IMPROVEMENTS TOWARDS DURABLE MANAGEMENT STRATEGIES OF HEMP POWDERY MILDEW THROUGH HOST RANGE DETERMINATION, FUNGICIDE USE, AND GENETIC HOST SUSCEPTIBILITY

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Ali R. Cala

August 2022

IMPROVEMENTS TOWARDS DURABLE MANAGEMENT STRATEGIES OF HEMP POWDERY MILDEW THROUGH HOST RANGE DETERMINATION, FUNGICIDE USE, AND GENETIC HOST SUSCEPTIBILITY

Ali R. Cala Ph. D.

Cornell University 2022

Hemp (*Cannabis sativa* L.) is an emerging crop in the United States with a market that is currently developing strong potential to grow. Several pathogens of hemp have emerged in recent years including powdery mildew, caused by *Golovinomyces ambrosiae*. Powdery mildews are a large group of obligate biotrophic plant pathogens that have major impacts on many crops worldwide. The highly polycyclic nature of powdery mildews can make disease management challenging, as disease pressure increases rapidly after initial infection. Therefore, the identification of effective management strategies is crucial for hemp production. The first goal of this research was to begin to determine the host range of *G. ambrosiae*. Most powdery mildews have a relatively narrow host range, but prior to this research, *G. ambrosiae* had been reported on several other crops. This work demonstrated that okra (*Abelmoschus esculentus*), sunnhemp (*Crotalaria juncea*), sunflower (*Helianthus annuuss*), some cucurbits (*Cucurbita pepo*) and some Zinnia (*Zinnia elegans*) are hosts of *G. ambrosiae* with varying levels of susceptibility. Through this work I was also able to identify several fungicides that were effective in controlling powdery mildew in the field. However,

those same products were not effective against the necrotrophic pathogen, *Botrytis cinerea*, the causal agent of gray mold on hemp. I was also able to determine that infection with *G. ambrosiae* or fungicide application largely did not impact cannabinoid production in hemp, while *B. cinerea* potentially had an impact. Lastly, this work sought to identify possible sources of genetic host resistance to *G. ambrosiae*. I was able to observe a wide range of disease susceptibility among the hemp germ plasma, including high cannabidiol (CBD), high cannabigerol (CBG), fiber, grain and dual-purpose hemp cultivars and accessions, throughout multiple field seasons and growth chamber inoculations. From these disease ratings, one resistant cultivar was identified and crossed with a susceptible cultivar to create a mapping population with the goal of identifying the responsible gene(s). The resulting F_1 and F_2 populations have been characterized for disease susceptibility. All this information contributes to the knowledge about *G. ambrosiae*, with the goal of improved disease management strategies and tools.

BIOGRAPHICAL SKETCH

Ali Rose Cala was born and raised in Geneva, New York where she attended Geneva High School. It was through her high school biology class that she found her interest and passion in biology. She pursued that interest by majoring in Biology and Molecular Bioscience and Biotechnology at Rochester Institute of Technology, graduating in 2017 with an immersion in Italian. During her undergraduate career, she sought out various research experiences starting with the position that introduced her to the field of plant pathology working on apple scab as a summer technician with Dr. Sara Villani and Dr. Kerik Cox at Cornell AgriTech. The following summer she studied abroad in Perth, Australia working on an ocean ecology research project, and worked as a summer technician with Dr. Brian Nault on an onion thrip project. At RIT, she worked on research projects with Dr. André Hudson focusing on understanding the lysine biosynthesis in bacteria. The applied nature of the research at Cornell AgriTech, combined with her interest in microbiology led to her to the Summer Scholar's Program where she worked with Dr. Chris Smart and Chase Crowell on a project characterizing host resistance in willow to the pathogen *Melamspora americana*. Ali then began graduate school in 2017 at Cornell University in the section of Plant Pathology and Plant-Microbe Biology under the advisement of Dr. Chris Smart, where she began studying various aspects of disease management of powdery mildew on hemp caused by *Golovinomyces ambrosiae*. Throughout her time at Cornell, she has held various leadership positions in the Plant Pathology and Plant-Microbe Biology Graduate Student Association (PPPMB-GSA) and the Student Associate of the Geneva Experiment Station (SAGES). She has also served as a workshop leader for the

Expanding Your Horizons conference at Cornell, leading a plant pathology workshop for middle school-aged girls. She has also served as the coordinator for the Student Seminar Exchange program for the Geneva plant pathology seminar series.

To my family, chosen and given.

ACKNOWLEDGMENTS

To my advisor, Chris Smart, I am forever grateful for all the needed support and guidance over the years of my PhD, including though many challenging and uncertain moments in time. Thank you for teaching me how to be a better scientist, teacher, mentor, and communicator while helping me find my confidence in all these areas. I am also grateful for my committee Larry Smart and Sara Pethybridge for contributing their expertise and perspectives which have helped me develop my projects over the years. I would like to thank several other faculty members at Cornell that have provided me with support and guidance including Kerik Cox, Steve Reiners, Marc Fuchs, and Brian Nault. I would also like to thank my undergraduate research advisor, Dr. André Hudson, and my high school English teacher Ryan DeWolfe, for remaining the biggest cheerleaders for me and all their students long after graduation.

To all the members of the Smart Lab, past and present, you have all been important in shaping my PhD experience, and my work would not have been done without all your help, support, and contributions. Thank you to Chase Crowell, Chris Peritore-Galve, Greg Vogel, Martha Sudermann, Mariami Bekauri, Holly Lange, Taylere Herrmann, Juan Luis González-Girón, Libby Indermaur, Patrick McMullen, Colin Day, and Garrett Giles.

On a personal note, I have made some wonderful friends here at Cornell that have made this experience so much fun and offered endless support both in and outside of the lab, particularly Maddie Flasco, Katrin Ayer, Chase Crowell, Juan Luis González-Girón, David Strickland, Larissa Osterbaan, Libby Indermaur, Anna Wunsch, Jess Choi, Martha Sudermann and Tori Hoyle. I also need to acknowledge my friends outside of

Cornell, particularly Cortney McArdell, Jess Gallagher, Taylor Chapman, Rachel Bratek, Morgan Riemer, Abby Allen, and Bethanny Warner who have offered nothing but love and encouragement over the years.

Thank you to my family, especially my parents who always believed in me and everything I've wanted to pursue. It's only because of your unwavering support throughout my life that I had the confidence and determination to pursue this PhD. Lastly, there are not enough words to express the gratitude that I have for my partner, Kevin Reed, for all that he has done for me, not only through my PhD, but for as long as we've known each other. I wouldn't want anyone else by my side through this journey and everything yet to come.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Cannabis sativa L. has a long history of human cultivation:

Cannabis sativa L. belongs to the plant family Cannabaceae which also includes the genus *Humulus* or hop of which there are three cultivated species (McPartland, 2018). *C. sativa* is considered to have begun its long history with humans as a "camp follower crop" (Small, 2017). Due to the various uses, such as its fibrous stem, edible seeds, and medicinal or psychoactive properties, and its ease of accessibility, it is likely that this plant would have been a favorable plant for collection by early humans. When the seeds were then discarded in the temporary camps, they would have found favorable conditions near water and nitrogen-rich manured soils and began to thrive (Small, 2017).

While *C. sativa* is known to have an ancient history with humans, the location of original domestication and origin of the species is not fully known. However, substantial archaeological evidence, subfossil pollen studies, and chloroplast marker analysis indicate that *C. sativa* originated and was first domesticated in China. (Li, 1974; McPartland et al., 2019; Osterberger et al., 2021). Additionally, in China, ancient artifacts indicate that *C. sativa* has been cultivated and its fiber used to make various materials such as rope, fishing nets, clothing and eventually the invention of paper (Li, 1974). It is also evident that *C. sativa* seeds were an important food source for humans, alongside other ancient grains, and was later used a source of cooking oil. Historical written works also suggest that the intoxicating and medicinal properties of *C. sativa*

have been well known and used in this way since the beginning of its cultivation (Li, 1974).

It is hypothesized that *C. sativa* was subsequently brought to Europe during the Bronze era and cultivated mainly for fiber use which continues for most of European history (Karus & Vogt, 2004; McPartland et al., 2018). Some evidence suggests that *C. sativa* was brought to India later (McPartland et al., 2019), while others hypothesize that India or other parts of southern Asia are in fact the origin of *C. sativa* cultivation (Zhang et al., 2018).

Hemp in the New World:

Hemp was brought to South America in the late 1500s (Fike, 2016) and then North America in early 1600s by Europeans (Miller, 1991). With the long history of the crop being grown mainly for fiber in Europe, this continued in the New World the next few hundred years (Fike, 2016; Fortenbery & Bennett, 2001; Roulac, 1997). However, by the early 1900s, other sources of fiber, such as cotton, became more competitive and hemp as a fiber source declined in popularity and production (Miller, 1991). Additionally, the Marihuana Tax Act of 1938 placed all *C. sativa* cultivation under the control of the USA Treasury Department, as concerns grew about the crop's use as a drug (USDA, 2000). Hemp production in the United States briefly increased due to demands during World War II as farmers were encouraged to grow "hemp for victory" (Fike, 2016; Robinson, 1942) but dropped again after the end of the war (Cherney & Small, 2016). Eventually, a complete ban of all *C. sativa*, regardless of psychoactive

properties (which is dependent on Δ^9-THC levels), was established with the 1970 Controlled Substance Act. From that point forward, all *C. sativa*, regardless of Δ^9-THC content, was treated as a Schedule I controlled substance by the US Drug Enforcement Agency (DEA) (USDA, 2000).

Hemp legalization in NY:

In more recent years, there has been a growing interest in the revitalization of *C. sativa* as a crop, for its many potential uses. This growing interest has led to recent legislation that differentiates between hemp and marijuana and allows for the legal production of hemp. Canada had similar *C. sativa* prohibition legislation in previously in place, but beginning in the 1990s, the Canadian government began passing legislation to allow commercial production of hemp. In 2014, the passing of the farm bill distinguished between marijuana, an illegal controlled substance, and hemp. In this bill, hemp is defined as *C. sativa L.* with less than 0.3% Δ^9-tetrahydrocannabdiol by weight. When this farm bill was passed, New York started their pilot program that focused on research into the marketing of hemp. The 2018 Farm Bill allows states to create licensing programs that allow farmers to grow hemp. New York State developed its pilot program in 2016, and since then interest in the crop has piqued growers' interest and been embraced by the public.

***Cannabis sativa* types and their uses:**

Cannabis sativa historically was and continues to be a popular crop among humans, partly due to the crop's versatility. The three main useful parts of *C.* sativa has been

cultivated for are its fiber, grain, and cannabinoids, and each of these come with a variety of uses. The desired use of the crop has resulted in distinct cultivated types of the plant that have been bred for a specific purpose. Fiber cultivars are the tallest with little branching and are planted at high densities. Traditionally, hemp fiber has been used for a wide variety of purposes including twine, textiles, fabrics, rope, etc. (Cherney & Small, 2016; Karus & Vogt, 2004; Li, 1974). Modern hemp fiber markets include products like natural fiber insulation and hempcrete (hemp-lime concrete) which can offer environmentally friendly versions of their conventional construction material counterparts (Domke & Mude, 2015).

While hemp seed has provided a source of human food for thousands of years, especially in China (Li, 1974), only recently have hemp breeding efforts begun selecting for grain production (Cherney & Small, 2016; Fike, 2016). The major modern uses for hemp grain or oil seed include human food in the form of seeds or oil, and a source animal feed (Callaway, 2004; Cherney & Small, 2016; Karus & Vogt, 2004).

Historically, *C. sativa* has also been used widely for both its medicinal and intoxicating properties, for which its production of cannabinoids are responsible. Cannabinoids are a class of secondary metabolites produced in the glandular stalked capitate trichomes of the female hemp flower (Livingston et al., 2020). The cannabinoid Δ^9-tetrahydrocannabdiol (THC) is responsible for the hallucinogenic and psychoactive effects of marijuana (*C. sativa* with greater than 0.3%), and currently remains federally prohibited in the United States. However, there are several other cannabinoids produced

by hemp that have potential desirable pharmaceutical uses, resulting from the interaction of these metabolites with the endocannabinoid signaling pathway in the human body (Pertwee, 2008; Pertwee et al., 2010). The major cannabinoids that are currently areas of study due to their potential medicinal uses include cannabidiol (CBD), cannabigerol (CBG) and cannabichromene (CBC) (Gloss & Vickrey, 2014; Mechoulam et al., 2002; Pertwee, 2006).

All cannabinoid production in *C. sativa* occurs through the same biosynthetic pathway, through the condensation of olivetolic acid and geranyl pyrophosphate to produce cannabigerolic acid (CBGA). The precursor to all other major cannabinoids is CBGA which is converted to the acid forms of either CBD, THC or CBC through an enzymatic reaction. Those acidic forms (CBDA, THCA, CBCA) can then be decarboxylated to their neutral forms non-enzymatically when exposed to heat. The enzymes cannabidiolic acid synthase (CBDAS) and tetrahydrocannabinolic acid synthase (THCAS) catalyze the reaction to convert CBGA to CBDA or THCA respectively. The complex genetic structure of CBDAS and THCAS has been elucidated (de Meijer et al., 2003; Grassa et al., 2018), and it has been determined that they may be modeled as a single allelic locus. This has led to a classification systemic comprised of chemotypes that describe the relative abundance of THC(A), CBD(A), and CBG(A). Chemotype I produces primarily THC(A), chemotype II produces CBD(A) and THC(A) in a ~1.5:1 ratio, and chemotype III produces primarily CBD(A) in a CBDA(A):THC(A) ratio of ~26:1 (de Meijer & Hammond, 2005; Mandolino et al., 2003; Stack et al., 2021). Regardless of chemotype classification, *C. sativa* cultivars that have been bred for

cannabinoid production share phenotypic characteristics. Due to the difficulties in determining the differences between chemotypes visually, genetic markers have been developed to rapidly differentiate them (Toth et al., 2020). In contrast to fiber hemp cultivars, cannabinoid *C. sativa* tend to be shorter and highly branched allowing the production of more female inflorescence, and therefore maximizing the cannabinoid production (McPartland, 2018).

Propagation methods of *Cannabis sativa*:

For cultivars that are used for cannabinoid production, because cannabinoids are mainly produced by unpollinated female inflorescence, a female only crop is highly desirable and are generally produced through the feminized seeds or clonal propagation Hemp clones are generated by taking stem cuttings of at least 6 inches and 3-5 nodes from lateral or apical branches from a mother plant. Stems are trimmed to 4 inches and excess foliage is removed. Cutting are sterilized in 10% bleach, coated in cloning gel (Rootech Original) with 0.55% Indole-3 butyric acid (IBA), and placed in a hemp cloner system with a cloning solution which contains Clonex (mL/L water) and Clear Rez (1oz/5gal water) at a pH of 5.8. The cloning solution pH is adjusted daily using General Hydroponics “pH UP” solution and Bloom city “pH down” professional grade pH adjustor. After approximately 2 weeks, a new solution is used containing Olivia’s cloning solution (60mL/1gal), RhizoBlast solution (0.5 oz/gal) and Clear Rez solution (1 oz/5gal). Once roots begin to form after about one week, plants can be transplanted into 50 cell flats and covered to humidity, and then transplanted into pots after about 2-3 weeks.

To produce feminized seeds, foliar sprays of silver thiosulfate (STS) is used on female plants to induce to production of male flowers. The resulting male flowers produce only genetically female pollen which subsequently result in all female seed (Lubell & Brand, 2018; Mohan Ram & Sett, 1982). *Cannabis sativa* that is clonally propagated or planted from feminized seed are often started in greenhouses before being transplanted. Conversely, *C. sativa* cultivars which are used for grain or fiber production are often directly seeded and are more densely planted than cannabinoid cultivars. Additionally, both males and female plants are required for seed production, so seed feminization is not necessary for grain and fiber production.

Golovinomyces ambrosiae is the causal agent of hemp powdery mildew:

With the passing of recent legislation that has allowed the cultivation of hemp in the United States, a market for hemp has developed and fluctuated in recent years. As hemp acreage has increased with growing interest in the crop for a variety of uses, problematic pathogens have also emerged. One of the first of these diseases was powdery mildew caused by *Golovinomyces ambrosiae* (formerly *Golovinomyces spadiceus*) (Qiu et al., 2020; Weldon et al., 2019). *Golovinomyces ambrosiae* are described as having amphigenous and caulicolous white mycelia consisting of 2-9 µm wide, thin-walled smooth and hyaline hyphae. The fungus is also characterized by nipple-shaped hyphal appressoria, and ellipsoid-oval, doliiform-subcylindrical conidia (Qiu et al., 2020). In recent years, *G. ambrosiae* has been reported causing disease on hemp in multiple states

and locations in the United States (Farinas & Hand, 2020; Szarka et al., 2019; Weldon et al., 2019).

Powdery mildews are obligate biotrophic ascomycete fungi of the family Erysiphaceae. This fungal family consists of powdery mildew-causing pathogens that can infect thousands of plant species, causing significant yield losses in various crops (Cao et al., 2014; Conner et al., 2003; Green et al., 2014). Powdery mildews initiate infection when either an asexual spore, called a conidium, or a sexual ascospore are able to germinate when they meet host tissue, typically the adaxial surface of a leaf. Hyphae can differentiate into an appressorium and eventually a penetration peg which penetrate host tissue mechanically and chemically. Subsequently a specialized structure called the haustorium forms, and the fungus is then able to absorb water and nutrients from a host plant (Voegele et al., 2001), as well as secrete small effector molecules to maintain fungal colonization (Godfrey et al., 2009; O'Connell & Panstruga, 2006; Voegele & Phytopathologie, 2006). Mycelia grow on and colonize the surface of the leaf and eventually differentiate into conidiophores which produce asexual conidia (Figure 1.1). Large numbers of conidia are produced within days of initial infection which are easily detached and dispersed by wind to cause a new infection. The extraction of nutrients can reduce overall plant health and vigor and leaf surface coverage eventually leads to early leaf abscission. Relatively warm temperatures and high humidity promote mycelial growth and trigger conidial production for most powdery mildews. Chasmothecia are the sexual structures of powdery mildew which allows for sexual recombination and are also overwintering structures. *Golovinomyces ambrosia*

chasmothecia have been observed (Szarka et al., 2019), but have not been reported in New York State. While the vegetative hyphae of other powdery mildew species can overwinter (Gent et al., 2018), the overwintering mechanism for *G. ambrosia* has not yet been determined. With the large number of conidia that are rapidly produced and easily dispersed, in addition to the potential for pathogen overwintering, severe powdery mildew epidemics are possible without the use of proper management strategies.

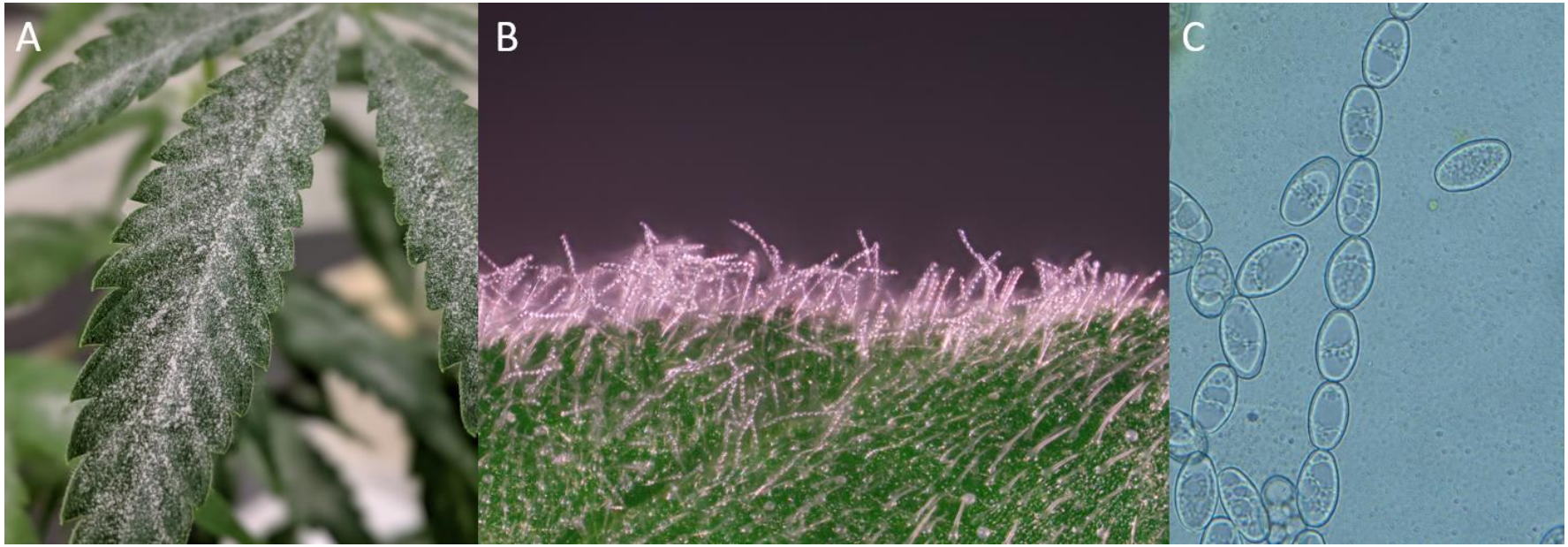

Figure 1.1 Powdery mildew of hemp (caused by *Golovinomyces ambrosiae*) produces asexual conidia on the surface of hemp leaves (A) and all above ground tissue. The asexual conidia are produced in chains from conidiophores (B and C) Photos B and C from Weldon et al. (2019).

Powdery mildew management strategies:

Common management strategies for powdery mildews include several cultural practices to reduce canopy humidity, such as pruning and expansion of row spacing. Pruning to remove infected tissue and leaf litter can also reduce sources of secondary inoculum. Additionally, powdery mildews tend to have relatively narrow host ranges, so crop rotation or crop diversification can reduce overall powdery mildew inoculum load. However, *G. ambrosiae* has been reported on a broader range of crops (Félix-Gastélum et al., 2019; Moparthi, Bradshaw, & Grove, 2018; Moparthi, Bradshaw, Frost, et al., 2018; Trigiano et al., 2018), and the extent of this powdery mildew species' host range is not fully understood.

There has also been success in controlling powdery mildews with the use of fungicides (Bowen et al., 1992; Garibaldi et al., 2011; Keinath & Dubose, 2004). However, there are limitations in products that are currently commercially available for use on hemp. Further, there is a lack of knowledge about the effectiveness of products against *G. ambrosiae* on hemp, and fungicide use on hemp diseases in general.

Disease resistance is a highly desirable trait for breeding, and there has been success in developing powdery mildew resistant crops (Büschges & Hollricher, 1997; Goyal et al., 2020; Henning et al., 2017). Qualitative resistance can be conferred through R genes, nucleotide-binding site leucine rich repeat (NBS-LRR) proteins, which are able to induce effector triggered immunity (ETI) through the recognition of pathogen effector proteins. These genes are dominantly inherited. To date, one NBS-LRR gene, *PM1*, has

been identified in a hemp cultivar (Mihalyov & Garfinkel, 2021). *Mildew Locus O* (*MLO*) genes are susceptibility genes that confer susceptibility to powdery mildews. These genes encode transmembrane proteins, and their functionality is not fully understood, though there is evidence for their role in establishment of interactions with beneficial mycorrhizal fungi (Jacott et al., 2020), possibly suggesting the exploitation of these proteins by powdery mildews to facilitate infection. Currently, two candidate *MLO* genes have been identified in *C. sativa* (Goyal et al., 2020)

Dissertation Research Goal:

The goal of this research was to increase our understanding of the biology of *G. ambrosiae* and develop disease management strategies of powdery mildew on hemp.

Specific Research Goals:

Chapter 2:

In this chapter, I sought to examine the host range of *Golovinomyces ambrosiae*, the causal agent of hemp powdery mildew. When *G. ambrosiae* was first reported on hemp in New York in 2019, the pathogen was called *G. spadiceus* and was also being reported on several other crops throughout the world including sunflowers and okra. In this chapter, several growth chamber inoculations and field trials were conducted with a variety of crops to determine whether *G. ambrosiae*, isolated from hemp would be able to infect those other crop species. Additionally, I sought to determine whether *G. ambrosiae* isolated from both okra and sunflower was able to infect hemp. I

hypothesized that *G. ambrosiae* has a relatively wide host range and would be able to infect a wide range of crops. This work will allow for more informed management strategies, such as crop rotation, to be employed to mitigate powdery mildew disease pressure in hemp and other *G. ambrosiae* host crops.

Chapter 3: The goal of this chapter was to determine the efficacy of several commercially available fungicides, including two biocontrol agents, against hemp powdery mildew, caused by *G. ambrosiae* and gray mold caused by *Botrytis cinerea*. Additionally, I was interested in determining whether the application of these products, or pathogen infection would have an impact on the production of cannabinoids in hemp. I hypothesized that some of the products would be effective in reducing disease severity in both pathosystems, and that pathogen infection, especially *B. cinerea* infection of hemp inflorescence, would have an impact on cannabinoid production. These studies will provide useful tools for current hemp growers in New York for controlling hemp diseases.

Chapter 4: This research was a collaborative effort with the Cornell University hemp breeding program to attempt to identify possible genetic sources of host resistance to hemp powdery mildew, caused by *G. ambrosiae*. Throughout multiple field seasons, hemp cultivars and accessions that have been included in hemp breeding program were screened for powdery mildew susceptibility through both controlled growth chamber inoculations and field ratings. As high CBD, high CBG, fiber, grain and dual-purpose

cultivars were evaluated, it was hypothesized that a range of susceptibility to hemp powdery mildew would be observed among the hosts screened. This work was important for identifying a potential source of genetic resistance which was evaluated in the following chapter.

Chapter 5: A potential source of genetic host resistance to hemp powdery mildew was evaluated in this chapter. The F_1 and F_2 populations of a breeding line derived from one powdery mildew-susceptible and one resistance parent were characterized for their relative susceptibility to *G. ambrosiae* in both controlled growth chamber inoculations and field ratings. Based on the susceptibility of the two parents, and the initial F_1 ratings, it was hypothesized that a source of qualitative genetic resistance was responsible for the observed powdery mildew resistance. Initial genetic analysis of the F_2 population indicated the presence of a susceptibility gene and current work will aim to characterize the functionality of that gene in relation to powdery mildew susceptibility and resistance.

Literature Cited:

Bowen, P., Menzies, J., Ehret, D., Samuels, L., & Glass, A. D. M. (1992). Soluble silicon sprays inhibit powdery mildew development on grape leaves. In *J. AMER. Soc. HORT. SCI* (Vol. 117, Issue 6).

Büschges, R., & Hollricher, K. (1997). The barley *MLO* gene: A novel control element of plant pathogen resistance. *Cell*, *88*, 695–705.

Callaway, J. C. (2004). Hempseed as a nutritional resource: An overview. *Euphytica*, *140*(1), 65–72. https://doi.org/10.1007/S10681-004-4811-6

Cao, X. R., Yao, D. M., Duan, X. Y., Liu, W., Fan, J. R., Ding, K. J., & Zhou, Y. L. (2014). Effects of powdery mildew on 1000-kernel weight, crude protein content and yield of winter wheat in three consecutive growing seasons. *Journal of Integrative Agriculture*, *13*(7), 1530–1537. https://doi.org/10.1016/S2095-3119(14)60806-6

Cherney, J., & Small, E. (2016). Industrial hemp in North America: production, politics and potential. *Agronomy*, *6*(4), 58. https://doi.org/10.3390/agronomy6040058

Conner, R. L., Kuzyk, A. D., & Su, H. (2003). Impact of powdery mildew on the yield of soft white spring wheat cultivars. *Canadian Journal of Plant Science*, *83*(4), 725–728. https://doi.org/https://doi.org/10.4141/P03-043

de Meijer, E. P. M., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V. M. C., Ranalli, P., & Mandolino, G. (2003). The inheritance of chemical phenotype in *Cannabis sativa* L. *Genetics*, *163*(1), 335. https://doi.org/10.1093/GENETICS/163.1.335

de Meijer, E. P. M., & Hammond, K. M. (2005). The inheritance of chemical phenotype in *Cannabis sativa* L. (II): Cannabigerol predominant plants. *Euphytica 2005 145:1*, *145*(1), 189–198. https://doi.org/10.1007/S10681-005-1164-8

Domke, P. v, & Mude, V. D. (2015). Natural fiber reinforced building materials. *IOSR Journal of Mechanical and Civil Engineering*, *12*(3), 104–107. https://doi.org/10.9790/1684-1232104107

Farinas, C., & Hand, F. P. (2020). First report of *Golovinomyces spadiceus* causing powdery mildew on industrial hemp (*Cannabis sativa*) in Ohio. *Plant Disease*, *104*(10), 2727–2727. https://doi.org/10.1094/PDIS-01-20-0198-PDN

Félix-Gastélum, R., Olivas-Peraza, D. D., Quiroz-Figueroa, F. R., Leyva-Madrigal, K. Y., Peñuelas-Rubio, O., Espinosa-Matías, S., & Maldonado-Mendoza, I. E. (2019). Powdery mildew caused by *Golovinomyces spadiceus* on wild sunflower in Sinaloa, Mexico. *Canadian Journal of Plant Pathology*, *41*(2), 301–309. https://doi.org/10.1080/07060661.2019.1577916

Fike, J. (2016). Industrial hemp: renewed opportunities for an ancient crop. *Critical Reviews in Plant Sciences*, *35*(5–6), 406–424. https://doi.org/10.1080/07352689.2016.1257842

Fortenbery, T. R., & Bennett, M. (2001). *Is industrial hemp worth further study in the US?*

Garibaldi, A., Gilardi, G., & Gullino, M. L. (2011). Effect of potassium silicate and electrical conductivity in reducing powdery mildew of hydroponically grown tomato. *Mediterranean Phytopathological Union*, *50*(2), 192–202. https://www-jstor-org.proxy.library.cornell.edu/stable/26458693?seq=1

Gent, D. H., Claassen, B. J., Twomey, M. C., Wolfenbarger, S. N., & Woods, J. L. (2018). Susceptibility of hop crown buds to powdery mildew and its relation to perennation of *Podosphaera macularis*. *Plant Disease*, *102*(7), 1316–1325. https://doi.org/10.1094/PDIS-10-17-1530-RE

Gloss, D., & Vickrey, B. (2014). Cannabinoids for epilepsy. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD009270.pub3

Godfrey, D., Zhang, Z., Saalbach, G., & Thordal-Christensen, H. (2009). A proteomics study of barley powdery mildew haustoria. *Proteomics*, *9*(12), 3222–3232. https://doi.org/10.1002/PMIC.200800645

Goyal, N., Bhatia, G., Sharma, S., Garewal, N., Upadhyay, A., Upadhyay, S. K., & Singh, K. (2020). Genome-wide characterization revealed role of NBS-LRR genes during powdery mildew infection in *Vitis vinifera*. *Genomics*, *112*(1), 312–322. https://doi.org/10.1016/J.YGENO.2019.02.011

Grassa, C. J., Wenger, J. P., Dabney, C., Poplawski, S. G., Motley, S. T., Michael, T. P., Schwartz, C. J., & Weiblen, G. D. (2018). A complete *Cannabis* chromosome assembly and adaptive admixture for elevated cannabidiol (CBD) content. *BioRxiv*, 458083. https://doi.org/10.1101/458083

Green, A. J., Berger, G., Griffey, C. A., Pitman, R., Thomason, W., & Balota, M. (2014). Genetic resistance to and effect of leaf rust and powdery mildew on yield and its components in 50 soft red winter wheat cultivars. *Crop Protection*, *64*, 177–186. https://doi.org/10.1016/J.CROPRO.2014.06.023

Henning, J. A., Gent, D. H., Townsend, M. S., Woods, J. L., Hill, S. T., & Hendrix, D. (2017). QTL analysis of resistance to powdery mildew in hop (*Humulus lupulus* L.). *Euphytica*, *213*(4), 1–13. https://doi.org/10.1007/S10681-017-1849-9/TABLES/2

Jacott, C. N., Charpentier, M., Murray, J. D., & Ridout, C. J. (2020). Mildew Locus O facilitates colonization by arbuscular mycorrhizal fungi in angiosperms. *The New Phytologist*, *227*(2), 343. https://doi.org/10.1111/NPH.16465

Karus, M., & Vogt, D. (2004). European hemp industry: Cultivation, processing and product lines. *Euphytica*, *140*(1), 7–12. https://doi.org/10.1007/S10681-004-4810-7

Keinath, A. P., & Dubose, V. B. (2004). Evaluation of fungicides for prevention and management of powdery mildew on watermelon. *Crop Protection*, *23*(1), 35–42. https://doi.org/10.1016/S0261-2194(03)00165-0

Li, H.-L. (1974). An archaeological and historical account of *Cannabis* in China. *Economic Botany*, *28*(4), 437–448.

Livingston, S. J., Quilichini, T. D., Booth, J. K., Wong, D. C. J., Rensing, K. H., Laflamme-Yonkman, J., Castellarin, S. D., Bohlmann, J., Page, J. E., & Samuels, A. L. (2020). Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. *Plant Journal*, *101*(1), 37–56. https://doi.org/10.1111/tpj.14516

Lubell, J. D., & Brand, M. H. (2018). Foliar sprays of silver thiosulfate produce male flowers on female hemp plants. *HortTechnology*, *28*(6), 743–747. https://doi.org/10.21273/HORTTECH04188-18

Mandolino, G., Bagatta, M., Carboni, A., Ranalli, P., & de Meijer, E. (2003). Qualitative and quantitative aspects of the inheritance of chemical phenotype in *Cannabis*. *Journal of Industrial Hemp*, *8*(2), 51–72. https://doi.org/10.1300/J237V08N02_04

McPartland, J. M. (2018). *Cannabis* systematics at the levels of family, genus, and species. *Cannabis and Cannabinoid Research*, *3*(1), 203–212. https://doi.org/10.1089/CAN.2018.0039

McPartland, J. M., Guy, G. W., & Hegman, W. (2018). Cannabis is indigenous to Europe and cultivation began during the Copper or Bronze age: a probabilistic synthesis of fossil pollen studies. *Vegetation History and Archaeobotany*, *27*(4), 635–648. https://doi.org/10.1007/S00334-018-0678-7/TABLES/1

McPartland, J. M., Hegman, W., & Long, T. (2019). *Cannabis* in Asia: its center of origin and early cultivation, based on a synthesis of subfossil pollen and archaeobotanical studies. *Vegetation History and Archaeobotany*, *28*(6), 691–702. https://doi.org/10.1007/S00334-019-00731-8/FIGURES/4

Mechoulam, R., Parker, L. A., & Gallily, R. (2002). Cannabidiol: An overview of some pharmacological aspects. *Journal of Clinical Pharmacology*, *42*(11 SUPPL.), 11–19. https://doi.org/10.1002/j.1552-4604.2002.tb05998.x

Mihalyov, P. D., & Garfinkel, A. R. (2021). Discovery and genetic mapping of PM1, a powdery mildew resistance gene in *Cannabis sativa* L. *Frontiers in Agronomy*, *3*, 66. https://doi.org/10.3389/FAGRO.2021.720215/BIBTEX

Miller, R. L. (1991). Hemp as a crop for Missouri farmers: markets, economics, cultivation, law. *Report to Agriculture Task Force Missouri House of Representatives*.

Mohan Ram, H. Y., & Sett, R. (1982). Induction of fertile male flowers in genetically female *Cannabis sativa* plants by silver nitrate and silver thiosulphate anionic complex. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *62*(4), 369–375. https://doi.org/10.1007/BF00275107

Moparthi, S., Bradshaw, M., Frost, K., Hamm, P. B., & Buck, J. W. (2018). First report of powdery mildew caused by *Golovinomyces spadiceus* on okra in the United States. *Plant Disease*, *102*(8), 1664–1664. https://doi.org/10.1094/PDIS-01-18-0179-PDN

Moparthi, S., Bradshaw, M. J., & Grove, G. G. (2018). First report of powdery mildew caused by *Golovinomyces spadiceus* on *Helianthus annuus*. *Plant Disease*, *102*(6), 1176. https://doi.org/10.1094/PDIS-09-17-1434-PDN

O'Connell, R. J., & Panstruga, R. (2006). Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. *New Phytologist*, *171*(4), 699–718. https://doi.org/10.1111/J.1469-8137.2006.01829.X

Osterberger, E., Lohwasser, U., Jovanovic, D., Ruzicka, J., & Novak, J. (2021). The origin of the genus *Cannabis*. *Genetic Resources and Crop Evolution*, *69*, 1438–1449. https://doi.org/10.1007/s10722-021-01309-y

Pertwee, R. G. (2006). Cannabinoid pharmacology: The first 66 years. *British Journal of Pharmacology*, *147*(SUPPL. 1). https://doi.org/10.1038/sj.bjp.0706406

Pertwee, R. G. (2008). The diverse CB1 and CB 2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. *British Journal of Pharmacology*, *153*(2), 199–215. https://doi.org/10.1038/sj.bjp.0707442

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P. H., di Marzo, V., Elphick, M. R., Greasley, P. J., Hansen, H. S., Kunos, G., Mackie, K., Mechoulam, R., & Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB1 and CB2. *Pharmacological Reviews*, *62*(4), 588–631. https://doi.org/10.1124/pr.110.003004

Qiu, P., Liu, S., Bradshaw, M., Rooney-latham, S., Takamatsu, S., Bulgakov, T. S., Tang, S., Feng, J., Jin, D., Aroge, T., Li, Y., & Wang, L. (2020). Multi-locus phylogeny and taxonomy of an unresolved, heterogeneous species complex within the genus *Golovinomyces ambrosiae*, *G. circumfusus* and *G. spadiceus*. *BMC Microbiology*, *20*(51), 1–16.

Robinson, B. B. (1942). *Hemp for Victory*. U.S. government.

Roulac, J. (1997). *Hemp Horizons: the comeback of the world's most promising plant*.

Small, E. (2017). *Cannabis: A Complete Guide*. CRC Press.

Stack, G. M., Toth, J. A., Carlson, C. H., Cala, A. R., Marrero-González, M. I., Wilk, R. L., Gentner, D. R., Crawford, J. L., Philippe, G., Rose, J. K. C., Viands, D. R., Smart, C. D., & Smart, L. B. (2021). Season-long characterization of high-cannabinoid hemp (*Cannabis sativa* L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. *GCB Bioenergy*, *13*(4), 546–561. https://doi.org/10.1111/gcbb.12793

Szarka, D., Tymon, L., Amsden, B., Dixon, E., Judy, J., & Gauthier, N. (2019). First report of powdery mildew caused by *Golovinomyces spadiceus* on industrial hemp

(*Cannabis sativa*) in Kentucky. *Plant Disease*, *103*(7), 1773–1773. https://doi.org/10.1094/pdis-01-19-0049-pdn

Toth, J. A., Stack, G. M., Cala, A. R., Carlson, C. H., Wilk, R. L., Crawford, J. L., Viands, D. R., Philippe, G., Smart, C. D., Rose, J. K. C., & Smart, L. B. (2020). Development and validation of genetic markers for sex and cannabinoid chemotype in *Cannabis sativa* L. *GCB Bioenergy*, *12*(3), 213–222. https://doi.org/10.1111/gcbb.12667

Trigiano, R. N., Boggess, S. L., & Bernard, E. C. (2018). First report of powdery mildew caused by *Golovinomyces spadiceus* on green and gold (*Chrysogonum virginianum*) in the United States. *Plant Disease*, *102*(1), 252–252. https://doi.org/10.1094/PDIS-08-17-1292-PDN

USDA. (2000). *Industrial hemp in the United States: Status and market potential*. https://www.ers.usda.gov/webdocs/publications/41740/15867_ages001e_1_.pdf?v=3478.1

Voegele, R. T., & Phytopathologie, L. (2006). *Uromyces fabae*: development, metabolism, and interactions with its host *Vicia faba*. *FEMS Microbiology Letters*, *259*(2), 165–173. https://doi.org/10.1111/J.1574-6968.2006.00248.X

Voegele, R. T., Struck, C., Hahn, M., & Mendgen, K. (2001). From the Cover: The role of haustoria in sugar supply during infection of broad bean by the rust fungus Uromyces fabae. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(14), 8138. https://doi.org/10.1073/PNAS.131186798

Weldon, W. A., Ullrich, M. R., Smart, L. B., Smart, C. D., & Gadoury, D. M. (2019). Cross infectivity of powdery mildew isolates originating from hemp (*Cannabis sativa*) and

Japanese hop (*Humulus japonicus*) in New York. *Plant Health Progress*, *21*(1), 47–53. https://doi.org/https://doi.org/10.1094/PHP-09-19-0067-RS

Zhang, Q., Chen, X., Guo, H., Trindade, L. M., Salentijn, E. M. J., Guo, R., Guo, M., Xu, Y., & Yang, M. (2018). Latitudinal adaptation and genetic insights into the origins of *Cannabis sativa* L. *Frontiers in Plant Science*, *871*, 1876. https://doi.org/10.3389/FPLS.2018.01876/BIBTEX

CHAPTER II

AN EXPLORATION OF THE HOST RANGE OF *GOLOVINOMYCES AMBROSIAE*, THE CAUSAL AGENT OF HEMP POWDERY MILDEW

Abstract:

With hemp emerging as a new crop in the United States, problematic diseases have emerged simultaneously, including powdery mildew caused by *Golovinomyces ambrosiae*, which was first identified in New York State in 2019. The pathogen has been reported on a broad range of host species including sunflower, okra, and the ornamental flower green and gold (*Chrysogonum virginianum*). It is unusual for powdery mildews to have such a broad host range and understanding the host range of a pathogen is important for determining management strategies for the disease. This study aimed to evaluate the host range of an isolate of *G. ambrosiae* from hemp in both an indoor walk-in growth chamber and a field trial. It was determined that okra, sunflower, zinnia, and sunnhemp are hosts of *G. ambrosiae*. While *G. ambrosiae* was also able to infect several cucurbit cultivars in the indoor growth chambers, in the field *Podosphaera xanthii* (cucurbit powdery mildew) was the predominant species causing disease on the cucurbit cultivars. Moreover, *G. ambrosiae* isolated from okra and sunflower were able to infect a hemp cultivar. This study expanded our knowledge of the powdery mildew species *G. ambrosiae*.

Introduction:

Hemp (*Cannabis sativa* L.) has long been grown by humans primarily for fiber, grain, and cannabinoids. However, growing hemp in the United States was completely banned since the mid-twentieth century. In more recent years, with the passing of federal farm bills, hemp (defined as having less than 0.3% of the psychoactive cannabinoid Δ9-tetrahydrocannabdiol by weight) has been legalized, and numerous states have developed programs for licensing growers (Fike, 2016). The production of hemp, especially for its production of other cannabinoids and secondary metabolites, was incredibly widespread in 2019, but has since stabilized to ~60,000 acres in the United States. This has resulted in both an increase in grower acreage, and the study of all areas of hemp and its metabolites. As the number of acres of hemp continue to increase throughout the country, several important pests and disease have emerged, including powdery mildew.

Due to the total ban of *C. sativa* cultivation for many years, there is a lack of studies and information about many hemp pathogens, and best practices for the management of diseases. The use of both conventional fungicides and biocontrol agents have been adopted for controlling powdery mildew in other crops, but there are a limited number of products that are currently commercially available for use on hemp, and minimal published work on the efficacy of those available fungicides against hemp diseases. Genetic host resistance plays a very important role in managing powdery mildew for many crops with successful deployment of nucleotide-binding site and leucine rich

repeat (NBS-LRR) resistance genes (Goyal et al., 2020; He et al., 2018), including in hop, *Humulus lupulus* L., (Henning et al., 2017) which is the most closely related cultivated crop to *C. sativa* L. Susceptibility *Mildew Locus O* (*MLO*) genes which, when mutated, confer qualitative resistance are also important sources of genetic resistance that act specifically against powdery mildew species (Kusch & Panstruga, 2017). While at least one NB-LRR resistance gene (Mihalyov & Garfinkel, 2021) and two candidate *MLO* genes (Pépin et al., 2021) have been identified in hemp, there still is a limited understanding of genetic host resistance in hemp to powdery mildew. Additionally, the introgression of identified resistance genes into marketable hemp cultivars and their subsequent deployment is not an immediate solution to managing powdery mildew. Therefore, it will be important for growers in New York State, and elsewhere, to implement cultural management strategies to limit disease pressure and potential yield loss.

Powdery mildews are a group of fungal obligate biotrophic plant pathogens that compose the ascomycete family, Erysiphaceae. There are many species of powdery mildews, which as a group are able infect over 10,000 plant species and can have significant impact of yield of crops (Cao et al., 2014; Conner et al., 2003; Green et al., 2014). Powdery mildew infection begins with the germination of either and ascospore (sexual spore) or conidia (asexual spore) on a host tissue, typically the surface of a leaf. From differentiated hyphae, an appressorium forms producing a penetration peg. The penetration peg uses both mechanical and chemical mechanisms to penetrate the host

tissue and forms a haustorium. The haustorium is a specialized structure, which is responsible for uptake of nutrients and water from the host (Voegele et al., 2001), while secreting small effector molecules which establish and maintain fungal colonization within the host (Godfrey et al., 2009; O'Connell & Panstruga, 2006; Voegele & Phytopathologie, 2006). As vegetative hyphae continue to grow epiphytically, they eventually differentiate into conidiophores which produce chains of asexual conidia. The large number of conidia that are produced within a few days of initial infection can disseminate through wind and reinfect new host tissue. The polycyclic nature of powdery mildews life cycles can cause a rapid increase in disease intensity within a field or greenhouse when environmental conditions are favorable, causing epidemics when proper management strategies are not in place. Powdery mildews are also able to overwinter through the production of hardy sexual structures called chasmothecia which form when two compatible mating types overlap in their growth, within which infectious ascospores are formed, allowing reinfection to occur from season to season. It's also been shown that mycelia of some powdery mildews can overwinter as well (Gent et al., 2018).

Powdery mildew on hemp was first identified in New York in 2019 and classified as *Golovinomyces spadiceus* (Weldon et al., 2019), but is now known as *Golovinomyces ambrosiae* (Qiu et al., 2020). Prior to renaming, *G. spadiceus* had been reported on a broad range of host species including sunflower (Félix-Gastélum et al., 2019; Moparthi et al., 2018), okra (Moparthi et al., 2018), and the ornamental flower green and gold

(Trigiano et al., 2018). *Golovinomyces ambrosiae* has also been reported on a variety of species in the Asteraceae family including sunflower species (Radisek et al., 2018; Trigiano et al., 2016; Zhao et al., 2018), daisy species (Choi et al., 2018; Mukhtar et al., 2022) and giant ragweed (Cho et al., 2011).

