POPULATION BIOLOGY OF *PHOMA BETAE* AND MANAGEMENT STRATEGIES FOR PHOMA LEAF SPOT OF TABLE BEET IN NEW YORK

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Lori Blair Koenick

May 2019

ABSTRACT

Phoma betae, causative agent of seedling, foliar, and root diseases, is an important pathogen of table and sugar beet worldwide. Little is known of the epidemiology and management of diseases caused by *P. betae* in New York (NY) table beet production systems. This research was conducted to understand the population biology of *P. betae* in table beet in NY and evaluate management options for the foliar disease caused by *P. betae,* Phoma leaf spot (PLS). Microsatellite and mating type markers were developed to genotype *P. betae* populations, and population biology analysis was performed to investigate hypotheses concerning pathogen biology and the role of various inoculum sources in PLS epidemics. High genetic diversity and moderate differentiation was observed among populations, along with evidence for a mixed reproductive mode. Development of these genetic tools will facilitate future studies targeting specific epidemiological questions. Field surveys were conducted in the summers of 2017 and 2018 to estimate prevalence, incidence, and severity of PLS epidemics in conventional and organic NY table beet production systems. PLS was found in 35 of 60 fields with incidence up to 31% in affected fields. The prevalence of PLS in organic table beet fields (74%) was higher than in conventional fields (49%). This research also focused on the potential of cultivar resistance to form part of an integrated management strategy for PLS in organic production. Eight popular table beet cultivars were evaluated for susceptibility to PLS and horticultural characteristics in mist chamber and small plot, replicated trials. Non-red cultivars (Avalanche, Boldor, and Chioggia Guardsmark) were consistently less susceptible to PLS than red cultivars (Falcon, Merlin, Red Ace, Rhonda, and Ruby Queen). Information from this research will be used to strengthen durable management strategies for this disease in NY.

BIOGRAPHICAL SKETCH

Lori grew up in Potomac, MD and received her high school diploma from Winston Churchill High School in 2012. She continued her education studying Applied and Environmental Microbiology at West Virginia University and graduated with a Bachelor of Science in May 2016. Throughout her undergraduate studies, Lori expanded her interest in microbiology and plant pathology through various classes, student organizations, and working in research labs. In summer 2014, Lori worked as an undergraduate research assistant in a nematology lab studying how to optimize management methods for dagger nematode populations in peach orchards. Throughout 2015 and 2016, Lori worked as an undergraduate research assistant in a forest pathology lab studying the biocontrol potential of various fungal symbionts of hemlock trees and ambrosia beetles. In summer 2016, Lori joined the lab of Dr. Sarah Pethybridge in the Plant Pathology and Plant-Microbe Biology Section of the School of Integrative Plant Science at Cornell University to complete her master's degree studying the importance of the fungal pathogen, *Phoma betae*, in NY table beet production.

Dedicated to my parents who have always given me the support
and confidence to pursue happiness.

ACKNOWLEDGMENTS

I would like to thank my thesis advisor, Dr. Sarah Pethybridge, for letting me complete my master's thesis in her lab, and her continuous support throughout my time at Cornell. I would also like to say a special thank you to my other committee members, Drs. Steve Reiners and Chris Smart, for their warmth and guidance throughout this process. In addition, I would like to thank all the current and past EVADE lab members for providing continuous encouragement and making the lab such a fun place to come into work every day. Thank you to Dr. Noel Knight for your patience in teaching me how to navigate academia. Thank you to Dr. Niloofar Vaghefi and David Strickland for training on numerous techniques. Thank you to Carol Bowden and Sean Murphy for your positivity and training in how to conduct mist chamber and field trials. Thank you to Adrienne Gorny for always having a listening ear available and support throughout this degree. Thank you to Matt Schwarzkopf for excellent summer assistance with mating type marker development. Thank you to Libby Cieniewicz, Larissa Osterbaan, Anna Wallis, Martha Sudermann, and Ali Cala for being incredible office mates and always keeping the sweets bowl full. Special shout outs to Zachary Stansell, Jane Petzoldt, Jenny Wilson, and Katrin Ayer for endless encouragement and affirmation throughout this degree. Thank you to Cornell Cooperative Extension Vegetable Specialists, Amy Ivy, Crystal Stewart, Elizabeth Buck, and Ethan Grunberg, for allowing me to experience table beet production in New York first hand. A special thank you to Vegetable Specialist, Julie Kikkert, who has taught me much about the vegetable processing industry in New York and has been a supportive collaborator throughout this entire degree. Thank you to Dr. Lindsey du Toit for encouraging collaboration and providing isolates of *P. betae*.

This work would not have been possible without funding from the Towards Sustainability Foundation Organic and Sustainability Grants Program, the New York State Vegetable Growers Association and Council, the United States Department of Agriculture National Institute of Food and Agriculture Federal Capacity project NYG 2018-19-113 and Hatch project NYG-625-424, and a fellowship from the Cornell Graduate School.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Phoma betae (syn. *Neocamarosporium betae*) (Ariyawansa et al. 2015) is an economically important seedborne pathogen of table beet worldwide that is responsible for damping off, foliar disease, and root decay. Increasing yields in table beet production relies upon an understanding of the etiology of priority diseases such as that caused by *P. betae*. The goal of this thesis research is to understand the population biology of *P. betae* and evaluate management options for use in New York (NY) table beet production.

Uses of Table Beet

Table beets (*Beta vulgaris* ssp. *vulgaris*) contain nutritious phytochemical compounds, betalains, and a high amount of inorganic nitrate (Ninfali and Angelino 2013; Clifford et al. 2015, 2017). Table beets are renowned for their production of betalain pigments that are responsible for different colors in the foliage and roots, and the earthy flavor of the root (Goldman and Navazio 2003). From 1980 to 2017, the lowest consumption of processed table beets per capita in the United States was in 2006 (USDA 2018). In 2017, consumption increased 32.5% from 2006 (USDA 2018). This newfound popularity can be attributed to a general increase in vegetable intake and an enhanced awareness of the health benefits offered by table beet consumption (Clifford et al. 2015, 2017; Navazio et al. 2010).

