the cytochrome subunits of menaquinone:cytochrome c reductase (bc complex) of *Bacillus subtilis*. The J. of Biol. Chem. 273:8860-8866.

Yu, J., Hederstedt, L., and Piggot, P. J. 1995. The cytochrome bc complex (menaquinone:cytochrome c reductase) in *Bacillus subtilis* has a nontraditional subunit organization. J. of Bacteriol. 177:6751–6760.