Due to the obligate biotrophic nature of powdery mildews and the resulting challenges of conducting controlled experiments, many powdery mildew species, including those in the *Golovinomyces* genus are understudied. While it is understood that most powdery mildews are host specific, infecting only one plant host, or a small number of closely related plants, it seems that *G. ambrosiae* has a relatively broad host range, being reported on a variety of plant families. While powdery mildew species with a broader host range is uncommon, there are some examples including *G. orontii* (formerly *Erysiphe orontii*) (Whipps et al., 1998) and *Podosphaera xanthii* (Yeh et al., 2020).

Crop rotation to a non-host crop is one important way that growers can manage powdery mildew over a long period of time by limiting pathogens survival from year to year, although the overwintering mechanism for *G. ambrosiae* in New York State is currently unknown. Additionally, growing crops with differences in their pathogen susceptibility near one another, can reduce disease intensity of that caused by a polycyclic pathogen like powdery mildew within a growing season. This is true in a field setting but is especially significant in a controlled environment setting like a greenhouse, where environmental conditions are highly favorable for powdery mildew infection and

spread. Therefore, determination of the host range of *G. ambrosiae* isolated from hemp is essential information needed to make planting decisions to minimize powdery mildew disease pressure on hemp and other possible hosts.

The objectives of this study were two-fold: first, begin to determine the scope of the host range of *G. ambrosiae* the causal agent of hemp powdery mildew. Second, to determine if there were differences in susceptibility among various hosts. This was accomplished though controlled inoculations of *G. ambrosia*, initially isolated from hemp, on a variety of crops in both a controlled growth chamber environment and in a field trial setting. *Golovinomyces ambrosia* was also isolated from both okra and sunflower and used for inoculations of hemp in this study.

Materials and Methods:

Greenhouse inoculation

In the spring of 2020, seven different crop species were screened as hosts of three different isolates of *G. ambrosiae* including 19002, and 19001, both of which are NY isolates previously described (Weldon et al., 2019), and isolate 19137, collected in 2019 from Ontario County, NY. Plants were grown in the greenhouse at 23°C day and 20°C night temperature with 14-hour light for four weeks at which time five plants of each crop were inoculated with each of the three *G. ambrosiae* isolates with each isolate in separate growth chambers in a randomized complete block design. Growth chamber temperature and light conditions were the same as the greenhouse. To make a liquid

inoculum, hemp leaves infected with one of the three isolates were washed in a solution of 1 L of distilled water and 100 µL of Tween-20. Plants were spray inoculated to runoff with a conidial suspension of 2 x 10^4 spores per mL and evaluated for disease incidence at 19 days post inoculation (dpi).

In the spring of 2021, the survey was expanded to include more species and cultivars of each crop type (Table 2.2). Four 3-week-old plants of each cultivar were inoculated with the *G. ambrosiae* isolate 19002 in a growth chamber in a randomized complete block design. Liquid inoculum was made and plants were inoculated as described above, at a concentration of concentration of 5×10^4 conidia per mL. Plants were evaluated at 20 dpi.

Field inoculations

In the summer of 2021, a field screening was conducted with 22 species (Table 2.3). Plants were seeded in 50-cell flats with Lambert's LM-1 Germination Mix. Seedlings were grown in a greenhouse with a day temperature of 24°C and 21°C night temperature with a 16-hour photoperiod until transplanted into the field on June 21, 2021. Each of the potential hosts were planted in five-plant plots replicated four times in a randomized block design in a Cornell research field in Geneva, NY.

Fields were prepared for planting by amending with 100 lb. nitrogren per acre of using 19-6-19 N-P-K. Raised beds were prepared in rows with 6ft on center spacing with

black plastic. Cucurbits were spaced 3 ft apart, while all other crops were spaced 1 ft apart, with 6 ft between plots. Plants were irrigated using dripline irrigation as necessary based on rainfall. No additional nitrogen was added, and plants were not sprayed for any other pests. Weed cloth between rows was used for weed control.

Once in the field, plants were inoculated twice with *G. ambrosiae* isolates which were grown on whole hemp plants in growth chambers. Field plants were initially inoculated on July 30, four weeks after planting. To do this a concentrated liquid inoculum was made by washing infected hemp leaves with a solution of 2 L of distilled water and 200 µL of Tween20 at a concentration of 1×10^5 conidia per mL. This concentrated inoculum was diluted and a total of 15 L of water was sprayed onto the plants with a backpack sprayer to runoff. Plants were inoculated a second time, 11 days after the first inoculation. Inoculum was produced as described above, with a final concentration of 6×10^4 conidia per mL. This concentrated solution was diluted in 15 L of water before being sprayed onto the plants.

Disease severity was assessed by rating for percent disease coverage per plot at 11-, 18-, 25-, 31- and 38- days after the initial inoculation. Data analysis was performed using R version 4.0.4 (R Core Team, 2021)in RStudio 1.4.1106 (R Studio Team, 2020). The mean area under the disease progress curve (AUDPC) for each cultivar was calculated using the R package agricolae (de Mendiburu, 2020). The mean AUDPC of cultivars the same crop (i.e. sunflowers, zinnia, okra) were compared to each other using an

analysis of variance (ANOVA) test ($p<0.05$), followed by a Tukey's HSD test ($p<0.05$) using the HSD.test function in the agricolae package (de Mendiburu, 2020) for pairwise comparison between cultivars.

Table 2.1. Plants inoculated with *Golovinomyces ambrosiae* in the summer of 2021 field trial.

Plant Species	Cultivar	Crop
Abelmoschus esculentus	Carmine Splendor	Okra
Abelmoschus esculentus	Jambalaya	Okra
Crotalaria juncea	*unknown*	Sunnhemp
Cucurbita maxima ssp. *Maxima*	Shokichi Green F_1	Kabocha squash
Cucurbita maxima ssp. *Maxima*	blue Hubbard	Hubbard squash
Cucurbita moschata	Waltham	Butternut squash
Cucurbita pepo var. *cylindrica*	Dunja F_1	Zucchini
Cucurbita pepo var. *cylindrica*	Magda F_1	Zucchini
Cucurbita pepo var. *cylindrica*	Tigress F_1	Zucchini
Cucurbita pepo var. *fastigata*	Spaghetti Squash	Spaghetti squash
Cucurbita pepo var. *pepo*	New England Pie	Pie pumpkin
Cucurbita pepo var. *turbinata*	Autumn Delight F_1	Acorn squash
Helianthus annuus	Autumn Beauty	Sunflower
Helianthus annuus	ProCut White Nite	Sunflower
Helianthus annuus	Royal Hybrid	Sunflower
Helianthus annuus	Double Quick Orange	Sunflower
Helianthus annuus	Firecracker F_1	Sunflower
Zinnia elegans	Lilliput mix	Zinnia
Zinnia elegans	Mini Zini Mix	Zinnia
Zinnia elegans	Zowie	Zinnia
Zinnia haageana	Jazzy Mix	Zinnia
Zinnia marylandica	Raspberry Lemonade Mix	zinnia

Identity of field-collected isolates

Powdery mildew samples were collected from the field experiment by using stickers to collect conidia from leaf surfaces in the field, a method developed by (Weldon et al., 2021). DNA was extracted from each sample using a protocol developed by (Healey et al., 2014) and modified by Xia Xu of the Lance Cadle-Davidson lab (personal communication). The samples were identified by PCR amplifying the intergenic spacer (IGS) region using the primers IGS-12a and NS1R (Carbone & Kohn, 1999). Amplicons were prepared for sequencing using the ExoSAP-IT™ PCR Product Cleanup kit as described by the manufacturer (Thermo Fisher Scientific, Waltham MA) and sequenced at the Cornell University Institute of Biotechnology. For each sample, consensus sequences were aligned using Geneious 2022.0.1 MUSCLE alignment software (https://www.geneious.com) and identification was based on BLAST search comparisons (Altschul et al., 1990).

Inoculation of hemp with **G. ambrosiae** *from other crops*

A powdery mildew isolate (21073) was collected from an okra plant (*Abelmoschus esculentus*) in Ontario County, NY in the summer of 2021. DNA was extracted, and the IGS region was PCR amplified and sequenced as described above. Powdery mildew conidia were transferred from the okra plant onto the hemp cultivar 'White CBG' using a paint brush. The hemp plant was placed in a growth chamber with a 14-hour photoperiod, 23°C day temperature and a night temperature of 20°C. The powdery mildew was able to infect and sporulate on the hemp plant. The conidia from the hemp

plant were then used to inoculate a new okra plant, also in a growth chamber under the same conditions (Figure 2.1).

Another powdery mildew isolate (21066) was collected in the summer of 2021 from a sunflower plant in Ontario County, NY. DNA was extracted, PCR amplified and sequenced as described above. Powdery mildew conidia were transferred to a hemp plant and then transferred back to a clean sunflower plant using the same method as described for the okra isolate.

Results:

Growth chamber inoculations

Plants inoculated with three different *G. ambrosia* isolates in the growth chamber in the spring of 2020 were evaluated with disease incidence 19 dpi. Of the seven crop species that were inoculated, three became infected with all three *G. ambrosiae* isolates: the okra cultivar 'Clemson Spineless #80', the zucchini cultivar 'Leopard squash', and the sunflower cultivar 'Dwarf Teddy Bear' (Table 2.1).

Table 2.2. Incidence of powdery mildew on various crops inoculated with three different isolates (19002, 19002, 19137) of *Golovinomyces ambrosiae* in a growth chamber in the spring 2020.

			Isolate		
			19001	19002	19137
Plant Species	**Cultivar**	**Crop**	**# Infected Plants/Total Plants**	**# Infected Plants/Total Plants**	**# Infected Plants/Total Plants**
Abelmoschus esculentus	Clemson Spineless #80	Okra	4/5	3/5	4/4
Cucurbita pepo	Crookneck squash	Yellow Squash	0/3	0/2	0/3
Citrullus lanatus	Crimson Sweet	Watermelon	0/5	0/5	0/5
Cucumis melo	Hales Best Jumbo	Cantaloupe	0/5	0/5	0/5
Cucurbita pepo	Leopard squash	Zucchini	5/5	5/5	5/5
Helianthus annuus	Dwarf Teddy Bear	Sunflower	5/5	2/5	1/5
Zinnia spp.	Mixture	Zinnia	0/5	0/5	0/5

The number of crop species and cultivars that were evaluated was expanded in the 2021 growth chamber trial, and crops were inoculated with only one powdery mildew isolate, 19002. In this trial, a different okra cultivar, 'Red Burgandy', was included and became infected with powdery mildew. Several *Cucurbita pepo* varieties were included, and all of them became infected with powdery mildew with exception of two: the cultivars 'Zephyr' (*Cucurbita pepo* var. recticollis) and 'Table Queen' (*Cucurbita pepo* var. turbinate). Several more sunflower and zinnia cultivars were also inoculated and all of them became infected expect for the zinnia cultivar 'Raspberry Lemonade Mix' (Table 2.2).

Over the two growth chamber trials, a total of 9 species and 22 cultivars were inoculated with G. *ambrosiae* and of those, 5 species and 15 cultivars were susceptible.

Table 2.3. Incidence of powdery mildew on plants inoculated with the *Golovinomyces ambrosiae* isolate 19002 in a growth chamber in 2021.

Plant Species	Cultivar	Crop	# Infected Plants/Total Plants
Abelmoschus esculentus	Red Burgundy	Okra	4/4
Crotalaria juncea	unknown	Sunnhemp	2/4
Cucurbita pepo var. *cylindrica*	Dunja F_1	Zucchini	4/4
Cucurbita pepo var. *cylindrica*	Magda F_1	Zucchini	4/4
Cucurbita pepo var. *cylindrica*	Tigress F_1	Zucchini	3/4
Cucurbita pepo var. *fastigata*	Unknown	Spaghetti Squash	1/4
Cucurbita pepo var. *pepo*	New England Pie	Pumpkin	4/4
Cucurbita pepo var. *recticollis*	Zephyr F_1	Summer Squash	0/4
Cucurbita pepo var. *turbinate*	Autumn Delight F_1	Acorn Squash	2/4
Cucurbita pepo var. *turbinate*	Table Queen	Acorn Squash	0/4
Helianthus annuus	Autumn Beauty	Sunflower	3/4
Helianthus annuus	ProCut	Sunflower	4/4
Zinnia elegans	Lilliput mix	Zinnia	1/4
Zinnia elegans	Mini Zini Mix	Zinnia	2/4
Zinnia marylandica	Raspberry Lemonade Mix	Zinnia	0/4

Field inoculation:

In the summer of 2021 field trial, 22 cultivars were evaluated for the susceptibility to the hemp powdery mildew pathogen *G. ambrosiae* (Table 2.3). For this trial, disease severity was assessed by rating the plots for percent disease coverage throughout the season. The date of first disease incidence, the average final rating and the mean AUDPC for each plant cultivar are shown in Table 2.4 to illustrate the disease progression throughout the season. At the final rating all cultivars in the field trial were infected with powdery mildew except for the zinnia cultivars 'Mini zini' mix (*Z. elegans*), 'Zowie' (*Z. elegans*) and 'Raspberry lemonade' mix (*Z. marylandica*).

The powdery mildew samples collected from the field were identified by sequencing PCR amplicons obtained using IGS primers (Table 2.5). Samples were collected from all plots where powdery mildew could be seen with the naked eye, but PCR amplification was not successful for all samples. Both okra cultivars were affected by powdery mildew and differed in disease severity (Table 2.4). The sample collected from cultivar 'Jambalaya' was successfully amplified and identified as *G. orontii* (Table 2.5). Powdery mildew was also observed in the sunnhemp plots (Table 2.4), and samples collected from those plots were identified as *G. ambrosiae* (Table 2.5). All the cucurbit cultivars became infected with powdery mildew with high amounts of variability in the disease severity between cultivars (Table 2.4). However, most of the samples collected from the cucurbit plots were identified as *Podosphaera xanthii*, cucurbit powdery mildew. *Golovinomyces ambrosiae* was identified in samples collected from plots of

the kabocha squash, cultivar ‘Shokichi Green’, and the zucchini cultivar ‘Tigress’. Another sample from a ‘Shokichi Green’ plot was identified as *G. orontii*. All sunflower cultivars included in this study showed signs of powdery mildew. ‘Procut White Nite’ was the most susceptible cultivar when AUDPC were compared (Table 2.4). All samples from sunflower that were successfully amplified through PCR were identified as *G. ambrosiae* (Table 2.5).

By the last rating, ‘Lilliput Mix’ and ‘Jazzy Mix’ were the only zinnia cultivars on which powdery mildew could be observed (Table 2.4). DNA from samples collected from ‘Jazzy mix’ plots were successfully amplified and identified as *G. ambrosiae* (Table 2.5). When samples were being collected, after the last disease rating, powdery mildew was observed in both ‘Mini zini’ mix and ‘Zowie’ plots for the first time. Samples were collected from those plots, and *G. ambrosiae* was identified from all samples (Table 2.5).

Table 2.4. Powdery mildew progression (as measured by area under the disease progress curve; AUDPC) and severity on field trials crops in 2021. The final disease rating was August 26, 2021.

	Date of 1st disease incidence	Mean final rating for disease severity	Mean AUDPC
Okra			
Carmine Slendor	August 6, 2021 (18 dpi)	11.8	131.6
Jambalaya	August 19, 2021 (31 dpi)	2.4	9.1
Sunnhemp	August 6, 2021 (18 dpi)	1.5	8.3
Cucurbits			
Shokichi Green F_1	August 6, 2021 (18 dpi)	20.0	102.2
Blue Hubbard	August 13, 2021 (25 dpi)	23.8	155.4
Waltham	August 13, 2021 (25 dpi)	17.5	139.6
Dunja F_1	August 26, 2021 (38 dpi)	5.2	18.1
Magda F_1	August 13, 2021 (25 dpi)	30.0	108.6
Tigress F_1	August 26, 2021 (38 dpi)	27.5	96.3
Spaghetti Squash	August 6, 2021 (18 dpi)	85.0	611.5
New England Pie	August 6, 2021 (18 dpi)	58.8	625.0
Autumn Delight F_1	August 13, 2021 (25 dpi)	10.0	43.3
Sunflowers			
Autumn Beauty	August 13, 2021 (25 dpi)	7.5	38.3
ProCut White Nite	August 13, 2021 (25 dpi)	22.5	200.6
Royal Hybrid	August 6, 2021 (18 dpi)	15.5	64.5

Double Quick Orange	August 19, 2021 (31 dpi)	8.0	39.5
Firecracker F_1	August 26, 2021 (38 dpi)	6.3	21.9
Zinnia			
Lilliput mix	August 13, 2021 (25 dpi)	0.5	8.3
Mini Zini Mix	NA	0.0	0.0
Zowie	NA	0.0	0.0
Jazzy Mix	August 6, 2021 (18 dpi)	15.0	194.5
Raspberry Lemonade Mix	NA	0.0	0

Table 2.5. Identification of powdery mildew samples collected from plants in field trial. Powdery mildew species were identified through sequencing of PCR products using IGS primers and BLAST searches of sequences.

Plant Collected from	Sample	Collection Date	Identification
Okra			
Jambalaya	02-02-01	September 20, 2021	*G. orontii*
Sunnhemp	03-02-01	September 20, 2021	*G. ambrosiae*
	03-02-02	September 20, 2021	*G. ambrosiae*
	03-02-04	September 20, 2021	*G. ambrosiae*
	03-04-01	August 16, 2021	*G. ambrosiae*
Cucurbits			
Shokichi Green F_1	04-01-01	August 16, 2021	*G. ambrosiae*
	04-04-01	August 16, 2021	*G. orontii*
Waltham	06-03-01	September 20, 2021	*P. xanthii*
Dunja F_1	07-02-01	September 20, 2021	*P. xanthii*
Magda F_1	08-02-01	September 20, 2021	*P. xanthii*
	08-02-02	September 20, 2021	*P. xanthii*
	08-03-01	September 20, 2021	*P. xanthii*
	08-03-02	September 20, 2021	*P. xanthii*
Tigress F_1	09-01-01	September 20, 2021	*P. xanthii*
	09-03-01	September 20, 2021	*P. xanthii*
	09-03-02	September 20, 2021	*P. xanthii*
	09-04-01	September 20, 2021	*G. ambrosiae*
Spaghetti Squash	10-02-02	August 16, 2021	*P. xanthii*
New England Pie	11-03-02	August 16, 2021	*P. xanthii*

Autumn Delight F_1	12-01-02	September 20, 2021	*P. xanthii*
	12-02-01	September 20, 2021	*P. xanthii*
Sunflowers			
ProCut White Nite	14-04-01	August 16, 2021	*G. ambrosiae*
	14-03-02	August 16, 2021	*G. latisporus*
Royal Hybrid	15-02-01	September 20, 2021	*G. ambrosiae*
Double Quick Orange	16-02-02	September 20, 2021	*G. ambrosiae*
	16-03-01	September 20, 2021	*G. ambrosiae*
Zinnia			
Mini Zini Mix	19-02-01	September 28, 2021	*G. ambrosiae*
	19-03-02	September 28, 2021	*G. ambrosiae*
	19-04-02	September 28, 2021	*G. ambrosiae*
Zowie	20-01-02	September 28, 2021	*G. ambrosiae*
	20-02-02	September 28, 2021	*G. ambrosiae*
	20-04-01	September 28, 2021	*G. ambrosiae*
	20-04-02	September 28, 2021	*G. ambrosiae*
Jazzy Mix	21-01-01	August 16, 2021	*G. ambrosiae*
	21-01-02	August 16, 2021	*G. ambrosiae*
	21-04-02	August 16, 2021	*G. ambrosiae*

Inoculation of hemp with* G. ambrosiae *from other crops:

Conidia from a powdery mildew isolate (21073) collected from an okra plant in a local garden were transferred to the hemp cultivar 'White CBG'. The powdery mildew was able to infect the hemp plant and sporulation occurred. When the conidia from the hemp plant were transferred to a clean okra plant, infection and sporulation occurred (Figure 2.1). Sequencing of PCR products determined that the powdery mildew identity was *G. ambrosiae*.

The powdery mildew isolate (21066) was also used to inoculate a 'White CBG' hemp plant. Infection was able to occur on the hemp plant, and conidia were transferred back to a clean sunflower plant. The sunflower plant also became infected with powdery mildew. Powdery mildew samples collected were sequenced and were determined to be *G. ambrosiae*.

Figure 2.1. Powdery mildew isolate 21073, originally collected from an okra plant, infecting the hemp cultivar 'White CBG' (A). The isolate was transferred to a clean okra plant and able to cause an infection and sporulate (B).

Discusssion:

This study has expanded our knowledge of the host range of the hemp powdery mildew pathogen *G. ambrosiae*. The growth chamber inoculations demonstrated that *G. ambrosiae* initially isolated from hemp infected and caused disease on a variety of crops that are not closely related to hemp. Specifically, it was demonstrated that the *G. ambrosiae* isolates 19001, 19002 and 19138 were able to infect okra, sunflowers, zinnia and the cucurbit species *C. pepo*. The *G. ambrosiae* isolate 19002 was also able to infect additional cultivars of okra, sunflower, zinnia and *C. pepo*. The ability of *G. ambrosiae* to infect sunflower and okra was consistent with the reports of *G. ambrosiae* in various locations (Félix-Gastélum et al., 2019; Moparthi, Bradshaw, & Grove, 2018; Moparthi, Bradshaw, Frost, et al., 2018). While a closely related powdery mildew species, *G. cichoracearum* is one of the causal agents of cucurbit powdery mildew and has been reported on hemp (Pépin et al., 2018), to our knowledge, *G. ambrosiae* has not been previously reported on cultivated cucurbit species. There is a recent report of *G. ambrosiae* on the wild species *Sicyos deppei* (Cucurbitaceae) in Mexico (Gregorio-Cipriano et al., 2022). Differences in resistance and susceptibility within the Cucurbitaceae to *G. ambrosiae* are evident as cucurbit genera *Citrullus* and *Cucumis* were screened in this experiment were not susceptible to *G. ambrosiae*. Future studies will screen additional cucurbit genera and species for susceptibility to *G. ambrosiae.* In addition to being a pathogen of cucurbits, G. *cichoracearum* has also been reported on zinnia (Park et al., 2011; Zhu et al., 2021), but Qiu et al. (2020) also report zinnia as a host of *G. ambrosiae*. This study confirms that zinnia is susceptible to *G. ambrosiae*,

with the exception of the ‘Raspberry Lemonade Mix’, which is a different species within the genus *Zinnia* and may have unique resistance or susceptibility genes.

The field inoculations in this experiment confirmed many of the results that were determined in the growth chamber experiments. The same sunflower and zinnia species that were susceptible to *G. ambrosiae* in the growth chamber also became infected in the field trial. Further, additional cultivars of sunflower, zinnia, okra, and sunn hemp were susceptible to *G. ambrosiae*.

While all the *C. pepo* cultivars appeared to be susceptible to *G. ambrosia* based on disease severity, through genetic identification of isolates collected from the field, it was revealed that many of the *C. pepo* individuals were infected with the powdery mildew species *P. xanthii*, though *G. ambrosiae* was also present in some samples collected from *C. pepo*. *Podosphaera xanthii* is a common causal agent of cucurbit powdery mildew in New York State, along with *G. cichoracerarum.* It is an extremely aggressive pathogen and is a major limiting factor for cucurbit production (Martínez-Cruz et al., 2014). While the growth chamber studies indicate that *C. pepo* cultivars are susceptible to *G. ambrosiae*, and *G. ambrosiae* was present on in some field samples collected from *C. pepo*, it is likely that *P. xanthii* is a much more aggressive pathogen on cucurbits and is therefore able to outcompete *G. ambrosiae* in a field setting when both pathogens are present. Cucurbit powdery mildew is very common and the plants in this study were infected by natural inoculum of *P. xanthii*.

Golovinomyces orontii was also identified in a small number of the samples collected from the field. This is likely since *G. orontii* and *G. ambrosiae* are very closely related species, and the resolution to differentiate them based on only the IGS sequence used in this study may be insufficient (Qiu et al., 2020). Additionally, it is possible that *G. orontii* was present in the field naturally and causing infection on those plants where it was identified.

In both the growth chamber and field trial it was demonstrated that zinnia species were susceptible to *G. ambrosiae*. However, in both the growth chamber and field, there were no observable powdery mildew symptoms on the cultivar 'Raspberry Lemonade Mix.' This cultivar was the only representative of the zinnia species *Zinnia marylandica*, one that is known to have resistance to other diseases including *Alternaria* blight (*Alternaria zinniae* Pape), and bacterial leaf spot (*Xanthomonas campestris* pv. zinnia) (Boyle & Wick, 1996; Spooner et al., 1991). Additional cultivars of this zinnia species should be screened to better understand the host range of *G. ambrosia* within the genus *Zinnia*.

In addition to demonstrating that *G. ambrosiae* originally isolated from hemp is pathogenic against a relatively broad range of hosts, this study also demonstrates that *G. ambrosiae* isolated from some of these other hosts are pathogenic on hemp. Specifically, two isolates of *G. ambrosiae* that were initially isolated from sunflower and okra were able to infect the hemp, validating the wide host range of this pathogen across isolates.

Understanding the host range of *G. ambrosiae*, the causal agent of hemp powdery mildew, will have implications on vegetable and specialty crops production and management strategies of powdery mildew in fields and greenhouses. Cultivating hemp near other *G. ambrosiae* host crops may increase powdery mildew disease severity and maximize an outbreak if the disease is not managed. While this study will allow more informed planting decisions to be made for powdery mildew disease management, additional work is needed as the scope of the host range is not fully known. Future studies will provide additional information on closely related species and cultivars that were not screened in this study.

Literature Cited:

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). *Basic local alignment search tool* (No. 215; pp. 403–410). Mol. Biol.

Boyle, T. H., & Wick, R. L. (1996). Responses of *Zinnia angustifolia* × *Z. violacea* backcross hybrids to three pathogens. *HortScience*, *31*(5), 851–854. https://doi.org/10.21273/HORTSCI.31.5.851

Cao, X. R., Yao, D. M., Duan, X. Y., Liu, W., Fan, J. R., Ding, K. J., & Zhou, Y. L. (2014). Effects of powdery mildew on 1000-kernel weight, crude protein content and yield of winter wheat in three consecutive growing seasons. *Journal of Integrative Agriculture*, *13*(7), 1530–1537. https://doi.org/10.1016/S2095-3119(14)60806-6

Carbone, I., & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia*, *91*(3), 553–556.

Cho, S. E., Park, J. H., Park, M. J., & Shin, H. D. (2011). First report of powdery mildew caused by *Golovinomyces ambrosiae* on *Ambrosia trifida* in Korea. *Plant Disease*, *95*(11), 1480–1480. https://doi.org/10.1094/PDIS-05-11-0422

Choi, I. Y., Ahn, M. S., Cho, S. E., Kim, J. Y., & Shin, H. D. (2018). First Report of powdery mildew caused by *Golovinomyces ambrosiae* on English daisy (*Bellis perennis*) in Korea. *Plant Disease*, *102*(10), 2035. https://doi.org/10.1094/PDIS-03-18-0476-PDN

Conner, R. L., Kuzyk, A. D., & Su, H. (2003). Impact of powdery mildew on the yield of soft white spring wheat cultivars. *Canadian Journal of Plant Science*, *83*(4), 725–728. https://doi.org/https://doi.org/10.4141/P03-043

de Mendiburu, F. (2020). *agricolae: Statistical Procedures for Agricultural Research.* (R package version 1.3-3.).

Félix-Gastélum, R., Olivas-Peraza, D. D., Quiroz-Figueroa, F. R., Leyva-Madrigal, K. Y., Peñuelas-Rubio, O., Espinosa-Matías, S., & Maldonado-Mendoza, I. E. (2019). Powdery mildew caused by *Golovinomyces spadiceus* on wild sunflower in Sinaloa, Mexico. *Canadian Journal of Plant Pathology*, *41*(2), 301–309. https://doi.org/10.1080/07060661.2019.1577916

Fike, J. (2016). Industrial hemp: renewed opportunities for an ancient crop. *Critical Reviews in Plant Sciences*, *35*(5–6), 406–424. https://doi.org/10.1080/07352689.2016.1257842

Gent, D. H., Claassen, B. J., Twomey, M. C., Wolfenbarger, S. N., & Woods, J. L. (2018). Susceptibility of hop crown buds to powdery mildew and its relation to perennation of *Podosphaera macularis*. *Plant Disease*, *102*(7), 1316–1325. https://doi.org/10.1094/PDIS-10-17-1530-RE

Godfrey, D., Zhang, Z., Saalbach, G., & Thordal-Christensen, H. (2009). A proteomics study of barley powdery mildew haustoria. *Proteomics*, *9*(12), 3222–3232. https://doi.org/10.1002/PMIC.200800645

Goyal, N., Bhatia, G., Sharma, S., Garewal, N., Upadhyay, A., Upadhyay, S. K., & Singh, K. (2020). Genome-wide characterization revealed role of NBS-LRR genes during powdery mildew infection in *Vitis vinifera*. *Genomics*, *112*(1), 312–322. https://doi.org/10.1016/J.YGENO.2019.02.011

Green, A. J., Berger, G., Griffey, C. A., Pitman, R., Thomason, W., & Balota, M. (2014). Genetic resistance to and effect of leaf rust and powdery mildew on yield and its components in 50 soft red winter wheat cultivars. *Crop Protection*, *64*, 177–186. https://doi.org/10.1016/J.CROPRO.2014.06.023

Gregorio-Cipriano, R., González, D., Félix-Gastelum, R., Chacón, S., Gregorio-Cipriano, R., González, D., & Chacón, S. (2022). Morphological and molecular identification of new records and new host plants of powdery mildews (Erysiphaceae) from Mexico. *Botany*, *00*, 1–17. https://doi.org/10.1139/CJB-2021-0209

He, H., Zhu, S., Zhao, R., Jiang, Z., Ji, Y., Ji, J., Qiu, D., Li, H., & Bie, T. (2018). Pm21, encoding a typical CC-NBS-LRR protein, confers broad-spectrum resistance to wheat powdery mildew disease. *Molecular Plant*, *11*, 879–882. https://doi.org/10.1016/j.molp.2018.03.004

Healey, A., Furtado, A., Cooper, T., & Henry, R. J. (2014). Protocol: a simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. *Plant Methods*, *10*(1), 21. https://doi.org/10.1186/1746-4811-10-21

Henning, J. A., Gent, D. H., Townsend, M. S., Woods, J. L., Hill, S. T., & Hendrix, D. (2017). QTL analysis of resistance to powdery mildew in hop (*Humulus lupulus* L.). *Euphytica*, *213*(4), 1–13. https://doi.org/10.1007/S10681-017-1849-9/TABLES/2

Kusch, S., & Panstruga, R. (2017). *Mlo*-based resistance: An apparently universal "weapon" to defeat powdery mildew disease. *Molecular Plant-Microbe Interactions*, *30*(3), 179–189. https://doi.org/https://doi.org/10.1094/MPMI-12-16-0255-CR

Martínez-Cruz, J., Romero, D., Dávila, J. C., & Pérez-García, A. (2014). The *Podosphaera xanthii* haustorium, the fungal Trojan horse of cucurbit-powdery mildew interactions. *Fungal Genetics and Biology*, *71*, 21–31. https://doi.org/10.1016/J.FGB.2014.08.006

Mihalyov, P. D., & Garfinkel, A. R. (2021). Discovery and genetic mapping of PM1, a powdery mildew resistance gene in *Cannabis sativa* L. *Frontiers in Agronomy*, *3*, 66. https://doi.org/10.3389/FAGRO.2021.720215/BIBTEX

Moparthi, S., Bradshaw, M., Frost, K., Hamm, P. B., & Buck, J. W. (2018). First report of powdery mildew caused by *Golovinomyces spadiceus* on okra in the United States. *Plant Disease*, *102*(8), 1664–1664. https://doi.org/10.1094/PDIS-01-18-0179-PDN

Moparthi, S., Bradshaw, M. J., & Grove, G. G. (2018). First report of powdery mildew caused by *Golovinomyces spadiceus* on *Helianthus annuus*. *Plant Disease*, *102*(6), 1176. https://doi.org/10.1094/PDIS-09-17-1434-PDN

Mukhtar, I., Cheng, Y., Khokhar, I., Chen, R., Li, R., Liang, C., Chen, X., & Chen, J. (2022). First report of powdery mildew caused by *Golovinomyces ambrosiae* on *Bidens pilosa* in China. *Plant Disease*, *106*(1). https://doi.org/10.1094/PDIS-05-21-0910-PDN

O'Connell, R. J., & Panstruga, R. (2006). Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. *New Phytologist*, *171*(4), 699–718. https://doi.org/10.1111/J.1469-8137.2006.01829.X

Park, M. J., Park, J. H., Kim, H. G., Lee, S. G., Koh, Y. J., Kim, B. S., Cha, B., Lee, H. B., & Shin, H. D. (2011). Outbreak of powdery mildew on *Zinnia elegans* by

Golovinomyces cichoracearum in Korea, 2008-2010. *The Plant Pathology Journal*, *27*(1), 85–88. https://doi.org/10.5423/PPJ.2011.27.1.085

Pépin, N., Hebert, F. O., & Joly, D. L. (2021). Genome-wide characterization of the *MLO* gene family in *Cannabis sativa* reveals two genes as strong candidates for powdery mildew susceptibility. *Frontiers in Plant Science*, *12*(729261). https://doi.org/10.3389/FPLS.2021.729261

Pépin, N., Punja, Z. K., & Joly, D. L. (2018). Occurrence of powdery mildew caused by *Golovinomyces cichoracearum* sensu lato on *Cannabis sativa* in Canada. *Plant Disease*, *102*(12), 2644. https://doi.org/10.1094/PDIS-04-18-0586-PDN

Qiu, P., Liu, S., Bradshaw, M., Rooney-latham, S., Takamatsu, S., Bulgakov, T. S., Tang, S., Feng, J., Jin, D., Aroge, T., Li, Y., & Wang, L. (2020). Multi-locus phylogeny and taxonomy of an unresolved, heterogeneous species complex within the genus *Golovinomyces ambrosiae*, *G. circumfusus* and *G. spadiceus*. *BMC Microbiology*, *20*(51), 1–16.

R Core Team. (2021). *R: A language and environment for statistical computing* (4.0.4). R Foundation for Statistical Computing.

R Studio Team. (2020). *RStudio: Integrated Development for R* (1.4.1106). RStudio, PBC.

Radisek, S., Jakse, J., Zhao, T. T., Cho, S. E., & Shin, H. D. (2018). First report of powdery mildew of *Helianthus tuberosus* caused by *Golovinomyces ambrosiae* in Slovenia. *Journal of Plant Pathology*, *100*(2), 331–331. https://doi.org/10.1007/S42161-018-0049-3

Spooner, D. M., Stimart, D. P., & Boyle, T. H. (1991). Zinnia marylandica (Asteraceae: Heliantheae), a new disease-resistant ornamental hybrid. *Brittonia*, *43*(1), 7–10. https://doi.org/10.2307/2807169

Trigiano, R. N., Bernard, E. C., Hadziabdic, D., Dattilo, A. J., & Wadl, P. A. (2016). First report of powdery mildew on whorled sunflower (*Helianthus verticillatus*) caused by *Golovinomyces ambrosiae*. *Plant Disease*, *100*(5), 1017. https://doi.org/10.1094/PDIS-11-15-1269-PDN

Trigiano, R. N., Boggess, S. L., & Bernard, E. C. (2018). First report of powdery mildew caused by *Golovinomyces spadiceus* on green and gold (*Chrysogonum virginianum*) in the United States. *Plant Disease*, *102*(1), 252–252. https://doi.org/10.1094/PDIS-08-17-1292-PDN

Voegele, R. T., & Phytopathologie, L. (2006). *Uromyces fabae*: development, metabolism, and interactions with its host *Vicia faba*. *FEMS Microbiology Letters*, *259*(2), 165–173. https://doi.org/10.1111/J.1574-6968.2006.00248.X

Voegele, R. T., Struck, C., Hahn, M., & Mendgen, K. (2001). From the Cover: The role of haustoria in sugar supply during infection of broad bean by the rust fungus Uromyces fabae. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(14), 8138. https://doi.org/10.1073/PNAS.131186798

Weldon, W. A., Knaus, B. J., Grünwald, N. J., Havill, J. S., Block, M. H., Gent, D. H., Cadle-Davidson, L. E., & Gadoury, D. M. (2021). Transcriptome-derived amplicon sequencing markers elucidate the U.S. *Podosphaera macularis* population structure

across feral and commercial plantings of *Humulus lupulus*. *Phytopathology*, *111*(1), 194–203. https://doi.org/https://doi.org/10.1094/PHYTO-07-20-0299-FI

Weldon, W. A., Ullrich, M. R., Smart, L. B., Smart, C. D., & Gadoury, D. M. (2019). Cross infectivity of powdery mildew isolates originating from hemp (*Cannabis sativa*) and Japanese hop (*Humulus japonicus*) in New York. *Plant Health Progress*, *21*(1), 47–53. https://doi.org/https://doi.org/10.1094/PHP-09-19-0067-RS

Whipps, J. M., Budge, S. P., & Fenlon, J. S. (1998). Characteristics and host range of tomato powdery mildew. *Plant Pathology*, *47*(1), 36–48. https://doi.org/10.1046/J.1365-3059.1998.00207.X

Yeh, Y. W., Wu, T. Y., Wen, H. L., Jair, H. W., Lee, M. Z., & Kirschner, R. (2020). Host plants of the powdery mildew fungus *Podosphaera xanthii* in Taiwan. *Tropical Plant Pathology 2020 46:1*, *46*(1), 44–61. https://doi.org/10.1007/S40858-020-00393-2

Zhao, T. T., Cho, S. E., Kim, J. Y., Choi, I. Y., & Shin, H. D. (2018). First Report of Powdery Mildew Caused by *Golovinomyces ambrosiae* on *Helianthus salicifolius* in Korea. *Https://Doi-Org.Proxy.Library.Cornell.Edu/10.1094/PDIS-12-17-1950-PDN*, *102*(7), 1453. https://doi.org/10.1094/PDIS-12-17-1950-PDN

Zhu, M., Ji, J., Duan, X., & Li, Y. F. (2021). First report of *Golovinomyces cichoracearum* causing powdery mildew on *Zinnia elegans* in China. *Plant Disease*, *105*(4), 1213. https://doi.org/10.1094/PDIS-11-20-2333-PDN

CHAPTER III

AN EVALUATION OF FUNGICIDE EFFICACY AGAINST *GOLOVINOMYCES AMBROSIAE* AND *BOTRYTIS CINEREA* ON HEMP AND THE IMPACT ON CANNABINOID PRODUCTION

Abstract:

Hemp is emerging as a new crop in New York State and across United States, but its expanded cultivation has come with challenges regarding disease management practices. Powdery mildew on hemp, caused by *Golovinomyces ambrosiae*, and gray mold caused by *Botrytis cinerea* have emerged as some of the major hemp diseases in New York State. Fungicides are important tools for growers to manage crop diseases like these, but to date there are few studies on the efficacy of control products against hemp diseases. Products tested in this study had active ingredients including *Bacillus amyloliquefaciens* strain D747, *B. mycoides* isolate J, potassium silicate, and azoxystrobin. *Bacillus amyloliquefaciens* and potassium silicate were effective in controlling powdery mildew severity, while none of the products tested impacted gray mold severity. This study also explored the effect of fungicide application and pathogen infection on cannabinoid levels in hemp. Biological fungicide treatments did not affect the profile of several cannabinoids including Δ9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), and cannabichrome (CBC). *Golovinomyces ambrosiae* infection did not impact major cannabinoid production, but *B. cinerea* may be associated with changes in some cannabinoid biosynthesis.

Introduction:

Since the passing of the 2018 farm bill which legalized hemp (defined as *Cannabis sativa* L. with less than 0.3% Δ^9-tetrahydrocannabdiol by weight), New York state has developed and expanded a hemp licensing program that has allowed farmers across the state to begin growing, processing, and selling hemp (Fike, 2016). Over time, the amount of hemp grown in the state has continued to fluctuate with 30,000 registered acres in 2021 (Ball, 2021). While the uses and possible markets for *C. sativa* are diverse, the production of cannabinoids is a major area of interest for growers in New York State. Cannabinoids are a class of secondary metabolites produced in high concentrations in the stalked capitate trichomes on the surface of unpollinated female hemp flowers (Livingston et al., 2020). The interactions between these cannabinoids and endocannabinoid signaling pathway in the human body and the resulting pharmaceutical effects are growing areas of study (Borgelt et al., 2013; Pertwee, 2006, 2008; Pertwee et al., 2010). Cannabidiol (CBD), Δ^9-tetrahydrocannabidiol (THC), cannabigerol (CBG), and cannabichrome (CBC) are some cannabinoids produced by hemp that are used in the health industry (Gloss & Vickrey, 2014; Mechoulam et al., 2002). Cannabigerolic acid (CBGA) is synthesized through the condensation of olivetolic acid and geranyl pyrophaphate (GPP) and is the precursor to other cannabinoids produced by hemp.

As the hemp industry continues to expand in New York State, best practices for the management of important pests and diseases continues to be an important area of study

and improvement. Powdery mildew and gray mold have both emerged as important disease of hemp in New York State (see Chapter II). *Golovinomyces ambrosiae* (Farinas & Hand, 2020; Qiu et al., 2020; Szarka et al., 2019; Weldon et al., 2019) and *Podospheara macularis* (Bates et al., 2021) have both been reported to cause powdery mildew on hemp, but only *G. ambrosiae* has been identified on hemp in New York State. Powdery mildews are obligate biotrophic fungi which absorb water and nutrients from a host plant through haustoria, reducing overall plant health and vigor and leaf surface coverage by the fungus eventually leads to early leaf drop (Glawe, 2008). Powdery mildews (Ascomycotina, Erysiphales) are found on crops world-wide, and are known to infect thousands of species of angiosperms (Glawe, 2008). On hemp, *G. ambrosiae* infection is obvious, as large numbers of conidia are produced and infected plant parts have a powdery white appearance (Figure 3.1A). The pathogen can infect all above ground parts of the hemp plant, including inflorescences. On hemp, powdery mildew is most commonly seen in greenhouse production, but we have frequently observed powdery mildew on hemp grown in the field in New York State. Selection of powdery mildew resistant cultivars, when available, and removal of infected tissue can help to reduce powdery mildew severity. Powdery mildews also grow best in a warm, humid environment, so improving air movement within a canopy can also be an effective management strategy.

Gray mold is caused by the necrotrophic ascomycete fungus *Botrytis cinerea*, which contains cell-wall degrading enzymes and toxins which degrade and rot the plant

(Nakajima & Akutsu, 2014; Rebordinos et al., 1996; Sasaki & Nagayama, 1996). *Botrytis cinerea* has a very broad host range and in hemp tends to infect the mature inflorescences (Figure 3.1B). Therefore, harvesting promptly can reduce the opportunity for pathogen infection. *Botrytis cinerea* also grows best in warm and wet conditions, so cultural practices such as improving air movement by increasing spacing between rows and removing leaves within plants will reduce leaf and tissue wetness (Bika et al., 2020).

In addition to cultural control methods, biological and chemical fungicides are important tools for managing crop diseases. However, there are a lack of published studies showing the efficacy of products labelled for use on hemp in New York State against major hemp disesaes. Further, many of the products labelled for use on hemp in New York are biological control agents. Previous studies have shown the efficacy of biological products containing *Bacillus* spp. as the active ingredient against hemp powdery mildew (Scott & Punja, 2020). However, the *Bacillus* species and strains included in this study have not yet been evaluated.

Figure 3.1. Symptoms of (A) powdery mildew caused by the obligate biotrophuc fungus *Golovinomyces ambrosiae* and (B) gray mold caused by the necrotrophic fungus *Botrytis cinerea* on hemp female inflorescence.

In this study we compared the efficacy of two *Bacillus* species – *Bacillus mycoides* isolate J and *Bacillus amyloliquefaciens* strain D747 – and one potassium silicate product. These two *Bacillus* species are commonly used by specialty crop growers and are legal for use on hemp in New York. Potassium silicate is labelled for use on hemp and has been shown to be active against powdery mildews (Tesfagiorgis et al., 2014), so was not evaluated for effectiveness against *B. cinerea.* Azoxystrobin (Quadris), a QoI fungicide that has been effective against many fungal pathogens, including other powdery mildew species (Keinath & Dubose, 2004; Romero et al., 2007), and *Botrytis* on various other hosts (Li-hua et al., 2009), was also included as a positive control. In addition to the efficacy of the fungicides that were applied, the impact of fungicide application on cannabinoid levels in hemp were also evaluated.

Materials and Methods:

Fungicide efficacy against hemp powdery mildew in 2020:

In the summer of 2020, commercially available fungicides, including several Organic Materials Review Institute (OMRI)-listed products were tested to determine their efficacy to control powdery mildew on hemp caused by *G. ambrosiae*. The products used for this trial, their active ingredients and rates are listed in Table 3.1. The hemp cultivar 'TJ's CBD' was used for this study which is known to be susceptible to *G. ambrosiae* (Stack et al., 2021; Weldon et al., 2019). Plants were clonally propagated by taking stem cuttings of at least 6 inches and 3-5 nodes from lateral or apical branches from a mother plant. Stems were trimmed to 4 inches and excess foliage was removed.

Cuttings were sterilized in 10% bleach, coated in cloning gel (Rootech Original) with 0.55% Indole-3 butyric acid (IBA), and placed in a hemp cloner system with a cloning solution which contains Clonex (mL/L water) and Clear Rez (1oz/5gal water) at a pH of 5.8. The cloning solution pH was adjusted daily using General Hydroponics "pH UP" solution and Bloom city "pH down" professional grade pH adjustor. After approximately 2 weeks, a new solution was used containing Olivia's cloning solution (60mL/1gal), RhizoBlast solution (0.5 oz/gal) and Clear Rez solution (1 oz/5gal). Once roots began to form after about one week, plants were transplanted into 50 cell flats and covered to maintain humidity, and then transplanted into pots after about 2-3 weeks. Plants were then grown in the greenhouse with a day temperature of 24°C and 21°C night temperature with a 16-hour photoperiod until transplanted in the field on June 15.

In the field, plants were separated by treatment in five plant plots each replicated four times in the field in a randomized complete block design. Fields were prepared for planting by amending with 100 lb. N per acre using 19-6-19 N-P-K Raised beds were prepared in rows with 6ft on center spacing with black plastic. Plants were spaced 3ft apart, with 6 ft between plots. Plants were irrigated using dripline irrigation as necessary based on rainfall. No additional nitrogen was added, and plants were not sprayed for any other pests. Weed cloth was used between rows for weed control.