The United States table beet industry currently harvests around 13,000 acres annually for processing into jars or cans, and for fresh market sales of roots and foliage (Reiners et al. 2018;

USDA 2012). Traditionally, table beet greens have also been used in salad mixes (Goldman and Navazio 2003). NY is currently the second largest producer of table beets in the United States producing around 2,500 acres for the processing industry and around 800 acres for the fresh market annually (USDA 2012). NY has an appropriate environment to grow beets with moderate climate, suitable soils, and rainfall. Table beet is a cool season crop that grows best in temperatures between 15.5 and 25°C (Navazio et al. 2010) and soils with good structure that allow for aeration and unrestricted root development, including well-drained sandy and silt loams (Reiners et al. 2018; Stivers 2001). Seed germinates best between 10 and 30°C (Reiners et al. 2018). Additionally, NY receives an average of 117 cm of rain annually spread throughout the cropping season (http://www.nrcc.cornell.edu). However, the relatively mild and wet environmental conditions typical of the NY cropping season are highly conducive for fungal diseases including those caused by *P. betae*.

Host Range and Distribution

Phoma betae is reported to have nearly a worldwide distribution occurring almost everywhere cultivated beets (table, sugar and fodder beet, and Swiss chard) are grown (Harveson et al. 2009; Shoemaker and Bissett 1998). For example, *P. betae* has been reported in all continents except for South America (Shoemaker and Bissett 1998). Historically, there has been a single report of *P. betae* on table beet in NY (Abawi et al. 1986). Leach and McDonald (1976) reported *P. betae* on sugar beet seed in the Pacific Northwest region of the United States, Canada, Italy, France, and Ireland. Byford (1972) reported *P. betae* on sugar beet seed in England. Bugbee (1982) reported *P. betae* on sugar beet in the Upper Midwest of the United States. Du Toit (2007) reported *P. betae* on table beet in Washington State. There have also been recent reports of *P. betae* on table beet in

Italy (Garibaldi et al. 2007; Gilardi et al. 2017). *Phoma betae* has also been reported on other hosts. Bugbee and Soine (1974) reported *P. betae* on lambsquarters (*Chenopodium album* L.). Recently, Avasthi et al. (2013) reported *P. betae* on *Aloe vera* in India and Bassimba et al. (2014) reported *P. betae* on spinach (*Spinacia oleracea* L.) in Spain. Jayanthi et al. (2014) reported *P. betae* as an endophyte on catmint (*Anisomeles malabarica* L.) in India and Kumaran et al. (2012) reported *P. betae* as an endophyte on maiden hair tree (*Ginkgo biloba* L.) in South Korea.

Symptoms

Phoma betae can affect table beet at all stages of development, including seedlings, foliage, and roots (Pethybridge et al. 2018). Root decay caused by *P. betae* may be exacerbated in storage (Bugbee 1982; Bugbee and Soine 1974). Foliar symptoms include tan-brown leaf spots with dark concentric rings (Figure 1.1A to 1.1C) (Pool and McKay 1915). Signs of *P. betae* are pycnidia, asexual fruiting bodies forming the dark concentric rings within the lesion (Figure 1.1B) (du Toit 2007). Phoma root rot typically begins in the center of the crown and spreads downward into the taproot (Figure 1.1D) (Bugbee 1982; Navazio et al. 2010; Harveson et al. 2009). Initial symptoms of root rot include wilting, and small brown depressed spots on the root surface near the crown (Edson 1915). More severe symptoms include black and dry rotted tissue. Phoma root rot symptoms can be mistaken for boron deficiency in sugar beets (Harveson et al. 2009). Pathogenicity mechanisms are relatively unknown for *P. betae*, although it has been reported that *P. betae* produces several non-specific phytotoxins, including betaenone C, capable of inducing necrosis (Sakai et al. 1985).

Figure 1.1 Symptoms of *Phoma betae* on table beet leaves and roots in New York. A) Phoma leaf spot lesion with dark concentric rings; B) Close-up of lesion at 63× magnification with pycnidia (arrows); C) Multiple lesions on a table beet leaf caused by *Phoma betae*; D) Root decay caused by *Phoma betae* surrounding the crown and signs of the pathogen include white fluffy mycelia on dark brown, dry-rot lesions.

Disease caused by *P. betae* can result in substantial losses (Leach and MacDonald 1976; Monte and García-Acha 1988b). For example, lesions on leaves can cause rejection in fresh market sales (Pethybridge et al. 2018). Seedling infection can reduce stand counts (Byford 1972; Pethybridge et al. 2018) and can cause abnormal root shape development (Abawi et al. 1986). Storage rot can reduce sucrose concentrations and result in significant economic losses (Bugbee and Cole 1981; Bugbee 1982; Fugate et al. 2012). *P. betae* has been described as "the most dangerous storage rot pathogen [of sugar beet]" because of its ability to infect beet at multiple stages of development (Bugbee 1982). Plants that are not affected by damping-off may still have latent infections that can lead to root rot after being placed in storage (Edson 1915). Knowledge of the signs and symptoms at the various phenological stages of the host can lead to successful pathogen identification, essential for implementing effective disease management strategies.

Pathogen Biology

Phoma betae has multiple ways of entering fields each growing season, including infected seed and infested soil, and wind and water dispersal (Figure 1.2) (Harveson et al. 2009; Pethybridge et al. 2018; Pool and McKay 1915). Dunning (1972) reported that *P. betae* was the most important seedling pathogen of sugar beet in Europe and conventional fungicide seed treatments were crucial for its control. It has also been reported that *P. betae* can survive internally and/or externally on sugar beet seed (Leach and MacDonald 1976). After field introduction, *P. betae* may persist on infested plant debris in soil for up to 26 months (Bugbee and Soine 1974). During the growing season, *P. betae* commonly spreads through rain-splash of conidia produced in pycnidia, asexual fruiting bodies, likely resulting in polycyclic epidemics (Harveson et al. 2009; Leach and MacDonald 1976). *P. betae* is capable of sexual reproduction, producing pseudothecia that release ascospores that can be dispersed long distances via wind (Bugbee 1979). The teleomorph (*Pleospora betae* (Berl.) Nevodovosky, syn = *Phoma bjoerlingii* Byford) is not commonly observed in the field, but most likely occurs in autumn on infested leaf debris (Bugbee 1979). The teleomorph has been observed on sugar beet seed stalks, allowing the pathogen to survive and disperse long distances in seed production areas (du Toit 2007; Harveson et al. 2009). The teleomorph has not been found on table beet in NY.

Disease depends on the virulence and aggressiveness of the pathogen, susceptibility of the host, and the environmental conditions of the interaction (Schumann and D'Arcy 2009). Temperatures between 14 and 18°C and high humidity are optimal conditions for disease caused by *P. betae* (du Toit 2007). Pycnidia of *P. betae* exude conidia within a cirrhus under moist conditions, and pre- and post-emergence damping-off is more likely to occur in cool and wet soils (Harveson et al.

2009). Monte and Garcia-Acha (1988a) reported the optimal temperature for germination of *P. betae* conidia is ~26°C under *in vitro* conditions.

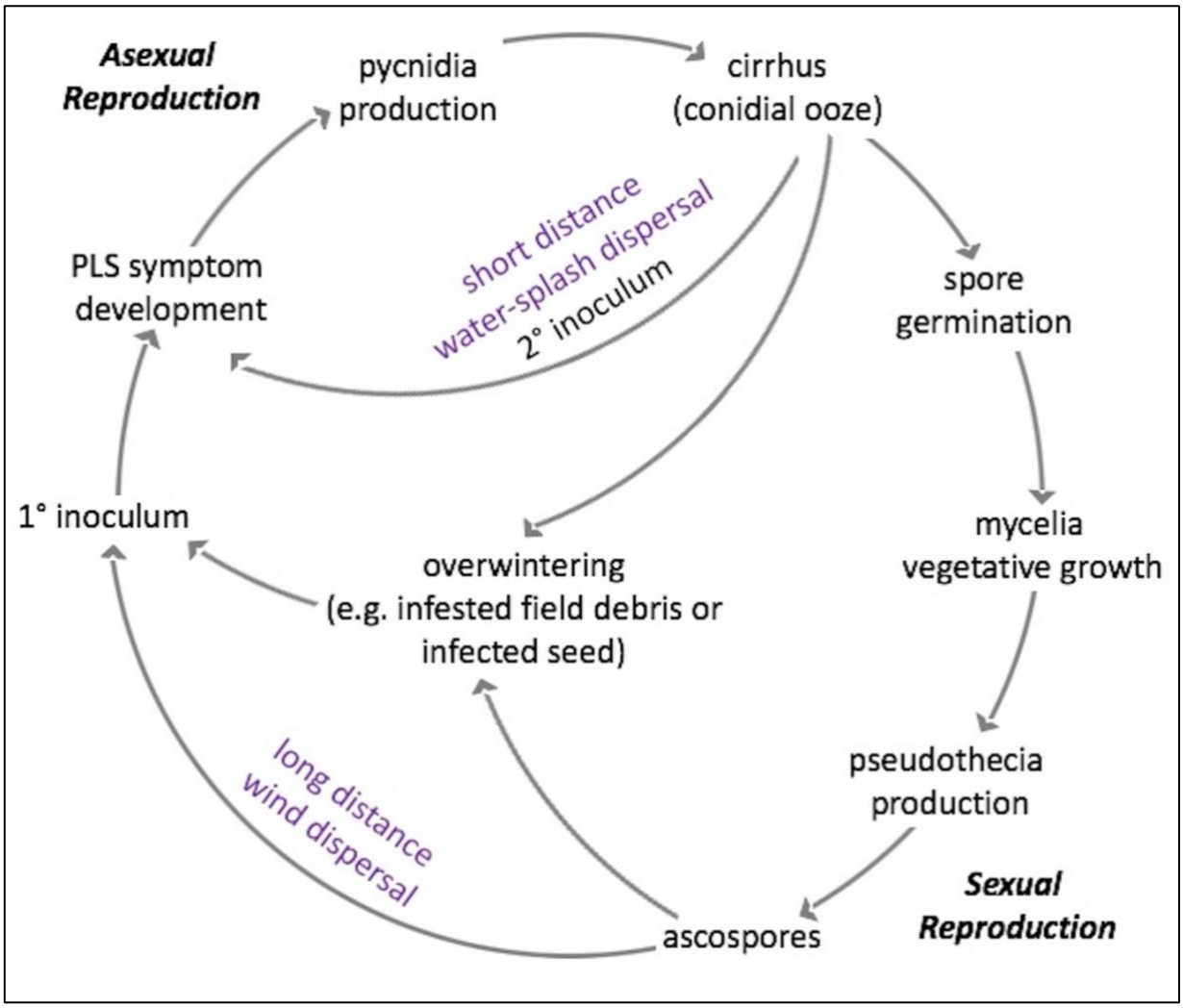

Figure 1.2 Lifecycle of *Phoma betae*, causative agent of Phoma leaf spot (PLS) on table beet.