When plants were established in the field, they were treated with their first fungicide treatment on July 27 (42 days after transplanting) with treatments applied weekly.

Fungicides were applied with a carbon dioxide backpack sprayer using a 3-nozzle boom with flat fan nozzles at a rate of 40 gal per acre. Because natural inoculum was apparently not present as no disease was seen in plots, the plants were inoculated on August 17 with the *G. ambrosiae* isolate, 19137 which was collected from hemp in Ontario County, NY in 2019. To inoculate the plants, 19137 was grown on hemp plants in growth chamber and conidia were washed from infected leaves with a solution of 1 L of water and 100 µL of Tween20 and diluted to a concentration of 1×10^5 conidia per mL. The concentrated spore solution was diluted in a total of 20 L and sprayed on to the plants until runoff with a backpack sprayer. Plants were inoculated two more times in the same way on August 30 and September 4. Disease severity was assessed by rating individual plants for percent disease coverage of the whole plant at 7-, 14-, 21-, 28-, and 35-days after the final inoculation.

Fungicide efficacy against Golovinomyces ambrosiae on hemp in 2021:

In the summer of 2021, the same fungicides evaluated in 2020 (Table 3.1) were tested for the effectiveness against hemp powdery mildew, *G. ambrosiae*. For this trial, the hemp cultivar 'White CBG' was used because it has been determined to be more susceptible than 'TJ's CBD.' Plants were seeded in 50-cell flats in with Lambert's LM 1 Germination Mix and grown in the greenhouse with the same conditions as 2020. Plants were transplanted into the field in the same randomized complete block design, as described above on June 21, 2021. Field preparation, row and plant spacing, irrigation, and weed control were the same as described for the 2020 trials.

Fungicide treatments started on July 21 and plants were treated on a weekly basis and applied as described above for the 2020 trial. The plants were then inoculated on July 22 with *G. ambrosiae* isolate 19002 (Weldon et al., 2019),using the same method as described above and plants were only inoculated one time. Disease severity was again assessed by rating individual plants for percent disease coverage of the whole plant at 12-, 19-, 26-, 34- and 40-days post inoculation.

Table 3.1. Treatments used to evaluate powdery mildew control in hemp in a field trial in Geneva, New York State. Each treatment was applied weekly starting July 20, 2020 and July 21 2021, which was 24-48 hours prior to inoculation.

Treatment	Brand Names	Brand	Rate
Bacillus mycoides isolate J (Bmj)	LifeGard	Certis	4.5 oz./100gal
Bacillus amyloliquefaciens strain D747 (BaD747)	Double Nickel	Certis	1 QT/acre
Potassium silicate	Sil-MATRIX	Certis	1% v/v
Bmj alternating with *BaD747*	LifeGard alternating with Double Nickel	Certis	4.5 oz./ 100 gal 1 QT/acre
Azoxystrobin	Quadris	Syngenta	15.5oz/acre
Untreated Control			

Fungicide efficacy against gray mold on hemp in 2020:

In the summer of 2020, several fungicides, including several OMRI-listed fungicide products were also evaluated for the effectiveness for controlling gray mold on hemp caused by *B. cinerea*. The products used, their active ingredients and rates are listed in

Table 3.2. The hemp cultivar ‘TJ’s CBD’ was used for this trial. Plants were clonally propagated alongside the plants used in the powdery mildew trial and grown in the greenhouse in the same conditions described above. Plants were transplanted in the field on June 15 in 5 plant plots for each treatment which was replicated four times in a randomized complete block design. The field was prepared in the same way as described above for the powdery mildew trial.

Fungicide treatments began on July 27, 2020 and continued weekly throughout the season and were applied as described previously for the powdery mildew trials. Plants were initially inoculated on September 3, once inflorescences were formed on plants. For inoculation, a *B. cinerea*, initially isolated from strawberry in Ontario County, NY in 2020, was grown on potato dextrose agar at room temperature with a 14-hour photoperiod. A concentrated liquid suspension was made by washing spores off the plates using 1 L of water and 100 µL of Tween20. The concentrated liquid suspension was diluted to a final concentration of 1×10^5 spores per mL in a final volume of 10 L of water. This inoculum was sprayed onto the plants until runoff using a backpack sprayer. Plants were also inoculated on September 9, September 18, September 25, and October 5. Individual plants were rated for the percent of the inflorescences that were infected. Ratings were done on 7-, 14- and 21-days after the final inoculation.

Table 3.2. Treatments used in the gray mold on hemp efficacy trial. Treatments were first applied two days prior to inoculation and continued once per week starting July 20, 2021 until harvest.

Treatment	Brand Names	Brand	Rate
Bacillus mycoides isolate J (Bmj)	LifeGard	Certis	4.5 oz./100gal
Bacillus amyloliquefaciens strain D747 (BaD747)	Double Nickel	Certis	1 QT/acre
Bacillus amyloliquefaciens strain D747 x2 (BaD747x2)	Double Nickel x2	Certis	2 QT/ acre
Bmj alternating with *BaD747*	LifeGard alternating with Double Nickel	Certis	4.5 oz./ 100 gal 1 QT/acre
Azoxystrobin	Quadris	Syngenta	15.5oz/acre
Untreated Control			

Data analysis of for fungicide efficacy:

All statistical analysis of were performed using R version 4.0.4 (R Core Team, 2021) in Rstudio 1.4.1106 (R Studio Team, 2020) Disease ratings from each of the fungicide trials were used to calculate the mean area under the disease progress curves (AUDPC) for each treatment using the R package agricolae (de Mendiburu, 2020) Within each trial, the mean AUPDC values for each treatment were compared using an analysis of variance test (ANOVA) ($p<0.05$). A Tukey's HSD test ($p<0.05$) was performed post-hoc using the HSD.test function in the agricolae package (de Mendiburu, 2020) for a pair-wise comparison between treatments.

Fungicide and disease impact on cannabinoid production in hemp:

Fungicide:

At the end of the 2020 and 2021 growing seasons, inflorescences were collected from hemp plants in each of the treatment plots in the both the powdery mildew and gray mold fungicide efficacy trials for cannabinoid analysis. The top 10 cm of mature terminal shoot tips were collected into paper bags from three randomly selected plants in each plot. The samples were air-dried and milled in a Ninja Pro food mill (Needham, MA) and cannabinoids were extracted and quantified using high-performance liquid chromatography (HPLC). The extraction and HPLC methods used were established previously by Stack et al. (2021a). For each sample, the following cannabinoids were quantified using this method: tetrahydrocannabinolic acid (THCA), Δ^9-tetrahydrocannabidiol (THC), cannabidiolic acid (CBDA), cannabidiol (CBD), cannabichromenic acid (CBCA), cannabichromene (CBC), cannabigerolic acid (CBGA), cannabigerol (CBG), tetrahydrocannabivarinic acid (THCVA), tetrahydrocannabivarin (THCV), cannanbidivarinic acid (CBDVA), cannabidivarin (CBDV), cannabicyclolic acid (CBLA), and cannabicyclol (CBL).

Disease:

At the end of the 2020 growing season, shoot tip samples were also collected from the untreated control plots in both the powdery mildew fungicide trial and the gray mold fungicide trials. In both trials, samples were collected from three plants per plot. In the powdery mildew trial, three samples from each of the four plots were taken from plants

with the greatest level of powdery mildew disease severity, for a total of 12 powdery mildew-infected samples on October 14, 2020. The top 10 cm of mature terminal shoot tips were collected as described above. In the gray mold trial, three samples (top 10 cm) in each plot were taken from shoot tips that were visibly infected with *B. cinerea* for a total of twelve *Botrytis*-infected samples on October 23, 2020. A separate plot of 'TJ's CBD' hemp was planted in the same field as each of these trials. These plants were originally seeded in the greenhouse and transplanted in the field as described previously. These plants were not treated with fungicides and were not inoculated with *G. ambrosiae* or *B. cinerea*. At the time of sample collection, the plants did not have symptoms or signs of powdery mildew infection. A total of 12 samples were collected from this plot to compare the samples affected with powdery mildew on October 14, 2020. Twelve more samples from this untreated and uninoculated plot were collected to compare to the *B. cinerea* infected samples October 23, 2020. Samples were processed and cannabinoids quantified using the same methods as described above.

Data analysis for cannabinoid production:

All data analysis was performed using R v4.0.4 (R Core Team, 2021) in RStudio v1.4.1106 (R Studio Team, 2020) and were based on the total potential cannabinoid percentages which were calculated based on the formulas described by (Stack et al., 2021) to control for variation in the decarboxylation of cannabinoids in their acidic forms. Mean cannabinoid values were calculated for each of the six fungicide treatments in each of the three trials and those values were compared between fungicide treatments

using an ANOVA ($p<0.05$), followed by a Tukey's HSD ($p<0.05$) pairwise comparison using the HSD.test function of the agricolae package (de Mendiburu, 2020).

To determine whether disease influenced cannabinoid production, the total potential cannabinoid percentages and means were calculated in the same way for the samples collected for these experiments. Mean cannabinoid percentages from samples infected with *G. ambrosiae* were compared to mean cannabinoid percentages from healthy samples using a t-test ($p<0.05$). Additionally, linear models were generated with total potential cannabinoids as the response variable and AUDPC as the explanatory variable. The same method was used to compare samples infected with *B. cinerea* and healthy samples.

To determine whether the overall cannabinoid profiles of hemp were impacted by fungicide treatment, non-metric multidimensional scaling (NMDS) was used with distance matrices based on Bray-Curtis using the metaMDS function in the vegan package (Oksanen et al., 2020) for each of the three fungicide trials. The same analysis was done to compare cannabinoid profiles of samples of pathogen-infected samples and healthy samples.

Results:

Fungicide efficacy against hemp powdery mildew

Fungicides (Table 3.1) were tested at a single application rate for efficacy against hemp powdery mildew caused by *G. ambrosiae*. Several of the fungicides tested in this study were effective in reducing hemp powdery mildew disease severity in both 2020 and 2021 (Figure 3.2). In both years, the conventional fungicide azoxystrobin was the most effective in reducing disease severity compared to the untreated control ($p < 0.05$). Potassium silicate (Sil-MATRIX), BaD747 (Double Nickel) and the treatment alternating Bmj (LifeGard) and BaD747 (Double Nickel) also significantly reduced disease severity in both years ($p<0.05$). The was a reduction in disease in plots treated with Bmj (LifeGard) in both years numerically but was not statistically lower than the untreated control. ($p>0.05$; Figure 3.2).

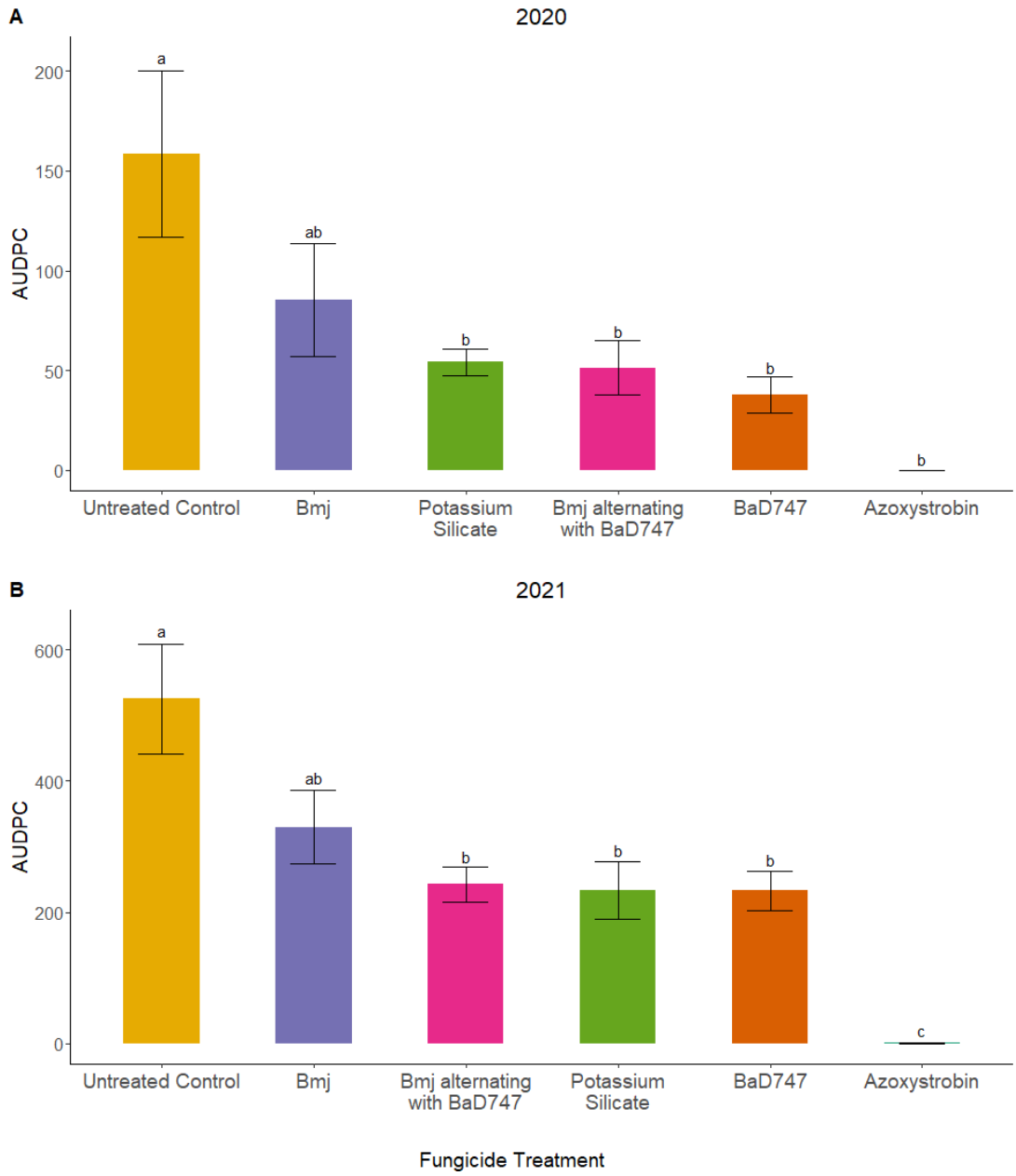

Figure 3.2. Disease severity of powdery mildew, caused by *Golovinomyces ambrosiae,* shown as area under the disease progress curve (AUDPC) for each fungicide treatment in both the 2020 (A) and 2021 (B) growing seasons. The active ingredient for each fungicide is listed on the X axis. Letters indicate Tukey's HSD groups ($p<0.05$).

Fungicide efficacy against grey mold on hemp

Fungicides were tested for efficacy against gray mold caused by *B. cinerea* on hemp inflorescence in 2020. The mean AUDPC of the untreated control treatment was 94.1, but the AUDPC for all six treatments were not statistically different from each other ($p>0.05$) and no treatments were not effective in reducing disease severity in 2020 (Figure 3.3).

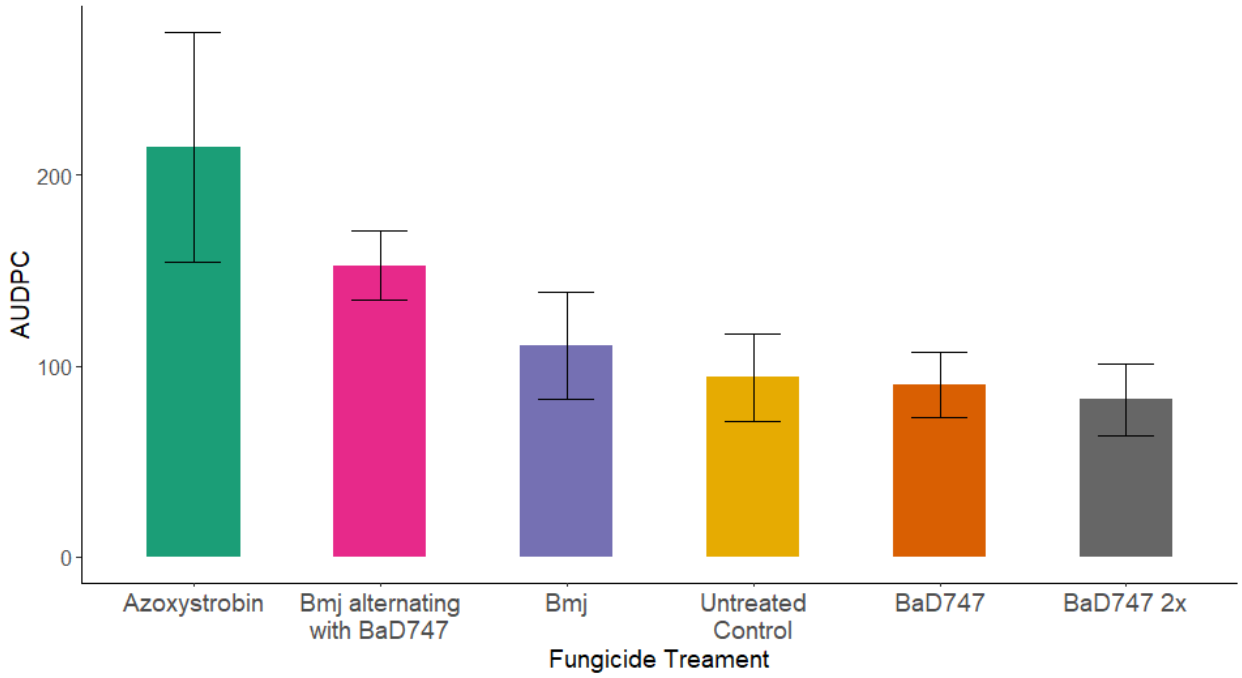

Figure 3.3. Gray mold, caused by *Botrytis cinerea*, disease severity on hemp shown as the area under the disease progress curve (AUDPC) for each fungicide treatment used during the 2020 growing season. The active ingredient for each fungicide is listed on the X axis. No significant differences were observed based on Tukey's HSD analysis.

Fungicide treatment impact on cannabinoid production:

Powdery mildew trial:

Cannabinoids were quantified in hemp inflorescences collected from plots in the powdery mildew trials in both 2020 and 2021. Based on non-metric multidimension scaling analysis using Bray-Curtis dissimilarity calculations, there were no overall differences between the powdery mildew fungicide treatments and the untreated control in both 2020 and 2021 (Figure 3.4, $p>0.05$). In both the 2020 and 2021 powdery mildew trials, there were no significant differences the total potential THC, CBD, CBG and CBC levels between treatments (Figure 3.5, ANOVA, $p>0.05$). There were also no differences in the CBD:THC ratios between fungicide treatments (Figure 3.5, ANOVA, $p>0.05$).

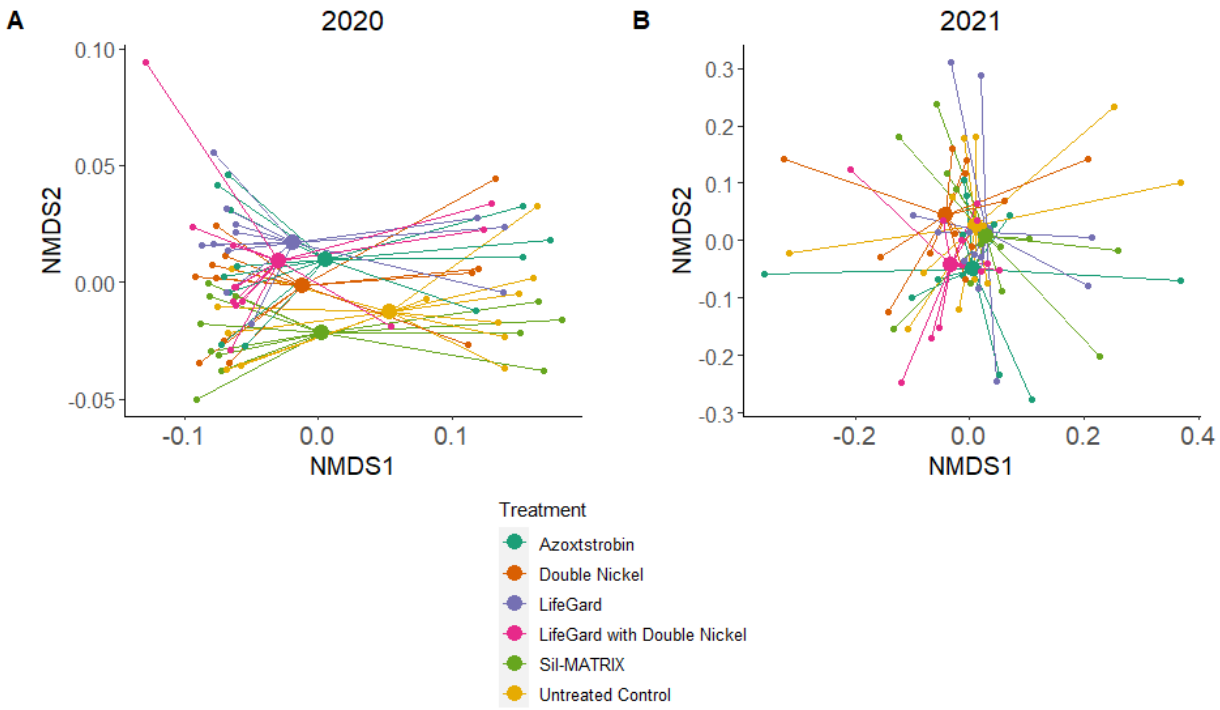

Figure 3.4. Non-metric multidimensional scaling (NMDS) of cannabinoid quantification based on Bray distance between hemp samples treated with fungicide treatments to control powdery mildew. There were no significant differences between samples in each of the fungicide treatments for both the 2020 (A) and the 2021 (B) growing season ($p>0.05$).

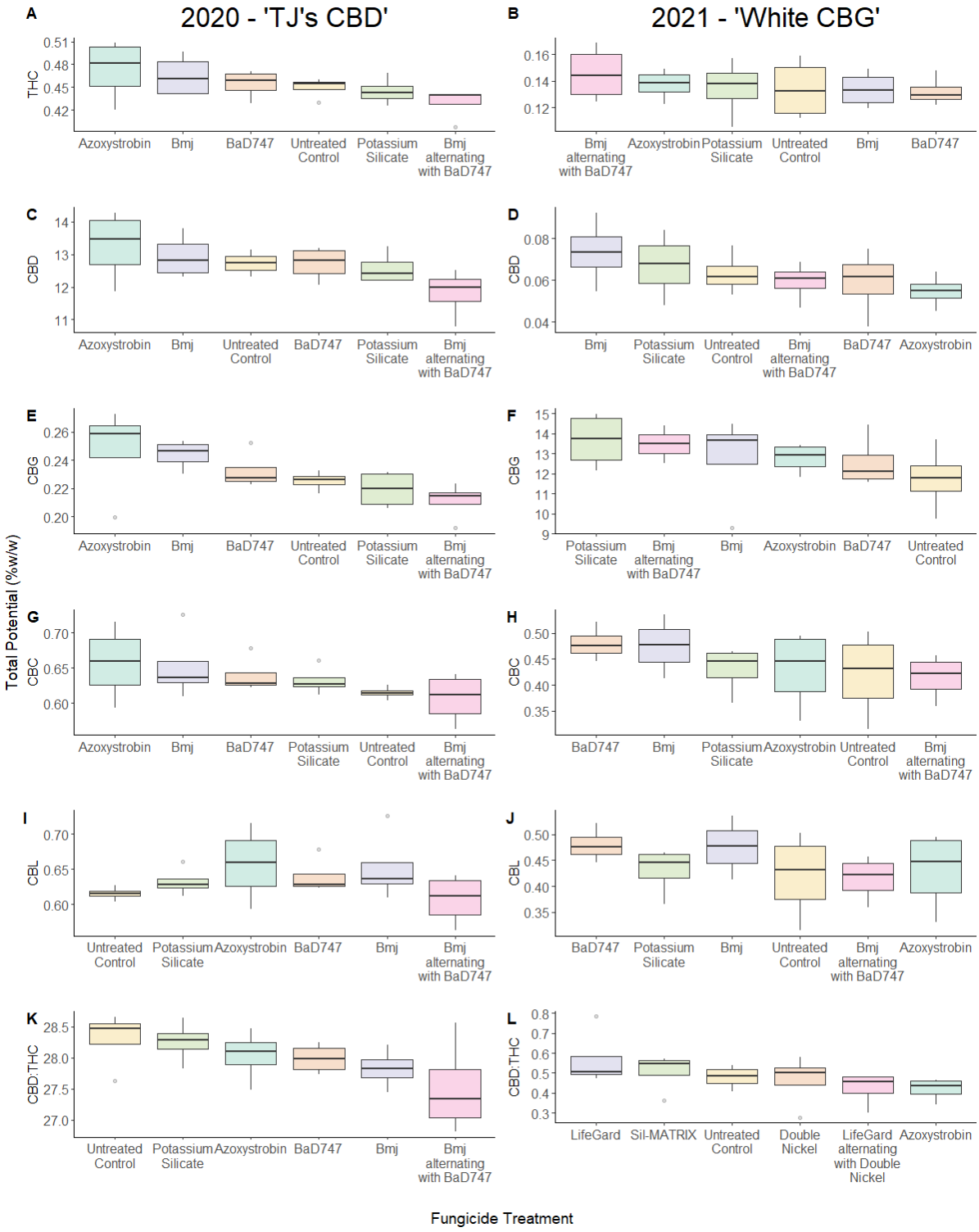
A
2020 - 'TJ's CBD'
B
2021 - 'White CBG'
THC
Azoxystrobin
Bmj
BaD747
Untreated Control
Potassium Silicate
Bmj alternating with BaD747
CBD
CBG
CBC
CBL
CBD:THC
LifeGard
Sil-MATRIX
Double Nickel
LifeGard alternating with Double Nickel
Total Potential (%w/w)
Fungicide Treatment

Figure 3.5. Quantification of key cannabinoids in hemp treated with fungicides to control powdery mildew. Boxplots indicate total potential percent THC (A and B), CBD (C and D), CBG (E and F), CBC (G and H), CBL (I and J), and CBD:THC (K and L) by weight in both the 2020 (A, C, E, G, I, K) and 2021 (B, D, F, H, J, L) growing season. Cultivar 'TJ's CBD' was used in 2020 (chemotype III), while 'White CBG' was used in 2021 (chemotype IV). There were no significant differences in total potential cannabinoids by treatment using a Tukey's HSD ($p<0.05$).

Gray mold trial:

Hemp inflorescences were collected from the gray mold trial for cannabinoid quantification in 2020. A non-metric multidimension scaling analysis using Bray-Curtis dissimilarity calculations were used to assess overall differences in cannabinoid levels between treatments, which showed there were statistical differences between fungicide treatments (Figure 3.6). In the gray mold trial, there were no differences in the total potential THC, CBD, CBG and CBC between each of the treatments in 2020 (Figure 3.7, ANOVA, $p>0.05$). There were higher levels of total potential CBL produced in hemp treated with Bmj (LifeGard) alternating with BaD747 (Double Nickel) and the Double Nickel at the 2 QT/acre rate (Figure 3.7, ANOVA, $p<0.05$). There were also significant differences in the CBD:THC ratios between fungicide treatments in the Botrytis trial (Figure 3.7, ANOVA, $p<0.05$).

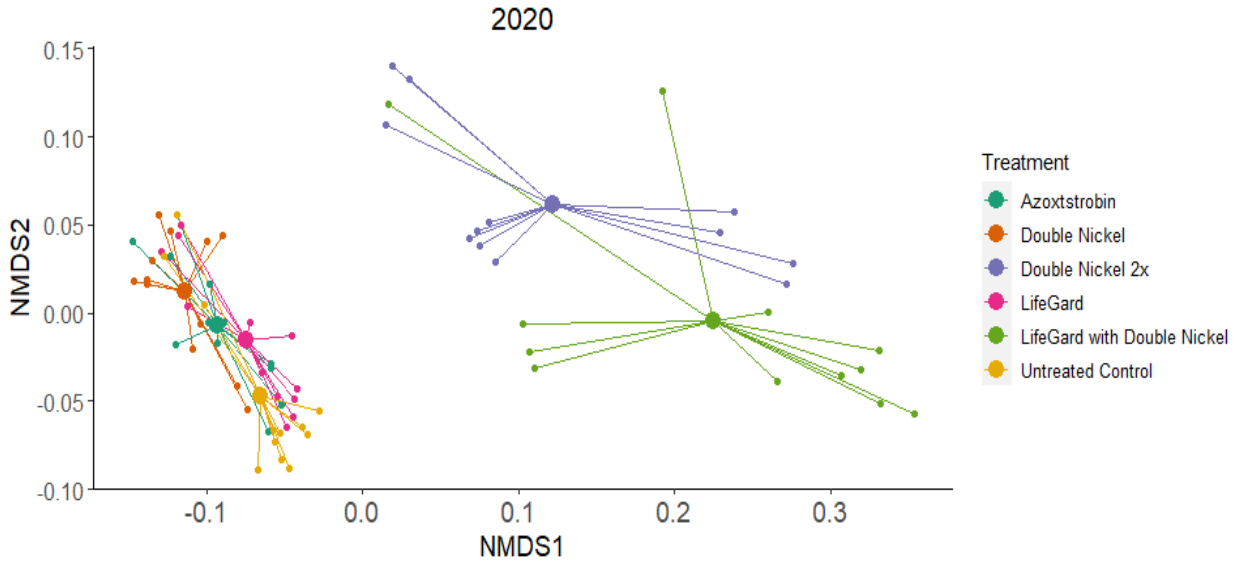

Figure 3.6. Non-metric multidimensional scaling (NMDS) of cannabinoid quantification based on Bray-Curtis distance between hemp samples treated with fungicide treatments to control gray mold. There were significant differences between fungicides ($p<0.05$).

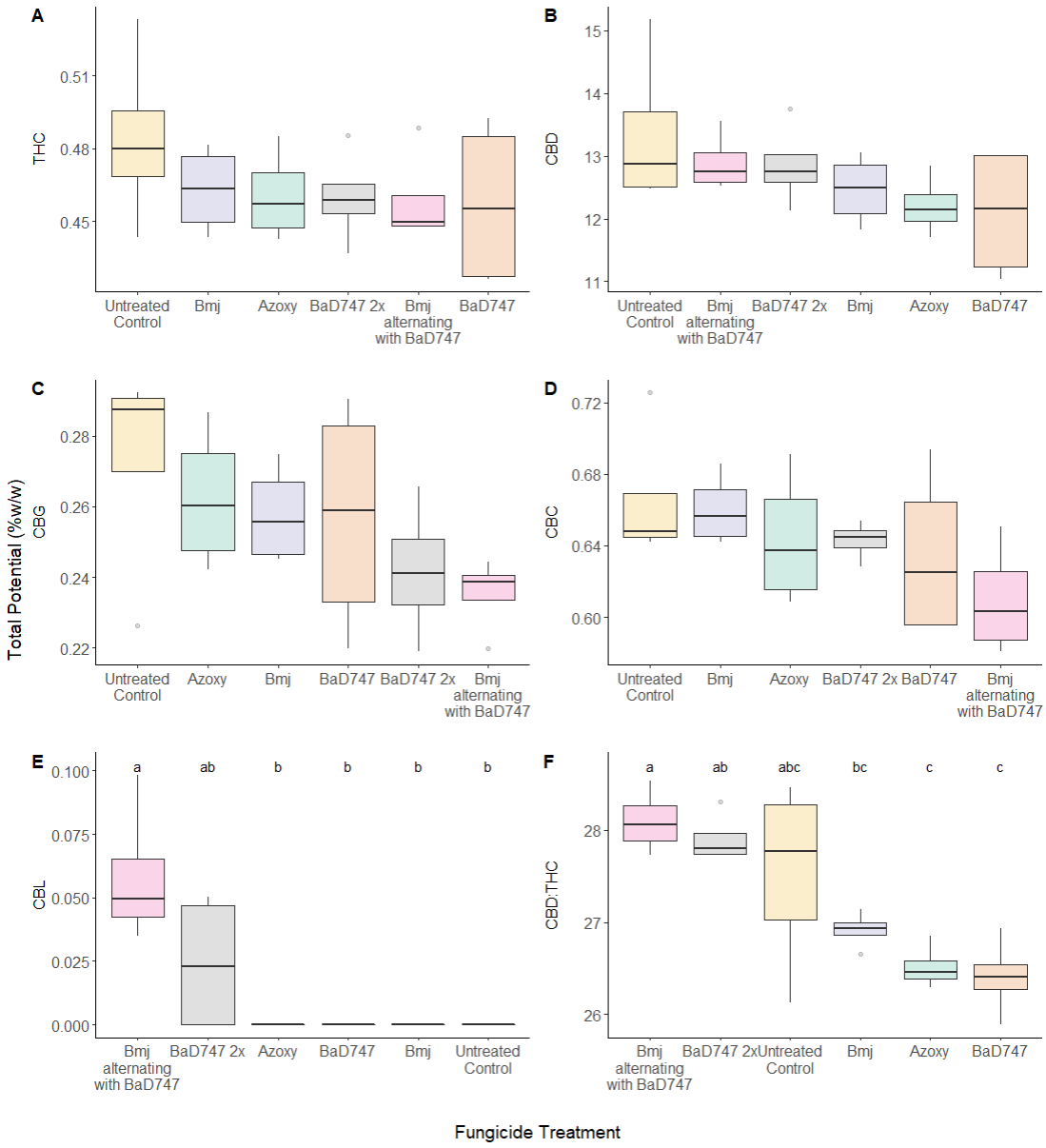

Figure 3.7. Quantification of key cannabinoids in hemp ('TJ's CBD') treated with fungicides to control gray mold caused by *Botrytis cinerea*. Boxplots indicate total potential percent THC (A), CBD (B), CBG (C), and CBC (D), CBL(E), by weight and the CBD:THC ration (F) in the 2020 growing season. There were no significant

differences in total potential THC, CBD, CBG, or CBC. There were differences in total potential CBL and CBD:THC and letters indicate Tukey's HSD groups ($p<0.05$)

Impact of pathogen infection of cannabinoid production:

Cannabinoids were quantified in hemp inflorescences to determine the impact of gray mold or powdery mildew. There were no overall differences in cannabinoid levels between hemp infected with *G. ambrosiae* and healthy hemp based on a Bray-Curtis Ordination analysis (Figure 3.8A, $p>0.05$). There were also no differences in the levels of total potential THC, CBD, CBG and CBC (Figure 3.9, ANOVA, $p>0.05$). Furthermore, the linear models demonstrated that cannabinoid levels did not depend on powdery mildew AUDPC ($p>0.05$). A Bray-Curtis Ordination analysis also showed no differences between the overall cannabinoid levels between hemp infected with *B. cinerea* and healthy hemp (Figure 3.8B, $p>0.05$). The levels of total potential THC, CBD and CBC were the same in hemp infected with *B. cinerea* and healthy hemp (Figure 3.10, ANOVA, $p>0.05$). Hemp infected with *B. cinerea* did have an increased amount of CBG compared to healthy hemp (Figure 3.10, $p<0.05$). Additionally, the linear models showed that cannabinoid levels did not depend on gray mold AUDPC ($p>0.05$).

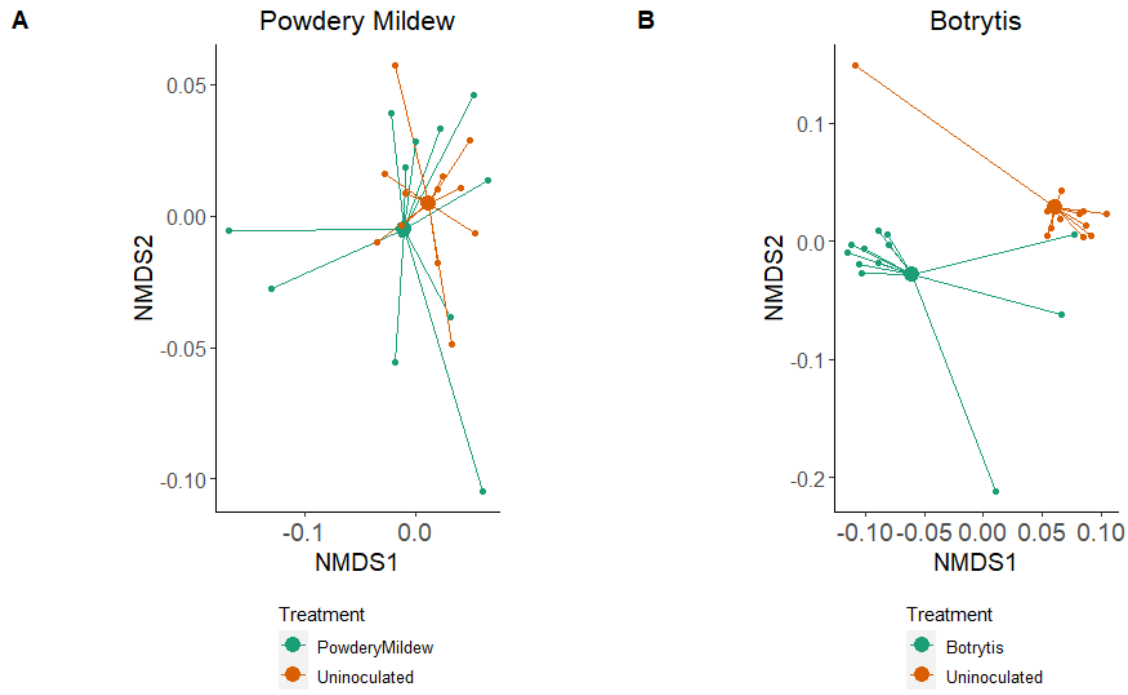

Figure 3.8. Non-metric multidimensional scaling (NMDS) of cannabinoid quantification based on Bray-Curtis distance between hemp samples inoculated with either (A) *Golovinomyces ambrosiae* (powdery mildew) or (B) *Botrytis cinerea* (gray mold) and uninoculated samples. There were no significant differences between samples inoculated with *G. ambrosiae* or *B. cinerea* and uninoculated samples ($p>0.05$).

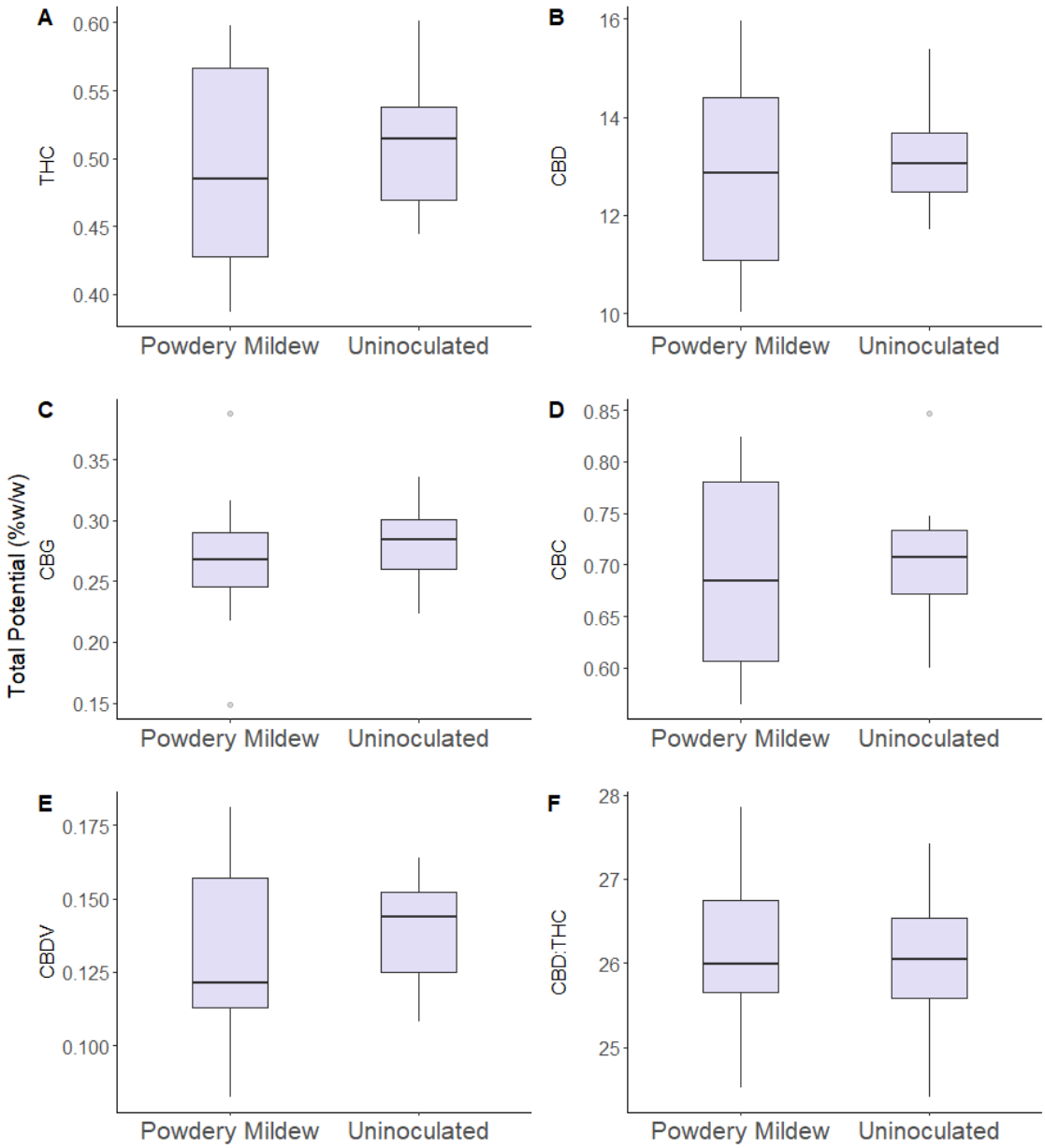

Figure 3.9. Quantification of cannabinoids in hemp ('TJ's CBD') inoculated with the powdery mildew pathogen *Golovinomyces ambrosiae*. Boxplots show the total potential percent THC (A), CBD (B), CBG (C), CBC (D), CBDV (E), and CBD:THC (F) by weight during the 2020 growing season. There were no differences in cannabinoids between inoculated and healthy samples ($p>0.05$).

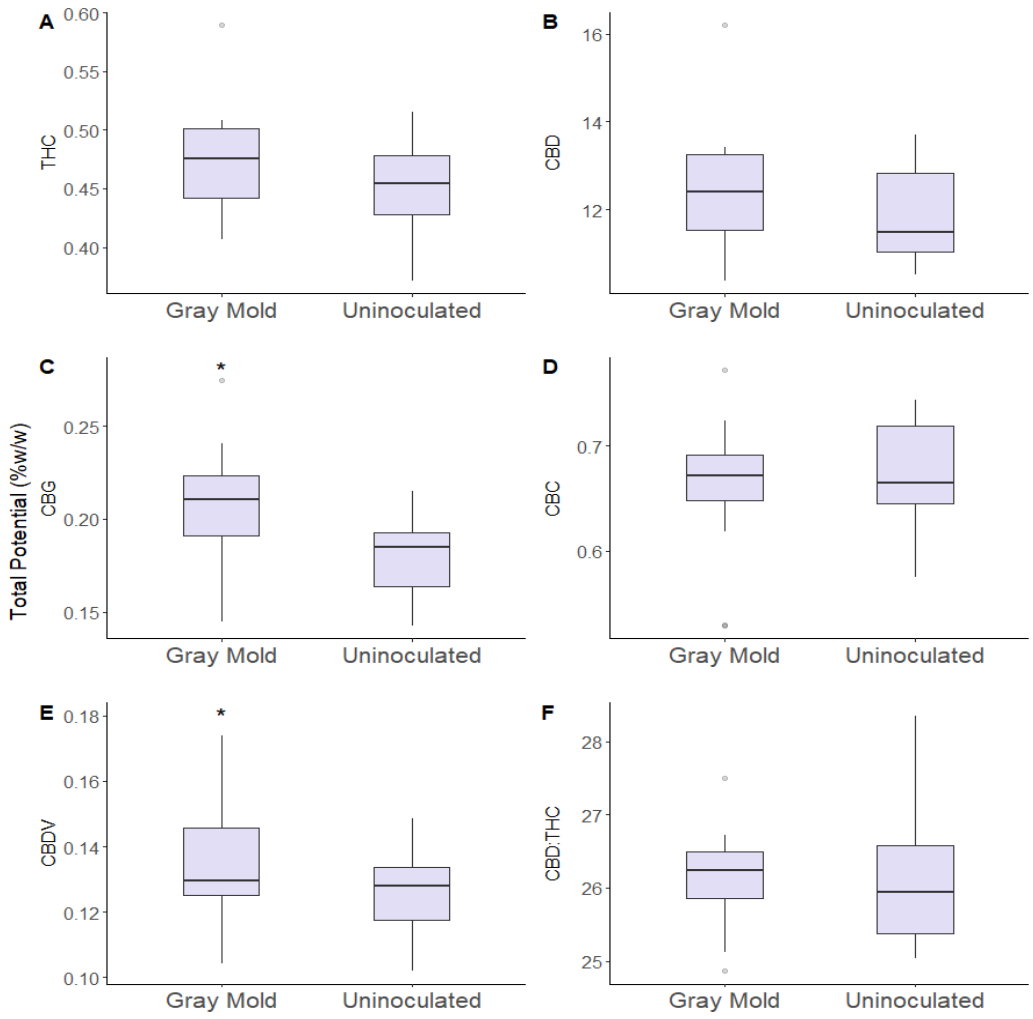

Figure 3.10. Quantification of cannabinoids in hemp ('TJ's CBD') inoculated with *Botrytis cinerea* (gray mold). Boxplots show the total potential percent THC (A), CBD (B), CBG (C), CBC (D), CBDV (E), and CBD:THC (F) by weight during the 2020 growing season. There were differences in total potential THC, CBD, CBC, or CBD:THC between inoculated and uninoculated samples ($p>0.05$). There was significantly greater percentage CBG and CBDV by weight in samples inoculated with *B. cinerea* compared to uninoculated samples based on a T-test ($p<0.05$), as indicated by an asterisk *.

Discussion:

As the hemp industry has developed and continues to change in New York State, several pathogens have emerged, causing significant disease, including powdery mildew caused by *G. ambrosiae* and gray mold caused by *B cinerea*. The use of fungicides are essential tools that can reduce disease pressure within a growing season. However, the lack of published studies on the efficacy of commercially available products for use on hemp, does not allow for informed decisions to be made about which available products will be effective against specific hemp pathogens. Furthermore, since cannabinoid production represents a major market class of hemp, it is important to understand the impacts of both fungicide application and pathogen infection on the production of those secondary metabolites in hemp.