Disease Management Practices

Table beet production practices balance pest and disease control with techniques to promote high yield and quality (Pethybridge et al. 2018). In addition to *P. betae*, a multitude of other foliar and root rot pathogens can infect table beet and cause crop loss. Another important foliar disease of table beet is Cercospora leaf spot (CLS) caused by the fungus, *Cercospora beticola* (Harveson

et al. 2009). The soilborne plant-pathogenic fungi, *Rhizoctonia solani*, *Aphanomyces cochlioides*, and *Pythium* spp. also cause significant seedling and root disease (Pethybridge et al. 2018). Distinguishing between pathogens and determining the loss associated with each disease can help inform disease management strategies (Harveson 2006). In NY, crop rotation is recommended with a minimum of three years between table beet for disease and weed control (Pethybridge et al. 2018). Weed control is important to prevent diseases surviving on alternative hosts between growing seasons and to achieve optimal yield (Reiners et al. 2018). Row spacing and plant density can have important implications for final root size and shape, and disease management (Kikkert et al. 2010; Shah and Stivers-Young 2004; Reiners et al. 2018). The selection of cultivars more resistant to disease can be an important factor in management, but susceptibility profiles are unknown for Phoma leaf spot (PLS).

In conventional systems, disease management relies on synthetic fungicides and cultural control practices. In organic production systems, disease management relies upon cultural strategies, and may include applications of Organic Materials Review Institute (OMRI)-approved products (Pethybridge et al. 2017). In organic and conventional production systems, seed treatments are important for managing disease and reducing the incidence of seedborne fungi, such as *P. betae*. Table beet seedballs can be multigerm, containing multiple viable seeds, or monogerm, containing one viable seed (Clark et al. 1967). However, monogerm varieties are not preferred due to the potential for low seedling vigor (Navazio et al. 2010). The position of *P. betae* in the seed affects the efficacy of seed treatments (Durrant et al. 1988; Leach and MacDonald 1976) and many treatments have been tested. In the early 1900s, steeping in ethyl mercury phosphate for 20 min was a common seed treatment to control *P. betae* on sugar beet seed (Cadogan 1952). Further research found that soaking red beet seed in thiram to be as effective and have higher germination

rates than an ethyl mercury phosphate steep (Durrant et al. 1988; Maude et al. 1969). Hot water treatment has long been used for successful control of seedborne *P. betae* on sugar beet (Edson 1915) and table beet (du Toit 2007); however, hot water treatment can be detrimental to germination (Durrant et al. 1988; Edson 1915; Herr 1971) and shelf-life. Monte and Garcia-Acha (1988a) found conidia of *P. betae* will not germinate at temperatures over 60°C, and suggests the most effective seed treatments include exposure to high temperatures for short times. Martin et al. (1984) evaluated a biofungicide seed treatment as viable management strategy for *P. betae.* Amending the soil or coating seedballs with the fungal competitor, *Laetisaria arvalis,* reduced the incidence of *P. betae* infection and other soilborne diseases. Seed treatments are typically effective, yet are not always feasible depending on production system constraints and effects on germination. It is best to use multiple complementary strategies for successful disease management in table beet production.

REFERENCES

Abawi, G. S., Crosier, D. C., Cobb, A. C., and Becker, R. F. 1986. Root rot of table beets in New York State. NY Food Life Sci. Bul. No. 115.

Ariyawansa, H. A., Thambugala, K. M., Manamgoda, D. S., Jayawardena, R., Camporesi, E., Boonmee, S., Wanasinghe, D. N., Phookamsak, R., Hongsanan, S., Singtripop, C., Chukeatirote, E., Kang, J.-C., Jones, E. B. G., and Hyde, K. D. 2015. Towards a natural classification and backbone tree for *Pleosporaceae*. Fung. Div. 71:85-139.

Avasthi, S., Gautam, A. K., and Bhadauria, R. 2013. First report of *Phoma betae* on *Aloe vera* in India. Arch. Phytopathol. Plant Prot. 46:1508-1511.

Bassimba, D. D. M., Mira, J. L., and Vicent, A. 2014. First report of leaf spot of spinach caused by *Pleospora betae* in Spain. Plant Dis. 98:1583.

Bugbee, W. M. 1979. *Phoma bjoerlingii* in the USA. Phytopathology 69:277-278.

Bugbee, W. M. 1982. Storage rot of sugar beet. Plant Dis. 66:871-873.

Bugbee, W. M., and Cole, D. F. 1981. The effect of seed infected with *Phoma betae* and sucrose yield of stored sugar beet. Phytopathology 71:357-359.

Bugbee, W. M., and Soine, O. C. 1974. Survival of *Phoma betae* in soil. Phytopathology 64:1258-1260.

Byford, W. J. 1972. The incidence of sugar beet seedling diseases and effects of seed treatment in England. Plant Pathol. 21:16-19.

Cadogan, J. P. 1952. Control of seedling diseases in beet. Beet Grow. 6:70-73.

Clark, B. E., Peck, N. H., Becker, R. F., and Kline, D. B. 1967. Predicting field stands of table beets. New York State Agr. Expt. Sta., Cornell Univ., NY Seed Res. Circ. 2:15.

Clifford, T., Constantinou, C. M., Keane, K. M., West, D. J., Howatson, G., and Stevenson, E. J. 2017. The plasma bioavailability of nitrate and betanin from *Beta vulgaris rubra* in humans. Eur. J. Nutr. 56:1245-1254.

Clifford, T., Howatson, G., West, D. J., and Stevenson, E. J. 2015. The potential benefits of red beetroot supplementation in health and disease. Nutrients 7:2801-2822.

du Toit, L. J. 2007. Crop profile for table beet seed in Washington. U.S Dept. Ag. Pest Management Centers. Online. <https://ipmdata.ipmcenters.org/documents/cropprofiles/WAbeetseed2007.pdf> Accessed 4 January 2018.