In 2020 and 2021, field trials were conducted to determine the efficacy of several commercially available products to control powdery mildew on hemp. In 2020, a field trial was also conducted to determine the efficacy of some of those same products against gray mold on hemp. In these trials, inflorescence samples were also analyzed to determine the effect of fungicide application and pathogen infection on cannabinoid production.

Potassium silicate was an OMRI-listed product that was included in the powdery mildew trials. This treatment reduced disease severity by 66% in 2020 and 56% in 2021, compared to untreated plots. It is often used as a source of soluble silica, an essential

micronutrient which can deposit in plant leaves and act as a physical barrier against pathogens (Samuels et al., 1991). This effect can be seen when applied as a soil ammendment, but also when applied as a foliar spray (Bowen et al., 1992). When applied as a soil fertilizer, it may also induce plant defenses against pathogens (Garibaldi et al., 2011; Tesfagiorgis et al., 2014; Yanar et al., 2013). The expected mode of action for potassium silicate depends on improving the resilience of living host tissue and would be expected to be ineffective against a necrotrophic pathogen like *B. cinerea*. Therefore, potassium silicate was not included in the gray mold trial.

Bacillus amyloliquefaciens strain D747 (Double Nickel) is a microbial biopesticide that was effective in reducing powdery mildew disease severity by 76% in 2020 and 56% in 2021 at a rate of 1 quart per acre, which were both significantly different from the untreated control plots. Despite treatment with both 1 quart per acre and 2 quarts per acre, neither treatment was effective in reducing the severity of gray mold. This species of *Bacillus* is a soil microbe that often colonizes plant roots and has been widely used as a soil treatment to reduce soil-borne root diseases (Romero et al., 2004). *Bacillus amyloliquefaciens* likely reduces disease severity through competitive exclusion by rapidly colonizing plant parts in many cases (Romero et al., 2004), but several strains have been shown to reduce disease severity of several powdery mildews on various plant hosts through the induction of systemic acquired resistance (Li et al., 2015; Yamamoto et al., 2015) and through the production of antifungal metabolites (Tanaka et al., 2017). Specifically, *B. amyloliquefaciens* strain D747 has demonstrated efficacy

in reducing disease severity of *Pseudomonas syringae* pv. *actinidiae* on kiwifruit (Biondi et al., 2021), *Cercospora* leaf spot on table beet (Pethybridge et al., 2017), and several others. Rotolo et al. (2018) showed that when *B. amyloliquefaciens* strain D474 was used alone, it was not effective in controlling gray mold on grapes, but disease severity was reduced when used in combination with other conventional fungicides. Therefore, while *B. amyloliquefaciens* strain D747 alone was not efficacious against gray mold on hemp, there is still the potential for it to be used effectively in a treatment plan for disease control.

Bacillus mycoides isolate J was included in both the powdery mildew trials and the gray mold trial within two different treatments: one alone, and one alternating with *B. amyloliquefaciens* strain D747. While *B. mycoides* isolate J alone seems to have moderately reduced the powdery mildew disease severity, it was not significantly different from the untreated control. There was also no difference in gray mold disease severity between the *B. mycoides* isolate J treated hemp and the untreated control. When *B. mycoides* isolate J was alternated with *B. amyloliquefaciens* strain D7474, powdery mildew disease severity was significantly lower than the untreated control in both years, likely suggesting that the efficacy of that treatment was due to the inclusion of *B. amyloliquefaciens* strain D747. However, that efficacy was not demonstrated against gray mold. *Bacillus mycoides* isolate J works by inducing plant defenses, creating induced resistance (IR) in the host plant (Bargabus, 2003; Bargabus et al., 2002). This type of resistance has been documented in many plants and gives an otherwise

susceptible host a broad-spectrum protection against a wide variety of pathogens (Albanchez et al., 2018; Li et al., 2015). *Bacillus mycoides* was first demonstrated its protective effects in sugar beet, which when applied as a foliar treatment, disease severity of *Cercospora* leaf spot caused by *Cercospora beticola* was reduced (Bargabus et al 2002). However, in this original experiment, the level of disease reduction was highly variable, reducing disease anywhere from 38 to 91 percent (Bargabus et al., 2002). The efficacy of *B. mycoides* isolate J has been variable against several pathogens in other systems and was shown to sometimes be more effective when used in combination with other biocontrol agents. (Guetsky, Shtienberg, Elad, & Dinoor, 2007; Guetsky, Shtienberg, Elad, Fischer, et al., 2007)

Azoxystrobin was the most effective treatment of those included in the trial to reduce disease severity of powdery mildew in both years, reducing the AUDPC by 99.9% in both 2020 and 2021. However, azoxystrobin was not effective in reducing the severity of gray mold in 2020. Azoxystrobin is a commonly used conventional broad spectrum, quinone outside inhibitor (QoI) fungicide. It is widely used in management plans to control a variety of fungal diseases, including powdery mildews and gray mold (Jiang et al., 2009; McGrath & Shishkoff, 2003). While azoxystrobin was the most effective treatment in controlling powdery mildew, is not currently labelled for use on hemp in New York State. It is possible that as hemp is more widely grown, more conventional fungicides will be available for use, however single-site fungicides like azoxystrobin should still be limited in their use to prevent the evolution of fungicide-resistance (Jiang

et al., 2009; Ma et al., 2003; C. Zhang et al., 2011). The *G. ambrosiae* isolate, 19002, that was used for inoculations in these field trials was sensitive to azoxystrobin, but resistance in other powdery mildew-causing fungi has been broadly detected after regular azoxystrobin for a relatively short period of time (Ishii et al., 2007; McGrath & Shishkoff, 2003). Further, QoI fungicide resistance is widely reported and relatively common in *B. cinerea* isolates (Harper et al., 2022; Jiang et al., 2009; Z. Zhang et al., 2009), which may explain why the azoxystrobin treatment was ineffective in reducing gray mold severity in the 2020 field trial.

Cannabinoid accumulation:

This study also aimed to determine whether fungicide treatment or pathogen infection would impact cannabinoid production in hemp. We found that cannabinoid levels were not impacted by the fungicide treatments in the powdery mildew trial, or by the *G. ambrosiae* infection itself. This supports previous studies that demonstrated cannabinoid production is based on genetics and not influenced by environmental stressors (Stack et al., 2021; Toth et al., 2021).

In the gray mold trials, however, CBL production and the CBD:THC ratio were significantly impacted by fungicide treatments. Specifically, there were significantly greater levels of CBL in plants treated with either Bmj alternating with BaD747 or those treated with BaD747 at the 2 Qt/acre rate, indicating that BaD747 could be impacting the production of CBL in hemp. However, this effect was not seen in the BaD747

treatment at the 1 Qt/acre rate, and there was little to no CBL produced by plants in all other treatments. Similarly, the Bmj alternating with BaD747 treatment had the highest CBD:THC ratio. However, both the Bmj treatment and the BaD747 at the 1 QT/acre rate had significantly lower CBD:THC ratios compared to the alternating treatment, making it difficult to determine which of the two treatments, if any, could be having the impact on the CBD:THC ratio. This experiment will be repeated in the future to verify results.

When looking at the impact of pathogen infection on cannabinoid production it was hypothesized that *B. cinerea* infection would be likely to have an impact on cannabinoid production, due to this pathogen primarily necrotizing floral tissue, where cannabinoid production occurs. We found that plants infected with *B. cinerea* had no significant differences in most cannabinoids compared to uninoculated controls, but significantly greater quantities of both total potential CBG and CBDV, demonstrating that there could be some interaction between the pathogen and the plant's cannabinoid biosynthetic pathway. In this pathway, CBGA is the precursor to THCA, CBDA, and CBCA, but CBGVA is the precursor for their homologs THCVA, CBDVA and CBCVA (Degenhardt et al., 2017; Fellermeier et al., 2001; Thomas et al., 2016). Therefore, these results imply that if there is an interaction, it is likely occurring prior to the enzymatic reaction that converts CBGA and CBDVA to their respective products. However, if this is the case, it would be expected that cannabinoids downstream in this biosynthetic pathway would also be impacted, which was not the case in this study. Additionally,

this study is limited to a single site, hemp cultivar and year, and it is possible that these results could vary based on these factors.

Conclusions:

In this study, we determined that several OMRI-listed products were effective in reducing hemp powdery mildew disease severity caused by *G. ambrosiae* in two different seasons. However, those same products were not effective in controlling the gray mold caused by *B. cinerea* on hemp. We also found that largely fungicide treatments did not influence cannabinoid production in hemp. While *G. ambrosiae* infection did not impact cannabinoid production, *B. cinerea* infection may be interacting with some cannabinoid biosynthesis in hemp.

Acknowledgements:

Thank you to the Jocelyn Rose research team, especially Stephen Snyder for performing the cannabinoid extractions and HPLC analysis for cannabinoid quantification that were used in this research.

Literature Cited:

Albanchez, E. G., García-Villaraco, A., Lucas, J. A., Gutierrez, F. J., & Ramos-Solano, B. (2018). Priming fingerprint induced by bacillus amyloliquefaciens qv15, a common pattern in arabidopsis thaliana and in field-grown blackberry. *Journal of Plant Interactions*, *13*(1), 398–408. https://doi.org/10.1080/17429145.2018.1484187/SUPPL_FILE/TJPI_A_1484187_SM1245.DOCX

Ball, R. A. (2021). *New York State Hemp Plan*.

Bargabus, R. L. (2003). *Systemic resistance induction by Bacillus mycoides isolate Bac J : the mode of action on Beta vulgaris (sugar beet)* [Dissertation]. Montana State University.

Bargabus, R. L., Zidack, N. K., Sherwood, J. E., & Jacobsen, B. J. (2002). Characterisation of systemic resistance in sugar beet elicited by a non-pathogenic, phyllosphere-colonizing *Bacillus mycoides*, biological control agent. *Physiological and Molecular Plant Pathology*, *61*(5), 289–298. https://doi.org/10.1006/PMPP.2003.0443

Bates, T. A., Block, M. H., Wiseman, M. S., Garfinkel, A. R., Gent, D. H., & Ocamb, C. M. (2021). First report of powdery mildew caused by *Podosphaera macularis* on hemp in Oregon. *Plant Health Progress*, *22*(4), 567–569. https://doi.org/https://doi.org/10.1094/PHP-04-21-0071-BR

Bika, R., Baysal-Gurel, F., & Jennings, C. (2020). *Botrytis cinerea* management in ornamental production: a continuous battle. *Canadian Journal of Plant Pathology*, *43*(3), 345–365. https://doi.org/10.1080/07060661.2020.1807409

Biondi, E., Gallipoli, L., Mazzaglia, A., Fuentealba, S. P., Kuz-Manovic, N., Bertaccini, A., Balestra, G. M., Biondi, E., Gallipoli, L., Mazzaglia, A., Fuentealba, S. P., Kuzmanović, N., Bertaccini, A., & Balestra, G. M. (2021). Bacillus-based products for management of kiwifruit bacterial canker. *Phytopathologia Mediterranea*, *60*(2), 215–228. https://doi.org/10.36253/PHYTO-12184

Borgelt, L. M., Franson, K. L., Nussbaum, A. M., & Wang, G. S. (2013). The pharmacologic and clinical effects of medical *Cannabis*. *Pharmacotherapy*, *33*(2), 195–209. https://doi.org/10.1002/phar.1187

Bowen, P., Menzies, J., Ehret, D., Samuels, L., & Glass, A. D. M. (1992). Soluble silicon sprays inhibit powdery mildew development on grape leaves. *J. AMER. Soc. HORT. SCI*, *117*(6), 906–912.

de Mendiburu, F. (2020). *agricolae: Statistical Procedures for Agricultural Research.* (R package version 1.3-3.).

Degenhardt, F., Stehle, F., & Kayser, O. (2017). The Biosynthesis of Cannabinoids. In *Handbook of Cannabis and Related Pathologies: Biology, Pharmacology, Diagnosis, and Treatment* (pp. 13–23). Elsevier Inc. https://doi.org/10.1016/B978-0-12-800756-3.00002-8

Farinas, C., & Hand, F. P. (2020). First report of *Golovinomyces spadiceus* causing powdery mildew on industrial hemp (*Cannabis sativa*) in Ohio. *Plant Disease*, *104*(10), 2727–2727. https://doi.org/10.1094/PDIS-01-20-0198-PDN

Fellermeier, M., Eisenreich, W., Bacher, A., & Zenk, M. H. (2001). *Biosynthesis of cannabinoids*. *1604*, 1596–1604.

Fike, J. (2016). Industrial hemp: renewed opportunities for an ancient crop. *Critical Reviews in Plant Sciences*, *35*(5–6), 406–424. https://doi.org/10.1080/07352689.2016.1257842

Garibaldi, A., Gilardi, G., & Gullino, M. L. (2011). Effect of potassium silicate and electrical conductivity in reducing powdery mildew of hydroponically grown tomato. *Mediterranean Phytopathological Union*, *50*(2), 192–202. https://www-jstor-org.proxy.library.cornell.edu/stable/26458693?seq=1

Glawe, D. A. (2008). The Powdery Mildews: A review of the world's most familiar (yet poorly known) plant pathogens. *Annual Reviews of Phytopathology*, *46*, 27–51. https://doi.org/10.1146/annurev.phyto.46.081407.104740

Gloss, D., & Vickrey, B. (2014). Cannabinoids for epilepsy. *Cochrane Database of Systematic Reviews*. https://doi.org/10.1002/14651858.CD009270.pub3

Guetsky, R., Shtienberg, D., Elad, Y., & Dinoor, A. (2007). Combining biocontrol agents to reduce the variability of biological control. *Phytopathology*, *91*(7), 621–627. https://doi.org/10.1094/PHYTO.2001.91.7.621

Guetsky, R., Shtienberg, D., Elad, Y., Fischer, E., & Dinoor, A. (2007). Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. *Phytopathology*, *92*(9), 976–985. https://doi.org/10.1094/PHYTO.2002.92.9.976

Harper, L. A., Paton, S., Hall, B., McKay, S., Oliver, R. P., & Lopez-Ruiz, F. J. (2022). Fungicide resistance characterized across seven modes of action in *Botrytis cinerea*

isolated from Australian vineyards. *Pest Management Science*, *78*(4), 1326–1340. https://doi.org/10.1002/PS.6749

Ishii, H., Fraaije, B. A., Sugiyama, T., Noguchi, K., Nishimura, K., Takeda, T., Amano, T., & Hollomon, D. W. (2007). Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. *Http://Dx.Doi.Org.Proxy.Library.Cornell.Edu/10.1094/PHYTO.2001.91.12.1166*, *91*(12), 1166–1171. https://doi.org/10.1094/PHYTO.2001.91.12.1166

Jiang, J., Ding, L., Michailides, T. J., Li, H., & Ma, Z. (2009). Molecular characterization of field azoxystrobin-resistant isolates of Botrytis cinerea. *Pesticide Biochemistry and Physiology*, *93*(2), 72–76. https://doi.org/10.1016/J.PESTBP.2008.11.004

Keinath, A. P., & Dubose, V. B. (2004). Evaluation of fungicides for prevention and management of powdery mildew on watermelon. *Crop Protection*, *23*(1), 35–42. https://doi.org/10.1016/S0261-2194(03)00165-0

Li, Y., Gu, Y., Li, J., Xu, M., Wei, Q., & Wang, Y. (2015). Biocontrol agent *Bacillus amyloliquefaciens* LJ02 induces systemic resistance against cucurbit powdery mildew. *Frontiers in Microbiology*, *6*(AUG), 883. https://doi.org/10.3389/FMICB.2015.00883/BIBTEX

Li-hua, J. I. N., Yu, C., Chang-jun, C., Jian-xin, W., & Ming-guo, Z. (2009). Activity of azoxystrobin and SHAM to four phytopathogens. *Agricultural Sciences in China*, *8*(7), 835–842. https://doi.org/10.1016/S1671-2927(08)60285-0

Livingston, S. J., Quilichini, T. D., Booth, J. K., Wong, D. C. J., Rensing, K. H., Laflamme-Yonkman, J., Castellarin, S. D., Bohlmann, J., Page, J. E., & Samuels, A.

L. (2020). Cannabis glandular trichomes alter morphology and metabolite content during flower maturation. *Plant Journal*, *101*(1), 37–56. https://doi.org/10.1111/tpj.14516

Ma, Z., Felts, D., & Michailides, T. J. (2003). Resistance to azoxystrobin in Alternaria isolates from pistachio in California. *Pesticide Biochemistry and Physiology*, *77*(2), 66–74. https://doi.org/10.1016/J.PESTBP.2003.08.002

McGrath, M. T., & Shishkoff, N. (2003). First report of the cucurbit powdery mildew fungus (*Podosphaera xanthii*) resistant to strobilurin fungicides in the United States. *Plant Disease*, *87*(8), 1007–1007. https://doi.org/10.1094/PDIS.2003.87.8.1007A

Mechoulam, R., Parker, L. A., & Gallily, R. (2002). Cannabidiol: An overview of some pharmacological aspects. *Journal of Clinical Pharmacology*, *42*(11 SUPPL.), 11–19. https://doi.org/10.1002/j.1552-4604.2002.tb05998.x

Nakajima, M., & Akutsu, K. (2014). Virulence factors of *Botrytis cinerea*. *Journal of General Plant Pathology*, *80*(1), 15–23. https://doi.org/10.1007/S10327-013-0492-0/TABLES/1

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2020). *Vegan: Community Ecology Package* (R package version 2.5-7).

Pertwee, R. G. (2006). Cannabinoid pharmacology: The first 66 years. *British Journal of Pharmacology*, *147*(SUPPL. 1). https://doi.org/10.1038/sj.bjp.0706406

Pertwee, R. G. (2008). The diverse CB1 and CB 2 receptor pharmacology of three plant cannabinoids: Δ9-tetrahydrocannabinol, cannabidiol and Δ9-tetrahydrocannabivarin. *British Journal of Pharmacology*, *153*(2), 199–215. https://doi.org/10.1038/sj.bjp.0707442

Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P. H., di Marzo, V., Elphick, M. R., Greasley, P. J., Hansen, H. S., Kunos, G., Mackie, K., Mechoulam, R., & Ross, R. A. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB1 and CB2. *Pharmacological Reviews*, *62*(4), 588–631. https://doi.org/10.1124/pr.110.003004

Pethybridge, S. J., Vaghefi, N., & Kikkert, J. R. (2017). Management of *Cercospora* leaf spot in conventional and organic table beet production. *Plant Disease*, *101*(9), 1642–1651. https://doi.org/https://doi-org.proxy.library.cornell.edu/10.1094/PDIS-04-17-0528-RE

Qiu, P., Liu, S., Bradshaw, M., Rooney-latham, S., Takamatsu, S., Bulgakov, T. S., Tang, S., Feng, J., Jin, D., Aroge, T., Li, Y., & Wang, L. (2020). Multi-locus phylogeny and taxonomy of an unresolved, heterogeneous species complex within the genus *Golovinomyces ambrosiae*, *G. circumfusus* and *G. spadiceus*. *BMC Microbiology*, *20*(51), 1–16.

R Core Team. (2021). *R: A language and environment for statistical computing* (4.0.4). R Foundation for Statistical Computing.

R Studio Team. (2020). *RStudio: Integrated Development for R* (1.4.1106). RStudio, PBC.

Rebordinos, L., Cantoral, J. M., Prieto, M. V., Hanson, J. R., & Collado, I. G. (1996). The phytotoxic activity of some metabolites of *Botrytis cinerea*. *Phytochemistry*, *42*(2), 383–387. https://doi.org/10.1016/0031-9422(95)00909-4

Romero, D., de Vicente, A., Zeriouh, H., Cazorla, F. M., Fernández-Ortuño, D., Torés, J. A., & Pérez-García, A. (2007). Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. *Plant Pathology*, *56*(6), 976–986. https://doi.org/10.1111/J.1365-3059.2007.01684.X

Romero, D., Pérez-García, A., Rivera, M. E., Cazorla, F. M., & de Vicente, A. (2004). Isolation and evaluation of antagonistic bacteria towards the cucurbit powdery mildew fungus *Podosphaera fusca*. *Applied Microbiology and Biotechnology*, *64*(2), 263–269. https://doi.org/10.1007/S00253-003-1439-8/TABLES/3

Rotolo, C., de Miccolis Angelini, R. M., Dongiovanni, C., Pollastro, S., Fumarola, G., di Carolo, M., Perrelli, D., Natale, P., & Faretra, F. (2018). Use of biocontrol agents and botanicals in integrated management of *Botrytis cinerea* in table grape vineyards. *Pest Management Science*, *74*(3), 715–725. https://doi.org/10.1002/PS.4767

Samuels, A. L., Glass, A. D. M., Ehret, D. L., & Menzies, J. G. (1991). Mobility and deposition of silicon in cucumber plants. *Plant, Cell & Environment*, *14*(5), 485–492. https://doi.org/10.1111/J.1365-3040.1991.TB01518.X

Sasaki, I., & Nagayama, H. (1996). β-Glucosidase of *Botrytis cinerea*: Its Involvement in the Pathogenicity of This Fungus. *Bioscience, Biotechnology, and Biochemistry*, *60*(1), 54–56. https://doi.org/10.1271/BBB.60.54

Scott, C., & Punja, Z. K. (2020). Evaluation of disease management approaches for powdery mildew on *Cannabis sativa* L. (marijuana) plants. *Canadian Journal of Plant Pathology*, *43*(3), 394–412. https://doi.org/10.1080/07060661.2020.1836026

Stack, G. M., Toth, J. A., Carlson, C. H., Cala, A. R., Marrero-González, M. I., Wilk, R. L., Gentner, D. R., Crawford, J. L., Philippe, G., Rose, J. K. C., Viands, D. R., Smart, C. D., & Smart, L. B. (2021). Season-long characterization of high-cannabinoid hemp (*Cannabis sativa* L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. *GCB Bioenergy*, *13*(4), 546–561. https://doi.org/10.1111/gcbb.12793

Szarka, D., Tymon, L., Amsden, B., Dixon, E., Judy, J., & Gauthier, N. (2019). First report of powdery mildew caused by *Golovinomyces spadiceus* on industrial hemp (*Cannabis sativa*) in Kentucky. *Plant Disease*, *103*(7), 1773–1773. https://doi.org/10.1094/pdis-01-19-0049-pdn

Tanaka, K., Fukuda, M., Amaki, Y., Sakaguchi, T., Inai, K., Ishihara, A., & Nakajima, H. (2017). Importance of prumycin produced by *Bacillus amyloliquefaciens* SD-32 in biocontrol against cucumber powdery mildew disease. *Pest Management Science*, *73*(12), 2419–2428. https://doi.org/10.1002/PS.4630

Tesfagiorgis, H. B., Laing, M. D., & Annegarn, H. J. (2014). Evaluation of biocontrol agents and potassium silicate for the management of powdery mildew of zucchini. *Biological Control*, *73*, 8–15. https://doi.org/10.1016/J.BIOCONTROL.2014.03.010

Thomas, B. F., & ElSohly, M. A. (2016). Biosynthesis and Pharmacology of Phytocannabinoids and Related Chemical Constituents. *The Analytical Chemistry of Cannabis*, 27–41. https://doi.org/10.1016/B978-0-12-804646-3.00002-3

Toth, J. A., Smart, L. B., Smart, C. D., Stack, G. M., Carlson, C. H., Philippe, G., & Rose, J. K. C. (2021). Limited effect of environmental stress on cannabinoid profiles in high-cannabidiol hemp (*Cannabis sativa* L.). *GCB Bioenergy*, *13*(10), 1666–1674. https://doi.org/10.1111/GCBB.12880

Weldon, W. A., Ullrich, M. R., Smart, L. B., Smart, C. D., & Gadoury, D. M. (2019). Cross infectivity of powdery mildew isolates originating from hemp (*Cannabis sativa*) and Japanese hop (*Humulus japonicus*) in New York. *Plant Health Progress*, *21*(1), 47–53. https://doi.org/https://doi.org/10.1094/PHP-09-19-0067-RS

Yamamoto, S., Shiraishi, S., Kawagoe, Y., Mochizuki, M., & Suzuki, S. (2015). Impact of *Bacillus amyloliquefaciens* S13-3 on control of bacterial wilt and powdery mildew in tomato. *Pest Management Science*, *71*(5), 722–727. https://doi.org/10.1002/PS.3837

Yanar, Y., Yanar, D., & Gebologlu, N. (2013). Control of powdery mildew (*Leveillula taurica*) on tomato by foliar sprays of liquid potassium silicate (K2SiO3). *African Journal of Biotechnology*, *10*(16), 3121–3123. https://doi.org/10.4314/ajb.v10i16.

Zhang, C., Liu, Y., & Ding, L. (2011). *Shift of sensitivity of* Botrytis cinerea *to azoxystrobin in greenhouse vegetables before and after exposure to the fungicide*. 293–302. https://doi.org/10.1007/s12600-011-0159-3

Zhang, Z., Zhu, Z., Ma, Z., & Li, H. (2009). A molecular mechanism of azoxystrobin resistance in *Penicillium digitatum* UV mutants and a PCR-based assay for detection

of azoxystrobin-resistant strains in packing- or store-house isolates. *International Journal of Food Microbiology*, *131*(2–3), 157–161. https://doi.org/10.1016/j.ijfoodmicro.2009.02.015.

CHAPTER IV

SUSCEPTIBLITY OF THE HEMP GERMPLASM TO THE *GOLOVINOMYCES AMBROSIAE*, THE CAUSAL AGENT OF HEMP POWDERY MILDEW

Abstract:

As the hemp (*Cannabis sativa* L.) market in the United States continues to develop, options for disease management will continue to be an area of interest for hemp growers. Powdery mildew, caused by *G. ambrosiae*, has emerged as a prevalent and impactful hemp disease. Genetic host resistance to diseases like powdery mildew are a highly desirable trait and target for breeding, but there is lack of knowledge of possible sources of genetic resistance in *C sativa*. The goal of this study was to characterize *G. ambrosiae* susceptibility of many hemp cultivars and identify possible sources of genetic host resistance. High-cannabidiol (CBD) and high-cannabigerol (CBG) hemp cultivars and breeding line accessions were evaluated in multiple growth chamber trials with controlled inoculations and field trials for their susceptibility to *G. ambrosiae* over multiple years. Grain, fiber and dual-purpose hemp cultivars were challenged with three different *G. ambrosiae* isolates in controlled growth chamber inoculations and evaluated for their susceptibility. Among the high-CBD and high-CBD cultivars and accessions, a range of susceptibility to *G. ambrosiae* was observed in the growth chamber and field trials, with multiple cultivars showing consistently high or low levels of susceptibility with high heritability, suggesting a potential sources of genetic host resistance. The

grain, fiber and dual-purpose cultivars displayed far less variability in powdery mildew susceptibility which did not vary significantly between *G. ambrosiae* isolates.

Introduction:

Hemp is a crop defined legally in the United States as *Cannabis sativa* L. having less than 0.3% Δ9-tetrahydrocannabinol by dry weight. It has long been cultivated by humans for many purposes, including for fiber, grain and cannabinoids. In recent years, the legality and regulations surrounding hemp, especially in the United States, has been rapidly evolving, resulting in fluctuations in the hemp market. Subsequently, there is a growing need for hemp research efforts to better understand best growing practices for hemp (Cherney & Small, 2016; Fike, 2016).

Soon after the modern era of hemp production began in New York State, hemp powdery mildew caused by *Golovinomyces ambrosiae*, formerly *G. spadiceus*, emerged as a prominent disease affecting hemp production (Weldon et al., 2019). Powdery mildew has also been reported as problematic disease in several other locations in the United States as well, as hemp production in those states have developed (Farinas & Hand, 2020; Szarka et al., 2019).

Golovinomyces ambrosiae is an obligate biotrophic fungus belonging to the ascomycete family Erysiphaceae, which is a large group composed of many powdery mildew-causing fungi that are able to infect many plant species (Glawe, 2008). Powdery mildew pathogens can infect host tissue through the germination of sexual ascospores or asexual

conidia, from which an appressorium differentiates and forms a penetration peg. The penetration peg can invade host tissue and form the haustorium, a specialized structure used for the uptake of nutrients from the host plant. Meanwhile, vegetative hyphae continue to grow on the surface of the host plant, typically the leaves, and eventually differentiate into conidiophores producing chains of asexual conidia. Conidia are the asexual fungal spores that can detach and spread through wind to infect new host tissue, causing secondary infections. Large numbers of conidia can be produced within days of initial infection, making powdery mildews polycyclic (Glawe, 2008). As a result, disease intensity can become extremely high in a field or greenhouse when environmental conditions are favorable (Glawe, 2008).

The fungi that cause powdery mildews rapidly grow and spread in warm and humid, but not wet, conditions, making them extremely common in greenhouses and other controlled environment conditions. Therefore, cultural practices such as increasing canopy airflow and reducing leaf wetness are crucial for reducing powdery mildew disease pressure. Pruning infected tissue to reduce secondary spread via conidia will also help to reduce overall disease pressure (Glawe, 2008; Scott & Punja, 2020).

In other crop systems, there has also been success managing powdery mildews with both conventional fungicide (Ishii et al., 2007; Karaoglanidis & Karadimos, 2006; Vielba-Fernández et al., 2020; Wicks & Hitch, 2002) and Organic Materials Review Institute (OMRI)-approved product applications (Cai et al., 2017; Garibaldi et al., 2011; Jiao et al., 2020; Shen et al., 2016; Tesfagiorgis et al., 2014; Tesfagiorgis & Annegarn,

2013). However, currently, there are a limited number of commercially available products that are labeled for use on hemp. Further, there are limited peer-reviewed publications demonstrating the efficacy of those labeled products against hemp diseases, including hemp powdery mildew (Scott & Punja, 2020).

In addition to cultural practices and chemical use, genetic host resistance has been effective for managing powdery mildew in various crop systems (Green et al., 2014; Helms Jorgensen, 1992; Kusch & Panstruga, 2017). Qualitative resistance conferred by major R-genes such as nucleotide-binding site leucine rich repeat (NBS-LRR) genes have been identified and deployed in other crops systems to work against powdery mildew (Goyal et al., 2020; He et al., 2018). These genes have a dominant mode of action, and the proteins transcribed from these genes function through the recognition of effector proteins secreted by pathogens and induction of effector-triggered immunity (ETI) in the plant host. ETI typically results in a hypersensitive response (HR), localized programmed cell death (Dangl et al., 2013; Jones & Dangl, 2006), limiting pathogen spread through host tissue. Genetic sources of qualitative resistance specifically against powdery mildews also include genes comprising the *Mildew Locus O* (*MLO*) gene family (Büschges & Hollricher, 1997; Czembor et al., 2016; Helms Jorgensen, 1992). These genes code for plasma membrane proteins, and while their specific mechanisms are not fully understood, they confer broad spectrum susceptibility to specific fungal species that cause powdery mildew (Bai et al., 2007; Humphry et al., 2011). Mutations of these genes renders them nonfunctional, resulting in qualitative resistance that has a recessive mode of action (Consonni et al., 2006). NBS-LRR confer

race-specific resistance and therefore can result in strong selection pressure for pathogen races that are able to overcome these R genes (Milgroom, 2017). *MLO* genes, however, confer broad spectrum resistance and limit the pathogen's ability to penetrate host tissue, resulting in increased durability of resistance compared with R genes (Humphry et al., 2011). To date, the NBS-LRR gene *PM1* has been identified in a *C. sativa* cultivar (Mihalyov & Garfinkel, 2021) through linkage mapping. Additionally, multiple candidate *MLO* genes, two of which are candidate susceptibility genes, have been identified *in silico* by Pépin et al. (2021).

Identification of additional possible sources of genetic host resistance will be an important aspect of hemp breeding programs. The objective of this study was to screen hemp germplasm to determine whether there are any potential sources of host resistance to hemp powdery mildew. This was done through multiple controlled inoculation experiments in growth chambers, as well as disease ratings of field variety trials over multiple years. High CBD, high CBG, grain, fiber and dual-purpose hemp cultivars were all included in this study. Additionally, consistency in host plant susceptibility to hemp powdery mildew in growth chamber and field screens was observed.

Materials and Methods:

Susceptibility of high CBD and high CBG cultivars to* Golovinomyces ambrosiae *in growth chamber inoculations:

In the spring of 2020, 30 high CBD hemp cultivars (Table 4.1) were screened for their susceptibility to *G. ambrosiae* in a growth chamber. Plants were seeded on May 11,

2020 in 50-cell flats with Lambert's LM-1 Germination Mix or clonally propagated. Clones were generated as previously described in Chapter III. Seedlings were grown a greenhouse with a 16-hour photoperiod, a day temperature of 24˚C and 21˚C night temperature. Plants were transplanted into 4-inch pots before being moved to a growth chamber.

In the growth chamber, three plants of each cultivar were arranged in randomized complete block design. Growth chamber control settings matched those of the greenhouse. Plants were inoculated with the previously described with *G. ambrosiae* isolate 19002 from New York State (Weldon et al., 2019). Liquid inoculum was made by washing powdery mildew-infected leaves with a solution of 1L of distilled water and 100 µL of Tween20. The plants were sprayed to runoff with a conidial suspension of 2×10^4 spores per mL. This screening was replicated in a separate growth chamber with three additional plants of each cultivar. Plants were arranged in a randomized complete block design and inoculated in the same way as the first replicate, and conditions were the same in both growth chambers.

Plants were evaluated for disease severity by rating individual plants for percent disease coverage of the whole plant at 0, 7-, 12-, 14- and 21-days post inoculation (dpi). This experiment was replicated using the same cultivars and *G. ambrosiae* isolate and this replicate was inoculated with a liquid conidial suspension at a concentration of 2×10^4 spores per mL.

Table 4.1. Metadata of high CBD cultivars from commercial sources and accessions from breeding lines that were screened for powdery mildew susceptibility through field trials in Geneva and Ithaca and growth chamber inoculations in 2020. Cultivars were either clonally propagated or started from feminized or dioecious seed.

Cultivar	Propagation method	Source
BaOx	Feminized seed	Ryes Creek
Berry Blossom	Feminized seed	Castetter Sustainability Group
Carolina Dream	Feminized seed	Ryes Creek
CJ 2	Feminized seed	Sunrise Genetics
CW EM-18	Feminized seed	Charlotte's Web
CW EM-28	Feminized seed	Charlotte's Web
CW EM-31	Feminized seed	Charlotte's Web
CW EM-73	Feminized seed	Charlotte's Web
Early Pearly	Female clone	Front Range Bioscience
FL 49	Female clone	Sunrise Genetics
FL 58	Female clone	Sunrise Genetics
FL 70	Female clone	Sunrise Genetics
GVA-H-19-1064-003	Female clone	Cornell-AC/DC selection
GVA-H-19-1066-001	Female clone	Cornell-Housewife selection
GVA-H-19-1067-001	Female clone	Cornell-Double Cherries selection
GVA-H-19-1068-003	Female clone	Cornell-Otto II selection
GVA-H-19-1077-008	Female clone	Cornell-Winterlake selection
GVA-H-19-1091	Dioecious seed	Cornell-A2R4 selection
GVA-H-19-1191	Feminized seed	Cornell-Nebraska selection
GVA-H-20-1030	Dioecious seed	Cornell-RNF selection
Hybrid #5	Female clone	Front Range Bioscience
Hybrid #9	Female clone	Front Range Bioscience
Lindorea	Feminized seed	Charlotte's Web
NS52	Feminized seed	Phytonyx
Rogue	Feminized seed	Industrial Seed Innovations
SB 1	Female clone	Sunrise Genetics
SR-1	Feminized seed	Industrial Seed Innovations
Sweetened	Feminized seed	Ryes Creek
The Grand (T2xT2)	Feminized seed	Boring Hemp
TJ's CBD	Female clone	Stem Holdings, OR
Umpqua	Feminized seed	Industrial Seed Innovations
Z 25	Female clone	Sunrise Genetics

Growth Chamber inoculations of High CBG cultivars:

In the summer of 2020, 11 high cannabigerol (CBG) and three high CBD hemp cultivars (Tables 4.1 and 4.2) were screened in growth chambers for their susceptibility to *G. ambrosiae* using the same procedure as described above. Plants were inoculated with a liquid conidial suspension of the *G. ambrosiae* isolate 19002 with a final conidial concentration of 2×10^4 spores per mL, and plants were sprayed until runoff. This screening was replicated in a second growth chamber with the same cultivars, and they were inoculated with the same isolate in the same way as described for the first replicate. Plants were evaluated for disease severity by rating for percent disease severity of the whole plant at 7-, 12-, 14-, 19- and 21-dpi.

Table 4.2. Metadata of high CBG hemp cultivars from commercial sources that were included in the growth chamber inoculations and field trial in 2020. Cultivars were either started from seed or clonally propagated. The cultivars 'Panakeia', 'Stardust' and 'Sweet Caroline' were not included in the field trial ratings, as many individuals were harvested by the last rating.

Cultivar	Propagation Method	Source
Auto CBG	Feminized seed	Oregon CBD
Black CBG	Female clone	Ryes Creek
CBG Delight	Feminized seed	Flura
First Class CBG	Female clone	Hydrogrow
Fountain of Youth	Female clone	Green Point Research
H5 CBG	Feminized seed	American Hemp Co
Panakeia	Female clone	Front Range Bioscience
Pure CBG	Female clone	Front Range Bio/Puregene
Stardust	Female clone	Ryes Creek
Sweet Caroline	Female clone	Ryes Creek
TJ's CBG	Feminized seed	Stem Holdings Agri
White CBG	Feminized seed	Oregon CBD

Field ratings of high CBD and high CBD cultivars in 2020:

In the summer of 2020, high CBD (Table 4.1) and high CBG cultivars (Table 4.2) were screened for their susceptibility to *G. ambrosiae*. Plants were seeded in 50 cell flats in Lambert's LM-1 Germination Mix or clonally propagated and grown in a greenhouse with the same conditions as described previously for the growth chamber screenings. In mid-June 2020, plants were transplanted into the field. Each cultivar was planted in 5 plant plots and replicated 4 times throughout the field in a randomized complete block design. The high CBD trail was planted in two locations: Geneva, NY (McCarthy farm)

and Ithaca, NY (Bluegrass Lane Turf and Ornamental Farm). The high CBG trial was planted only in the Geneva location on the same farm (McCarthy farm).

At each site, fields were prepared by applying 150 lbs. N per acre as 19- 19- 19 N-P-K (Phelps Supply Inc.). Raised beds with black mulch plastic and drip irrigation were prepared with row space every 6ft on center, and plants were spaced 4ft apart within rows. Fertilizer was applied twice during the growing season with 25 lbs. per application of Jack's 12-4-16 Hydro FedED RO. Weed cloth was used for weed control at both locations.

Toward the end of the growing season, plants in all fields at Geneva and Ithaca locations became naturally infected with *G. ambrosiae* and were evaluated for disease severity. Individual plants were rated for percent disease coverage and ratings within each plot were averaged. The high CBD trial in Geneva was rated 1-, 21-, and 35- days after initial symptoms were observed in the field. The trial in Ithaca was rated 1-, 22-, and 36- after initial symptoms were observed in the field. The high CBG trial was rated 1-, 20-, and 35- days after symptoms were first observed.

The powdery mildew pathogen was collected from infected plants in the field using stickers to collect conidia from leaf surfaces as described by (Weldon et al., 2021). DNA was extracted as previously described (Healey et al., 2014) and PCR amplified using intergenic spacer region primers (Carbone & Kohn, 1999). Amplicons were prepared

using Exo-SAP-IT^{TM} PCR Product Cleanup kit using the manufacture's protocol (Thermo Fisher Scientific, Waltham MA) and subsequently sequenced at the Cornell University Institute of Biotechnology. Consensus sequences were aligned using Geneious 2022.0.1 MUSCLE alignment software (https://www.geneious.com) and compared to the NCBI database using BLAST (Altschul et al., 1990) to identify the pathogen present.

Field ratings of high CBD and high CBD cultivars in 2021:

In the summer of 2021, high CBD and high CBG hemp cultivars (Table 4.3) were screened for their susceptibility to *G. ambrosiae* in the field. Eight high CBD cultivars ('FL 49,' FL 58,' FL 70,', GVA-H-19-1066-001, GVA-H-19-1067-001, 'Rogue,' 'TJ's CBD' and 'Z 25') and three high CBG ('Pure CBG', 'Sweet Caroline' and 'White CBG') screened in 2021were also screened in 2020. Twenty-one of the cultivars were screened for the first time in 2021. Some cultivars (Table 4.3) were seeded in 50 cells flats in Lambert's LM-1 germination mix and seedlings were grown greenhouse conditions as described previously for 2021 trials while others (Table 4.3) were clonally propagated from stem cuttings. Plants were transplanted into five-plant plots and replicated four times in the field in a randomized complete block design. For this trial, high CBD and high CBG cultivars were planted in the same field in one location in Geneva, NY (McCarthy farm). The field was prepared by applying 120 lbs. N per acre as 19- 19- 19 N-P-K (Phelps Supply Inc.). Raised beds with black mulch plastic and drip irrigation were prepared with row spacing every 6ft on center, and plants were

spaced 6ft apart within rows. Fertilizer was applied twice during the growing season with 25 lbs. per application of Jack's 12-4-16 Hydro FedED RO. Weed cloth was used for weed control.

At the end of the growing season, plants became infected with *G. ambrosiae* from natural inoculum and were evaluated for disease severity. Individual plants were rated for percent disease coverage only one time before they were harvested.

Table 4.3. Metadata of high CBD and high CBG hemp cultivars from commercial sources or accessions from Cornell hemp breeding lines included in a field trial and screened for powdery mildew susceptibility in 2021. Plants were either seeded or clonally propagated.

Cultivar	Propagation Method	Source	Cultivar type
Apollo	Feminized seed	Davis Farms	High CBD
Bubbatonic	Feminized seed	Kayagene	High CBD
Eighty-Eight	Feminized seed	Davis Farms	High CBD
FL 49	Female clone	Sunrise Genetics	High CBD
FL 58	Female clone	Sunrise Genetics	High CBD
FL 70	Female clone	Sunrise Genetics	High CBD
Fruity Petals	Feminized seed	Front Range Bioscience	High CBD
Golden Kush (NS52)	Feminized seed	Phytonyx	High CBD
GVA-H-19-1066-001	Female clone	Cornell Hemp	High CBD
GVA-H-19-1067-001	Female clone	Cornell Hemp	High CBD
GVA-H-21-1002	Feminized seed	Cornell Hemp	High CBD
Hempress	Feminized seed	Point3 Farma	High CBD
Lifter	Feminized seed	Oregon CBD	High CBD
Lifter Seedless	Feminized seed	Oregon CBD	High CBD
Magic Bullet #5	Feminized seed	Point3 Farma	High CBD
PhotoCBD	Feminized seed	Phylos	High CBD
Pine Walker Seedless	Feminized seed	Oregon CBD	High CBD
Pure CBG	Feminized seed	Alterra Hemp	High CBG
Purple Emperor	Feminized seed	Davis Farms	High CBD
Rogue	Feminized seed	Arcadia Biosciences	High CBD
Skipper	Feminized seed	Davis Farms	High CBD
Sour Kush	Feminized seed	Kayagene	High CBD
Sour Lifter Seedless	Feminized seed	Oregon CBD	High CBD
Sour Suver Haze Seedless	Feminized seed	Oregon CBD	High CBD
Suver Haze	Feminized seed	Oregon CBD	High CBD
Suver Haze Seedless	Feminized seed	Oregon CBD	High CBD
Sweet Caroline	Female clone	Ryes Creek	High CBG
Tangerine	Feminized seed	Atlas Seeds	High CBD

TJ's CBD	Female clone	Stem Holdings Agri	High CBD
Truckee	Feminized seed	Arcadia Biosciences	High CBD
Umpqua	Feminized seed	Arcadia Biosciences	High CBD
Valerie 17	Feminized seed	Front Range Bioscience	High CBD
Valerie 29	Feminized seed	Front Range Bioscience	High CBD
White CBG	Feminized seed	Oregon CBD	High CBG
White CBG Seedless	Feminized seed	Oregon CBD	High CBG
Z 25	Female clone	Sunrise Genetics	High CBD

Susceptibility of grain and fiber hemp cultivars to *Golovinomyces ambrosiae*:

In the winter of 2020, 23 grain, fiber and dual-purpose hemp cultivars (Table 4.4) were screened for their susceptibility to three isolates of *G. ambrosiae*. Plants were seeded in 50-cells flats in Lambert's LM-1 germination media and grown in a greenhouse with day temperatures of 24°C, night temperatures of 21°C and a 16-hour photoperiod. Plants were transplanted to four-inch pots before being moved to growth chambers for inoculation. Plants were divided into three separate growth chambers with 5 plants of each cultivar in each chamber. Within each growth chamber plants were arranged into a randomized complete block design with one plant in each block and five blocks. Each growth chamber was inoculated with one of three *G. ambrosiae* isolates: 19002, 19137 and 19001. The isolates 19001 and 19002 are NY isolates that were previously described (Weldon et al., 2019) and 19137 is an isolate that was collected from Ontario County, NY in 2019.

A liquid inoculum at a concentration of 2×10^4 spores per mL was made by washing infected hemp leaves with a solution of one liter of water and 100 µL of Tween-20. Plants were spray inoculated to runoff with the conidial suspension. Disease severity was evaluated by rating individual plants for percent disease coverage of the whole plant at 12-, 19-, 26-dpi. This experiment was replicated with the same cultivars and *G. ambrosiae* isolates and a with a liquid spore inoculum concentration of 2×10^4 spores per milliliter. These 23 grain and fiber cultivars were also planted in the field in Geneva, NY (Research North farm). In 2020, the field was prepared by plowing and disking and

an application of 75 lbs N per acre as 22-9-9 N-P-K. Plants were directly seeded into the field using a cone seeder. Plots were 6 rows at 7.5 inch spacing and were 3.75 ft × 20 feet. Prowl H2O was applied after planting for weed control. In 2021, four fields in Geneva (two at Crittendon North farm and two at Research North farm) were prepared by plowing, disking and applications of 50 lbs N per acre with 19-19-19 N-P-K. Cultivars were directed seeded into the field with a cone seeder and plots were as described in 2020. The field was top dressed once through the growing season with ammonium sulphate at 21-0-0-24 N-P-K-S. Prowl H2O was used again for weed control. Biweekly field surveys starting at 4 weeks post seeding until harvest were performed by walking through each plot of the replicated trial.

Table 4.4. Metadata grain, fiber and dual-purpose hemp cultivars which were screened for susceptibility to three *G. ambrosiae* isolates in growth chambers in 2020. All were grown from seed obtained from various sources listed.

Cultivar	Source
Bialobrzeskie	Bija Hemp
Canda	Parkland Industrial Hemp Growers
Carmagnola	Schiavi Seed
Carmagnola Selezionata	Schiavi Seed
CFX-1	Hemp Genetics International
CFX-2	Hemp Genetics International
CRS-1	Hemp Genetics International
Earlina 8	UNISeeds
Eletta Campana	Schiavi Seeds
Fedora 17	UNISeeds
Felina 32	UNISeeds
Ferimon	UNISeeds
Fibranova	Schiavi Seeds
Futura 75	UNISeeds
Grandi	Hemp Genetics International
Helena	Cornell Hemp
Joey	Parkland Industrial Hemp Growers
Katani	Hemp Genetics International
Tygra	Cornell Hemp
USO-31	UNISeeds
Wojko	Assocanapa
X-59	Legacy Hemp

Data Analysis:

All data analyses were performed using R version 2.0.4 (R Core Team, 2021) in RStudio 1.4.1106 (R Studio Team, 2020). Within each growth chamber or field trial, the means of area under the disease progress curves (AUDPC) were calculated using the R package agricolae (de Mendiburu, 2020) for each cultivar. The mean AUDPC for each cultivar were compared using an analysis of variance (ANOVA, $P<0.05$), and a Tukey's HSD test was subsequently performed for a pairwise comparison using the HSD.test function in the agricolae package (de Mendiburu, 2020). For the 2021 high CBD/CBG field trial, because there was only one disease rating, mean disease severity ratings were calculated for each cultivar and compared using and ANOVA ($P<0.05$) and a Tukey's HSD test was performed post-hoc using the same methods described above.

Results:

Growth chamber inoculations of high CBD cultivars:

Thirty high CBD hemp cultivars were evaluated in a growth chamber for their susceptibility to *G. ambrosiae*, the causal agent of hemp powdery mildew. The AUDPC of each of the cultivars were compared showing that there was a large amount of variation in powdery mildew severity among all the cultivars in both replicates (Figure 4.1). The cultivars 'NS52,' 'CW-EM 28,' 'Umpqua,' and TJ's CBD' were among those with the highest disease ratings in both replicates. The cultivars 'SB-1,' GVA-H-19-1068-003, and 'FL 58' had some of the lowest disease ratings in both replicates.

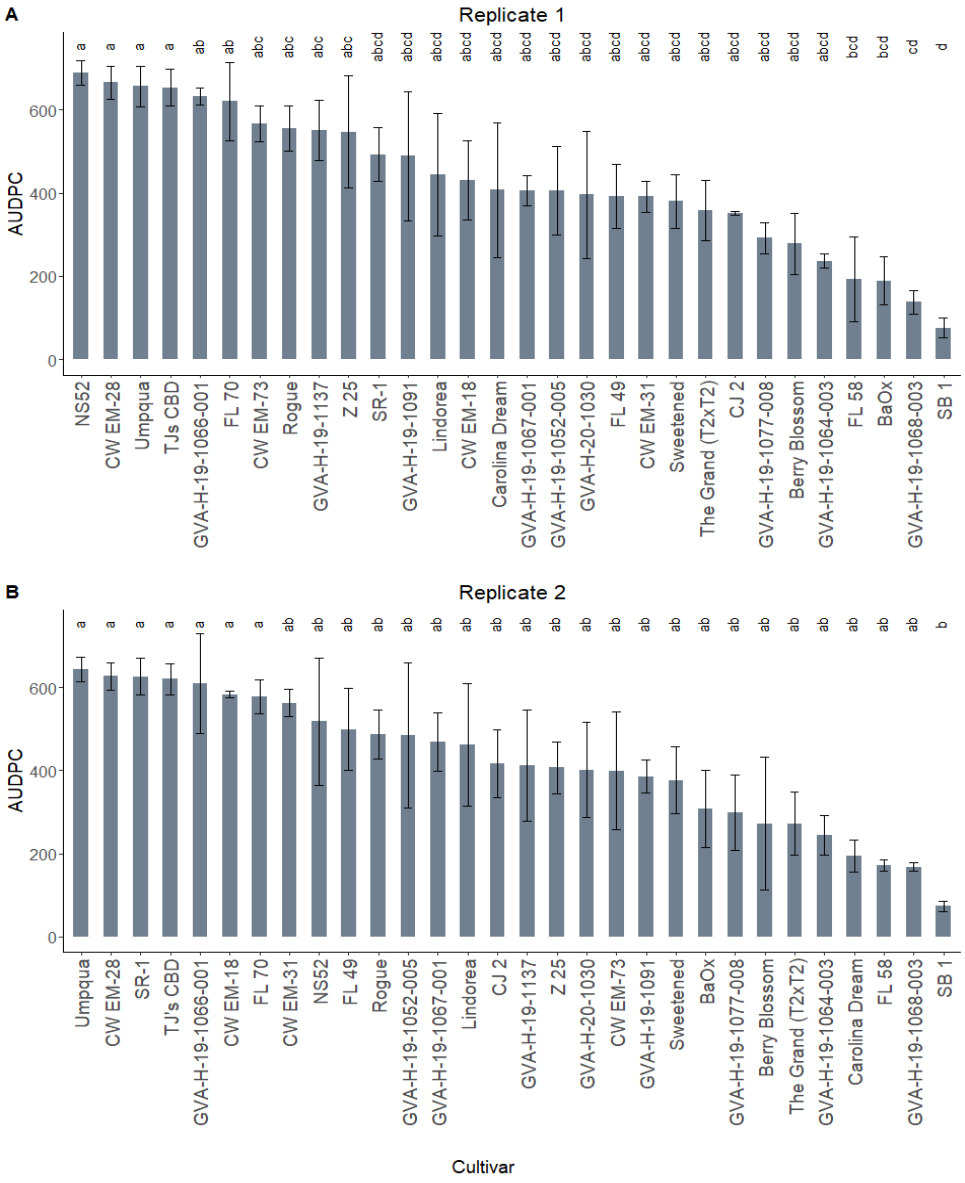

Figure 4.1. Disease severity of powdery mildew on high cannabidiol cultivars in a growth chamber environment in 2020 inoculated with the *Golovinomyces ambrosiae* isolate 19002. Disease severity was evaluated by calculating the area under the disease progress curve (AUDPC) for both replicate one (A) and two (B). Letters indicate Tukey's HSD groups ($p<0.05$).

Growth chamber inoculations of high CBG cultivars:

Eleven high CBG and three additional high CBD cultivars were evaluated for *G. ambrosiae* susceptibility in a growth chamber, which varied among the cultivars as reflected in the AUDPC. The cultivar 'White CBG' had the greatest AUDPC in both replicates and was significantly greater than all but four other cultivars in the first replicate (Figure 4.2, $p<0.05$). 'TJ's CBG' also consistently had greater disease severity numerically in both replicates, but was not significantly greater than many of the other cultivars. The cultivar 'Auto CBG' had significantly lower disease severity in both replicates (Figure 4.2, $p<0.05$). The other cultivars varied in their disease severity between replicates (Figure 4.2).

***Field ratings of high CBD and high CBG cultivars in 2020*:**

High CBD and high CBG cultivars were evaluated for powdery mildew disease severity in the summer of 2020, many of which were the same cultivars that were evaluated in the growth chambers. Similar to the growth chamber inoculations, the cultivars in the field trials displayed wide variations in the levels of powdery mildew disease severity between cultivars.

For the high CBD cultivars, there was overall greater disease pressure in the Geneva field location compared to the Ithaca location, but the variation in disease severity can still be observed in the Ithaca location. The cultivar 'CW EM-28' had significantly greater powdery mildew disease severity compared to the other cultivars in both locations (Figure 4.3, $p<0.05$), followed closely behind by the cultivars 'Umpqua,' 'SR-

1,' and 'NS52.' These results are consistent with the results of the growth chamber inoculations. Also similar to the growth chamber inoculations, 'FL 58,' 'SB 1,' and GVA-H-19-1068-003 were among the cultivars with the lowest powdery mildew disease severity in both locations (Figure 4.3, $p<0.0.5$).

Among the high CBG cultivars, several the cultivars that were planted in this field trial were harvested before the end of the field ratings and therefore were not included in data analysis. Therefore, a total of nine high CBG were evaluated for their susceptibility to *G. ambrosiae* in the field setting in 2020. Similar to the growth chamber inoculations, variation in the powdery mildew disease severity was observed. 'White CBG' displayed significantly greater powdery mildew disease severity in the field setting than all other CBG cultivars tested, and 'Black CBG' had the lowest level of disease but was not significantly different from most other cultivars (Figure 4.4, $p<0.05$).

The powdery mildew pathogen collected from each field was identified as *G. ambrosiae* using IGS PCR followed by amplicon sequencing.

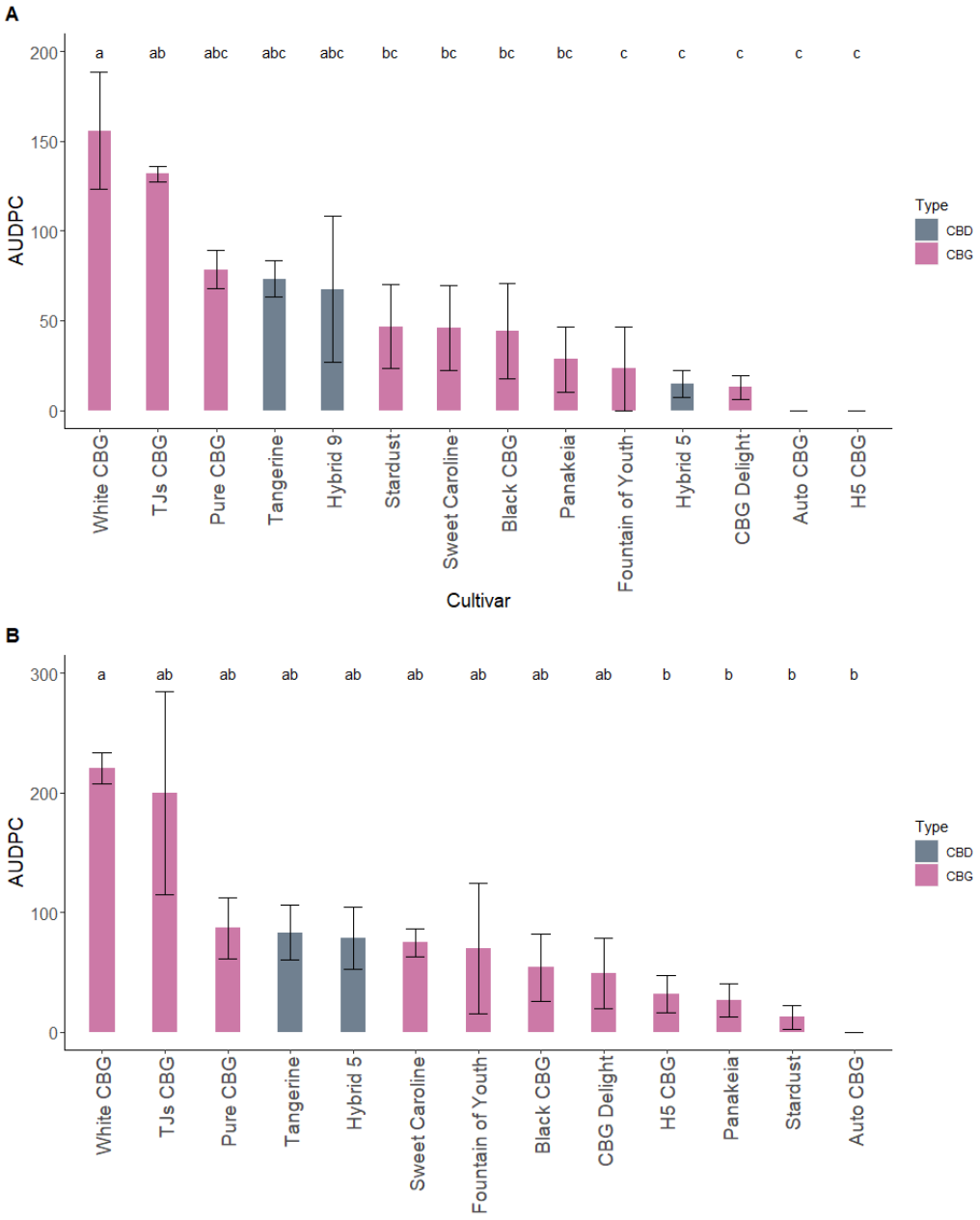

Figure 4.2. Powdery mildew disease severity on high cannabigerol and high cannabiodiol hemp cultivars in a growth chamber environment. Area under the disease progress curves (AUDPC) represent disease severity in plots for replicates one (A) and two (B). Letters indicate Tukey's HSD groups ($p<0.05$).

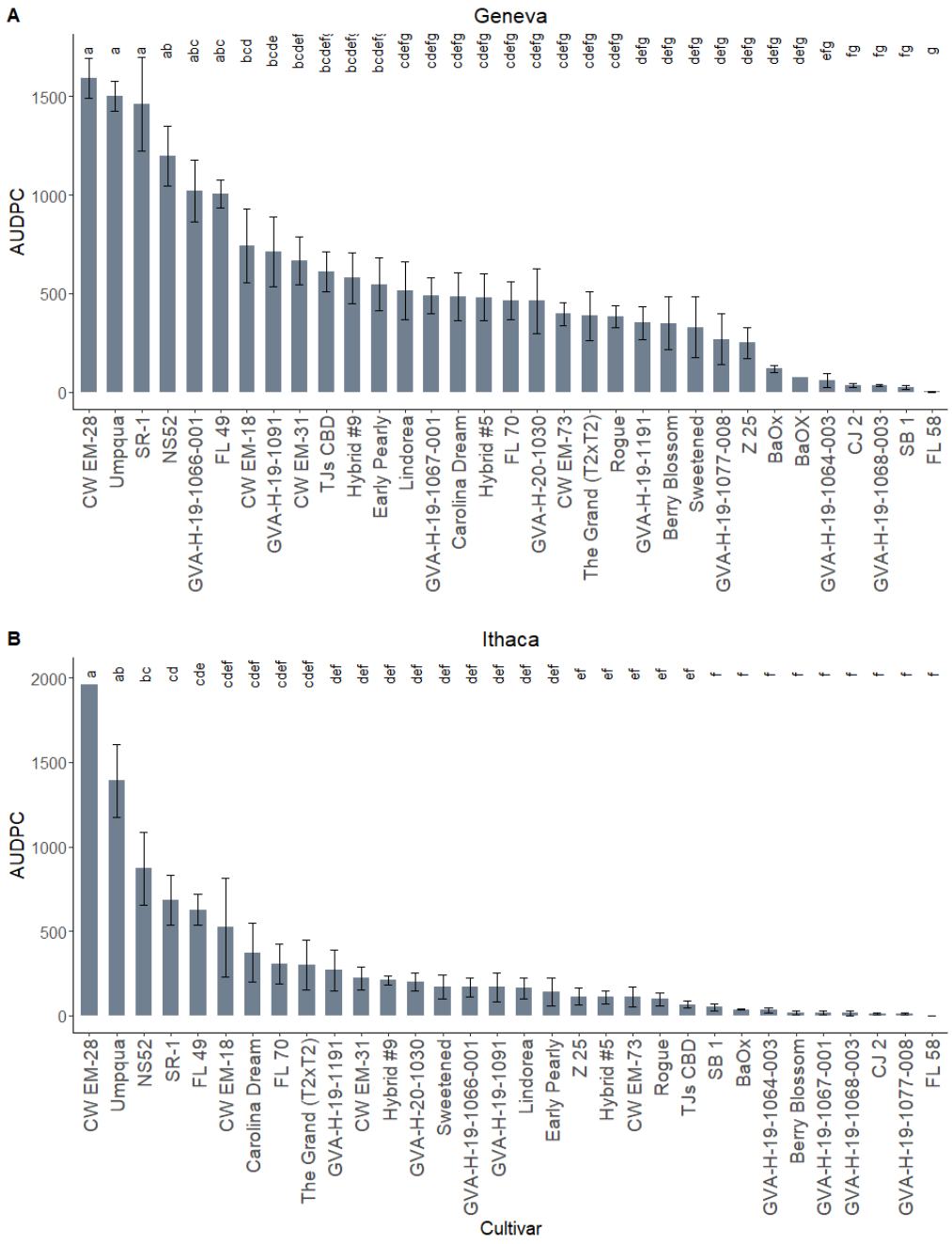

Figure 4.3. Disease severity of powdery mildew shown as area under the disease progress curve (AUPDC) on high cannabidiol hemp cultivars at planting locations in Geneva (A) and Ithaca (B) in 2020. Letters indicate Tukey's HSD groups ($p<0.05$).

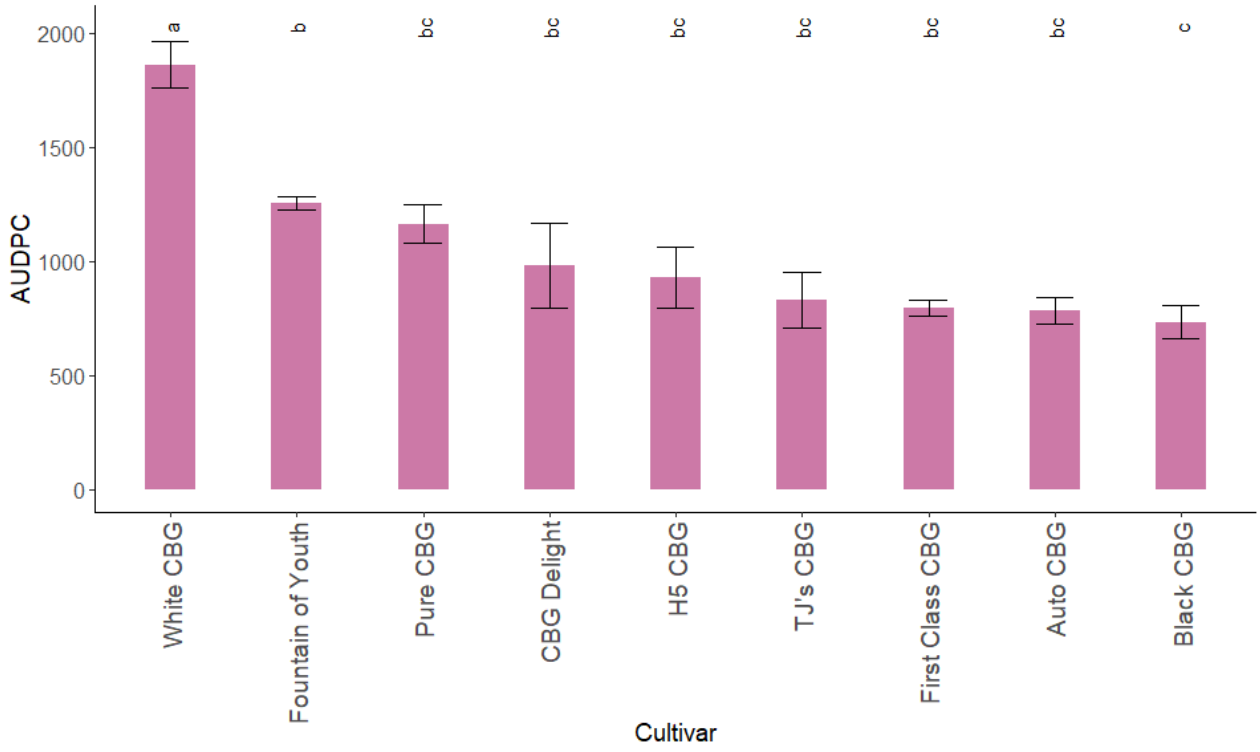

Figure 4.4. Powdery mildew disease severity shown as average area under the disease progress curve (AUDPC) calculations on high cannabigerol hemp cultivars in a field trial in 2020. Letters indicate Tukey's HSD groups ($p<0.05$).

Field ratings of high CBD and high CBG cultivars in 2021:

In the summer of 2021, both high CBD and high CBG hemp cultivars were planted in the same field and evaluated for their susceptibility to powdery mildew. Powdery mildew disease developed late in the season in 2021, only allowing for one disease rating. The percentage disease coverage ratings were compared among cultivars. In this field trial, 'Sweet Caroline' had a significantly greater disease rating than all other cultivars (Figure 4.5, $p<0.05$). The cultivars 'White CBG Seedless,' 'White CBG' and 'Golden Kush' also had significantly greater ratings than the other cultivars. Similar to the previous growth chamber inoculations and field ratings, "FL 58' ('FL 58') was among the cultivars with the lowest disease ratings. 'Pure CBG' was a high CBG cultivar that was evaluated for the first time in this trial and showed similarly low disease ratings to 'FL 58' (Figure 4.5). The powdery mildew pathogen was collected from each field and identified as *G. ambrosiae* using IGS PCR followed by amplicon sequencing.

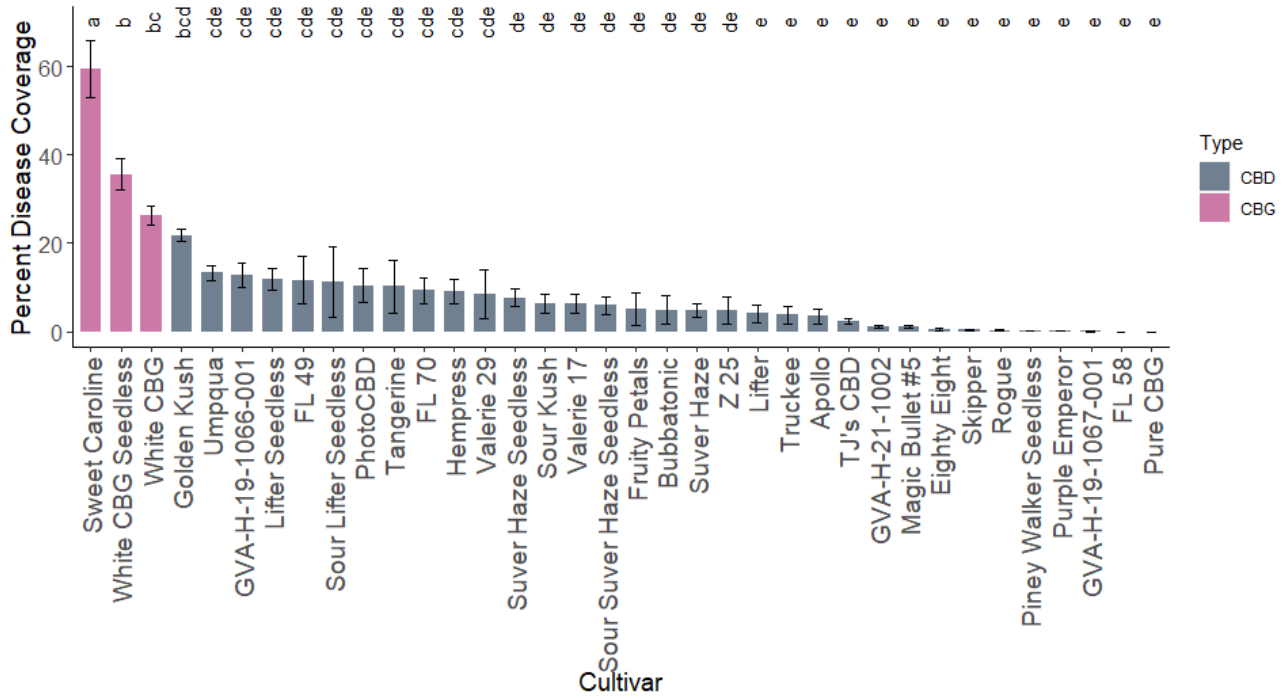

Figure 4.5. Disease severity of powdery mildew on high cannabidiol and high cannabigerol hemp cultivars in a field trial in 2021. Disease severity is represented by a single rating of mean percent disease coverage in each five-plant plot. Letters indicate Tukey's HSD groups ($p<0.05$).

Growth chamber inoculations of grain and fiber cultivars:

Twenty-three grain, fiber and dual-purpose hemp cultivars were evaluated for their susceptibility to three different *G. ambrosiae* isolates through growth chamber inoculations (Table 4.4). The AUDPC of each cultivar was compared for each of the three isolates. The *G. ambrosiae*- susceptible high CBD cultivar 'TJ's CBD' was included as a control for the inoculations. In the first replicate of this experiment, 'TJ's

CBD' had significantly greater disease ratings compared to the other cultivars when inoculated with isolates 19137 and 19001 and had the greatest disease rating, but was not significantly different from 11 other cultivars when inoculated with 19002 (Figure 4.6ACE, $p<0.0.5$). Among the rest of the grain, fiber and dual-purpose cultivars, overall, there were a few other cultivars that had significantly greater powdery mildew disease severity compared to the other cultivars ($p<0.05$), but those cultivars were not consistent between isolates, and between replicates of the same isolate (Figure 4.6ACE). In the second replicate there were no significant differences in the powdery mildew severity among all of the cultivars for each of the three *G. ambrosiae* isolates (Figure 4.6BDF). No powdery mildew was observed on the field-planted grain and fiber cultivars.

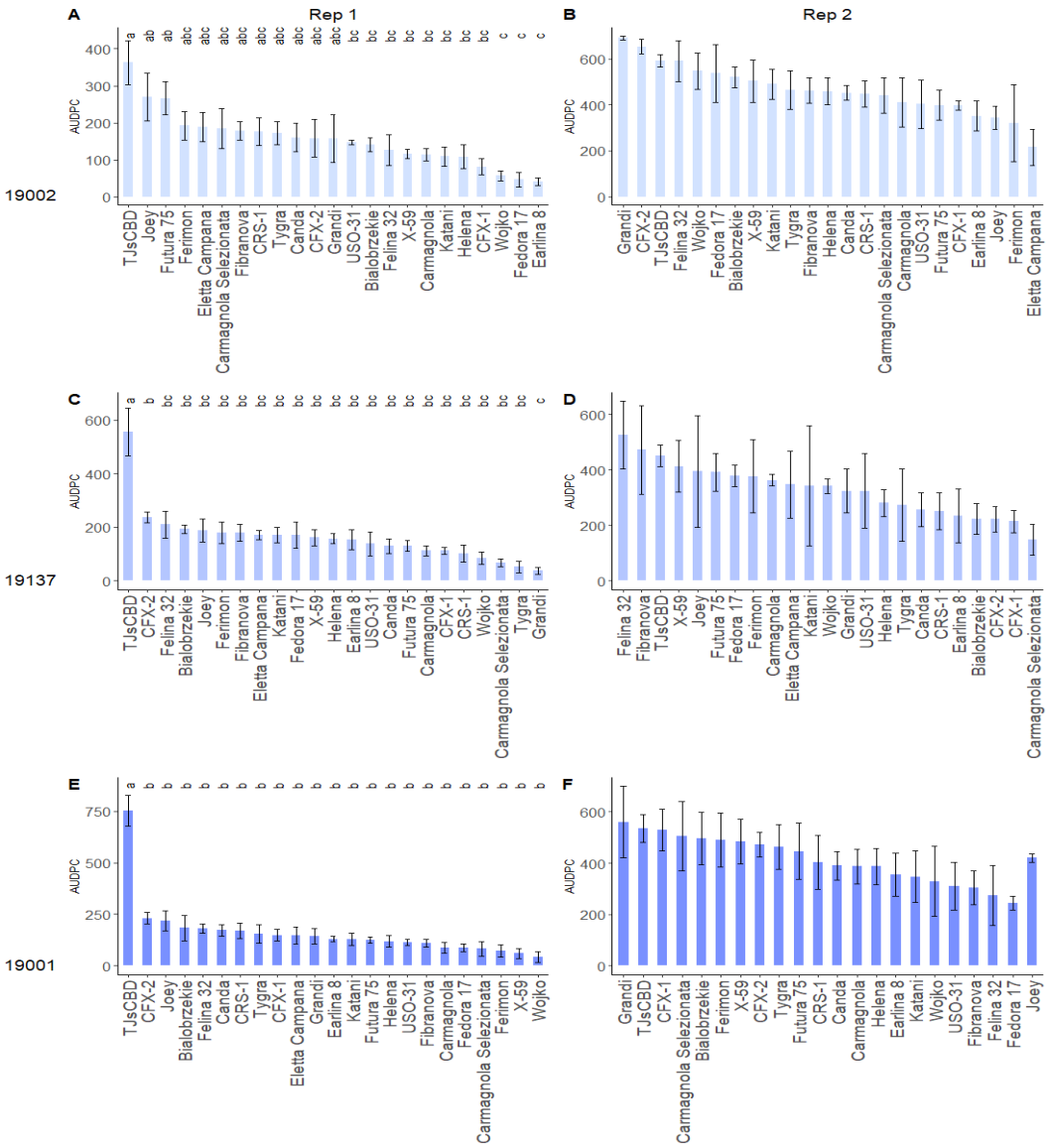

Figure 4.6. Powdery mildew disease severity on grain and fiber trials in a growth chamber environment inoculated with the *Golovinomyces ambrosiae* isolates 19002 (A and B), 19137 (C and D), and 19001 (E and F). Disease severity for each cultivar is represented by an average area under the disease progress curve (AUDPC) value for both replicate one (A, C, E) and two (B, D, F). Letters indicate Tukey's HSD groups ($p<0.05$).

Discussion:

***Golovinomyces ambrosiae* susceptibility of high CBD and high CBD hemp cultivars:**

High CBD and high CBG hemp cultivars were screened for their susceptibility to hemp powdery mildew caused by *G. ambrosiae* through replicated growth chamber inoculations and multiple field season disease ratings. Considering all these experiments and field ratings, there was wide variation i disease severity between cultivars. However, consistent high or low disease ratings were observed for a number of the evaluated cultivars. The high CBD cultivars 'CW EM-28,' 'Umpqua,' 'SR-1' and 'NS52' were consistently the most susceptible to powdery mildew compared to the other high CBD cultivars. Similarly, 'White CBG' was consistently the most susceptible high CBG cultivar. Among the high CBD cultivars, 'FL 58' consistently demonstrated very low levels of disease in both the growth chamber and in field trials over multiple years and multiple locations. The low level of disease on 'FL 58' is consistent with previous field trial powdery mildew severity ratings of high CBD cultivars (Stack et al., 2021). The high CBD cultivars 'SB-1' and 'GVA-H-19-1068-003' were also less susceptible to powdery mildew in both the growth chamber and field trials. 'White CBG' was the only high CBG cultivar that was consistently significantly more susceptible to powdery mildew across growth chamber and field trials in both years.

One purpose of this study was to determine whether there could be a source of genetic resistance to powdery mildew among the hemp breeding germplasm. The distinct differences between the most and least susceptible cultivars suggests that there are

potential sources of qualitative resistance. Both NBS-LRR resistance genes (Goyal et al., 2020; He et al., 2018) as well as mutated *MLO* (Büschges & Hollricher, 1997; Helms Jorgensen, 1992) genes have played important roles in the management of various powdery mildew species in a variety of crop systems. The consistently extremely low levels of disease on the cultivar 'FL 58' suggest a potential source of qualitative resistance to *G. ambrosiae*, but further genetic analysis will be needed to understand the possible mechanism of resistance. 'White CBG' also stood out among all the high CBG and high CBD cultivars as being the most susceptible cultivar in these ratings. The presence of a novel susceptibility *MLO* gene in 'White CBG' could be a potential future target for mutation to confer resistance. However, additional genetic analyses are required to determine the mechanism that is conferring such extreme powdery mildew susceptibility and identification of potential breeding targets.

In addition to the extreme differences between highly susceptible and less susceptible or possible resistant cultivars, there was a large degree of variation in disease susceptibility among the other high CBD and high CBG cultivars in all the growth chamber and field trials. This variation could suggest potential sources of quantitative disease resistance (Niks et al., 2015; Poland et al., 2009; St. Clair, 2010), or microenvironments within the growth chambers and fields used in this study. While qualitative or complete resistance is often desirable for their effectiveness against a given pathogen, the deployment of these genes creates a strong selection pressure for the pathogen to overcome resistance (Milgroom, 2017). Therefore, the deployment of quantitative resistance can often have greater durability (Brown, 2015; Poland et al.,

2009; Stuthman et al., 2007). While quantitative resistance genes will not result in a disease-free crop, they can be deployed strategically alongside other disease management methods to reduce overall disease severity (Brown, 2015; Milgroom, 2017).

Susceptibility of grain and fiber hemp cultivars to *Golovinomyces ambrosiae*:

Grain, fiber and dual-purpose hemp cultivars were evaluated for their susceptibility to three separate NYS *G. ambrosiae* isolates in growth chambers. When inoculated with each of the three *G. ambrosiae* isolates, there was not as much variation in disease severity between cultivars compared to the high CBD and high CBG cultivars. This lack of extremes in phenotypes likely indicates a lack of variation in qualitative genetic host resistance to *G. ambrosiae* among the cultivars that were tested. In the first replicate, the high CBD cultivar 'TJ's CBD' had significantly greater disease severity than the rest of the cultivars tested, indicating that overall, these cultivars are less susceptible to *G. ambrosiae*. This could potentially indicate the presence of quantitative resistance genes (Niks et al., 2015; Poland et al., 2009; St. Clair, 2010) and would explain why powdery mildew was not observed in any of the grain and fiber field trials. However, this difference was not observed in the second replicate of this growth chamber experiment.

When comparing disease susceptibility in a controlled environment growth chamber and the field, cultivars that were most resistant to *G. ambrosiae* in the growth chamber

were also most resistant in the field. Cultivars such as FL-58 which were completely or nearly resistant in the field, were seen to have some lesions in the growth chamber. Overall, the variation in AUDPC values in the growth chamber was far less than in the field. Our hypothesis is that plants in the growth chamber were inoculated at a much younger age than we typically saw natural inoculum in the field. Additionally, plants in the growth chamber were only rated for 21 days, rather than over the course of the entire field season. Even with these differences, those plants that were the most susceptible in the growth chamber were generally most susceptible in the field, and this was true of host resistance as well. Additionally, major differences were not observed when inoculating with three different isolates of *G. ambrosiae* in the growth chamber, however this was not repeated in the field. Overall, this study supports the idea that screening hemp for resistance to *G. ambrosiae* in a controlled environment is appropriate for high-throughput screening of germplasm for resistance to powdery mildew. Furthermore, mapping resistance/susceptibility developing markers linked to those is a viable strategy for breeding powdery mildew resistant cultivars.

Acknowledgements:

Thank you to Ryes Creek, Castetter Sustainability Group, Sunrise Genetics, Charlotte's Web, Front Range Bioscience, Phytonyx, Industrial Seed Innovations, Boring Hemp, Stemp Holdings OR, Oregon CBD, Flura, Hydrogrow, Green Point Research. American Hemp Co., Puregene, Stem Holdings Agri., Davis Farms, Kayagene, Point 3 Farma, Phylos, Alterra Hemp, Arcadia Biosciences, Atlas Seeds, Bija Hemp, Parkland

Industrial Hemp Growers, Schiavi Seed, Hemp Genetics International, NISeeds, Assocanapa, and Legacy Hemp for providing cultivars which were included in these experiments.

Literature Cited:

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). *Basic local alignment search tool* (No. 215; pp. 403–410). Mol. Biol.

Bai, Y., Pavan, S., Zheng, Z., Zappel, N. F., Reinstädler, A., Lotti, C., de Giovanni, C., Ricciardi, L., Lindhout, P., Visser, R., Theres, K., & Panstruga, R. (2007). Naturally occurring broad-spectrum powdery mildew resistance in a Central American tomato accession Is caused by loss of *MLO* function. *Molecular Plant-Microbe Interactions*, *21*(1), 30–39. https://doi.org/10.1094/MPMI-21-1-0030

Brown, J. K. M. (2015). Durable resistance of crops to disease: A Darwinian perspective. *Annual Review of Phytopathology*, *53*, 513–539. https://doi.org/10.1146/ANNUREV-PHYTO-102313-045914

Büschges, R., & Hollricher, K. (1997). The barley *MLO* gene: A novel control element of plant pathogen resistance. *Cell*, *88*, 695–705.

Cai, X. C., Liu, C. H., Wang, B. T., & Xue, Y. R. (2017). Genomic and metabolic traits endow *Bacillus velezensis* CC09 with a potential biocontrol agent in control of wheat powdery mildew disease. *Microbiological Research*, *196*, 89–94. https://doi.org/10.1016/J.MICRES.2016.12.007

Carbone, I., & Kohn, L. M. (1999). A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia*, *91*(3), 553–556.

Cherney, J., & Small, E. (2016). Industrial hemp in North America: production, politics and potential. *Agronomy*, *6*(4), 58. https://doi.org/10.3390/agronomy6040058

Consonni, C., Humphry, M. E., Hartmann, H. A., Livaja, M., Durner, J., Westphal, L., Vogel, J., Lipka, V., Kemmerling, B., Schulze-Lefert, P., Somerville, S. C., & Panstruga, R. (2006). Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nature Genetics*, *38*(6), 716–720. https://doi.org/10.1038/ng1806

Czembor, J. H., Hodowli, I., & Roslin, A. (2016). Transfer of the *MLO* resistance gene into the genome of winter barley. *Progress in Plant Protection*, *56*(3), 379–387. https://doi.org/10.14199/ppp-2016-061

Dangl, J. L., Horvath, D. M., & Staskawicz, B. J. (2013). Pivoting the plant immune system from dissection to deployment. *Science*, *341*(6147), 746–751. https://doi.org/10.1126/SCIENCE.1236011

de Mendiburu, F. (2020). *agricolae: Statistical Procedures for Agricultural Research.* (R package version 1.3-3.).

Farinas, C., & Hand, F. P. (2020). First report of *Golovinomyces spadiceus* causing powdery mildew on industrial hemp (*Cannabis sativa*) in Ohio. *Plant Disease*, *104*(10), 2727–2727. https://doi.org/10.1094/PDIS-01-20-0198-PDN

Fike, J. (2016). Industrial hemp: renewed opportunities for an ancient crop. *Critical Reviews in Plant Sciences*, *35*(5–6), 406–424. https://doi.org/10.1080/07352689.2016.1257842

Garibaldi, A., Gilardi, G., & Gullino, M. L. (2011). Effect of potassium silicate and electrical conductivity in reducing powdery mildew of hydroponically grown tomato.

Mediterranean Phytopathological Union, *50*(2), 192–202. https://www-jstor-org.proxy.library.cornell.edu/stable/26458693?seq=1

Glawe, D. A. (2008). The Powdery Mildews: A review of the world's most familiar (yet poorly known) plant pathogens. *Annual Reviews of Phytopathology*, *46*, 27–51. https://doi.org/10.1146/annurev.phyto.46.081407.104740

Goyal, N., Bhatia, G., Sharma, S., Garewal, N., Upadhyay, A., Upadhyay, S. K., & Singh, K. (2020). Genome-wide characterization revealed role of NBS-LRR genes during powdery mildew infection in *Vitis vinifera*. *Genomics*, *112*(1), 312–322. https://doi.org/10.1016/J.YGENO.2019.02.011

Green, A. J., Berger, G., Griffey, C. A., Pitman, R., Thomason, W., & Balota, M. (2014). Genetic resistance to and effect of leaf rust and powdery mildew on yield and its components in 50 soft red winter wheat cultivars. *Crop Protection*, *64*, 177–186. https://doi.org/10.1016/J.CROPRO.2014.06.023

He, H., Zhu, S., Zhao, R., Jiang, Z., Ji, Y., Ji, J., Qiu, D., Li, H., & Bie, T. (2018). Pm21, encoding a typical CC-NBS-LRR protein, confers broad-spectrum resistance to wheat powdery mildew disease. *Molecular Plant*, *11*, 879–882. https://doi.org/10.1016/j.molp.2018.03.004

Healey, A., Furtado, A., Cooper, T., & Henry, R. J. (2014). Protocol: A simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. *Plant Methods*, *10*(1), 1–8. https://doi.org/10.1186/1746-4811-10-21/COMMENTS

Helms Jorgensen, J. (1992). Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. In *Euphytica* (Vol. 63, Issue 0). Kluwer Academic Publishers.

Humphry, M., Reinstädler, A., Ivanov, S., Bisseling, T., & Panstruga, R. (2011). Durable broad-spectrum powdery mildew resistance in pea er1 plants is conferred by natural loss-of-function mutations in PsMLO1. *Molecular Plant Pathology*, *12*(9), 866–878. https://doi.org/10.1111/J.1364-3703.2011.00718.X

Ishii, H., Fraaije, B. A., Sugiyama, T., Noguchi, K., Nishimura, K., Takeda, T., Amano, T., & Hollomon, D. W. (2007). Occurrence and molecular characterization of strobilurin resistance in cucumber powdery mildew and downy mildew. *Http://Dx.Doi.Org.Proxy.Library.Cornell.Edu/10.1094/PHYTO.2001.91.12.1166*, *91*(12), 1166–1171. https://doi.org/10.1094/PHYTO.2001.91.12.1166

Jiao, R., Munir, S., He, P., Yang, H., Wu, Y., Wang, J., He, P., Cai, Y., Wang, G., & He, Y. (2020). Biocontrol potential of the endophytic Bacillus amyloliquefaciens YN201732 against tobacco powdery mildew and its growth promotion. *Biological Control*, *143*, 104160. https://doi.org/10.1016/J.BIOCONTROL.2019.104160

Jones, J. D. G., & Dangl, J. L. (2006). The plant immune system. *Nature*, *444*(7117), 323–329. https://doi.org/10.1038/nature05286

Karaoglanidis, G. S., & Karadimos, D. A. (2006). Efficacy of strobilurins and mixtures with DMI fungicides in controlling powdery mildew in field-grown sugar beet. *Crop Protection*, *25*(9), 977–983. https://doi.org/10.1016/J.CROPRO.2006.01.005

Kusch, S., & Panstruga, R. (2017). *Mlo*-based resistance: An apparently universal "weapon" to defeat powdery mildew disease. *Molecular Plant-Microbe Interactions*, *30*(3), 179–189. https://doi.org/https://doi.org/10.1094/MPMI-12-16-0255-CR

Mihalyov, P. D., & Garfinkel, A. R. (2021). Discovery and genetic mapping of PM1, a powdery mildew resistance gene in *Cannabis sativa* L. *Frontiers in Agronomy*, *3*, 66. https://doi.org/10.3389/FAGRO.2021.720215/BIBTEX

Milgroom, M. (2017). Durability and the deployment of disease resistance. In M. G. Milgroom (Ed.), *Population Biology of Plant Pathogens: Genetics, Ecology, and Evolution* (pp. 249–273). The American Phytopathological Society. https://doi.org/10.1094/9780890544525.010

Niks, R. E., Qi, X., & Marcel, T. C. (2015). Quantitative resistance to biotrophic filamentous plant pathogens: concepts, misconceptions, and mechanisms. *Annual Review of Phytopathology*, *53*, 445–470. https://doi.org/10.1146/ANNUREV-PHYTO-080614-115928

Pépin, N., Hebert, F. O., & Joly, D. L. (2021). Genome-wide characterization of the *MLO* gene family in *Cannabis sativa* reveals two genes as strong candidates for powdery mildew susceptibility. *Frontiers in Plant Science*, *12*(729261). https://doi.org/10.3389/FPLS.2021.729261

Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C., & Nelson, R. J. (2009). Shades of gray: the world of quantitative disease resistance. *Trends in Plant Science*, *14*(1), 21–29. https://doi.org/10.1016/J.TPLANTS.2008.10.006

R Core Team. (2021). *R: A language and environment for statistical computing* (4.0.4). R Foundation for Statistical Computing.

R Studio Team. (2020). *RStudio: Integrated Development for R* (1.4.1106). RStudio, PBC.

Scott, C., & Punja, Z. K. (2020). Evaluation of disease management approaches for powdery mildew on *Cannabis sativa* L. (marijuana) plants. *Canadian Journal of Plant Pathology*, *43*(3), 394–412. https://doi.org/10.1080/07060661.2020.1836026

Shen, G. H., Xue, Q. H., Tang, M., Chen, Q., Wang, L. N., Duan, C. M., Xue, L., & Zhao, J. (2016). Inhibitory effects of potassium silicate on five soil-borne phytopathogenic fungi in vitro. *Journal of Plant Diseases and Protection*, *117*(4), 180–184. https://doi.org/10.1007/BF03356358

st. Clair, D. A. (2010). Quantitative disease resistance and quantitative resistance loci in breeding. *Annual Review of Phytopathology*, *48*, 247–268. https://doi.org/10.1146/ANNUREV-PHYTO-080508-081904

Stack, G. M., Toth, J. A., Carlson, C. H., Cala, A. R., Marrero-González, M. I., Wilk, R. L., Gentner, D. R., Crawford, J. L., Philippe, G., Rose, J. K. C., Viands, D. R., Smart, C. D., & Smart, L. B. (2021). Season-long characterization of high-cannabinoid hemp (*Cannabis sativa* L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. *GCB Bioenergy*, *13*(4), 546–561. https://doi.org/10.1111/gcbb.12793

Stuthman, D. D., Leonard, K. J., & Miller-Garvin, J. (2007). Breeding crops for durable resistance to disease. *Advances in Agronomy*, *95*, 319–367. https://doi.org/10.1016/S0065-2113(07)95004-X

Szarka, D., Tymon, L., Amsden, B., Dixon, E., Judy, J., & Gauthier, N. (2019). First report of powdery mildew caused by *Golovinomyces spadiceus* on industrial hemp (*Cannabis sativa*) in Kentucky. *Plant Disease*, *103*(7), 1773–1773. https://doi.org/10.1094/pdis-01-19-0049-pdn

Tesfagiorgis, H. B., & Annegarn, H. J. (2013). Evaluation of adjuvants for enhanced management of *Podosphaera xanthii* using potassium silicate and biocontrol agents. *Crop Protection*, *48*, 69–75. https://doi.org/10.1016/J.CROPRO.2013.03.002

Tesfagiorgis, H. B., Laing, M. D., & Annegarn, H. J. (2014). Evaluation of biocontrol agents and potassium silicate for the management of powdery mildew of zucchini. *Biological Control*, *73*, 8–15. https://doi.org/10.1016/J.BIOCONTROL.2014.03.010

Vielba-Fernández, A., Polonio, Á., Ruiz-Jiménez, L., de Vicente, A., Pérez-García, A., & Fernández-Ortuño, D. (2020). Fungicide resistance in powdery mildew fungi. *Microorganisms*, *8*(9), 1431. https://doi.org/10.3390/MICROORGANISMS8091431

Weldon, W. A., Knaus, B. J., Grünwald, N. J., Havill, J. S., Block, M. H., Gent, D. H., Cadle-Davidson, L. E., & Gadoury, D. M. (2021). Transcriptome-derived amplicon sequencing markers elucidate the U.S. *Podosphaera macularis* population structure across feral and commercial plantings of *Humulus lupulus*. *Phytopathology*, *111*(1), 194–203. https://doi.org/https://doi.org/10.1094/PHYTO-07-20-0299-FI

Weldon, W. A., Ullrich, M. R., Smart, L. B., Smart, C. D., & Gadoury, D. M. (2019). Cross infectivity of powdery mildew isolates originating from hemp (*Cannabis sativa*) and Japanese hop (*Humulus japonicus*) in New York. *Plant Health Progress*, *21*(1), 47–53. https://doi.org/https://doi.org/10.1094/PHP-09-19-0067-RS

Wicks, T. J., & Hitch, C. J. (2002). Integration of strobilurins and other fungicides for the control of powdery mildew on grapes. *Australian Journal of Grape and Wine Research, 8*(2), 132–139. https://doi.org/10.1111/J.1755-0238.2002.TB00222.X

CHAPTER V

CHARACTERIZATION OF A HEMP BREEDING POPULATION TO IDENTIFY GENETIC HOST RESISTANCE TO POWDERY MILDEW CAUSED BY *GOLOVINOMYCES AMBROSIAE*

Abstract:

Genetic host resistance is a highly desirable breeding trait and can be an important disease management tool. As hemp (*Cannabis sativa* L.) continues to grow as an area of interest for consumers, growers, and researchers in the United States, so does the interest in developing disease-resistant hemp cultivars. In recent years, with the development of the hemp market in the United States, powdery mildew, caused by *Golovinomyces ambrosiae* has emerged as an important disease impacting hemp production in greenhouse and field settings. This study aims to identify and characterize possible sources of genetic host resistance to powdery mildew through field ratings and subsequent evaluation of a mapping population resulting from a cross between powdery mildew susceptible and powdery mildew resistant parent cultivars. Through the initial field ratings in this study, the powdery mildew-resistant cultivar 'FL 58' was identified and crossed with the susceptible cultivar 'TJ's CBD' to generate an F_1 population. From that F_1 population, susceptible and resistant accessions were identified through growth chamber inoculations and field screenings, which were self-pollinated to create an F_2 population. From the evaluation of the F_2 population in the field, I found that approximately one quarter of each mapping family were resistant to powdery mildew,

indicating a possible sources of recessive qualitative host resistance. Through preliminary genetic analysis, a candidate *MLO* gene was identified and there are ongoing QTL analyses to confirm this result.