Dunning, R. A. 1972. Sugar-beet pest and disease incidence and damage, and pesticide usage. J. I.I.R.B. 6:19-34.

Durrant, M. J., Payne, P. A., Prince, J. W. F., and Fletcher, R. 1988. Thiram steep seed treatment to control *Phoma betae* and improve the establishment of the sugar-beet plant stand. Crop Prot. 7:319-326.

Edson, H. A. 1915. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. J. Agric. Res. 4:135-168.

Fugate, K. K., Ferrareze, J. P., Bolton, M. D., Deckard, E. L., and Campbell, L. G. 2012. Postharvest jasmonic acid treatment of sugarbeet roots reduces rot due to *Botrytis cinerea*, *Penicillium claviforme*, and *Phoma betae*. Postharvest Biol. Technol. 65:1-4.

Garibaldi, A., Gilardi, G., Bertetti, D., and Gullino, M. L. 2007. First report of leaf spot and root rot caused by *Phoma betae* on *Beta vulgaris* subsp. *vulgaris* (garden beet group) in Italy. Plant Dis. 91:1515.

Gilardi, G., Gisi, U., Garibaldi, A., and Gullino, M. L. 2017. Effect of elevated atmospheric CO_2 and temperature on the chemical and biological control of powdery mildew of zucchini and the Phoma leaf spot of leaf beet. Eur. J. Plant Pathol. 148:229-236.

Goldman, I. L., and Navazio, J. P. 2003. History and breeding of table beet in the United States. Plant Breed. Rev. 22:357-388.

Harveson, R. M. 2006. Identifying and distinguishing seedling and root rot diseases of sugar beets. Plant Health Prog. 7:39.

Harveson, R. M., Hanson, L. E., and Hein, G. L. 2009. Compendium of Beet Diseases and Pests, 2nd Edition. APS Press, St. Paul, MN.

Herr, L. J. 1971. Hot water treatment for elimination of seed-borne *Phoma betae* and other microbial contaminants from sugar beet seed. J. Amer. Soc. Sugar Beet Technol. 16:568-574.

Jayanthi, G., Karthikeyan, K., and Muthumary, J. 2014. Pervasiveness of endophytic fungal diversity in *Anisomeles malabarica* from Aliyar, Western Ghats, South India. Mycosphere 5:830-840.

Kikkert, J. R., Reiners, S., and Gugino, B. K. 2010. Row width, population density, and harvest date effects on marketable yield of table beet. HortTechnol. 20:560-567.

Kumaran, R. S., Choi, Y. K., Lee, S., Jeon, H. J., Jung, H., and Kim, H. J. 2012. Isolation of taxol, an anticancer drug produced by the endophytic fungus, *Phoma betae*. Afr. J. Biotechnol. 11:950-960.

Leach, L. D., and MacDonald, J. D. 1976. Seed-borne *Phoma betae* as influenced by area of sugar beet production, seed processing and fungicidal seed treatments. J. Amer. Soc. Sugar Beet Technol. 19:4-15.

Martin, S. B., Abawi, G. S., and Hoch, H. C. 1984. Influence of the antagonist *Laetisaria arvalis* on infection of table beets by *Phoma betae*. Phytopathology 74:1092-1096.

Maude, R. B., Vizor, A. S., and Shuring, C. G. 1969. The control of fungal seed–borne diseases by means of a thiram seed soak. Ann. Appl. Biol. 64:245-257.

Monte, E., and García-Acha, I. 1988a. Germination of conidia in *Phoma betae*. Trans. Br. Mycol. Soc. 91:133-139.

Monte, E., and García-Acha, I. 1988b. Vegetative and reproductive structures of *Phoma betae in vitro*. Trans. Br. Mycol. Soc. 90:233-245.

Navazio, J., Colley, M., and Zyskowski, J. 2010. Principles and practices of organic beet seed production in the Pacific Northwest. Org. Seed Alliance. Available at: https://seedalliance.org/publications/principles-practices-organic-beet-seed-production-pacific-northwest/.

Ninfali, P., and Angelino, D. 2013. Nutritional and functional potential of *Beta vulgaris cicla* and *rubra*. Fitoterapia 89:188-199.

Pethybridge, S. J., Vaghefi, N., and Kikkert, J. R. 2017. Management of Cercospora leaf spot in conventional and organic table beet production. Plant Dis. 101:1642-1651.

Pethybridge, S., Kikkert, J., Hanson, L., and Nelson, S. 2018. Challenges and prospects for building resilient disease management strategies and tactics for the New York table beet industry. Agronomy. 8:112.

Pool, V. W., and McKay, M. B. 1915. *Phoma betae* on the leaves of sugar beet. J. Agric. Res. 4:169-177.

Reiners, S., Wallace, J., Curtis, P., Helms, M., Landers, A., McGrath, M., Nault, B., and Seaman, A. 2018. Beets. Pages 113-120 in: Cornell Integrated Crop and Pest Management Guidelines for Commercial Vegetable Production. Cornell Cooperative Extension.

Sakai, R., Mino, Y., Ichihara, A., and Sakamura, S. 1985. Phytotoxicity of the new metabolites produced by *Phoma betae*, the cause of Phoma root rot and leaf spot in sugar beet (*Beta vulgaris* L.). Annu. Phytopath. Soc. Japan 51:219-222.

Schumann, G. L., and D'Arcy, C. J. 2009. Essential Plant Pathology. 2nd Edition. APS Press, St. Paul, MN.

Shah, D. A., and Stivers-Young, L. 2004. Factors associated with decay in table beets identified by a statistical analysis of grower and processor records. HortTechnol. 14:25-29.

Shoemaker, R. A., and Bissett, J. 1998. *Pleospora betae*. Can. J. Plant Pathol. 20:206-209.

Stivers, L. 2001. Crop profile: beets in New York. U.S. Dept. of Pest Management Centers. Online. http://pmep.cce.cornell.edu/fqpa/crop-profiles/beet.html. Accessed 7 January 2019.

U.S. Department of Agriculture (USDA) - Economic Research Service (ERS). 2018. 2018. Vegetables and Pulses Yearbook Data. Available at: https://www.ers.usda.gov/data-products/vegetables-and-pulses-data/vegetables-and-pulses-yearbook-tables/.

U.S. Department of Agriculture (USDA) - National Agricultural Statistics Service (NASS). 2012. 2012 Census of Agriculture. Available at: https://www.nass.usda.gov/Publications/AgCensus/2012/Full_Report/Volume_1,_Chapter_1_US/.

CHAPTER 2

*GENETIC DIVERSITY AND DIFFERENTIATION IN *PHOMA BETAE* POPULATIONS ON TABLE BEET IN NEW YORK AND WASHINGTON STATES

Abstract

Phoma betae is an important seedborne pathogen of table beet worldwide that is capable of causing foliar, root and damping-off diseases. Ten microsatellite markers and mating type markers were developed to investigate the genetics of *P. betae* populations in table beet root crops in New York (NY) and in table beet seed crops in Washington (WA), from where table beet seed is predominantly sourced. The markers were used to characterize 175 isolates comprising five *P. betae* populations (two from NY and three from WA), and were highly polymorphic with an allelic range of 4 to 33 and an average of 11.7 alleles per locus. All populations had high genotypic diversity (Simpson's complement index = 0.857 to 0.924) and moderate allelic diversity (Nei's unbiased gene diversity = 0.582 to 0.653). Greater differentiation observed between populations from the two states compared to populations within the same state suggested that an external inoculum source, such as windblown ascospores, may be homogenizing the populations. However, most genetic diversity (87%) was among individual isolates within populations (Φ_{PT} = 0.127; P = 0.001), suggesting that local within field inoculum source(s), such as infested field debris or infected weeds, may also be important in initiating disease outbreaks. Standardized index of association, proportion of compatible pairs of loci, and mating type ratio calculations showed evidence for a mixed reproduction mode in all populations. These findings could be useful in

designing more effective management strategies for diseases caused by *P. betae* in table beet production.

---

*Koenick, L. B., Vaghefi, N., Knight, N. L., du Toit, L., and Pethybridge, S. J. 2019. Genetic diversity and differentiation in *Phoma betae* populations on table beet in New York and Washington State. Plant Disease (In Press) https://doi.org/10.1094/PDIS-09-18-1675-RE.

Introduction

The United States table beet (*Beta vulgaris* L. subsp. *vulgaris*) industry currently supplies approximately 116,000 tons of table beets for processing and fresh markets annually [United States Department of Agriculture National Agricultural Statistics Service (USDA NASS) 2012]. New York (NY) is the second largest producer of table beets in the United States, producing around 30,000 tons annually (USDA NASS 2012). In NY, table beets are grown using conventional and organic production practices in a broad range of farming systems from small, mixed crop farms to broad acre, monoculture fields (Pethybridge et al. 2017, 2018; Stivers 2001). Seed used to establish table beet fields for processing in NY is predominantly produced in the maritime Pacific Northwest region of the United States [western Washington (WA) and western Oregon (OR)] and New Zealand (du Toit 2007). Seed used to establish fields in smaller scale, conventional and organic production typically is sourced from a range of merchants based in the United States (Julie Kikkert, *personal communication*).

Phoma betae (syn. *Neocamarosporium betae*) (Ariyawansa et al. 2015) is an important pathogen of table beet, sugar beet, and Swiss chard worldwide, and can negatively affect crop stands by causing damping-off (Edson 1915) and/or reducing photosynthetic leaf area as a result of Phoma leaf spot (Edson 1915; Leach and MacDonald 1976; Pool and McKay 1915). On leaves, *P. betae* causes large, circular, tan-colored leaf spots which can coalesce rapidly to cause defoliation (Pool and McKay 1915; Harveson et al. 2009; Pethybridge et al. 2018). The pathogen also reduces root quality, such that infected roots decay in storage (Abawi et al. 1986; Bugbee 1982; Bugbee and Cole 1981; Edson 1915; Pethybridge et al. 2018). On roots, rot typically starts in the center of the crown and spreads into the taproot (Bugbee 1982; Edson 1915), appearing as dark brown, dry lesions that can lead to shrinkage (Edson 1915; Harveson et al. 2009). The

pathogen also infects roots through wounds. In NY and WA, the prevalence of foliar and root diseases caused by *P. betae* varies with growing conditions. In NY, surveys have shown Phoma leaf spot to be present predominantly in fields grown with organic production practices (Appendix). In 2017, Phoma leaf spot was found in 73% of table beet root fields in NY grown with organic production practices (n = 11) and was found in 71% of conventional fields (n = 17) (Appendix). In 2016, Phoma leaf spot was found in 25% of table beet seed crop fields surveyed in WA (n = 11) (du Toit 2017).

P. betae is a haploid ascomycete (Aveskamp et al. 2009, 2010; de Gruyter et al. 2013), capable of asexual and sexual reproduction. Asexual reproduction contributes to rapid spread of the disease through polycyclic epidemics resulting from rain or irrigation splash of conidia produced by pycnidia on diseased plant material (Harveson et al. 2009; Leach and MacDonald 1976; Monte and García-Acha 1988), while sexually produced ascospores released from pseudothecia promote longer distance, aerial dispersal (Bugbee 1979). *P. betae,* therefore, has multiple modes of initiating epidemics each season (Bugbee 1982; Harveson et al. 2009). Ascospores may be transported by wind currents and, hence, may initiate infections across multiple fields in a region (Bugbee 1979). *P. betae* may also be seedborne and, therefore, infected seeds can be an important pathway for introduction of inoculum and initiation of epidemics (Edson 1915; Herr 1971; Leach and MacDonald 1976; Mangan 1971). Seed treatments, such as thiram and hot water, are effective management practices for limiting the potential for seedborne inoculum to initiate infections in beet crops (Maude et al. 1969). The pathogen can also survive on infested plant debris in a field between growing seasons. In the Red River Valley of North Dakota and Minnesota, Bugbee and Soine (1974) reported *P. betae* could survive up to 26 months in the soil on infested sugar beet plant debris, and that *P. betae* was not found in fields after three years of rotation to non-host crops.