*Portions of this chapter were published in: Stack, G. M., Toth, J. A., Carlson, C. H., Cala, A. R., Marrero-González, M. I., Wilk, R. L., Gentner, D. R., Crawford, J. L., Philippe, G., Rose, J. K. C., Viands, D. R., Smart, C. D., & Smart, L. B. (2021). Season-long characterization of high-cannabinoid hemp (Cannabis sativa L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. GCB Bioenergy, 13(4), 546–561. https://doi.org/10.1111/gcbb.12793

Introduction:

Genetic host resistance can be an incredibly powerful disease management approach. Successful deployment of qualitative R gene resistance against powdery mildew has made significant impacts on yield in other systems (Green et al., 2014). Nucleotide-binding site leucine rich repeat (NBS-LRR) proteins are a family of proteins that are often responsible for qualitative disease resistance. NBS-LRR proteins are responsible for the recognition, either directly or indirectly (Lee & Yeom, 2015; Van Der Hoorn & Kamoun, 2008), of pathogen effectors, subsequently initiating effector-triggered immunity (ETI). ETI results in a hypersensitive response, a programmed cell death which limits the pathogen (Goyal et al., 2020; He et al., 2018) spread to healthy tissue. This form of qualitative resistance can cause a high amount of selection pressure for

pathogen races that are able to overcome resistance, thereby potentially reducing their long-term durability (Brown, 2015; Dangl et al., 2013; Milgroom, 2017).

Mildew Locus O (*MLO*) genes are another source of qualitative resistance that work specifically against powdery mildews. Functional *MLO* genes are susceptibility genes that play an important role in establishing pathogen-host interactions that confer resistance to powdery mildew through loss-of-function mutations. Silencing of these genes through various methods have resulted in powdery mildew resistance phenotypes (Bai et al., 2007; Helms Jorgensen, 1992; Humphry et al., 2011; Niks et al., 2015). *MLO* is also a gene family which has diversified into seven clades (I through VII) all of which encode for transmembrane proteins (Devoto et al., 1999) and are present across angiosperm genomes (Acevedo-Garcia et al., 2017; Kusch et al., 2016). The *MLO* clades IV and V encode for proteins that are responsible for powdery mildew susceptibility in monocots and dicots, respectively (Acevedo-Garcia et al., 2017; Kusch et al., 2016) and their functionality has been demonstrated previously(Bai et al., 2007; Freialdenhoven et al., 1996; Piffanelli et al., 2002; Zheng et al., 2013). However, several clade IV *MLO* genes have been demonstrated to play a role in the establishment of beneficial relationships with mycorrhizal fungi (Hilbert et al., 2020; Jacott et al., 2020) suggesting the exploitation of these genes by powdery mildew species (Jacott et al., 2020; Pépin et al., 2021).

Powdery mildew resistance conferred by loss-of-function *MLO* susceptibility genes seems to be broad spectrum against their respective powdery mildew species. Therefore, this source of genetic resistance is not race-specific and could possibly result in long-term durability (Bai et al., 2007; Humphry et al., 2011; Stuthman et al., 2007), making this a desirable target for resistance breeding.

Like many areas of hemp research, currently there is little known about sources of genetic host resistance to powdery mildew. Thus far, two candidate *MLO* genes have been identified and characterized in silico in one *C. sativa* accession (Pépin et al., 2021), though the functionality of those candidate genes related to powdery mildew infection in vivo have not yet been characterized. Additionally, an NBS-LRR gene (PM1) has been identified in a *C. sativa* cultivar with observed relative powdery mildew resistance through linkage mapping (Mihalyov & Garfinkel, 2021).

This study aims to use available hemp germplasm to identify sources for genetic resistance to hemp powdery mildew caused by *G. ambrosiae*. Through replicated field trial ratings of powdery mildew disease severity, one resistant cultivar ('FL 58') and one susceptible cultivar ('TJ's CBD') were identified and selected to be crossed, to create a mapping population. The resulting F_1 and F_2 populations were evaluated through growth chamber inoculations and field ratings. Approximately 25% of the F_2 population individuals were resistant individuals, indicating a source of qualitative resistance. The intermediate phenotypes observed in the remaining F_2 individuals could

indicate potential sources of quantitative resistance as well. Preliminary results indicate the presence of an *MLO* gene associated with the observed resistance in this population.

Materials and Methods:

Powdery mildew severity of high CBD hemp cultivars in 2019 field trials:

Two research field sites, in Geneva and Ithaca NY, were used to evaluate the *G.* a*mbrosiae* susceptibility of high-CBD hemp cultivars and accessions. Methods for these trials are described in (Stack et al., 2021). At the end of the growing season, all the plants at both sites were visually rated for severity of powdery mildew infection based on a continuous scale of 0–100% leaf area showing disease symptoms. For each field site, ratings for the plants within each plot were averaged, and an unbalanced, one-way ANOVA test was used to determine whether there was a significant effect of cultivar on percent coverage in powdery mildew. The data for the Ithaca site were log (% + 1) transformed to ensure the normality of the residuals. When the effect of cultivar was found to be significant, a post-hoc Tukey's HSD analysis was used to test pairwise differences between cultivars.

F_1 population

From the powdery mildew disease susceptibility ratings that were done in the summer of 2019, powdery mildew-resistant cultivar, ‘FL 58’, and a powdery mildew-susceptible cultivar, ‘TJ’s CBD’ were selected to be parents in a cross to generate a population to be used to map the resistance observed in ‘FL 58’. These two parents were crossed, and

the resulting seeds were planted to generate an F_1 population. Because both parents were clonally propagated female plants, 'TJ's CBD' plants were sprayed with silver thiosulfate to produce pollen (Lubell & Brand, 2018; Mohan Ram & Sett, 1982). Pollen producing 'TJ's CBD' plants were placed in an enclosed greenhouse with 'FL 58' plants (all female) and fans provided wind that dispersed the pollen from 'TJ's CBD' to 'FL 58' for seed production. The resulting F_1 population seeds from this cross were planted in Lambert's LM-1 Germination Mix and grown in a greenhouse with day temperatures of 24 °C days and 21 °C nights, and a 16-hour photoperiod. Selections from this F_1 population were clonally propagated for further screenings.

Six clonally propagated plants of each F_1 selection were transplanted into four-inch pots, moved to a growth chamber and arranged in a randomized complete block design. A liquid inoculum of the *G. ambrosiae* isolate 19002 was made by washing infected hemp leaves in a solution of 1 L of distilled water and 100 µL of Tween20. Plants were spray-inoculated to runoff with a conidial suspension of 2×10^5 spores per milliliter and evaluated for disease severity at 7-, 12-, 14-, 19-, 21- and 25-days post inoculation. Statistical analyses were performed using R version 4.0.4 (R Core Team, 2021) in RStudio 1.4.1106 (R Studio Team, 2020). Specifically, RStudio was used to calculate mean area under the disease progress curve (AUDPC) for each F_1 selection using the R package agricolae (de Mendiburu, 2020) and post-hoc compared by performing a Tukey's Honest Significant Difference (HSD) test ($p<0.05$) using the HSD.test function in the agricolae package (de Mendiburu, 2020).

Four clonal ramets of each F_1 selections were also planted in plots, with only one plot per accession, in a field trial in the summer of 2020. Plants became naturally infected by the end of the growing season and individuals were rated for powdery mildew disease severity three times, once a week, starting after symptoms were visible. A mean AUDPC was calculated for each plot using the R version 4.0.4 (R Core Team, 2021) package agricolae (de Mendiburu, 2020) in Rstudio 1.4.1106 (R Studio Team, 2020) for each plot. Because there were no replications of the plots in the field, statistical analysis could not be done to compare disease severity of the accessions planted in the field.

F_2 population evaluation:

Based on the ratings in both the growth chamber and the field, the F_1 selections GVA-H-19-1166-002 and GVA-H-19-1166-005 were selected to be crossed as the most susceptible and least susceptible among available F_1 pregeny, respectively. These two selections were self-pollinated, and the resulting seeds were planted in Lambert's LM-1 Germination Mix and grown in a greenhouse with conditions the same as described above in the summer of 2021, producing seeds of two F_2 populations. The family produced by selfing GVA-H-19-1166-002 was designated as the GVA-H-21-1004, and the F_2 family from the self of GVA-H-19-1166-005 was designated as the GVA-H-21-1005. Seed of GVA-H-21-1004 (21-1004 for shorthand) were planted to produce 378 individuals and seeds of the GVA-H-21-1005 (21-1005 for shorthand) family resulted in 305 individuals. These were transplanted into a field trial on July 6, 2022 on the Gates

West Farm in Geneva, NY. The original parents ‘FL 58’ and ‘TJ’s CBD’ were clonally propagated in the same greenhouse environment and transplanted randomly throughout the field among the F_2 progeny.

All plants in this experiment were inoculated with the NY *G. ambrosiae* isolate, 19002 (Weldon et al., 2019) on August 16, 2022. A liquid inoculum was made by washing infected hemp leaves with a solution of 100 µL of Tween20 per 1 L of water. A liquid conidial suspension at a concentration of 9×10^3 spores per milliliter was sprayed onto each of the plants until runoff with a backpack sprayer.

Individual plants were evaluated for their susceptibility to *G. ambrosiae* and rated for powdery mildew severity at 16-, 23-, 31-, 39-, and 44-days post-inoculation (dpi). Using R version 4.0.4 (R Core Team, 2021) in RStudio 1.4.1106v (R Studio Team, 2020), the AUDPC were calculated for each individual using the R package agricolae (de Mendiburu, 2020).

Results:

Susceptibility of high CBD cultivars to* G. ambrosiae *in 2019 field variety trials:

There was a wide distribution in the severity of powdery mildew infection by cultivar at the two sites, although there was more disease in the Geneva trial than the Ithaca trial (Figure 5.1). For some cultivars, disease severity by cultivar varied by site: 'NY Cherry' had no signs of powdery mildew in the Ithaca trial, but nearly 20% mean leaf area with powdery mildew in the Geneva trial. Despite the abundance of powdery mildew disease

in both trials, especially in the Geneva trial, no powdery mildew lesions were observed on any of the 'FL 58' plants (Figure 5.1). Cultivar ‘TJ’s CBD’ was susceptible in both locations with about 40% of the leaf area covered in powdery mildew in the Geneva trial (Figure 5.1).

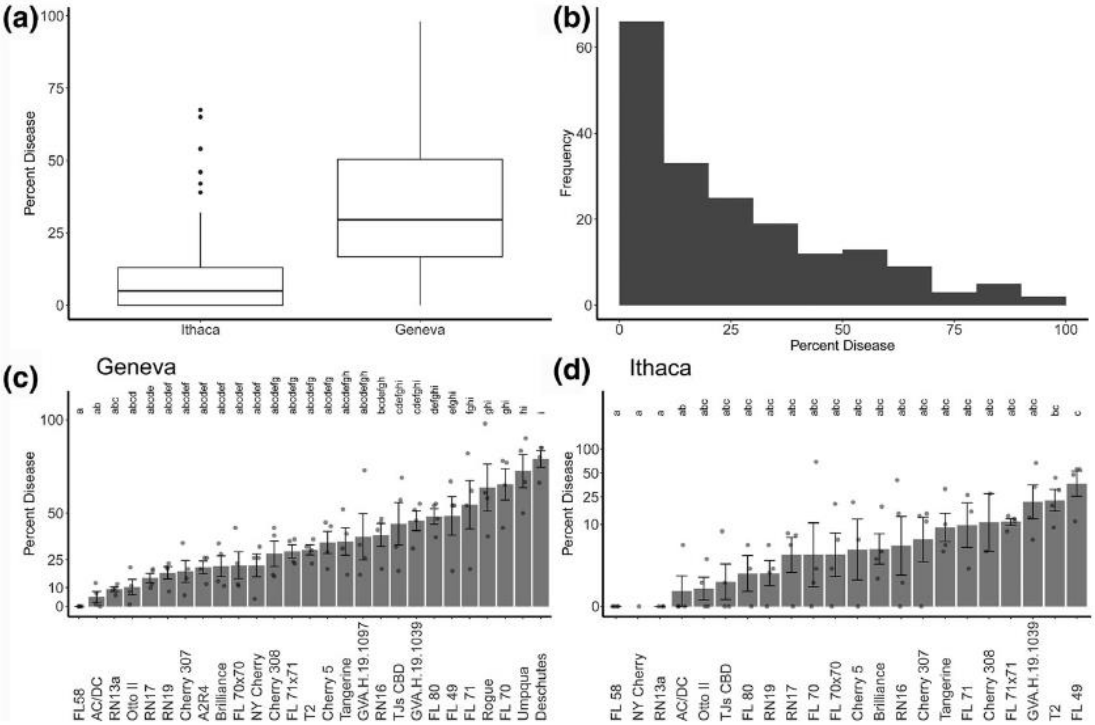

Figure 5.1. Visual ratings of percent leaf area in each plot with powdery mildew (b) at the end of September for two field sites: Geneva, NY (a and c) and Ithaca, NY (a and d). Data were log (%+1) transformed for the analysis of the Ithaca trial and plotted on a log scale (d). Letters indicate statistically significant differences between cultivars based

on a post-hoc Tukey's HSD test. Y-axis breaks correspond to 0%, 10%, 25%, 50%, and 100% coverage for panels (c) and (d). Reprinted from Stack et al. (2021).

F_1 population:

The hemp cultivars 'FL 58' and 'TJ's CBD were selected to be crossed to create a powdery mildew resistance mapping population. Progeny in the F_1 population resulting from this cross were evaluated for their susceptibility to *G. ambrosiae* in both a growth chamber and field trial. Of the individuals screened in the growth chamber, the F_1 individual GVA-H-19-1166-005 was the least susceptible to *G. ambrosia* and individual GVA-H-19-1166-002 was among the most susceptible (Figure 5.2A). Additionally, GVA-H-19-1166-002 was the first accession to show powdery mildew symptoms.

The same progeny individuals that were evaluated in the growth chamber and some additional F_1 progeny individuals were evaluated in a field setting. While the plots of these F_1 progeny were not replicated and therefore statistical analysis was not possible, the F_1 progeny GVA-H-19-1166-005 numerically had the lowest level of observed powdery mildew based on AUDPC (Figure 5.2B). The F_1 individual GVA-H-1166-002 was among the progeny with greatest overall powdery mildew severity rating (Figure 5.2B).

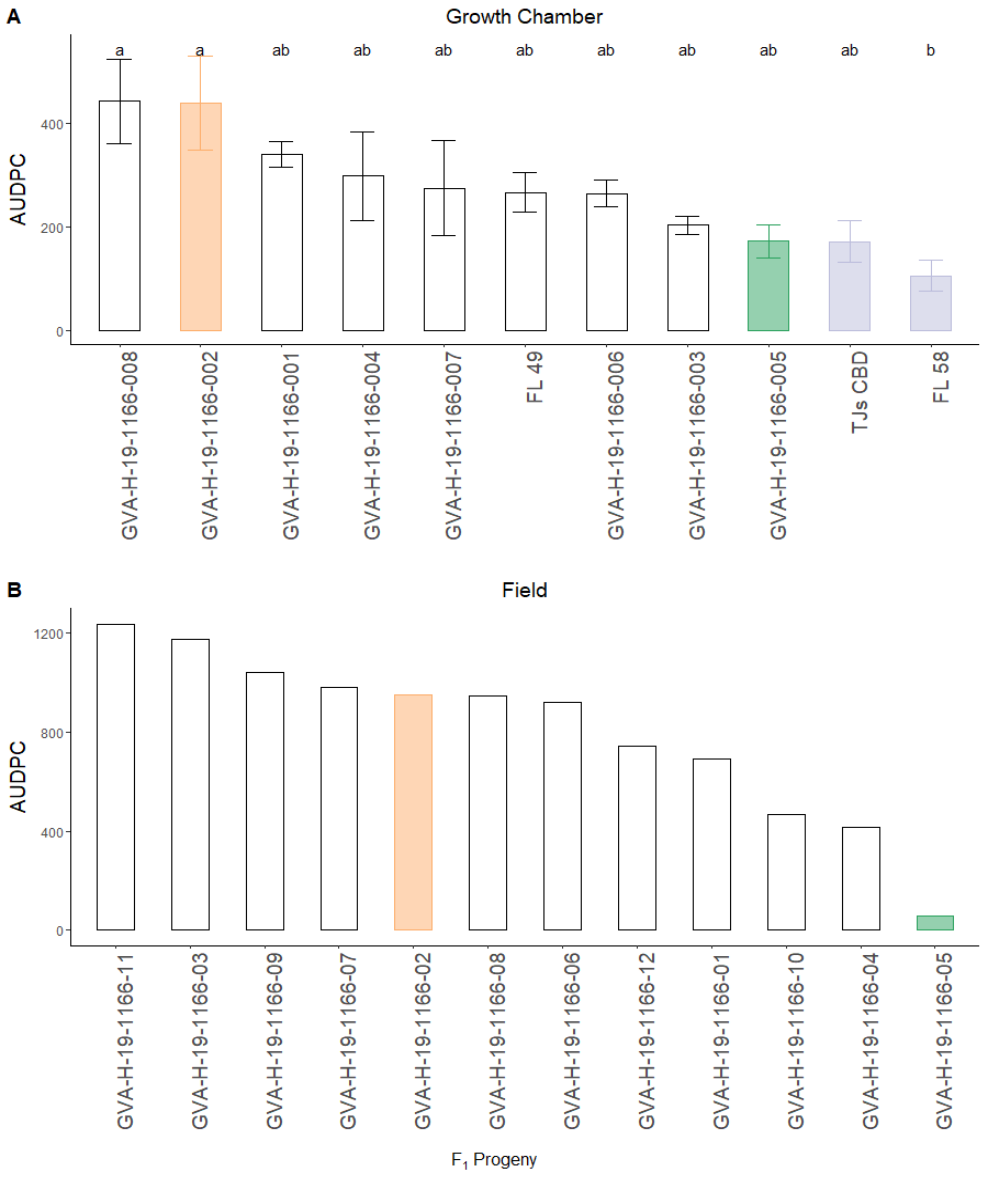

Figure 5.2. Powdery mildew severity ratings of F_1 progeny generated from a cross between a powdery mildew resistant cultivar ('FL 58') and a powdery mildew susceptible cultivar ('TJ's CBD'). Disease severity was evaluated by calculating the mean area under the disease progress curve (AUDPC) in a growth chamber environment inoculated with isolate *G. ambrosiae* 19002 (A), and in field trial plots with natural inoculum (B). Letters indicate Tukey's HSD groups ($p>0.05$).

Evaluation of *G. ambrosiae* susceptibility of F_2 mapping population:

The F_1 progeny GVA-H-19-1166-005 and GVA-H-19-1166-002 were self-pollinated and the resulting F_2 populations were evaluated in a field trial. The parents from the original cross, 'FL 58' and 'TJ's CBD' were also included and evaluated in this field trial. The AUDPC were calculated to determine the powdery mildew disease severity of each individual planted in the field. All 11 'FL 58' clonal ramets that were evaluated had a disease rating of zero in the field (Figure 5.3A), while all the 'TJ's CBD' clonal ramets were infected with powdery mildew, with varying levels of disease severity (Figure 5.3B). One quarter of the individuals (25.57%) in the 21-1005 family had a disease severity rating of zero (Figure 5.3C). The same was true for approximately one quarter (26.19%) in the 21-1004 family (Figure 5.3D). Powdery mildew severity varied considerably among the remaining individuals in both F_2 families (Figure 5.3CD).

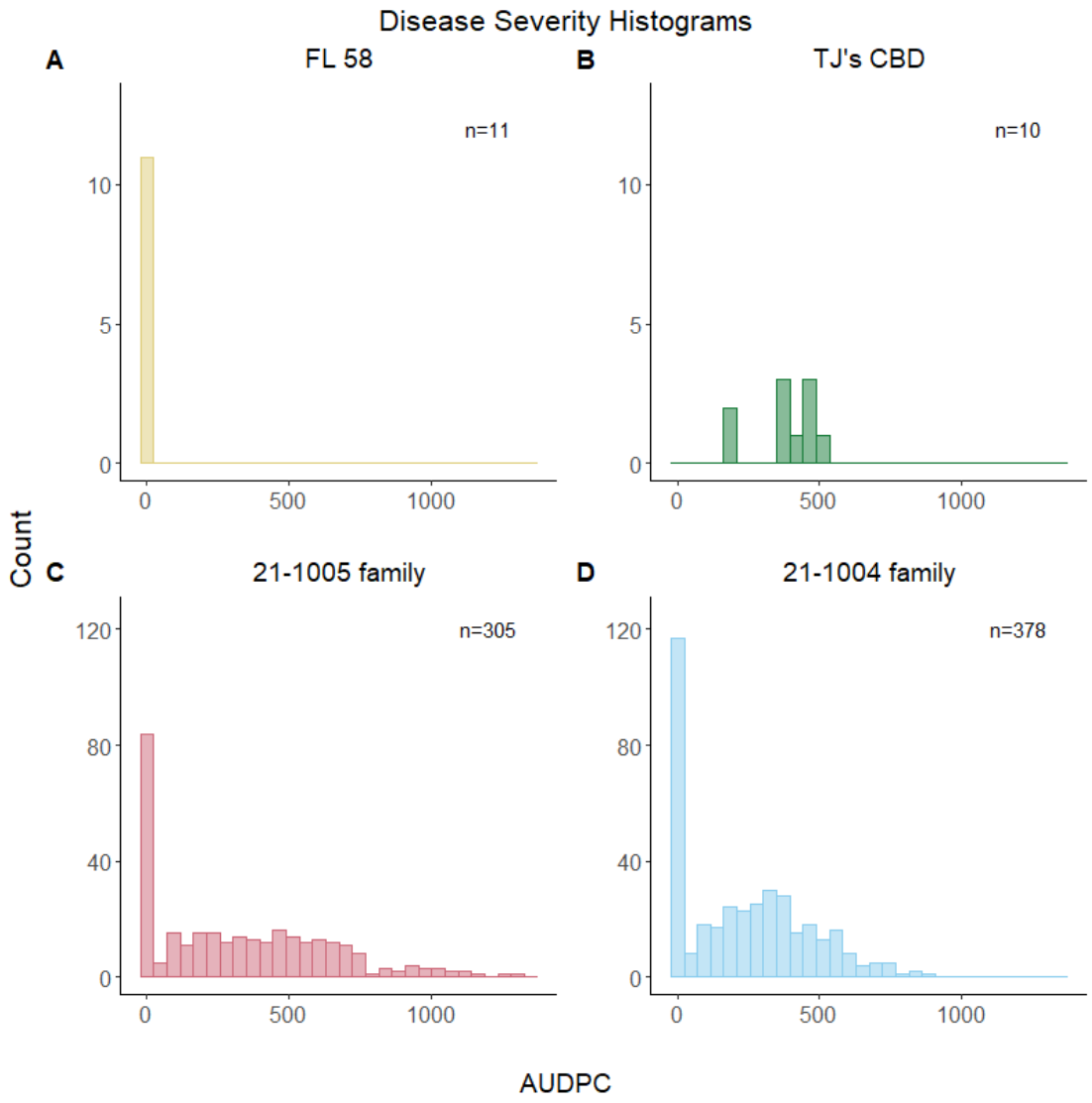

Figure 5.3. Histograms of powdery mildew severity on individuals in an F_2 mapping population. (A) clonal ramets of the powdery mildew resistant cultivar ('FL58') parent (B) clonal ramets of the powdery mildew susceptible cultivar ('TJ's CBD') parent (C) F_2 individuals in the 21-1005 family (D) F_2 individuals in the 21-1004 family. Disease severity was evaluated by calculating the area under the disease progress curve for each individual in the population (x-axes) with less disease on the left (AUDPC of 0) and more disease on the right-hand side of each graph.

Discussion:

The *G. ambrosiae* susceptible hemp cultivar 'TJ's CBD' and the resistant hemp cultivar 'FL 58' were crossed to produce an F_1 population. The resulting F_1 progeny were evaluated for their susceptibility to *G. ambrosiae* in a growth chamber and field trial in the summer of 2020. Two F_1 progeny were selected, one powdery mildew-susceptible (GVA-H-19-1166-002) and one less susceptible accession (GVA-H-1166-005) and self-pollinated to produce two F_2 mapping populations.

The individuals of each of the F_2 populations were evaluated for susceptibility to *G. ambrosiae* in the field in 2021. One quarter of the individuals in both of the F_2 families were resistant to *G. ambrosiae*, with powdery mildew disease ratings of zero. This proportion of individuals rated as resistant suggests a qualitative mechanism of genetic resistance to *G. ambrosiae*. Nucleotide-binding site, leucine rich repeat (NBS-LRR) genes are common sources of dominantly inherited pathogen resistance. NBS-LRR R-genes function through the recognition of pathogen effectors and subsequent induction of effector triggered immunity (ETI). To date, one NB-LRR gene conferring resistance to *G. ambrosiae* has been identified in one hemp cultivar (Mihalyov & Garfinkel, 2021). *MLO* genes confer susceptibility to various powdery mildew species. When mutated, nonfunctional forms of these genes result in broad-spectrum resistance to powdery mildew can (Bai et al., 2007; Freialdenhoven et al., 1996; He et al., 2018; Humphry et al., 2011; Piffanelli et al., 2002). This form of resistance has been documented, characterized, and deployed in several systems.

Of the individuals in both F_2 families with a non-zero rating, there was a broad, continuous distribution of disease severity ratings. This range of susceptibility could indicate additional quantitative resistance mechanisms underlying qualitative resistance to in susceptibility to *G. ambrosiae* (Poland et al., 2009; St. Clair, 2010). With the available phenotypic and genetic data from this mapping population, further QTL analysis may be possible to identify quantitative resistance loci which are related to the quantitative disease resistance observed in this population. From this, DNA-based markers may be developed to be in used for marker assisted selection in future breeding efforts.

Ongoing and future work:

Tissue samples from each F_2 individual planted in the field trial were collected during the field season. DNA was extracted from each tissue sample and genotyped using an Illumina SNP array. Currently, in collaboration with the hemp breeding program, a QTL analysis is being performed with the goal of mapping regions of the genome that are associated with susceptibility to *G. ambrosiae*. Based on initial genotyping results, powdery mildew severity mapped to a single, major effect locus with the most significant SNP on chromosome 1 near *CsMLO1*, a gene previously identified through in silico analysis of the CBDRx-CS10 genome for its potential role in powdery mildew susceptibility (Pépin et al., 2021). The initial data support the hypothesis that 'FL 58' may have a recessive, mutated MLO susceptibility gene that leads to resistance in

homozygous genotypes. Further QTL analysis that includes genotype data from all individuals of both F_2 families is underway to confirm this hypothesis.

Growth chamber inoculations of individuals in conjunction with quantitative reverse transcription polymerase chain reaction (q-RTPCR) analyses are also currently ongoing to determine if there are modifications in gene expression of the candidate *MLO* gene following inoculation with *G. ambrosiae*. If it is determined that a mutated *MLO* gene is responsible for the qualitative resistance to powdery mildew that is observed in the hemp cultivars 'FL 58,' this will be an important step in understanding host-pathogen interactions between *G. ambrosiae* and *C. sativa*, and eventually breeding powdery mildew resistant *C. sativa*.

Literature Cited:

Acevedo-Garcia, J., Spencer, D., Thieron, H., Reinstädler, A., Hammond-Kosack, K., Phillips, A. L., & Panstruga, R. (2017). *MLO*-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach. *Plant Biotechnology Journal*, *15*(3), 367–378. https://doi.org/10.1111/pbi.12631

Bai, Y., Pavan, S., Zheng, Z., Zappel, N. F., Reinstädler, A., Lotti, C., de Giovanni, C., Ricciardi, L., Lindhout, P., Visser, R., Theres, K., & Panstruga, R. (2007). Naturally occurring broad-spectrum powdery mildew resistance in a Central American tomato accession Is caused by loss of *MLO* function. *Molecular Plant-Microbe Interactions*, *21*(1), 30–39. https://doi.org/10.1094/MPMI-21-1-0030

Brown, J. K. M. (2015). Durable resistance of crops to disease: A Darwinian perspective. *Annual Review of Phytopathology*, *53*, 513–539. https://doi.org/10.1146/ANNUREV-PHYTO-102313-045914

Dangl, J. L., Horvath, D. M., & Staskawicz, B. J. (2013). Pivoting the plant immune system from dissection to deployment. *Science*, *341*(6147), 746–751. https://doi.org/10.1126/SCIENCE.1236011

de Mendiburu, F. (2020). *agricolae: Statistical Procedures for Agricultural Research.* (R package version 1.3-3.).

Devoto, A., Piffanelli, P., Nilsson, I. M., Wallin, E., Panstruga, R., von Heijne, G., & Schulze-Lefert, P. (1999). Topology, subcellular localization, and sequence diversity of the *MLO* family in plants. *Journal of Biological Chemistry*, *274*(49), 34993–35004. https://doi.org/10.1074/jbc.274.49.34993

Freialdenhoven, A., Peterhansel, C., Kurth, J., Kreuzaler, F., & Schulze-Lefert, P. (1996). Identification of genes required for the function of non-race-specific *MLO* resistance to powdery mildew in barley. *The Plant Cell*, *8*(1), 5–14. https://doi.org/10.1105/TPC.8.1.5

Goyal, N., Bhatia, G., Sharma, S., Garewal, N., Upadhyay, A., Upadhyay, S. K., & Singh, K. (2020). Genome-wide characterization revealed role of NBS-LRR genes during powdery mildew infection in *Vitis vinifera*. *Genomics*, *112*(1), 312–322. https://doi.org/10.1016/J.YGENO.2019.02.011

Green, A. J., Berger, G., Griffey, C. A., Pitman, R., Thomason, W., & Balota, M. (2014). Genetic resistance to and effect of leaf rust and powdery mildew on yield and its components in 50 soft red winter wheat cultivars. *Crop Protection*, *64*, 177–186. https://doi.org/10.1016/J.CROPRO.2014.06.023

He, H., Zhu, S., Zhao, R., Jiang, Z., Ji, Y., Ji, J., Qiu, D., Li, H., & Bie, T. (2018). Pm21, encoding a typical CC-NBS-LRR protein, confers broad-spectrum resistance to wheat powdery mildew disease. *Molecular Plant*, *June*, 879–882. https://doi.org/10.1016/j.molp.2018.03.004

Helms Jorgensen, J. (1992). Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. In *Euphytica* (Vol. 63, Issue 0). Kluwer Academic Publishers.

Hilbert, M., Novero, M., Rovenich, H., Mari, S., Grimm, C., Bonfante, P., & Zuccaro, A. (2020). *MLO* differentially regulates barley root colonization by beneficial endophytic

and mycorrhizal fungi. *Frontiers in Plant Science*, *10*, 1678. https://doi.org/10.3389/FPLS.2019.01678/BIBTEX

Humphry, M., Reinstädler, A., Ivanov, S., Bisseling, T., & Panstruga, R. (2011). Durable broad-spectrum powdery mildew resistance in pea er1 plants is conferred by natural loss-of-function mutations in *PsMLO1*. *Molecular Plant Pathology*, *12*(9), 866–878. https://doi.org/10.1111/J.1364-3703.2011.00718.X

Jacott, C. N., Charpentier, M., Murray, J. D., & Ridout, C. J. (2020). Mildew Locus O facilitates colonization by arbuscular mycorrhizal fungi in angiosperms. *New Phytologist*, *227*(2), 343–351. https://doi.org/10.1111/NPH.16465

Kusch, S., Pesch, L., & Panstruga, R. (2016). Comprehensive phylogenetic analysis sheds light on the diversity and origin of the *MLO* family of integral membrane proteins. *Genome Biology and Evolution*, *8*(3), 878–895. https://doi.org/10.1093/gbe/evw036

Lee, H. A., & Yeom, S. I. (2015). Plant NB-LRR proteins: tightly regulated sensors in a complex manner. *Briefings in Functional Genomics*, *14*(4), 233–242. https://doi.org/10.1093/BFGP/ELV012

Lubell, J. D., & Brand, M. H. (2018). Foliar sprays of silver thiosulfate produce male flowers on female hemp plants. *HortTechnology*, *28*(6), 743–747. https://doi.org/10.21273/HORTTECH04188-18

Mihalyov, P. D., & Garfinkel, A. R. (2021). Discovery and genetic mapping of PM1, a powdery mildew resistance gene in *Cannabis sativa* L. *Frontiers in Agronomy*, *3*, 66. https://doi.org/10.3389/FAGRO.2021.720215/BIBTEX

Milgroom, M. (2017). Durability and the deployment of disease resistance. In M. G. Milgroom (Ed.), *Population Biology of Plant Pathogens: Genetics, Ecology, and Evolution* (pp. 249–273). The American Phytopathological Society. https://doi.org/10.1094/9780890544525.010

Mohan Ram, H. Y., & Sett, R. (1982). Induction of fertile male flowers in genetically female *Cannabis sativa* plants by silver nitrate and silver thiosulphate anionic complex. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *62*(4), 369–375. https://doi.org/10.1007/BF00275107

Niks, R. E., Qi, X., & Marcel, T. C. (2015). Quantitative resistance to biotrophic filamentous plant pathogens: concepts, misconceptions, and mechanisms. *Annual Review of Phytopathology*, *53*, 445–470. https://doi.org/10.1146/ANNUREV-PHYTO-080614-115928

Pépin, N., Hebert, F. O., & Joly, D. L. (2021). Genome-wide characterization of the *MLO* gene family in *Cannabis sativa* reveals two genes as strong candidates for powdery mildew susceptibility. *Frontiers in Plant Science*, *12*(729261). https://doi.org/10.3389/FPLS.2021.729261

Piffanelli, P., Zhou, F., Casais, C., Orme, J., Jarosch, B., Schaffrath, U., Collins, N. C., Panstruga, R., & Schulze-Lefert, P. (2002). The barley *MLO* modulator of defense and cell death Is responsive to biotic and abiotic stress stimuli. *Plant Physiology*, *129*(3), 1076–1085. https://doi.org/10.1104/PP.010954

Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C., & Nelson, R. J. (2009). Shades of gray: the world of quantitative disease resistance. *Trends in Plant Science*, *14*(1), 21–29. https://doi.org/10.1016/J.TPLANTS.2008.10.006

R Core Team. (2021). *R: A language and environment for statistical computing* (4.0.4). R Foundation for Statistical Computing.

R Studio Team. (2020). *RStudio: Integrated Development for R* (1.4.1106). RStudio, PBC.

st. Clair, D. A. (2010). Quantitative disease resistance and quantitative resistance loci in breeding. *Annual Review of Phytopathology*, *48*, 247–268. https://doi.org/10.1146/ANNUREV-PHYTO-080508-081904

Stack, G. M., Toth, J. A., Carlson, C. H., Cala, A. R., Marrero-González, M. I., Wilk, R. L., Gentner, D. R., Crawford, J. L., Philippe, G., Rose, J. K. C., Viands, D. R., Smart, C. D., & Smart, L. B. (2021). Season-long characterization of high-cannabinoid hemp (*Cannabis sativa* L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. *GCB Bioenergy*, *13*(4), 546–561. https://doi.org/10.1111/gcbb.12793

Stuthman, D. D., Leonard, K. J., & Miller-Garvin, J. (2007). Breeding crops for durable resistance to disease. *Advances in Agronomy*, *95*, 319–367. https://doi.org/10.1016/S0065-2113(07)95004-X

Van Der Hoorn, R. A. L., & Kamoun, S. (2008). From guard to decoy: A new model for perception of plant pathogen effectors. *The Plant Cell*, *20*(8), 2009. https://doi.org/10.1105/TPC.108.060194

Weldon, W. A., Ullrich, M. R., Smart, L. B., Smart, C. D., & Gadoury, D. M. (2019). Cross infectivity of powdery mildew isolates originating from hemp (*Cannabis sativa*) and Japanese hop (*Humulus japonicus*) in New York. *Plant Health Progress*, *21*(1), 47–53. https://doi.org/https://doi.org/10.1094/PHP-09-19-0067-RS

Zheng, Z., Nonomura, T., Appiano, M., Pavan, S., Matsuda, Y., Toyoda, H., Wolters, A. M. A., Visser, R. G. F., & Bai, Y. (2013). Loss of function in *MLO* orthologs reduces susceptibility of pepper and tomato to powdery mildew disease caused by *Leveillula taurica*. *PLOS ONE*, *8*(7), e70723. https://doi.org/10.1371/JOURNAL.PONE.0070723

CHAPTER VI

CONCLUSIONS

The goal of this research was to understand various aspects of *Golovinomyces ambrosiae,* the causal agent of hemp powdery mildew, in order to of better understand the pathogen and potential disease management strategies.

In chapter two, I explored the host range of *G. ambrosiae* through both controlled growth chamber inoculations and field studies of various potential hosts. Through the growth chamber inoculations, sunflowers (*Helianthus annuus*), okra (*Abelmoschus esculentus*), zinnia (*Zinnia spp.*), and some cucurbits (*Cucurbita pepo*) were identified as hosts of a *G. ambrosiae* isolate that was originally isolated from hemp. In the field studies, the number of cultivars of each of these crops screened was expanded. Through this, I determined that cultivars varied significantly in their susceptibility to *G. ambrosiae,* with one cultivar of zinnia appearing to have some resistance. Through genotyping of powdery mildew isolates collected in the field however, I determined that the primary powdery mildew species that was affecting the cucurbits was *Podosphaera xanthii,* the causal agent of cucurbit powdery mildew. While I am confident that *C. pepo* is a host of *G. ambrosiae,* based on growth chamber inoculations, it seems likely that *P. xanthii* can out-compete *G. ambrosiae* in a field setting when both are present. In this chapter, I was also able to demonstrate that *G. ambrosiae* isolates from both okra and sunflower were able to infect the hemp cultivar 'White CBG." The results of this study confirm the reports of *G. ambrosiae* causing powdery mildew infection on a

variety of crops (Félix-Gastélum et al., 2019; Moparthi, Bradshaw, & Grove, 2018; Moparthi, Bradshaw, Frost, et al., 2018) and indicate that *G. ambrosiae* has a broader host range than most powdery mildew species. Hopefully, this information will allow those growing hemp, and other *G. ambrosiae*-susceptible crop hosts, to make informed decisions about crop rotation and planting locations. My research was limited in the number of crops and cultivars that were able to be screened, so there is room for future work to continue screening related species that could be potential hosts of *G. ambrosiae*. The disease ratings in the field study also showed variability in susceptibility among crops and crop cultivars, indicating a possible source of genetic host resistance in those crops, which could be further investigated.

In chapter 3, I tested the efficiency of several fungicides against hemp powdery mildew and gray mold. Through field trials over multiple years, I was able to identify multiple OMRI-listed products which were effective in reducing overall powdery mildew disease severity. The conventional fungicide, azoxystrobin, which I used as a positive control due to its efficacy against other powdery mildew species (Li-hua et al., 2009) was also effective against powdery mildew, but is not currently labelled for hemp use in New York State. In contrast, none of the treatments used to treat *B. cinerea* were effective in reducing disease severity. While more fungicide treatment options for those growing hemp continues to become available, their continues to be a lack of literature demonstrating the efficacy of products against hemp diseases. Therefore, the results of my work will allow growers to know that there are some effective treatments available

to them for powdery mildew mitigation. On the other hand, knowing that these treatments are not effective for *Botrytis* management will still help a grower make a more informed spraying decision. In this chapter I also tested whether fungicide treatment or pathogen infection would impact hemp cannabinoid production and found that overall, fungicide treatment did not impact cannabinoid production. I also determined that while *G. ambrosiae* infection did not impact cannabinoid production, there may be an impact of *B. cinerea* infection on concentrations of some minor cannabinoids. There is still more research to be done in this area, to expand efficacy trials to include several other fungicides to treat both hemp powdery mildew and *B. cinerea* on hemp. Further, spray timing, differences in spray frequency or application rates were not evaluated for any of the products in this study. Additionally, the extent to which *B. cinerea* is impacting cannabinoid production and the possible mechanisms could be further explored.

In chapter four, I evaluated the susceptibility of hemp germplasm to *G. ambrosiae* in growth chamber inoculations and field trials. I evaluated High CBD, high CBD, grain, fiber and dual-purpose cultivars and accessions throughout multiple field seasons. Through field ratings and growth chamber inoculations, the high CBD and high CBG cultivars displayed wide variation in their susceptibility to *G. ambrosiae*, and several cultivars consistently displayed either high or low disease severity over multiple field seasons and growth chamber inoculations. The extremes of high or low disease severity suggested a possible source of qualitative genetic host resistance present in the evaluated

hemp cultivars, which I explored further in chapter 5. The intermediate phenotypes, however, suggest the possibility of sources of quantitative host resistance which has not yet been explored further. Among the grain and fiber cultivars which I evaluated in a growth chamber setting and challenged with three different *G. ambrosiae* isolates, some variation in disease severity was observed, but not to the extent which was observed among the high CBD and CBG cultivars. Further, the three *G. ambrosiae* isolates did not display significant differences in their virulence against those cultivars. Additional isolates need to be collected and used to challenge the two known sources of resistance to begin to define possible races of this species.

Chapter five was a collaborative effort, focused on evaluating mapping populations resulting from the cross of a powdery mildew-susceptible cultivar, 'TJ's CBD' and a resistant cultivar, 'FL 58' (Stack et al., 2021). The relative powdery mildew susceptibility of these two cultivars was determined through field ratings in two sites in 2019, where 'FL 58' stood out, as it remained disease-free in two fields which were both heavily infected with powdery mildew. I evaluated a small number of F_1 progeny resulting from this population for their disease severity and two F_1 individuals were selected to be self-pollinated to generate the two F_2 populations. About 400 individuals from each of the two F_2 populations were planted in a field trial and I evaluated them for their susceptibility to *G. ambrosiae*. The proportion of individuals that displayed disease-resistant phenotypes indicated the likelihood of qualitative genetic host resistance. Additionally, QTL analysis of the F_2 populations is currently underway,

with preliminary results indicating a possible *Mildew Locus O* (*MLO*) gene associated with disease resistance. Further work is being done currently to confirm this finding, and to begin to characterize this susceptibility gene and its functionality. If it is confirmed that this candidate *MLO* gene is non-functional and responsible conferring resistance to *G. ambrosiae* in hemp, it will be an important source of genetic resistance that can be introgressed into breeding lines and eventually deployed commercially.

Throughout my work, there were many challenges related to working with an obligate biotrophic pathogen, of which there is little known. In order to perform all of the controlled inoculation experiments, *G. ambrosiae* isolates were grown on whole living plants and kept separate in their respective growth chambers. Because inoculum needs to be grown on whole plants, to grow enough inoculum for large growth chamber experiments or field trials, many large, healthy plants were required. Further, powdery mildew conidia have a poor survival rate in water, so spraying liquid conidial suspensions immediately is imperative. For field trials, this issue was addressed by making inoculum in the field, spraying immediately and counting spores while the field is being inoculated – this method is explained in Appendix I. This does not allow the spore concentration to be adjusted before inoculating, limiting the ability to have a consistent spore concentration between experiments, but ensures that the maximum number of viable spores are sprayed onto the plants.

Additionally, when beginning this work, there were no established protocols for extraction of DNA from *G. ambrosiae*, specifically. Further, the genome of *G. ambrosiae* has not been sequenced. Throughout my work, a protocol established by the L. Cadle-Davidson research team was used to extract DNA from isolates and field samples, and this protocol is described in Appendix II. However, this protocol results in low DNA yields and therefore created challenges when performing PCR and other further analyses. Creating PCR species-specific primers has also been problematic, not only as a result of the lack of a sequenced genome, but also because low DNA extraction yield also makes troubleshooting with primers difficult. There is potential for further work to focus on an extraction protocol that results in higher yields of high-quality DNA to be used for further genetic work of this powdery mildew species.

Overall, my body of work contributes to further understanding of *G. ambrosiae*, the causal agent of hemp powdery mildew. Through a better understanding of the pathogen host range and efficacious fungicides, hemp growers can make informed decisions about their disease managements practices. Further, sources of qualitative genetic host resistance to powdery mildew will be an important breeding tool.