Infected weeds can also serve as an inoculum source between growing seasons. *P. betae* has been reported to infect the roots of *Chenopodium album* (lambsquarters) in fields and sugar beet storage yards (Bugbee and Soine 1974). Most information on the biology of *P. betae* has resulted from studies conducted on sugar beet, also classified as *B. vulgaris*. Sugar beets are cultivated using similar production practices to table beet, yet grown predominantly for the production of sucrose (Harveson et al. 2009). *P. betae* is of increasing importance to NY table beet production, because of the increase in organic production, which negates the use of thiram as a seed treatment (Maude et al. 1969). Limited literature is available on the relative importance of different sources of *P. betae* inoculum initiating epidemics in NY table beet production.

Knowledge of the genetic diversity, differentiation, and reproductive biology of pathogen populations supports the design of durable strategies and tactics for disease management. Population biology is the interdisciplinary subject combining population genetic and epidemiological concepts to study the ecological and evolutionary dynamics of plants and plant pathogens at the population level (Milgroom 2001). Population biology studies can provide indirect evidence to test hypotheses regarding pathogen biology and epidemiology (McDonald 2015; McDonald and Mundt 2016). Milgroom (2015) defined population genetics as the study of evolutionary processes (mutation, selection, recombination, random genetic drift, and migration) that generate and maintain genetic variation within and among populations. These studies use patterns of allele frequencies as genetic evidence to derive information about the evolutionary processes shaping populations (Carbone and Kohn 2004; Grünwald et al. 2017). Information from population biology studies has been used to evaluate the relative contribution of inoculum sources for some pathogens such as *Phaeosphaeria nodorum* on winter wheat (Bennett et al. 2007) and *Rhizoctonia solani* on potato (Muzhinji et al. 2018). For example, if primary inoculum is dominant

from a source external to the field of interest, such as infected seed or windblown ascospores, low population differentiation among fields is expected. If primary inoculum is arising from within fields, such as infested overwintering plant debris or weedy host reservoirs, high population differentiation among fields is predicted (Li and Brewer 2016). Population biology studies have also been used to test hypotheses regarding pathogen origin (Goss et al. 2014), migration pathways (Banke and McDonald 2005; Estoup and Guillemaud 2010), patterns of emergence and reemergence (Grünwald and Goss 2011; Grünwald et al. 2012), and reproductive biology (Milgroom et al. 2014; Short et al. 2014; Vaghefi et al. 2017b). To the best of our knowledge, information on the population biology of *P. betae* currently is not available.

Investigating the structure and evolutionary history of populations requires selectively-neutral genetic markers (Grünwald et al. 2017). Microsatellites or simple sequence repeats (SSRs) are a popular choice for population biology studies due to their high degree of polymorphism, reproducibility, locus-specificity, abundance, and neutrality (Bruford and Wayne 1993; Ellegren 2004). In combination, microsatellites and mating type markers can be used to investigate pathogen reproductive strategy, which can have considerable implications on population structure, disease inoculum sources and management strategies (McDonald and Linde 2002; Milgroom 1996).

Asexual (clonal) reproduction produces genomes identical to the parent genome and allows for swift dispersal of successful genotypes (Milgroom 1996; Taylor et al. 1999). In contrast, sexual reproduction involves recombination in eukaryotic organisms. Recombination facilitates the exchange of genetic material among organisms, and hence contributes to the rapid formation of novel multilocus genotypes (MLGs) and the purging of deleterious mutations from populations (Milgroom 1996; 2015). The mating type (*MAT1*) locus controls sexual reproduction in

ascomycetes (Coppin et al. 1997; Debuchy and Turgeon 2006). The *MAT1* locus in most ascomycetes carries either one or two mating type alleles (*MAT1-1* and *MAT1-2*), both of which are usually required for sexual reproduction (Coppin et al. 1997). The *MAT1-1* and *MAT1-2* open reading frames (ORFs) encode proteins with an alpha box domain and DNA-binding domain of a high mobility group (HMG) type, respectively (Debuchy and Turgeon 2006). Based on the structure of the mating type locus, ascomycetes are classified as heterothallic (self-incompatible) or homothallic (self-compatible). For heterothallic fungi, two isolates carrying alternate mating types are required for sexual reproduction. Therefore, an equal ratio of *MAT1-1* and *MAT1-2* isolates in populations can provide evidence for random mating (Milgroom 2015; Zhan et al. 2002). The mating type locus has not been characterized for *P. betae* and the sexual form of *P. betae* has not been observed in NY (Pethybridge, *unpublished data*).

The objectives of this study were to develop a reproducible and polymorphic microsatellite library for *P. betae*, characterize the *MAT1* locus for *P. betae*, and use both microsatellite and mating type genetic markers to examine the diversity, differentiation, and reproductive strategy of *P. betae* populations in table beet fields grown for seed in WA and roots in NY. Microsatellites were selected to facilitate relatively rapid and cost-effective screening of diversity within *P. betae* populations (Milgroom 2015).