Literature Cited:

Félix-Gastélum, R., Olivas-Peraza, D. D., Quiroz-Figueroa, F. R., Leyva-Madrigal, K. Y., Peñuelas-Rubio, O., Espinosa-Matías, S., & Maldonado-Mendoza, I. E. (2019). Powdery mildew caused by *Golovinomyces spadiceus* on wild sunflower in Sinaloa, Mexico. *Canadian Journal of Plant Pathology*, *41*(2), 301–309. https://doi.org/10.1080/07060661.2019.1577916

Li-hua, J. I. N., Yu, C., Chang-jun, C., Jian-xin, W., & Ming-guo, Z. (2009). Activity of azoxystrobin and SHAM to four phytopathogens. *Agricultural Sciences in China*, *8*(7), 835–842. https://doi.org/10.1016/S1671-2927(08)60285-0

Moparthi, S., Bradshaw, M., Frost, K., Hamm, P. B., & Buck, J. W. (2018). First report of powdery mildew caused by *Golovinomyces spadiceus* on okra in the United States. *Plant Disease*, *102*(8), 1664–1664. https://doi.org/10.1094/PDIS-01-18-0179-PDN

Moparthi, S., Bradshaw, M. J., & Grove, G. G. (2018). First report of powdery mildew caused by *Golovinomyces spadiceus* on *Helianthus annuus*. *Plant Disease*, *102*(6), 1176. https://doi.org/10.1094/PDIS-09-17-1434-PDN

Stack, G. M., Toth, J. A., Carlson, C. H., Cala, A. R., Marrero-González, M. I., Wilk, R. L., Gentner, D. R., Crawford, J. L., Philippe, G., Rose, J. K. C., Viands, D. R., Smart, C. D., & Smart, L. B. (2021). Season-long characterization of high-cannabinoid hemp (*Cannabis sativa* L.) reveals variation in cannabinoid accumulation, flowering time, and disease resistance. *GCB Bioenergy*, *13*(4), 546–561. https://doi.org/10.1111/gcbb.12793

APPENDIX I

Powdery Mildew Spray Inoculation

Ali Cala, Smart Lab

Cornell University

Materials:

- Living plant(s) infected with powdery mildew isolate that are actively sporulating
- Tween-20
- Water
- Hemacytometer
- Pipettors & tips (10-20 µL)
- Compound microscope
- Hand tally counter
- Nitrile gloves

Greenhouse/Growth chamber inoculations:

- Spray bottle
- 1L bottle with cap

Field inoculations:

- Backpack sprayer(s)
- Large beaker
- 50 mL tube with cap

Inoculum preparation:

Greenhouse/Growth chamber inoculations:

1. Create a solution of water and Tween-20 with a concentration of 1 L of distilled water and 100 µL of Tween-20 (~1 drop Tween 20/1L water) in a bottle with a screw cap.
    a. For a growth chamber experiment, no more than 1 L is usually needed, but this will vary depending on the number of plants to be inoculated, and the size of the plants.
2. Add leaves with (preferably heavy) powdery mildew sporulation to the water and tween solution.
    a. The number of leaves needed, will vary depending on the size of the infected leaves, and how heavily infected they are.
3. Close the cap of the bottle and shake to wash the conidia off the leaves.
4. Take samples from the solution to count conidia using the hemocytometer.
    a. The concentration should be ~1×10^5 spores/mL
5. Depending on the initial concentration, the solution may need to be diluted, or more infected leaves may need to be added to adjust the solution to the desired concentration.

a. Steps 4 & 5 should be done as quickly and efficiently as possible to ensure the most accurate count and most concentrated spore solution possible, as the powdery mildew spores will burst in the water in a short amount of time.

6. Once the spore solution is at the desired concentration, pour the solution into the spray bottle to be used for the spray, being careful to leave the leaves behind in the bottle.

***Field inoculations**:*

Because powdery mildew spores tend to burst quickly in water, when preparing inoculum for a field trial, the inoculum should be prepared in the field so it can be sprayed immediately. This presents challenges in getting an accurate spore count, as well as obtaining the desired spore concentrations.

1. Materials should be brought to the field to prepare inoculum.
2. Add water to a large beaker along with the appropriate amount of Tween-20 (100 µL of Tween-20/1 L of water).
    a. Water from field irrigation can be used for this if available.
    b. ~250 mL is usually an appropriate amount of water.

3. Add infected leaves to the water + Tween 20 solution and using a gloved hand, swirl the leaves to mix and *gently* rub conidia off the leaves.
    a. The number of leaves used will depend on the size of the field trial, the size of the plants to be inoculated, the size of the infected plants, and how heavily infected the plants are.
    b. For reference, most of the leaves on ~3-4 heavily infected, large plants should be enough inoculum for a field trial where 120 hemp plants need to be inoculated.
4. Once inoculum is made, a small sample of inoculum should be taken in a 50 mL tube and taken back to the lab for spores to be counted using a hemocytometer.
    a. It is best to have a second person to do this so inoculum can be sprayed simultaneously.
    b. This inoculum will be diluted, so keep this in mind when calculating final spore counts.
5. Add the rest of the inoculum to the backpack sprayer, being sure to filter out all plant material.
6. Add more water to the backpack sprayer to bring volume to ~10 L
    a. The amount will vary depending on the size of the trial.

Inoculation methods

Greenhouse, growth chamber & field:

1. Spray each plant to run off, covering the entire plant.

APPENDIX II

Modified by Xia Xu (Lance Cadle-Davidson lab, USDA-ARS, GGRU, Geneva, NY)

Genomic DNA Extraction from Grape Powdery Mildew

Modified from:

Protocol: a simple method for extracting next-generation sequencing quality genomic DNA from recalcitrant plant species. Haley A, Furtado A, Cooper T, Henry RJ. Plant Methods. 2014, 10:21

Buffers and Reagents

1. Extraction Buffer:

100 mM Tris-HCl pH 8.0

25 mM EDTA pH 8.0

1.5 M NaCl

2% CTAB

DNA Extraction Buffer

Reagent	Stock Conc.	Final Conc.	10 mL need	30 mL need	40 mL need
Tris-HCl	**1 M (pH8.0)**	**100 mM**	**1 mL**	**3 mL**	**4 mL**
EDTA	**0.5 M (pH8.0)**	**25 mM**	**0.5 mL**	**1.5 mL**	**2 mL**
NaCl	**5 M**	**1.5 M**	**3 mL**	**9 mL**	**12 mL**
CTAB		**2 % (w/v)**	**200 mg**	**600 mg**	**800 mg**
H2O*				**~ 11 mL**	**~ 18 mL**
Total (mL)			**10 mL**	**30 mL**	**30 mL**

Notes: (1) DNA extraction buffer should incubate in 65°C for at least 20 min.

(2) *First add less H2O and keep in 65°C for a while (solu. is clear), then add more to the vol.

CTAB: Hexadecyltrimethylammoniumbromide (FW=364.46) (Sigma: H6269-500G)

2. Chloroform-Isoamylalcohol (CIA) 24:1 (v/v) (50 ml)

3. 95% Ethanol (100ml) (store at -20°C)

4. 5M sodium chloride (NaCl)

5. DNase-free RNase an enzyme (100 mg/ml or 10 mg/ml), optional

6. 70 % Ethanol (v/v)

7. EB buffer (10 mM Tris-Cl, pH8.5) or nuclease-free water, or 0.1 mM EDTA (pH8.0).

Powdery Mildew (PM) collection and grinding beads:

Collection: Using clear plastic tape, about 1cm x 1.8cm size to collect PM, store in 2 ml tube

(https://www.eppendorf.com/US-en/; Cat. No.: 022363352, 500 pcs/$54.00)

Grinding beads: (1) Stainless grinding ball: SPEXSamplePrep 2150

(www.SPEXSAMPLEPREP.com);

(2) Silica beads, 400 micron; SP-2179 (www.opsdiagnostics.com)

The other reagents' ordering information:

1. EDTA, 0.5M (pH 8.0), Molecular Biology Grade: http://www.promega.com/; Cat. No.: V4231, 100 ml/$15.00.
2. **Tris-HCl, 1M Solution, pH 8.0:** http://www.affymetrix.com/; Cat. No.: 22638 500 ML, $38.00.
3. DNase-free RNase an enzyme: https://www.qiagen.com/us/ ; Cat No.: 19101; 100 mg/ml, 2.5 ml for $211.00.

DNA extraction Protocol (DNA extraction is performed at room temperature after grinding)

Things to do right at the beginning:

1. Turn on the two water baths at 65C (one bath for extraction buffer, one bath for incubating 2mL tubes)
2. Check that you have 95% EtOH in the -20C freezer
3. Check that you have enough of all other reagents
4. Pre-label all tubes that you will need for the extraction
5. For repeater pipette, either gather new tubes for each reagent or clean previous tubes with 95% EtOH

Extraction Steps:

1. For each collected PM, use 400ul extraction buffer.

2. Prepare extraction buffer and pre-warm at 65 ^{o}C for at least 20 min, and keep warm while grinding PM.

3. Grind PM samples in liquid nitrogen using grinder mill: Each 2mL microcentrifuge tube, add 1 stainless grinding ball and a few silica beads; Use GENO/GRINDER: 1x RATE, 300 strikes/min, and 3 x 30 sec.

4. Add 400ul extraction buffer to each tube, vortex to mix well.

5. Incubate tubes in a 65 ^{o}C for 20 min. (I use heat block with 800 rpm shaking; otherwise need invert tube every 5 min.) If RNase A is added to the extraction buffer (see step 7 for amount), then extend this to 30 minutes and you can skip step 7.

6. Centrifuge the sample tubes for 5 min at maximum speed for bench-top centrifuge. Transfer the supernatant into a new tube, spin down 2 min again and move clear supernatant to new 2ml tube.

7. Add 1µl of 100 mg/ml RNase A, and incubate at 37 ^{o}C for 10-15 min (I use heat block with 800 rpm shaking; otherwise need invert tube two times). This step is optional, and not needed for AmpSeq genotyping.

8. Add equal volume of CIA (24:1 v/v) (300ul) and invert tube for several times. Centrifuge 10 min at maximum speed. Pipet supernatant (~ 250ul) to new 1.5 ml-tubes.

APPENDIX III

Phytobiomes Journal • 2020 • 4:351-363 https://doi.org/10.1094/PBIOMES-06-20-0046-R

OPEN ACCESS phytobiomesjournal.org APS

Phytobiomes Journal

A Transdisciplinary Journal of Sustainable Plant Productivity

RESEARCH

Evaluating the Microbiome of Hemp

Samuel E. Barnett,[1] Ali R. Cala,[2] Julie L. Hansen,[3] Jamie Crawford,[3] Donald R. Viands,[3] Lawrence B. Smart,[4] Christine D. Smart,[2,†] and Daniel H. Buckley[1]

[1] Soil and Crop Sciences Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, U.S.A.
[2] Plant Pathology and Plant-Microbe Biology Section, School of Integrative Plant Science, Cornell University, Cornell AgriTech, Geneva, NY 14456, U.S.A.
[3] Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY 14853, U.S.A.
[4] Horticulture Section, School of Integrative Plant Science, Cornell University, Cornell AgriTech, Geneva, NY 14456, U.S.A.

Accepted for publication 29 August 2020.

ABSTRACT

Plant microbiomes contribute to plant fitness and crop yields both bacterial and fungal community composition varied through a variety of mechanisms. Determining variability in significantly among plant compartments. Rhizosphere microbiome composition among individuals of a species, and communities were largely similar to the bulk soil communities but identifying core microbiome membership, are essential first steps root tissue, leaf, and flower communities had distinct compositions. for exploring host–microbe interactions. Members of a core We identified candidate core microbiome members of each plant microbiome are microorganisms that are tightly associated with compartment (bacterial core taxa: root tissue [n = 6], leaves [n = and are found widespread across individuals of a plant genotype or 11], and flowers [n = 7]; fungal core taxa: rhizosphere [n = 1], species. Hemp (Cannabis sativa L.) is an economically important leaves [n = 14], and flowers [n = 2]). Many of these candidate core crop that has gained a resurgence following its removal from the microbiome members were related to organisms previously list of controlled substances by the U.S. government. Despite associated with plant growth promotion or pathogen resistance in renewed interest in this crop, the microbiome of hemp has not been various plants. The core microbiome identified in this study can be well studied. We analyzed the bacterial and fungal communities further investigated to improve cultivation of this important crop. associated with four plant compartments (rhizosphere, root tissue,
leaf surface, and flowers) of C. sativa 'Anka' across six fields in the Keywords: bacteria, Cannabis sativa, crop, fungi, hemp, Finger Lakes region of New York, United States. We found that microbiome, microorganism, phyllosphere, rhizosphere

Plant-associated microbes are key players in the health and productivity of their plant hosts (Turner et al. 2013). Plant microbiomes include bacteria and fungi within rhizosphere soil, within root tissues (root epiphytes, ectophytes, and endophytes), and upon aboveground structures such as leaves and flowers

† Corresponding author: C. D. Smart; cds14@cornell.edu

Author contributions: C.D.S. and A.R.C. designed the experiment with consultation from D.H.B. A.R.C., J.L.H., J.C., D.R.V., L.B.S., and C.D.S. designed and implemented hemp cultivation and sample collection. A.R.C. and S.E.B. performed the experiments and collected the results. S.E.B. analyzed the data and wrote the manuscript with much input from A.R.C., C.D.S., and D.H.B.. All authors contributed to editing the manuscript.

Funding: Support was provided by the New York State Department of Agriculture and Markets (grant number C00195GG).

The author(s) declare no conflict of interest.

(phyllosphere) (Turner et al. 2013). Some of these microbes perform beneficial functions for their hosts, including fixation of atmospheric nitrogen (Franche et al. 2009), improving access to soil nutrients (Colombo et al. 2014; Gyaneshwar et al. 2002; Whiting et al. 2001), production of plant hormones (Boller 1995; Ort´ızCastro et al. 2009), protecting against pathogens through competitive exclusion (Innerebner et al. 2011), production of antimicrobial compounds (Lievens et al. 1989; Urquhart and Punja 2002; Weller 1988), and plant defense priming (Pieterse et al. 2014). The microbiome's role in plant health has made it a target for study in economically important crops such as maize, soybean, and rice (de Cassia Silva et al. 2016; Edwards et al. 2015; Kuklinsky-Sobral´ et al. 2004; Peiffer et al. 2013; Wallace et al. 2018).

High-throughput DNA sequencing has promoted numerous studies describing the microbiomes of important agricultural plant species (Busby et al. 2017). These early reports are important steppingstones for understanding how microbiomes affect plant growth, identifying the impact of plant traits on microbiome composition, and developing methods for manipulating microbiomes to increase crop yields or disease resistance (Busby et al. 2017). Microbiome considerations are especially important when developing sustainable cropping systems (Toju et al. 2018).

Hemp is likely to be an important crop in the United States, with economic value in many industries such as textiles and fibers, food products, personal care products, and health and wellness products (Cherney and Small 2016). Hemp refers to cultivars of Cannabis sativa with less than 0.3% dry weight tetrahydrocannabinol (THC) (Small and Cronquist 1976). Recent U.S. government legislation has removed hemp from the list of controlled substances, reducing roadblocks to cultivation and promoting agricultural development for this crop (115th Congress 2018). Despite the anticipated growth of hemp cultivation, little is known about its microbiome. Almost all previous studies examining the microbiome or generally microbes associated with C. sativa focus on high-THC cultivars grown primarily for recreational or medicinal use (Comeau et al. 2020; McKernan et al. 2015; Winston et al. 2014). Others focused on examining activities of isolated C. sativa endophytes (Gautam et al. 2013; Kusari et al. 2013; Scott et al. 2018).

Here, we present one of the first characterizations of both bacterial and fungal communities associated with multiple plant compartments of hemp. We used high-throughput sequencing of bacterial (16S ribosomal RNA [rRNA] gene) and fungal (internal transcribed spacer 1 [ITS1]) biomarkers to describe the microbiome of the C. sativa 'Anka' grown across six field sites in the Finger Lakes region of New York, United States. We examined the microbes inhabiting the rhizosphere, root tissue, leaf surface, and flower surfaces, aiming to identify the core microbiome of Anka grown in this region. Results from our study will provide a reference for future research in defining functionality of microbial symbionts of hemp and developing sustainable microbiome manipulations to improve crop yields.

MATERIALS AND METHODS

Plant cultivation and sampling. Plants used in this study were part of a larger cultivar trial (35 entries) and were planted in six secure research field locations around the Finger Lakes region of New York (Table 1). Plot trials (1.22 by 6.10 m) were planted with a tractor-mounted 6-row cone seeder. In McGowan Early, McGowan Late, East Ithaca, and Research North fields, nitrogen was applied at7.85 g m^{-2} 3 weeks after planting. At Crittenden North, nitrogen at

11.21 g m was applied prior to planting. No nitrogen was applied at Freeville. Each plot was replicated five times in a randomized complete block design. All microbiome sampling was done on C. sativa 'Anka' (Uniseeds, Ontario, CA, U.S.A.), which is a monecious cultivar used for dual-purpose grain and fiber production. The seeding rate was pure live seed at 2.24 g m.

Five plants were collected per field (one plant per replicate plot) when plants were in full flower (Table 1) and most plants were over 2 m in height. For each collection, the entire plant was dug up and placed in a large plastic bag. Bulk soil was collected to a depth of 10cm within 1 m of the selected plant and placed in a Ziplock bag. Samples were driven to the laboratory for immediate processing or placed in a cold room (4C) and processed within 24 h. To process plants, large soil aggregates were first removed from the roots; then, the rhizosphere soil was gently removed from roots and collected. The roots were cut into 4-cm-long pieces using a razor blade. Rhizosphere and bulk soil samples were sieved to 2 mm and stored at −20C prior to DNA extraction. Roots were frozen with liquid nitrogen and crushed using a mortar and pestle, then stored at −20C prior to DNA extraction.

For leaf and flower samples, we specifically targeted collection of bacterial and fungal epiphytes. Cotton swabs were dipped in sampling buffer (100 mM potassium phosphate, pH 7; 10 mM EDTA; and 0.05% Triton), then rubbed over either the leaf or flower surface for 10 s The swab was then returned to a tube with 1 ml of sampling buffer. To concentrate the flower and leaf wash samples for DNA extraction, they were lyophilized at −40C, then rehydrated in 200 ml of molecular-grade water. Samples were stored at −20C prior to DNA extraction.

DNA extraction, amplification, and sequencing. We extracted DNA from samples with the PowerMag Microbiome RNA/DNA Isolation Kit (catalog number 27500-4-EP; MO BIO Laboratories Inc., Carlsbad, CA, U.S.A.), using the manufacturer's instructions with the following modifications. We used 0.25 g of bulk or rhizosphere soil per soil DNA extraction. For root tissue extraction, between 0.10 and 0.25 g of powdered root tissue was used due to limited material. All 200 ml of rehydrated wash solution was used for extraction from leaf and flower washes. All samples underwent bead beating for 2.5 min using a Mini-Beadbeater-96 (catalog number 1001; Biospec Products, Bartlesville, OK, U.S.A.).

Prior to amplification, we quantified DNA concentrations using a Quant-iT PicoGreen assay (number P7589; Thermo Fisher Scientific, Waltham, MA, U.S.A.). High-concentration samples were diluted to 1.0 ng ml with molecular-grade water to normalize PCR template concentrations. To capture the bacterial community composition, we amplified the V4 region of the 16S rRNA gene using the 515f/806r dual-indexing primer set (Kozich et al. 2013). To capture the fungal community composition, we amplified the ITS1 region using the nBITS2f/58A2r primer set

TABLE 1

Description of field sites, hemp planting dates, sample collection dates, and grain yield

Site name	Location	Soil type	Planting date	Collection date	Grain yield
East Ithaca	Ithaca, NY	Arkport fine sandy loam	14 June 2017	2 August 2017	582.20
McGowan	Ithaca, NY	Niagara silt loam	9 June 2017	26 July 2017	2,340.80
McGowan Late	Ithaca, NY	Niagara silt loam	29 June 2017	9 August 2017	1,608.60
Research North	Geneva, NY	Lima loam	28 June 2017	10 August 2017	624.22
Crittenden North	Geneva, NY	Odessa silt loam	10 July 2017	21 August 2017	780.27
Freeville	Freeville, NY	Howard gravelly loam	12 July 2017	23 August 2017	NA

(Koechli et al. 2019). The nBITS2f/58A2r primer set used the same dual-indexing scheme as the 515f/806r primer set. Triplicate PCR assays contained 2.0 ml of template, 2.5 ml of pooled 10× forward and reverse primers, 13.1 ml of Q5 master mix (catalog number M0494L; New England Biolabs, Ipswich, MA, U.S.A.) containing 1:0.025 (vol/ vol) 4× PicoGreen reagent, and 7.4 ml of molecular-grade water. PCR conditions were 95C for 2 min; followed by 30 cycles of 95C for 20 s, 55C for 15 s, and 72C for 10 s; and followed by 72C for 5 min. We normalized PCR product concentrations across samples, replicates pooled, with the Invitrogen SequalPrep Normalization Plate Kit (catalog number A1051001; Thermo Fisher Scientific, Waltham, MA, U.S.A.), then pooled 16S rRNA gene and ITS1 libraries separately. Libraries were sequenced at the Cornell Biotechnology Resource Center (Ithaca, NY, U.S.A.) on the Illumina MiSeq platform with the paired-end 2-by-250-bp V2 kit for the 16S rRNA gene library and the paired-end 2-by-300-bp V3 kit for ITS1 library. Raw demultiplexed reads are accessible on the short-read archive with BioProject accession number PRJNA607742.

Sequence processing. For both amplicon libraries, we merged paired end reads using PEAR (Zhang et al. 2014) with default settings, then demultiplexed using a custom script. For the 16S rRNA gene amplicon library, we performed alignment-based quality filtering with mothur (Schloss et al. 2009) with the Silva SEED database release 128 as a reference, discarding homopolymers greater than 8 bp. Reads mapping to mitochondria and chloroplasts were removed. We clustered operational taxonomic units (OTUs) at 97% sequence identity with UPARSE clustering from USEARCH (Edgar 2010). Taxonomy was assigned using the uclust algorithm through QIIME (Caporaso et al. 2010) with the SILVA release 128 database as reference. OTUs not classified as bacteria were removed.

For the ITS1 amplicon library, we used mothur (Schloss et al. 2009) for quality filtering, with a maximum homopolymer length of 8 bp but without alignment-based filtering. We then filtered and trimmed sequences to just the ITS1 region using ITSx (BengtssonPalme et al. 2013). To improve OTU clustering, all sequences were set to an equal length by adding ambiguous bases (N) to the ends of each sequence (Palmer et al. 2018). Using vsearch (Rognes et al. 2016), we preclustered sequences at 98% sequence identity and filtered out chimeric sequences. We then clustered final OTUs at 97% sequence identity. Taxonomy was assigned using the sintax classifier through vsearch with the USEARCH formatted UNITE reference database, release 01.12.2017. OTUs not classified as fungi were removed. The sequence processing pipeline, representative OTU sequences, OTU and taxonomy tables, and metadata are available at https://github.com/seb369/hemp_microbiome (archived via Zenodo) (Barnett 2020).

Data analysis. We performed all data analysis in R version 3.6.2 (R Core Team 2018) and performed each analysis separately for fungi and bacteria. Code for all analyses are available at https://github.com/seb369/hemp microbiome. All root tissue samples were removed from the fungal amplicon library because the vast majority of reads matched the C. sativa ITS1 region, leaving too few actual fungal reads for analysis. We further removed samples with less than 2,000 bacterial reads or 1,000 fungal reads prior to analysis because these cutoffs typically indicated poorly sequenced samples. This read cutoff removed some replicate plants; thus, although 30 plants were originally sampled, often less than 30 plants were analyzed. Read counts varied across samples, from 2,425 to 223,698 bacterial reads and 1,396 to 58,300 fungal reads. In order to account for different sequencing depths between samples, we rarefied read counts to that of the lowest read count sample (bacterial = 2,425 and fungal = 1,396) using the rarefy even depth function from package phyloseq (McMurdie and Holmes 2013). Rarefaction was used to normalize sequence counts because it is among the most robust methods for normalizing uneven sample sizes (Weiss et al. 2017). One drawback to this normalization method is that it results in random loss of some OTUs within samples despite

their detection originally. This may disproportionately lead to false-negative detection of low-abundance OTUs or taxa susceptible to primer bias. Species evenness was calculated for each sample and the effect of plant compartment on evenness was determined by analysis of variance (ANOVA). The post hoc Tukey test of evenness was run between plant compartments using function glht from package multcomp (Hothorn et al. 2008).

To compare overall bacterial or fungal community structure across plant compartment and field locations, we ran a permutational multivariate ANOVA (PERMANOVA) on the Bray-Curtis dissimilarity metric with the function adonis from package vegan (Oksanen et al. 2018). The PERMANOVA models incorporated plant compartment, field, and their interaction as variables. We generated ordinations based on the Bray-Curtis dissimilarity metric using function ordination from package phyloseq (McMurdie and Holmes 2013). To examine the interaction between plant compartment and field site on community composition, we ran separate PERMANOVA on Bray-Curtis dissimilarity within each plant compartment with field as the sole variable, with P values corrected for multiple comparisons using Bonferroni correction. This analysis was to determine whether field site was an important factor in community composition for each plant compartment.

Although we were interested in the effect of the microbial community compositions on crop productivity, we were limited in our data on crop yield, because this value was measured for fields and not individual plants. No yield data were collected from the Freeville field. To see whether or not community composition differences corresponded to differences in grain yield, we averaged the Bray-Curtis dissimilarity across all sample comparisons between each field pair and then calculated the Pearson correlation coefficient between these community dissimilarity measures and the difference in grain yield between the field pairs, with P values corrected for multiple comparisons using Bonferroni correction. This analysis was performed separately for each plant compartment.

We examined the variation in relative abundance for highly abundant phyla or classes (>1% of the bacterial or fungal community) across plant compartments using the Kruskal-Wallis rank sum test with P values adjusted for multiple comparisons with Bonferroni correction. We used a nonparametric test in this case due to the nonnormal nature of these data (Weiss et al. 2017). Separate pairwise comparisons across compartments were then run for taxa with significant abundance variation using the Dunn test function from package FSA (Ogle et al. 2020).

To identify members of the hemp microbiome that may be of further interest, we applied three different approaches. First, we identified the most abundant OTUs within each plant compartment by averaging each OTU's relative abundance across all plants. Relative abundance of these OTUs used the rarefied OTU table. Second, we identified ubiquitous OTUs within each plant compartment (i.e., detected on 100% of plants). In this case, we used unrarefied OTU tables because uneven sampling depth should have little effect on the type 2 error (i.e., false identification of ubiquitous OTUs). We note that it is likely that some low-abundance yet ubiquitous OTUs were not detected in some samples with lower sequencing depth (i.e., type 1 error). Third, we used DESeq2 (Love et al. 2014) to estimate the $\log_2$-fold change enrichment of the OTUs between each plant compartment and the bulk soil. For each DESeq2 analysis, we used unrarefied OTU tables filtered to include only OTUs represented by at least five reads in at least one sample of the plant compartment-bulk soil set. We also used a one-sided test, a $\log_2$-fold change threshold of 0.25, the Wald significance test, the Benjamini and Hochberg P value adjustment for multiple comparisons, and a P value cutoff of 0.1. We defined the core microbiome as bacterial and fungal OTUs that were ubiquitous in hemp plants and enriched significantly in a plant compartment relative to bulk soil. Presence in the core hemp microbiome does not indicate strength or direction of plant–microbe

interaction, nor does it indicate exclusivity to hemp; it merely indicates that the microbes are enriched in hemp across a wide range of soil type and field conditions.

RESULTS

Plant compartments harbor different bacterial communities. We identified 8,913 bacterial OTUs across all samples after rarefying, comprising 39 different phyla. We found that plant compartment explained the most variation in microbiome composition (PERMANOVA: $R^2 = 0.32310$, df = 4, P = 0.001), followed by the interaction of plant compartment and field site ($R^2 = 0.16247$, df = 20, P = 0.001), while field site alone explained the least variation in community composition ($R^2 = 0.08029$, df = 5, P = 0.001). Rhizosphere microbiomes were most similar to those of bulk soil, while root, leaf, and flower microbiomes were all fairly dissimilar in community composition (Fig. 1A). Flower bacterial communities had high variability in community composition, even within a field (Fig. 1A). Field site explained significant variation in bacterial community composition within all plant compartments (bulk soil: $R^2 = 0.4309$, df = 5, adjusted P = 0.005; rhizosphere soil: $R^2 = 0.43205$, df = 5, adjusted P = 0.005; root tissue: $R^2 = 0.35325$, df = 5, adjusted P = 0.005; leaves: $R^2 = 0.26795$, df = 5, adjusted P = 0.025; flowers: $R^2 = 0.32542$, df = 5, adjusted P = 0.005). However, we found no relationship between bacterial community dissimilarity and crop yield at field scale (rhizosphere soil: Pearson's r = −0.1056, df = 8, adjusted P = 1; root tissue: Pearson's r = 0.2609, df = 8, adjusted P = 1; leaves: Pearson's r = −0.5691, df = 8, adjusted P = 0.3440; flowers: Pearson's r = −0.3933, df = 8, adjusted P = 1).

Different bacterial phyla (class for Proteobacteria) were favored in different plant compartments (Fig. 2), with the most abundant taxa (>1% of the bacterial community) varying significantly in relative abundance across plant compartments (Kruskal-Wallis, all Bonferroni adjusted P < 0.001). Notably, based on post hoc tests (Dunn tests, P < 0.05), no phyla or class varied significantly in relative abundance between bulk and rhizosphere soils. Actinobacteria were in higher relative abundance in the soil, rhizosphere, and root tissue than in either the leaves or flowers. Betaproteobacteria were in greater abundance in root tissue than in any other plant compartment. Alphaproteobacteria were more abundant on leaves than in any other plant compartment. Bacteroidetes were more abundant in root tissue and on leaves than the other plant compartments. Firmicutes and Gammaproteobacteria both had strikingly high abundances on the flowers compared with bulk soil, rhizosphere soil, and leaves, with Gammaproteobacteria also in high abundance in the root tissue (Fig. 2).

Plant compartments harbor different fungal communities. We identified 982 fungal OTUs across all samples after rarefying, comprising 9 phyla. We found that plant compartment explained the most variation in fungal community composition (PERMANOVA: $R^2 = 0.47902$, df = 3, P = 0.001), followed by the interaction of plant compartment and field site ($R^2 = 0.15231$, df = 13, P = 0.001), while field site alone explained the least variation in community composition ($R^2 = 0.14361$, df = 5, P = 0.001). Rhizosphere fungal communities were most similar to bulk soil communities, while the fungal communities found on leaves resembled those found on flowers (Fig. 1B). Field site explained significant variation in fungal community composition within all plant compartments (bulk soil: $R^2 = 0.60938$, df = 4, adjusted P = 0.004; rhizosphere soil: $R^2 = 0.60956$, df = 5, adjusted P = 0.004; leaves: $R^2 = 0.4716$, df = 5, adjusted P = 0.004; flowers: $R^2 = 0.52618$, df = 5, adjusted P = 0.004). We found no relationship between fungal community dissimilarity and crop yield at field scale (rhizosphere soil:

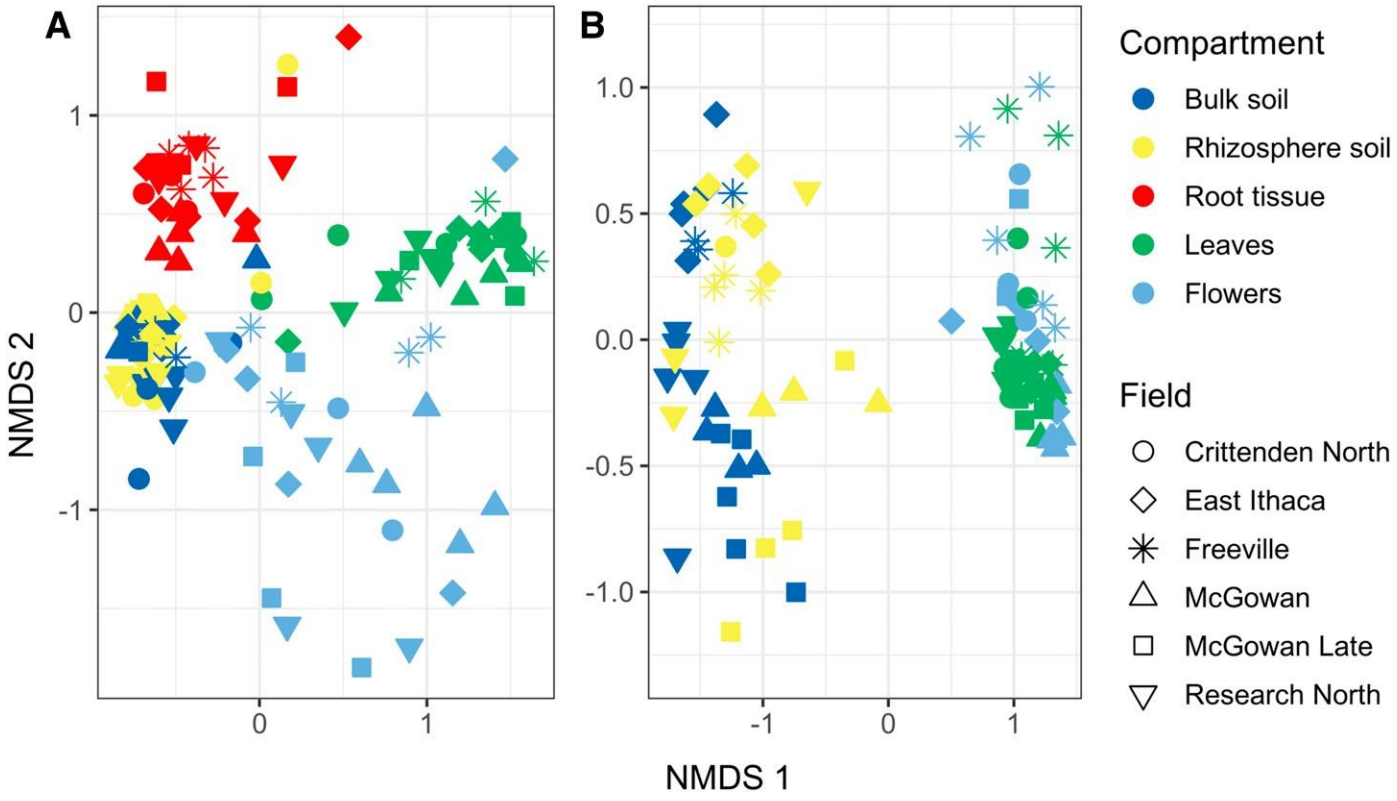

Fig. 1. Both A, bacterial (stress = 0.16) and B, fungal (stress = 0.11) communities vary by plant compartment (bacterial: $R^2 = 0.32310$, df = 4, P = 0.001; fungal: $R^2 = 0.47902$, df = 3, P = 0.001), field location (bacterial: $R^2 = 0.08029$, df = 5, P = 0.001; fungal: $R^2 = 0.14361$, df = 5, P = 0.001), and their interaction (bacterial: $R^2 = 0.16247$, df = 20, P = 0.001; fungal: $R^2 = 0.15231$, df = 13, P = 0.001). NMDS = nonmetric multidimensional scaling. Ordinations generated based on Bray-Curtis dissimilarity.

Pearson's r = 0.0390, df = 4; adjusted P = 1; leaves: Pearson's r = 0.2667, df = 8, adjusted P = 1; flowers: Pearson's r = 0.1869, df = 1, adjusted P = 1).

Fungal taxa were favored differently across plant compartments (Fig. 3), with the most abundant classes (>1% of the fungal community) varying significantly in relative abundance across plant compartments (Kruskal-Wallis, all Bonferroni adjusted P < 0.001). Notably, based on post hoc tests (Dunn tests, P < 0.05), no class varied significantly in relative abundance between bulk and rhizosphere soils. Classes Sordariomycetes, Eurotiomycetes, Mortierellomycetes, and Leotiomycetes were in greater relative abundance in

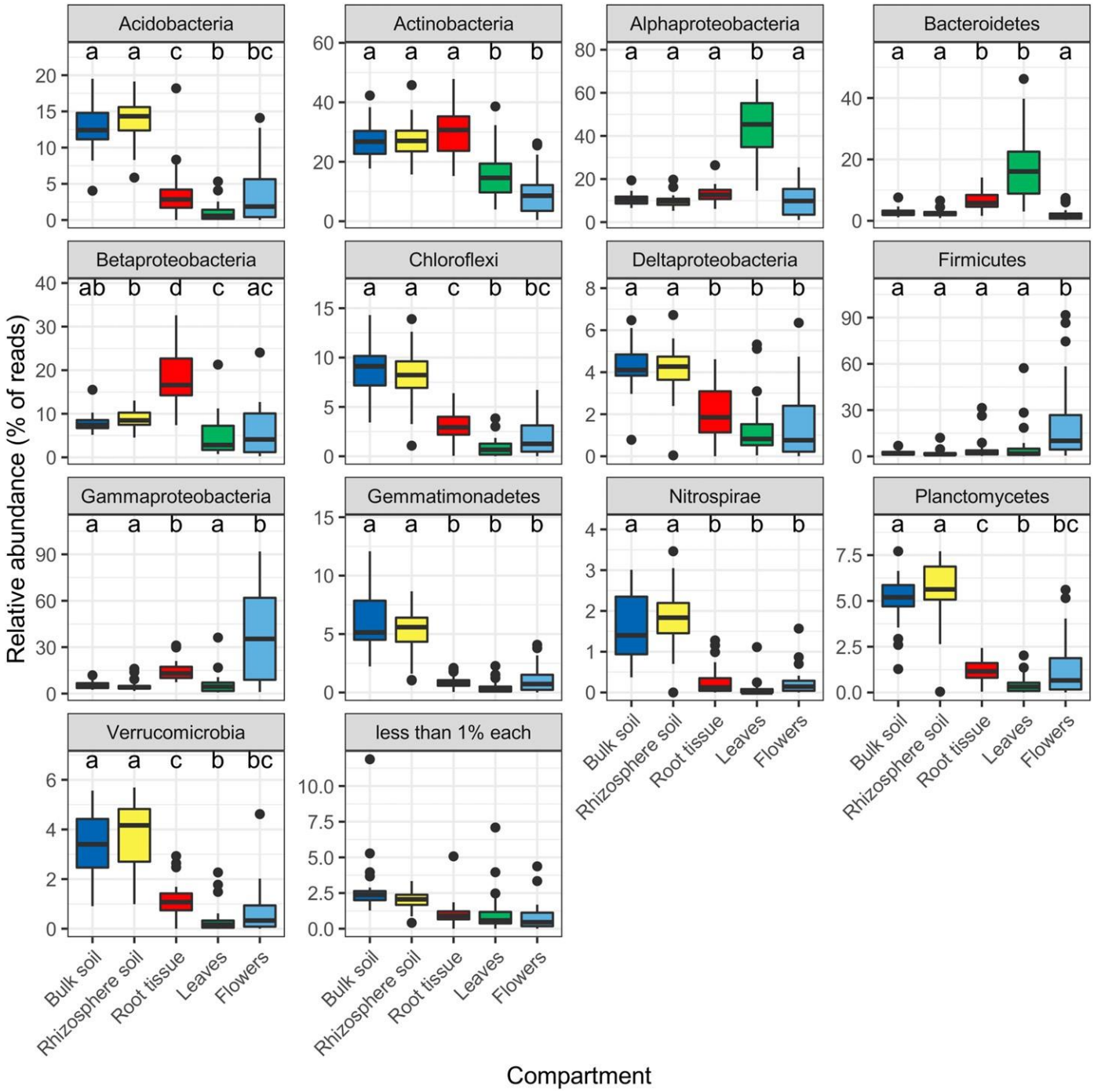

Fig. 2. Relative abundances of all highly abundant bacterial phyla or classes (>1% of the rarefied community) differ significantly across plant compartments (Kruskal-Wallis with Bonferroni correction for multiple comparisons: all P < 0.05). Proteobacteria operational taxonomic units (OTUs) have been grouped at the class level. For each phylum or class, all relative abundance values from the rarefied OTU table for each member OTU was summed for each sample. Post hoc Dunn test pairwise comparison grouping among plant compartment is indicated by lowercase letters (P < 0.05).

bulk and rhizosphere soil communities relative to those of leaves or flowers. In contrast, classes Exobasidomycetes, Microbotryomycetes, and Tremellomycetes were more abundant on leaves and flowers. Isolates of Dothideomycetes were also more abundant on the leaves than any other plant compartment (Fig. 3). The top five most abundant bacterial and fungal OTUs differ across plant compartments. We examined the top five most abundant fungal and bacterial OTUs within each plant compartment (Tables 2 and 3). We found that the portion of the communityrepresented by the top five OTUs differed across plant compartment. The top five most abundant bacterial OTUs in each compartment comprised approximately 7% of the bulk and rhizosphere soil communities and 19, 47, and 42% of the root tissue, leaf, and flower communities, respectively (Table 2). A similar trend was observed for the top five most abundant fungal OTUs, which comprised 38 and 37% of the bulk and rhizosphere soil communities, respectively, and approximately 74 and 77% of the leaf and flower communities, respectively (Table 3). Accordingly, Pielou's species evenness varied significantly across plant compartment for both bacterial and fungal communities (ANOVA; bacterial: $df = 4$, $F = 60.657$, $P < 0.001$; fungal: $df = 3$, $F = 53.059$, $P < 0.001$). Leaves and flowers had lower bacterial and fungal evenness than bulk and rhizosphere soils. In addition, root tissues had lower bacterial evenness than bulk and rhizosphere soils but higher evenness than leaves or flowers (Fig. 4) (Post hoc Tukey; all $P < 0.05$).

The top five most abundant bacterial OTUs found in root tissues, leaves, and flowers were all enriched significantly in these compartments compared with their relative abundance in bulk soil (DESeq2, $P < 0.1$) (Table 2). In contrast, the most abundant

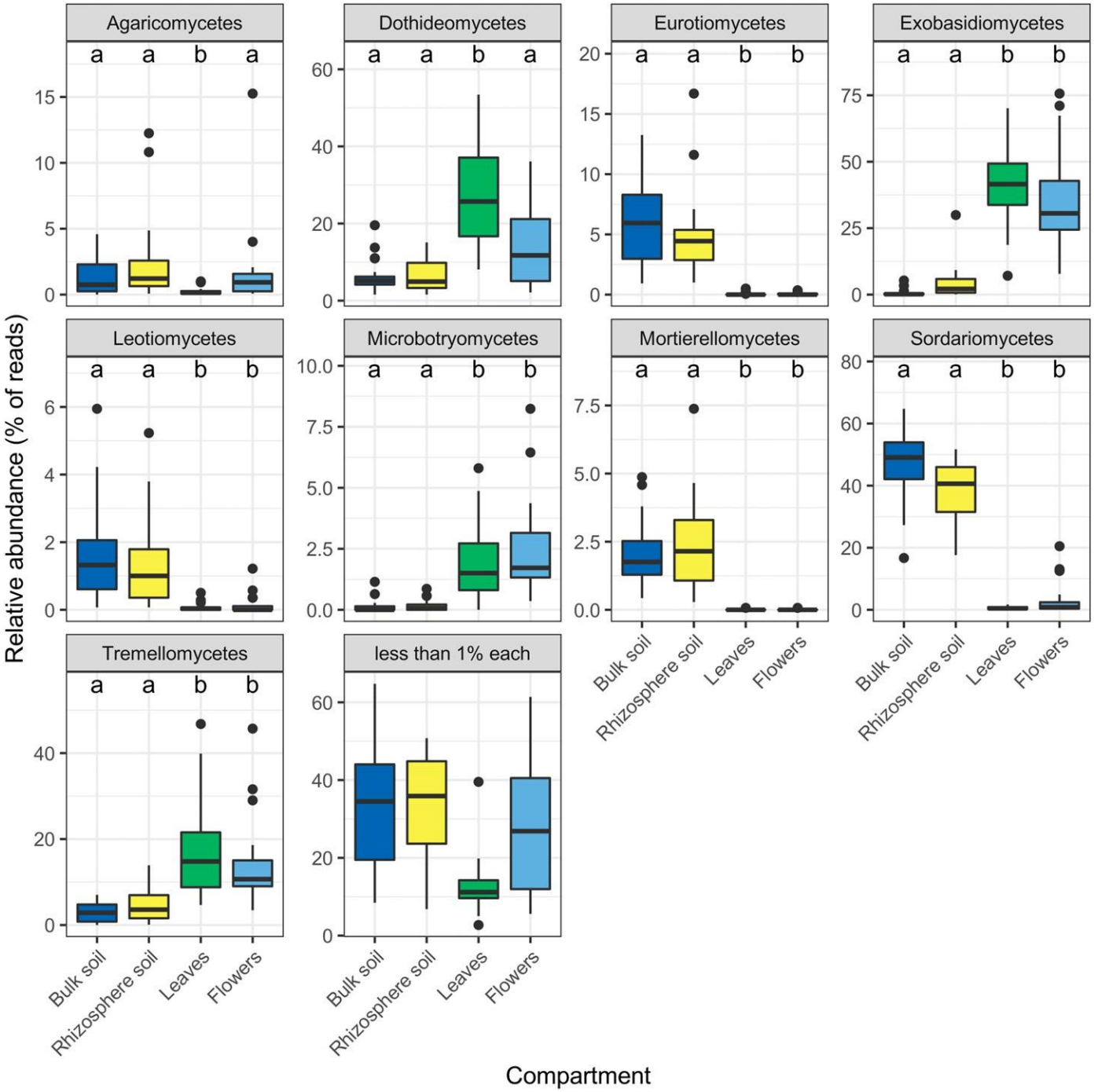

Fig.3. Relative abundances of all highly abundant fungal classes (>1% of the rarefied community) differ significantly across plant compartments (KruskalWallis with Bonferroni correction for multiple comparisons: all $P < 0.05$). For each class, all relative abundance values from the rarefied operational taxonomic unit (OTU) table for each member OTU was summed for each sample. Post hoc Dunn test pairwise comparison grouping among plant compartment is indicated by lowercase letters ($P < 0.05$).

TABLE 2

Bacterial core operational taxonomic units (OTUs) (Core?), identified as ubiquitous in each plant compartment (Ubiq plant) and enriched in the plant samples compared with bulk soil (Log_2 FC), as well as the top five most abundant OTUs (Rank) within each compartment[a]

Compartment (samples), OTU[b]	Abundance (SE) (%)[c]	Log_2 FC (SE)	Rank	Ubiq plant	Ubiq soil	Core?	Best classification (level)
Bulk soil (26)							
OTU.10	1.77 (0.25)	NA	1	NA	Yes	No	Pseudarthrobacter (genus)
OTU.28	1.67 (0.14)	NA	2	NA	No	No	Chloroflexi, KD4-96 (class)
OTU.21	1.29 (0.13)	NA	3	NA	Yes	No	Janibacter (genus)
OTU.66	1.23 (0.23)	NA	4	NA	Yes	No	Comamonadaceae (family)
OTU.39	1.12 (0.14)	NA	5	NA	Yes	No	Lysobacter (genus)
Rhizosphere soil (28)							
OTU.10	2.00 (0.22)	NS	1	Yes	Yes	No	Pseudarthrobacter (genus)
OTU.28	1.63 (0.12)	NS	2	No	No	No	Chloroflexi, KD4-96 (class)
OTU.21	1.20 (0.16)	NS	3	No	Yes	No	Janibacter (genus)
OTU.66	1.00 (0.11)	NS	4	Yes	Yes	No	Comamonadaceae (family)
OTU.63	0.85 (0.07)	NS	5	No	No	No	Acidobacteria, subgroup 6 (class)
Root tissue (27)							
OTU.24	5.69 (0.79)	4.10 (0.38)	1	No	No	No	Streptomyces (genus)
OTU.17	4.94 (1.33)	8.62 (0.72)	2	No	No	No	Halieaceae (family)
OTU.49	3.47 (0.65)	2.46 (0.32)	3	Yes	Yes	Yes	Comamonadaceae (family)
OTU.18	2.68 (0.60)	1.53 (0.29)	4	Yes	Yes	Yes	Massilia (genus)
OTU.117	2.43 (0.45)	2.75 (0.35)	5	Yes	Yes	Yes	Aquabacterium (genus)
OTU.74	1.09 (0.23)	2.69 (0.46)	14	Yes	No	Yes	Rhizobium (genus)
OTU.91	1.05 (0.35)	2.62 (0.52)	17	Yes	No	Yes	Pseudomonas (genus)
OTU.8	0.59 (0.12)	2.92 (0.33)	32	Yes	Yes	Yes	Sphingomonas (genus)
Leaves (28)							
OTU.8	13.14 (1.88)	6.70 (0.39)	1	Yes	Yes	Yes	Sphingomonas (genus)
OTU.5	12.81 (1.99)	7.65 (0.46)	2	Yes	No	Yes	Sphingomonas (genus)
OTU.9	8.55 (1.73)	7.46 (0.57)	3	Yes	No	Yes	Methylobacterium (genus)
OTU.11	6.53 (1.09)	7.17 (0.59)	4	Yes	No	Yes	Hymenobacter (genus)
OTU.23	5.63 (1.78)	8.30 (0.67)	5	Yes	No	Yes	Hymenobacter (genus)
OTU.16	4.91 (0.83)	4.98 (0.44)	6	Yes	Yes	Yes	Microbacteriaceae (family)
OTU.37	2.28 (0.36)	2.36 (0.42)	8	Yes	Yes	Yes	Microbacteriaceae (family)
OTU.25	1.67 (0.45)	7.53 (0.75)	9	Yes	No	Yes	Hymenobacter (genus)
OTU.3	1.53 (0.34)	5.57 (0.61)	11	Yes	No	Yes	Pseudomonas (genus)
OTU.137	0.49 (0.09)	6.47 (0.58)	30	Yes	No	Yes	Sphingomonas (genus)
OTU.1896	0.23 (0.04)	4.60 (0.53)	44	Yes	No	Yes	Sphingomonas (genus)
Flowers (26)							
OTU.7	12.56 (3.19)	6.21 (0.59)	1	No	No	No	Pseudomonas (genus)
OTU.4	11.67 (4.41)	9.92 (0.72)	2	No	No	No	Lactococcus lactis (species)
OTU.12	6.33 (2.01)	8.03 (0.73)	3	No	No	No	Pantoea (genus)
OTU.20	6.23 (2.00)	5.85 (0.62)	4	No	No	No	Enterobacter (genus)
OTU.6	5.53 (3.59)	2.06 (0.56)	5	Yes	Yes	Yes	Bacillus cereus (species)
OTU.13	2.85 (0.85)	2.02 (0.32)	7	Yes	Yes	Yes	Ralstonia (genus)

OTU.61	1.22 (0.55)	1.52 (0.41)	10	Yes	Yes	Yes	Bacillus (genus)
OTU.5	1.05 (0.36)	4.94 (0.55)	11	Yes	No	Yes	Sphingomonas (genus)
OTU.8	1.01 (0.33)	4.07 (0.46)	12	Yes	Yes	Yes	Sphingomonas (genus)
OTU.9568	0.72 (0.18)	6.74 (0.56)	16	Yes	No	Yes	Bradyrhizobium (genus)
OTU.9	0.37 (0.13)	3.41 (0.54)	24	Yes	No	Yes	Methylobacterium (genus)

[a] The number of samples used for these analyses differed across plant compartments due to removal of samples with low sequence counts. Relative abundance, as percentage of rarefied reads, was averaged across all samples. Column Log_2 FC indicates the log_2-fold change in read count between plant compartment and bulk soil for OTUs that were enriched significantly (Benjamini and Hochberg adjusted $P < 0.1$), calculated with DESeq2 on unrarefied read counts. NS indicates nonstatistically significant enrichment. NA indicates measures that are not applicable for the bulk soil. Bold values are used for emphasis. OTU taxonomy is given as the highest classification level determined from the SILVA database. [b] Plant compartment (number of samples) and OTU ID. [c] Percent relative abundance and standard error (SE).

TABLE 3

Fungal coreoperational taxonomicunits(OTUs) (Core?),identifiedas ubiquitous ineachplantcompartment (Ubiqplant)andenrichedinthe plant samples compared with bulk soil (Log_2 FC), as well as the top five most abundant OTUs (Rank) within each compartment[a]

Compartment (samples),OTU[b]	Abundance (SE) (%)[c]	Log_2 FC (SE)	Rank	Ubiq plant	Ubiq soil	Core?	Best classification (level)
Bulk soil (22)							
OTU_13	13.97 (2.28)	NA	1	NA	Yes	NA	Verticillium dahlia (species)
OTU_8	6.93 (0.98)	NA	2	NA	Yes	NA	Fusarium (genus)
OTU_34	6.84 (1.50)	NA	3	NA	Yes	NA	Fungi (kingdom)
OTU_309	5.08 (1.52)	NA	4	NA	Yes	NA	Fungi (kingdom)
OTU_33	4.79 (2.07)	NA	5	NA	Yes	NA	Hypocreales (order)
Rhizosphere soil (21)							
OTU_13	10.94 (1.60)	NS	1	Yes	Yes	No	V. dahlia (species)
OTU_34	9.57 (1.81)	NS	2	Yes	Yes	No	Fungi (kingdom)
OTU_8	7.63 (1.57)	NS	3	Yes	Yes	No	Fusarium (genus)
OTU_21	4.66 (1.45)	2.85 (0.50)	4	No	No	No	Tilletiopsis washingtonensis (species)
OTU_38	4.31 (0.69)	NS	5	Yes	Yes	No	Didymella dimorpha (species)
OTU_27	0.46 (0.18)	2.43 (0.41)	46	Yes	No	Yes	Bullera alba (species)
Leaves (30)							
OTU_21	36.25 (3.11)	5.88 (0.42)	1	Yes	No	Yes	T. washingtonensis (species)
OTU_174	16.75 (1.67)	5.58 (0.42)	2	Yes	No	Yes	Epicoccum (genus)
OTU_27	11.64 (1.64)	6.42 (0.41)	3	Yes	No	Yes	B. alba (species)
OTU_115	5.27 (0.68)	2.09 (0.25)	4	Yes	Yes	Yes	Ascomycota (phylum)
OTU_125	4.15 (0.59)	3.37 (0.44)	5	Yes	No	Yes	Fungi (kingdom)
OTU_84	2.21 (0.44)	5.72 (0.61)	8	Yes	No	Yes	Neoascochyta (genus)
OTU_118	1.74 (0.33)	5.91 (0.50)	9	Yes	No	Yes	Alternaria infectoria (species)
OTU_331	0.88 (0.12	6.38 (0.51)	10	Yes	No	Yes	Dioszegia hungarica (species)
OTU_338	0.80 (0.12)	6.21 (0.53)	11	Yes	No	Yes	Basidiomycota (phylum)
OTU_333	0.75 (0.16)	5.68 (0.51)	12	Yes	No	Yes	Sporobolomyces ruberrimus (species)
OTU_346	0.70 (0.13)	5.58 (0.47)	13	Yes	No	Yes	Fungi (kingdom)
OTU_359	0.60 (0.07)	5.45 (0.54)	16	Yes	No	Yes	Pleosporales (order)
OTU_332	0.36 (0.08)	3.56 (0.63)	20	Yes	No	Yes	Filobasidium (genus)
OTU_360	0.27 (0.04)	5.01 (0.52)	27	Yes	No	Yes	Mycosphaerellaceae (family)
Flowers (19)							
OTU_21	32.30 (5.16)	NS	1	Yes	No	No	T. washingtonensis (species)
OTU_115	22.33 (3.35)	NS	2	Yes	Yes	No	Ascomycota (phylum)
OTU_27	10.80 (2.24)	2.07 (0.49)	3	Yes	No	Yes	B. alba (species)
OTU_174	8.18 (1.99)	NS	4	Yes	No	No	Epicoccum (genus)
OTU_3520	3.22 (2.26)	NS	5	No	No	No	T. washingtonensis (species)
OTU_333	1.43 (0.31)	2.53 (0.68)	8	Yes	No	Yes	S. ruberrimus (species)

[a] The number of samples used for these analyses differed across plant compartments due to removal of samples with low sequence counts. Relative abundance, as percent of rarefied reads, was averaged across all samples. Column Log_2 FC indicates the log_2-fold change in read count between plant compartment and bulk soil for OTUs that were enriched significantly (Benjamini and Hochberg adjusted $P < 0.1$), calculated with DESeq2 on unrarefied read counts. NS indicates nonstatistically significant enrichment. NA indicates measures that are not applicable for the bulk soil. Bold values are used for emphasis. OTU taxonomy is given as the highest classification level determined from the UNITE database.

[b] Plant compartment (number of samples) and OTU ID. [c] Percent relative abundance and standard error (SE).

bacterial OTUs in the bulk and rhizosphere soil communities were highly similar, and none of the top-abundance rhizosphere taxa were enriched significantly relative to bulk soil (DESeq2, $P > 0.1$) (Table 2). Of the OTUs observed in root tissue, the most enriched was a member of the family Halieaceae (OTU.17; $\log_2$-fold change = 8.62 ± 0.72). This OTU had very low abundance in the bulk soil (and was below the detection limit in multiple samples) but

was the second most abundant within root tissue (4.94 ± 1.33%). Similarly, an OTU classified as Lactococcus lactis (OTU.4) was highly enriched on the flowers compared with soil ($\log_2$-fold change = 9.92 ± 0.72) and highly abundant on the flowers (11.67 ± 4.41%).

The top five most abundant fungal OTUs found in leaves were also enriched significantly compared with their relative abundancein bulk soil; however, only one from the flowers was enriched significantly. The top five most abundant fungal OTUs had similar abundance in bulk and rhizosphere soils, with only one highabundance rhizosphere taxon, OTU_21 (classified as Tilletiopsis washingtonensis), enriched significantly in the rhizosphere relative to bulk soil ($\log_2$-fold change = 2.85 ± 0.50) (Table 3). Of the top five most abundant fungal OTUs found on the leaves and flowers, four are shared between the two plant compartments. These include OTUs matching T. washingtonensis (OTU_21) which is found widely throughout the hemp samples, an OTU matching Bullera alba (OTU_27), an unclassified Epicoccum sp. (OTU_174), and an unclassified Ascomycota sp. (OTU_115).

The core microbiome of hemp. We looked for both bacterial and fungal OTUs that may be part of a core microbiome of hemp. We defined a member of the core microbiome for a given plant compartment as an OTU that was ubiquitous across all plants in all sampling locations and enriched significantly in their plant compartment relative to the bulk soil.

We identified 20 candidate core bacterial OTUs across our hemp plants, all of which were identified in the root tissue, on the leaf surface, or in the flowers (Table 2). There were six core bacterial OTUs from the root tissue, all of which were members of the phylum Proteobacteria. There were 11 core bacterial OTUs from the leaf surface, made up of members of the Proteobacteria, Actinobacteria, and Bacteroidetes. In all, 9 of the 11 most abundant OTUs on the leaves were part of this core group. The two most abundant of these OTUs (OTU.8 and OTU.5, representing 13.14 ± 1.88 and 12.81 ± 1.99% of the leaf bacterial community, respectively) were classified in the genus Sphingomonas. There were seven core bacterial OTUs from the flowers, made up of members of the Proteobacteria and Firmicutes. Three of the core flower OTUs were also core leaf OTUs (OTU.5, OTU.8, and OTU.9). OTU.8 was a core OTU in the root tissue, leaf surface, and flower. None of the ubiquitous bacterial OTUs in the rhizosphere were enriched significantly in the rhizosphere compared with the bulk soil.

When we applied our core microbiome definition to fungal OTUs, we found 14 candidate core OTUs (Table 3). All core fungal OTUs were classified as Basidiomycota or Ascomycota, or unclassified at the phylum level. We found only one core fungal OTU in the rhizosphere soil, OTU_27, classified as B. alba. This OTU was also a core OTU on the leaves and in the flowers. There were 14 core fungal OTUs found on the leaves, including all of the top 5 most abundant OTUs. By far the most abundant core fungal OTU on the leaves was OTU_21, classified as T. washingtonensis, making up 36.25 ± 3.11% of the leaf fungal community. This OTU was also highly abundant (32.30 ± 5.16%) and ubiquitous in the flowers, though not significantly enriched in that compartment compared with the bulk soil. There were only two core fungal OTUs found in the flowers, both of which were also core on the leaves.

Bacteria and fungi enriched significantly in the rhizosphere compared with bulk soil. We found little evidence for a core hemp rhizosphere microbiome, with only one core fungal OTU identified. Furthermore, for the most part, the top

five most abundant bacterial and fungal OTUs were shared between the rhizosphere and bulk soil (Tables 1 and 2). To expand our search for bacterial and fungal OTUs that may be enriched in the hemp rhizosphere, we used DESeq2 to look for OTUs that were enriched significantly relative to the bulk soil but not necessarily ubiquitous or highly abundant in the rhizosphere. We found only one bacterial and two fungal OTUs enriched significantly in the rhizosphere. The bacterial OTU was classified in the family Pseudonocardiaceae (OTU.79; $\log_2$-fold change = 1.49 ± 0.29, P = 0.06). The two fungal OTUs matched T. washingtonensis (OTU_21; $\log_2$-fold change = 2.85 ± 0.50, P = 2.65E-5) and B. alba (OTU_27; DESeq2 $\log_2$-fold change = 2.43 ± 0.41, P = 2.65E-5). These two fungal OTUs were also identified within the core microbiome of leaves and flowers.

DISCUSSION

Characterizing the microbes associated with an economically valuable crop is an important first step in understanding how the interactions between the plant and its microbiome influence crop yield and health. Here, we examined the bacteria and fungi

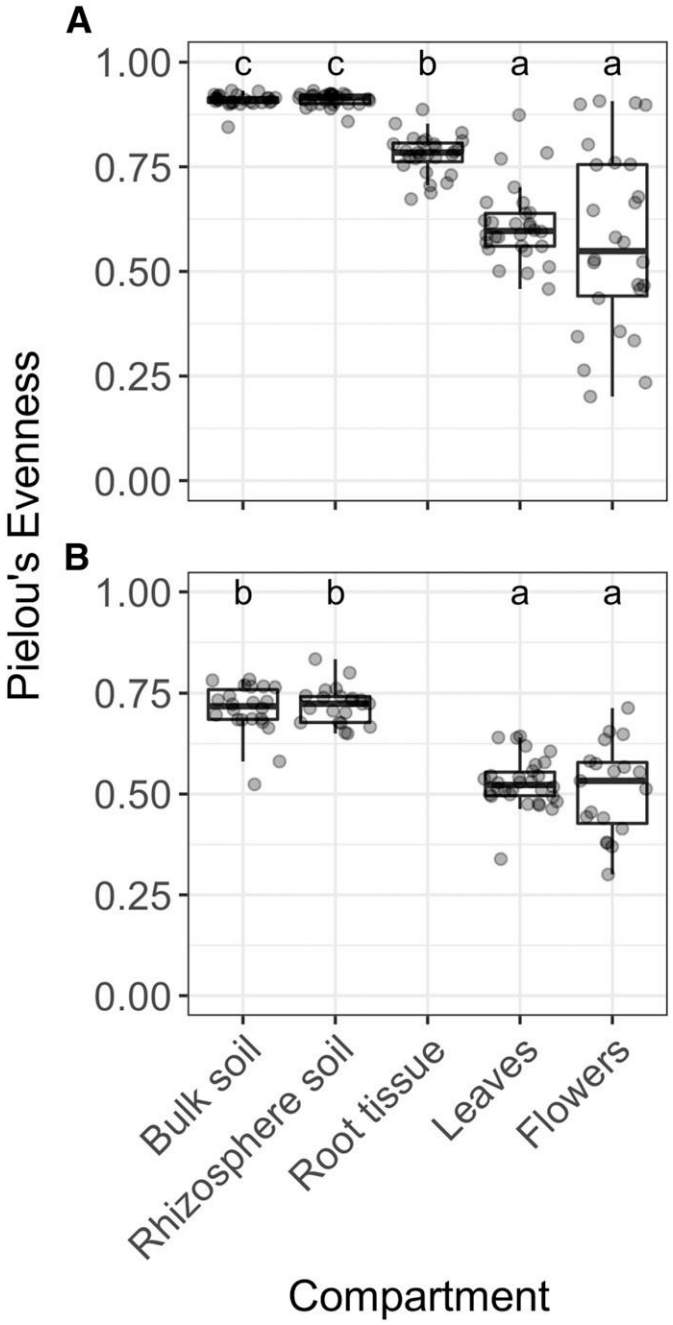

Fig. 4. Pielou's evenness for A, bacterial and B, fungal communities varies significantly across plant compartments (analysis of variance; bacterial: df = 4, F statistic = 60.657, P < 0.001; fungal: df = 3, F statistic = 53.059, P < 0.001). Post hoc Tukey tests show that, in both cases, leaves and flowers have lower species evenness than bulk and rhizosphere soils. Pairwise comparison grouping among plant compartment is indicated by lowercase letters.

associated with the rhizosphere, root tissue, leaf surface, and flowers of the hemp cultivar Anka. We identified the dominant taxa across plant compartments and characterized the core microbiome of this cultivar. We found that plant compartment (rhizosphere, root, leaf, or flower) had strong effects on the bacterial and fungal taxa present (Figs. 1, 2, and 3). We observed that the

OTUs most highly abundant in the roots, leaves, and flowers occurred at low abundance in soils, suggesting selective enrichment within these plant compartments (Tables 1 and 2). The top five most abundant taxa also made up a greater proportion of the community in the roots, leaves, and flowers than the top most abundant taxa in the bulk or rhizosphere soils. This observation suggests that the root tissue and the phyllosphere are dominated by few highly abundant OTUs, which was further confirmed by the low evenness in these plant compartments (Fig. 4). Reduced microbial community evenness in plant compartments has been documented in other plant species (Bodenhausen et al. 2013).

We also found that field site explained significant variation in community composition within each plant compartment. The field site effect was strongest for bulk and rhizosphere soil communities. It is likely that a number of factors drive this site-specific variation, particularly soil chemistry. Although we did not have the data resolution to tease apart how soil chemistry may influence aboveand belowground microbial communities associated with hemp, we note that most of our sites differed in their soil type (Table 1). Our knowledge of the hemp microbiome would benefit from studies linking environmental factors to microbial community composition, especially factors related to management practices such as tillage and fertilization. Altogether, these findings suggest that, although local environmental factors can influence the hemp microbiome, certain microbes are highly enriched in hemp regardless of field site. We did not observe any relationship between bacterial or fungal community composition and crop yield across field sites.

We found little evidence for a core microbiome in the hemp rhizosphere when we examined either the top five most abundant OTUs or ubiquitous OTUs in the rhizosphere soils. Only one fungal OTU was determined to be a core OTU by our definition (OTU_27). We expanded our search to look at all OTUs that were enriched significantly in the rhizosphere soil compared with the bulk soil. Only one bacterial OTU and two fungal OTUs were enriched significantly in the rhizosphere when compared with bulk soil. OTU.79 was classified as an Actinobacteria within the family Pseudonocardiaceae and, when aligned to the NCBI RefSeq database, matched genus Lechevalieria (BLAST, 100% identity). This genus includes species commonly observed in soil and rhizosphere habitats. The two fungal OTUs (OTU.21 and OTU.27) enriched in the rhizosphere were classified as T. washingtonensis and B. alba, respectively. These two OTUs were also ubiquitous and enriched significantly, relative to bulk soil, on hemp leaves (both OTUs) and flowers (OTU_27). These observations suggest that these fungi have high affinity for hemp. Most bacterial and fungal OTUs that were observed widely in the hemp rhizosphere were also ubiquitous in the bulk soil and were not enriched in rhizosphere relative to the bulk soil (Tables 2 and 3). A number of the microbes that were highly abundant in the rhizosphere belong to taxa commonly found in plants; however, we only included in the core hemp microbiome those OTUs that were enriched significantly in the hemp rhizosphere relative to bulk soil. Altogether, our results suggest that hemp does not have a strong immediate effect on overall microbial community composition in the rhizosphere. This result is surprising because plants are widely known to significantly influence their rhizosphere microbial community (Berendsen et al. 2012). However, environmental factors (Peiffer et al. 2013), particularly agricultural practices (Schmidt et al. 2019), influence strength of plant effect on rhizosphere microbiome composition. Other cultivars of C. sativa have previously displayed weak effects on their rhizosphere microbiomes, suggested to be due to root decay (Winston et al. 2014). Alternative reasons for an apparent weak plant effect may include the plant development stage at sampling or root structure and rhizosphere sampling method. Future studies should more closely examine the influence of hemp on rhizosphere microbial composition, including analysis of root exudates and community succession over hemp growth stages.

T. washingtonensis is a widespread yeast-like fungus often isolated from the phyllosphere of plants (Urquhart and Punja 2002). It has been studied as a biocontrol agent against powdery mildew (Urquhart and Punja 2002; Urquhart et al. 1994); however, it has also been suggested as the causative agent of white haze on apple fruit (Baric et al. 2010). B. alba is another widespread yeast-like fungus with at least one isolate found to produce antifungal compounds (Golubev et al. 1997). Although these fungi have not been studied in the context of hemp, their rhizosphere enrichment and characterization as dominant members of the microbiome of hemp leaves and flowers makes them intriguing targets for further study in this system. Their potential as biocontrol agents of common diseases such as powdery mildew, a disease present on C. sativa (McPartland and Cubeta 1997; Weldon et al. 2020), may implicate these fungi as factors of disease resistance in this crop.

In our characterization of the core root microbiome of hemp, we found evidence for further plant growth or health-promoting microbes in close association with the plants. From the root tissue, we found six core OTUs. One of these, OTU.49, matched species within the family Comamonadaceae, genus Variovorax (BLAST to NCBI RefSeq, 100% identity with NR_114214.1, NR_169353.1, NR_169352.1, NR_041588.1, and NR_112562.1). Members of this genus are common in soils and some species are associated with plant growth promotion (Han et al. 2011; Schmalenberger et al. 2008). OTU.18 was classified within the Oxalobacteraceae genus Massilia. Species of this genus have been implicated as mediators of plant growth promotion in numerous plant species (Ofek et al. 2012). OTU.117, classified to the Burkholderiales genus Aquabacterium, have been detected in high abundance in the rhizosphere of numerous plant species (Bai et al. 2020; Schmalenberger and Tebbe 2003; Wang et al. 2014) as well as ground and drinking water (Guo et al. 2019; Kalmbach et al. 2000). Similarly, relatives of the other three core root OTUs classified to genera Rhizobium, Pseudomonas, or Sphingomonas have been widely found to be associated with plants in various ways. Although not a core bacterium by our definition because it was not ubiquitous in hemp roots, OTU.17, classified within the family Halieaceae, was very highly enriched in the root tissue of many hemp plants. Its high enrichment in this particular plant compartment suggests an interaction between the bacterium and hemp roots. To the best of our knowledge, little is known about members of this family in terms of plant interactions and, therefore, further study of this group may be of particular interest. Due to a limitation of experimental design, we were unable to examine fungi in close association with the root tissue; however, future studies using methods to remove host DNA prior to sequencing may identify fungi in close association with hemp roots.

Our analysis of the core phyllosphere bacterial OTUs of hemp revealed well-known phyllosphere organisms, including microbes known to promote plant growth and disease resistance. Of the 11 core leaf bacterial OTUs, the top five most abundant were classified to the genera Sphingomonas, Methylobacterium, and Hymenobacter. These genera are widely observed in plant phyllospheres (Delmotte et al. 2009; Grady et al. 2019; Rastogi et al. 2013; Turner et al. 2013; Vorholt 2012; Wellner et al. 2011). Certain Sphingomonas strains have been observed to confer resistance to the leaf pathogen Pseudomonas syringae (Innerebner et al. 2011). Similarly, numerous studies have observed that Methylobacterium spp. can promote plant growth and yield as a result of methanol cycling (Abanda-Nkpwatt et al. 2006) and the production of auxins (Ivanova et al. 2001), cytokinins (Ivanova et al. 2000), and vitamins (Trotsenko et al. 2001).

The community composition on hemp flowers varied widely among plants. The hemp flower is a dynamic environment, with structural and chemical changes occurring as the plant ages and interacts with pollinators. This dynamic environment may play a role in the high variability observed across plants. Despite this variability, we identified seven core bacterial OTUs in the flower. Some taxa discussed previously included the genera

Sphingomonas and Methylobacterium. We also found two members of the genus Bacillus, one of which was classified to the species Bacillus cereus. Although this genus is widespread in soil and plant microbiomes, B. cereus strains found in the phyllosphere have been shown to promote plant growth and pathogen resistance (Kloepper et al. 2004; Saleem et al. 2017). OTU.13 was classified to the genus Ralstonia, a notable genus for containing a number of prominent plant pathogens. This particular OTU, however, matches isolates of Ralstonia pickettii (BLAST to NCBI RefSeq, 100% identity with NR_114126.1, NR_043142.1, and NR_113352.1). Strains of this species have been proposed as a biocontrol agent against diseasecausing Ralstonia spp. (Wei et al. 2013). Although not a core OTU by our definition, one of the bacterial OTUs with the greatest enrichment in the flower samples relative to the bulk soil was OTU.4, classified as Lactococcus lactis. The genus Lactococcus includes fermentative aerotolerant anaerobes, which are often observed on plants and which are essential for the production of fermented plant products (Di Cagno et al. 2013). Because many of the phytochemicals of interest (e.g., cannabinoids) from hemp are produced in the flowers, these highly abundant Lactococcus spp. as well as the core bacterial OTUs discussed here may play a role in phytochemical yield and quality.

Most fungal OTUs of interest in the phyllosphere were shared between leaves and flowers. These shared core fungal OTUs included T. washingtonensis and Bullera alba (discussed previously). A leaf core fungal member, OTU_174, classified to the genus Epicoccum, was also highly abundant and ubiquitous in the flower community, though not significantly enriched in that compartment. The genus Epicoccum is often observed in soils and in the plant phyllosphere and some strains promote disease resistance in grapevines (Del Frari et al. 2019). OTU_118, a core fungal OTU on the leaf, was classified as Alternaria infectoria (OTU_118). This species, along with close relatives, has been shown to cause diseases such as leaf blight in wheat and other grains (Perello and Sisterna´ 2006; Prasada and Prabhu 1962). A. infectoria can also cause cutaneous diseases in humans, usually associated with immunocompromised individuals (Dubois et al. 2005; Lopes et al. 2013). The effect of this fungal species on hemp or hemp products has yet to be determined; however, isolates of the Alternaria genus have been isolated from hemp cultivar Anka leaves in other studies (Scott et al. 2018). We also identified a core leaf fungal OTU classified to the genus Neoascochyta (OTU_84). Members of this genus are widely found associated with plants and include plant pathogens, particularly for grasses (Chen et al. 2017; Golzar et al. 2019).

Our results show that numerous microbes are enriched within the roots, leaves, and flowers of hemp. Many of the microbes enriched on hemp plants are observed widely on various plant species and are linked to plant growth promotion and disease resistance, while a few have been implicated as possible plant pathogens, though not necessarily in C. sativa. Future research should include more

C. sativa cultivars to understand cultivar-specific variability of the microbiome. Rhizosphere microbiome variability has previously been observed across cultivars of C. sativa with high THC content (Winston et al. 2014) and those of varying THC and cannabidiol levels (Comeau et al. 2020). Field trials connecting the microbiome communities and crop yields or disease resistance should also be a focus for future research.

The microbes found within plant compartments and the mechanisms by which plants enrich for their microbiomes vary significantly across time and across plant growth stages (Copeland et al. 2015; Walters et al. 2018). The rhizosphere and endosphere of C. sativa cultivars have been shown to vary across plant growth stages as well (Comeau et al. 2020). Because our study determined microbiome composition at flowering, we may be missing core hemp microbiome members important at other stages. Future studies aimed at further elucidating the core hemp microbiome would benefit from more time points at other key stages of growth. As we show here, some members of the hemp microbiome are similar to those that promote

growth in other plant species. Specifically, understanding how the microbiome composition influences crop yield parameters such as fiber or grain quantity or quality and phytochemical production may lead to better cultivation practices. Modulation to the hemp microbiome to aid in fiber processing, specifically retting, has been proposed (Law et al. 2020). Similarly, microbes found on the hemp flowers may play a role in quality of the phytochemicals (Taghinasab and Jabaji 2020) and may be important for preventing contamination by opportunistic human pathogens. Identifying microbiome members with specific disease resistance capabilities allows for development of new sustainable biocontrol agents. As cultivation of hemp becomes more widespread, there will be many opportunities to study and improve upon our understanding of the diverse microbiome of this valuable crop.

ACKNOWLEDGMENTS

We thank S. Higgins for advice on development of the fungal ITS1 processing pipeline and H. Lange and M. Bekauri for technical assistance in collecting and sampling hemp plants.

LITERATURE CITED

115th Congress. 2018. H.R.2 – Agriculture Improvement Act of 2018. https://www.congress.gov/bill/115th-congress/house-bill/2

Abanda-Nkpwatt, D., Musch, M., Tschiersch, J., Boettner, M., and Schwab, W.¨ 2006. Molecular interaction between Methylobacterium extorquens and seedlings: Growth promotion, methanol consumption, and localization of the methanol emission site. J. Exp. Bot. 57:4025-4032.

Bai, Y., Ren, P., Feng, P., Yan, H., and Li, W. 2020. Shift in rhizospheric and endophytic bacterial communities of tomato caused by salinity and grafting. Sci. Total Environ. 734:139388.

Baric, S., Lindner, L., Marschall, K., and Dalla Via, J. 2010. Haplotype diversity of Tilletiopsis spp. causing white haze in apple orchards in Northern Italy.
Plant Pathol. 59:535-541.

Barnett, S. 2020. seb369/hemp_microbiome: First release of hemp microbiome project data. https://zenodo.org/record/4001760

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe,
A., De Wit, P., Sanchez-Garc´´ıa, M., Ebersberger, I., de Sousa, F., Amend, A. S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K.,
Bertrand, Y. J. K., Sanli, K., Eriksson, K. M., Vik, U., Veldre, V., and Nilsson, R. H. 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4: 914-919.

Berendsen, R. L., Pieterse, C. M. J., and Bakker, P. A. H. M. 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17: 478-486.

Bodenhausen, N., Horton, M. W., and Bergelson, J. 2013. Bacterial communities associated with the leaves and the roots of Arabidopsis thaliana. PLoS One 8:
e56329.

Boller, T. 1995. Chemoperception of microbial signals in plant cells. Annu. Rev. Plant Physiol. 46:189-214.

Busby, P. E., Soman, C., Wagner, M. R., Friesen, M. L., Kremer, J., Bennett, A., Morsy, M., Eisen, J. A., Leach, J. E., and Dangl, J. L. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. PLoS Biol. 15:e2001793.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Turnbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7:335-336.

Chen, Q., Hou, L. W., Duan, W. J., Crous, P. W., and Cai, L. 2017. Didymellaceae revisited. Stud. Mycol. 87:105-159.

Cherney, J. H., and Small, E. 2016. Industrial hemp in North America: Production, politics and potential. Agronomy (Basel) 6:58.

Colombo, C., Palumbo, G., He, J.-Z., Pinton, R., and Cesco, S. 2014. Review on iron availability in soil: Interaction of Fe minerals, plants, and microbes. J. Soils Sediments 14:538-548.

Comeau, D., Novinscak, A., Joly, D. L., and Filion, M. 2020. Spatio-temporal and cultivar-dependent variations in the cannabis microbiome. Front. Microbiol. 11:491.

Copeland, J. K., Yuan, L., Layeghifard, M., Wang, P. W., and Guttman, D. S. 2015. Seasonal community succession of the phyllosphere microbiome. Mol. Plant-Microbe Interact. 28:274-285.

de Cassia Silva, U., de Oliveira, C. A., de Paula Lana, U. G., Gomes, E. A., and´ dos Santos, V. L. 2016. Growth promotion features of the maize microbiome: From an agriculture perspective. Pages 345-374 in: Plant-Microbe Interaction: An Approach to Sustainable Agriculture. D. K. Choudhary, A. Varma, and N. Tuteja, eds. Springer Singapore, Singapore.

Del Frari, G., Cabral, A., Nascimento, T., Boavida Ferreira, R., and Oliveira, H. 2019. Epicoccum layuense a potential biological control agent of escaassociated fungi in grapevine. PLoS One 14:e0213273.

Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., von Mering, C., and Vorholt, J. A. 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. Proc. Natl. Acad. Sci. U.S.A. 106:16428-16433.

Di Cagno, R., Coda, R., De Angelis, M., and Gobbetti, M. 2013. Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiol. 33: 1-10.

Dubois, D., Pihet, M., Le Clec'h, C., Croue, A., Beguin, H., Bouchara, J. P., and´ Chabasse, D. 2005. Cutaneous phaeohyphomycosis due to Alternaria infectoria. Mycopathologia 160:117-123.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460-2461.

Edwards, J., Johnson, C., Santos-Medell´ın, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., Eisen, J. A., and Sundaresan, V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc. Natl. Acad. Sci. U.S.A. 112:E911-E920.

Franche, C., Lindstrom, K., and Elmerich, C. 2009. Nitrogen-¨ fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35-59.

Gautam, A. K., Kant, M., and Thakur, Y. 2013. Isolation of endophytic fungi from Cannabis sativa and study their antifungal potential. Arch. Phytopathol. Plant Prot. 46:627-635.

Golubev, W., Ikeda, R., Shinoda, T., and Nakase, T. 1997. Antifungal activity of Bullera alba (Hanna) Derx. Mycoscience 38:25-29.

Golzar, H., Thomas, G., Jayasena, K. W., Wright, D., Wang, C., and Kehoe, M.
2019. Neoascochyta species cause leaf scorch on wheat in Australia. Australas. Plant Dis. Notes 14:1.

Grady, K. L., Sorensen, J. W., Stopnisek, N., Guittar, J., and Shade, A. 2019. Assembly and seasonality of core phyllosphere microbiota on perennial biofuel crops. Nat. Commun. 10:4135.

Guo, L., Wang, G., Sheng, Y., Shi, Z., and Sun, X. 2019. Groundwater microbial communities and their connection to hydrochemical environment in Golmud, Northwest China. Sci. Total Environ. 695:133848.

Gyaneshwar, P., Naresh Kumar, G., Parekh, L. J., and Poole, P. S. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83-93.

Han, J.-I., Choi, H.-K., Lee, S.-W., Orwin, P. M., Kim, J., LaRoe, S. L., Kim, T., O'Neil, J., Leadbetter, J. R., Lee, S. Y., Hur, C., Spain, J. C., Ovchinnikova, G., Goodwin, L., and Han, C. 2011. Complete genome sequence of the metabolically versatile plant growth-promoting endophyte Variovorax paradoxus S110. J. Bacteriol. 193:1183-1190.

Hothorn, T., Bretz, F., and Westfall, P. 2008. Simultaneous inference in general parametric models. Biometrical J. 50:346-363.

Innerebner, G., Knief, C., and Vorholt, J. A. 2011. Protection of Arabidopsis thaliana against leaf-pathogenic Pseudomonas syringae by Sphingomonas strains in a controlled model system. Appl. Environ. Microbiol. 77: 3202-3210.

Ivanova, E. G., Doronina, N. V., Shepelyakovskaya, A. O., Laman, A. G., Brovko, F. A., and Trotsenko, Y. A. 2000. Facultative and obligate aerobic Methylobacteria synthesize cytokinins. Microbiology 69:646-651.

Ivanova, E. G., Doronina, N. V., and Trotsenko, Y. A. 2001. Aerobic Methylobacteria are capable of synthesizing auxins. Microbiology 70: 392-397.

Kalmbach, S., Manz, W., Bendinger, B., and Szewzyk, U. 2000. In situ probing reveals Aquabacterium commune as a widespread and highly abundant bacterial species in drinking water biofilms. Water Res. 34:575-581.

Kloepper, J. W., Ryu, C.-M., and Zhang, S. 2004. Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94: 1259-1266.

Koechli, C., Campbell, A. N., Pepe-Ranney, C., and Buckley, D. H. 2019. Assessing fungal contributions to cellulose degradation in soil by using highthroughput stable isotope probing. Soil Biol. Biochem. 130:150-158.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79:5112-5120.

Kuklinsky-Sobral, J., Araujo, W. L., Mendes, R., Geraldi, I. O., Pizzirani-´ Kleiner, A. A., and Azevedo, J. L. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ. Microbiol. 6:1244-1251.

Kusari, P., Kusari, S., Spiteller, M., and Kayser, O. 2013. Endophytic fungi harbored in Cannabis sativa L.: Diversity and potential as biocontrol agents against host plant-specific phytopathogens. Fungal Divers. 60:137-151.

Law, D. A., McNees, R. C., and Moe, A. L. 2020. The microbiology of hemp retting in a controlled environment: Steering the hemp microbiome towards more consistent fiber production. Agronomy (Basel) 10:492.

Lievens, K. H., van Rijsbergen, R., Leyns, F. R., Lambert, B. J., Terming, P., Swings, J., and Joos, H. J.-P. 1989. Dominant rhizosphere bacteria as a source for antifungal agents. Pestic. Sci. 27:141-154.

Lopes, L., Borges-Costa, J., Soares-Almeida, L., Filipe, P., Neves, F., Santana, A., Guerra, J., and Kutzner, H. 2013. Cutaneous alternariosis caused by Alternaria infectoria: Three cases in kidney transplant patients. Health Care (Don Mills) 1:100-106.

Love, M. I., Huber, W., and Anders, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15:550.

McKernan, K., Spangler, J., Zhang, L., Tadigotla, V., Helbert, Y., Foss, T., and Smith, D. 2015. Cannabis microbiome sequencing reveals several mycotoxic fungi native to dispensary grade Cannabis flowers. F1000 Res. 4:1422.

McMurdie, P. J., and Holmes, S. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8: e61217.

McPartland, J. M., and Cubeta, M. A. 1997. New species, combinations, host associations and location records of fungi associated with hemp (Cannabis sativa). Mycol. Res. 101:853-857.

Ofek, M., Hadar, Y., and Minz, D. 2012. Ecology of root colonizing Massilia (Oxalobacteraceae). PLoS One 7:e40117.

Ogle, D. H., Wheeler, P., and Dinno, A. 2020. FSA: Fisheries stock analysis. https://github.com/droglenc/FSA

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. 2018. vegan: Community Ecology Package. https://cran.r-project.org/web/packages/vegan/index.html

Ort´ız-Castro, R., Contreras-Cornejo, H. A., Mac´ıas-Rodr´ıguez, L., and Lopez-´ Bucio, J. 2009. The role of microbial signals in plant growth and development. Plant Signal. Behav. 4:701-712.

Palmer, J. M., Jusino, M. A., Banik, M. T., and Lindner, D. L. 2018. Nonbiological synthetic spike-in controls and the AMPtk software pipeline improve mycobiome data. PeerJ 6:e4925.

Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., Buckler, E. S., and Ley, R. E. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. Proc. Natl. Acad. Sci. U.S.A. 110: 6548-6553.

Perello, A. E., and Sisterna, M. N. 2006. Leaf blight of wheat caused by´ Alternaria triticina in Argentina. Plant Pathol. 55:303.

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., and Bakker, P. A. H. M. 2014. Induced systemic resistance by beneficial microbes. Annu. Rev. Phytopathol. 52:347-375.

Prasada, R., and Prabhu, A. S. 1962. Leaf blight of wheat caused by a new species of Alternaria. Indian Phytopathol. 15:292-293.

Rastogi, G., Coaker, G. L., and Leveau, J. H. J. 2013. New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. FEMS Microbiol. Lett. 348:1-10.

R Core Team. 2018. R: A Language and Environment for statistical Computing. The R Foundation, Vienna, Austria. https://www.r-project.org/

Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahe, F. 2016. VSEARCH:´ A versatile open source tool for metagenomics. PeerJ 4:e2584.

Saleem, M., Meckes, N., Pervaiz, Z. H., and Traw, M. B. 2017. Microbial interactions in the phyllosphere increase plant performance under herbivore biotic stress. Front. Microbiol. 8:41.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., and Weber, C. F. 2009. Introducing mothur: Open-source, platform-independent, communitysupported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75:7537-7541.

Schmalenberger, A., Hodge, S., Bryant, A., Hawkesford, M. J., Singh, B. K., and Kertesz, M. A. 2008. The role of Variovorax and other Comamonadaceae in sulfur transformations by microbial wheat rhizosphere communities exposed to different sulfur fertilization regimes. Environ. Microbiol. 10:1486-1500.

Schmalenberger, A., and Tebbe, C. C. 2003. Bacterial diversity in maize rhizospheres: Conclusions on the use of genetic profiles based on PCRamplified partial small subunit rRNA genes in ecological studies. Mol. Ecol. 12:251-262.

Schmidt, J. E., Kent, A. D., Brisson, V. L., and Gaudin, A. C. M. 2019. Agricultural management and plant selection interactively affect rhizosphere microbial community structure and nitrogen cycling. Microbiome 7:146.

Scott, M., Rani, M., Samsatly, J., Charron, J. B., and Jabaji, S. 2018. Endophytes of industrial hemp (Cannabis sativa L.) cultivars: Identification of culturable bacteria and fungi in leaves, petioles, and seeds. Can. J. Microbiol. 64: 664-680.

Small, E., and Cronquist, A. 1976. A practical and natural taxonomy for Cannabis. Taxon 25:405-435.

Taghinasab, M., and Jabaji, S. 2020. Cannabis microbiome and the role of endophytes in modulating the production of secondary metabolites: An overview. Microorganisms 8:355.

Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., Onoda, Y., Yoshida, K., Schlaeppi, K., Bai, Y., Sugiura, R., Ichihashi, Y., Minamisawa, K., and Kiers, E. T. 2018. Core microbiomes for sustainable agroecosystems. Nat. Plants 4:247-257.

Trotsenko, Y. A., Ivanova, E. G., and Doronina, N. V. 2001. Aerobic methylotrophic bacteria as phytosymbionts. Microbiology 70:623-632.

Turner, T. R., James, E. K., and Poole, P. S. 2013. The plant microbiome. Genome Biol. 14:209.

Urquhart, E. J., Menzies, J. G., and Punja, Z. K. 1994. Growth and biological control activity of Tilletiopsis species against powdery mildew (Sphaerotheca fuliginea) on greenhouse cucumber. Phytopathology 84:341-351.

Urquhart, E. J., and Punja, Z. K. 2002. Hydrolytic enzymes and antifungal compounds produced by Tilletiopsis species, phyllosphere yeasts that are antagonists of powdery mildew fungi. Can. J. Microbiol. 48:219-229.

Vorholt, J. A. 2012. Microbial life in the phyllosphere. Nat. Rev. Microbiol. 10: 828-840.

Wallace, J. G., Kremling, K. A., Kovar, L. L., and Buckler, E. S. 2018. Quantitative genetics of the maize leaf microbiome. Phytobiomes J. 2:208-224.

Walters, W. A., Jin, Z., Youngblut, N., Wallace, J. G., Sutter, J., Zhang, W., Gonzalez-Peña, A., Peiffer, J., Koren, O., Shi, Q., Knight, R., Glavina del Rio,´ T., Tringe, S. G., Buckler, E. S., Dangl, J. L., and Ley, R. E. 2018. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. Proc. Natl. Acad. Sci. U.S.A. 115:7368-7373.

Wang, H., Wang, S. D., Jiang, Y., Zhao, S. J., and Chen, W. X. 2014. Diversity of rhizosphere bacteria associated with different soybean cultivars in two soil conditions. Soil Sci. Plant Nutr. 60:630-639.

Wei, Z., Huang, J., Tan, S., Mei, X., Shen, Q., and Xu, Y. 2013. The congeneric strain Ralstonia pickettii QL-A6 of Ralstonia solanacearum as an effective biocontrol agent for bacterial wilt of tomato. Biol. Control 65:278-285.

Weiss, S., Xu, Z. Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone,C., Zaneveld, J. R., Vazquez-Baeza, Y., Birmingham, A., Hyde, E.´ R., and Knight, R. 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5:27.

Weldon, W. A., Ullrich, M. R., Smart, L. B., Smart, C. D., and Gadoury, D. M.
2020. Cross-infectivity of powdery mildew isolates originating from hemp (Cannabis sativa) and Japanese hop (Humulus japonicus) in New York. Plant Health Prog. 21:47-53.

Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.

Wellner, S., Lodders, N., and Kampfer, P. 2011. Diversity and biogeography of¨ selected phyllosphere bacteria with special emphasis on Methylobacterium spp. Syst. Appl. Microbiol. 34:621-630.

Whiting, S. N., de Souza, M. P., and Terry, N. 2001. Rhizosphere bacteria mobilize Zn for hyperaccumulation by Thlaspi caerulescens. Environ. Sci. Technol. 35:3144-3150.

Winston, M. E., Hampton-Marcell, J., Zarraonaindia, I., Owens, S. M., Moreau, C. S., Gilbert, J. A., Hartsel, J., Kennedy, S. J., and Gibbons, S. M. 2014. Understanding cultivar-specificity and soil determinants of the Cannabis microbiome. PLoS One 9:e99641.

Zhang, J., Kobert, K., Flouri, T., and Stamatakis, A. 2014. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics 30:614-620.