Materials and Methods

Fungal isolate sampling strategy and DNA extraction. A total of 198 *P. betae* isolates were used in this study. Twenty-three isolates collected from table beet were used for microsatellite library development (Table 2.1). These included isolates from NY and WA; and three reference strains from international culture collections.

A total of 175 *P. betae* isolates was used for genotyping populations from NY and WA (70 from NY and 105 from WA). Two populations of 35 *P. betae* isolates each were collected from table beet root fields in NY (NY1 and NY2) in 2017. Population NY1 was collected in a field (cultivar Ruby Queen) in Ontario County and population NY2 was collected in a field (cultivar Red Ace) in Essex County. Three populations of 35 *P. betae* isolates each were collected from table beet seed fields (proprietary identity of cultivars) in western WA (WA1, WA2, and WA3) in 2016 in Skagit County. The term population is used in this study to refer to a group of isolates collected from the same table beet field.

In each population, each isolate originated from a single Phoma leaf spot lesion, and all isolates in that population originated from different leaves in the same table beet field. Isolates collected in NY were obtained by removing three pycnidia followed by placement into 200 µl sterile water in the same 1.5 ml tube. The tube was vortexed vigorously and 100 µl of the conidial suspension was spread on 2% water agar (Hardy Diagnostics, Santa Maria, CA) amended with 0.02% (w/v) ampicillin (Sigma-Aldrich, St. Louis, MO) and 0.02% (w/v) streptomycin (Sigma-Aldrich). After 24 h, germinated conidia were excised and placed on clarified V8 (CV8) juice [10% (v/v) CV8 juice (Campbell's Soup Co., Camden, NJ), 0.5% (w/v) $CaCO_3$] agar (Jeffers 2015; Miller 1955). Isolates collected from seed crops in western WA were each obtained by surface-sterilizing a piece of symptomatic leaf material in 95% ethyl alcohol, drying the tissue thoroughly, and plating half of each lesion onto 2% water agar and the other half onto half-strength potato dextrose agar. Fungal isolates with morphological characteristics resembling *P. betae* (mycelial color and growth characteristics, pycnidia production, and presence of holdfasts at the interface of the 2% water agar and surface of the Petri plate [International Seed Testing Association (ISTA) 1982]) were streaked onto new plates of 2% water agar to obtain single-conidium isolates.

For DNA extraction, single-conidium isolates were grown in CV8 broth (10% (v/v) CV8 juice) for 4 to 7 days. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI) following the manufacturer's instructions. For each isolate, 50 to 120 mg of wet mycelium was ground in Nuclei Lysis Solution in a 2 ml microcentrifuge tube with two 4.5 mm diameter, zinc-plated, spherical balls (Daisy premium grade BBs, Rogers, AR) using a tissue homogenizer (TissueLyser, QIAGEN, Valencia, CA). Quantity of extracted DNA was determined using a Nanodrop spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, DE). The quality of the DNA samples was evaluated through gel electrophoresis on a 1% (w/v) agarose gel in Tris-acetate-EDTA amended with 0.001% (v/v) GelRed (Biotium, Inc., Hayward, CA). Product size was determined against a 100-bp DNA Ladder (Axygen, Inc., Union City, CA).

Microsatellite marker development. Microsatellite marker development followed the general protocol used for *Cercospora beticola* by Vaghefi et al. (2017a). A *de novo* genome assembly for isolate Pb1 (Vaghefi et al. 2019) was used as the scaffold for identification of microsatellite motifs in Tandem Repeat Finder v.408 (Benson 1999). The search was restricted to sequences with tri- to hexa-nucleotide repeat motifs, with a minimum score of 50, a matching point of two, and a copy number of at least five. Forty loci were selected initially for primer design. To minimize the probability of physical linkage of loci, only one microsatellite per contig was selected for primer design. Moreover, the genome within 5 kb of the microsatellite motifs was searched in the Basic Local Alignment Search Tool (BLAST) against the nuclear ribosomal DNA (nrDNA) database in GenBank at the National Center for Biotechnology Information (NCBI) to ensure that loci were not linked to genic regions. Microsatellite primers for each of the 40 motifs were designed using the program Primer3 v.0.4.0 (Untergasser et al. 2012) with melting temperatures between 58 and 60°C, and product sizes ranging from 90 to 450 bp to allow for multiplexing. Each

primer pair was tested initially using a subset of eight *P. betae* isolates with a polymerase chain reaction (PCR) assay. The assay was conducted in a total volume of 15 µl and contained 1× Standard PCR buffer (New England Biolabs, Inc., Ipswich, MA), 1.5 mM $MgCl_2$ (New England Biolabs), 0.1 mM dNTPs (New England Biolabs), 0.1 µM of each primer (Integrated DNA Technologies, Skokie, IL), 0.8 U *Taq* polymerase (New England Biolabs), and 8 to 10 ng of template DNA. The PCR conditions included an initial denaturation for 10 min at 95°C; followed by 34 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 20 s, and extension at 68°C for 30 s; followed by a final extension at 68°C for 5 min. Ten primer pairs were selected for evaluation based on amplification of single bands of expected size for all isolates visualized on a 1% agarose gel containing 0.001% (v/v) GelRed (Biotium Inc.).

Microsatellite evaluation for polymorphism and multiplexing. The 5' end of the forward primer for each of the markers was labeled with one of four fluorophores (6-FAM, NED, PET and VIC; Applied Biosystems, Foster City, CA) to allow for multiplexing. PCR assays were performed on 23 *P. betae* isolates (Table 2.1) as described above with an annealing temperature of 58°C. Fragment analysis of the fluorescently-labeled PCR products was done on an ABI 3730xl DNA Analyzer at the Biotechnology Resource Center, Institute of Biotechnology, Cornell University, Ithaca, NY, with a GeneScan-600 LIZ size standard (Applied Biosystems). Alleles were scored using the Microsatellite Plugin Version 1.4 in Geneious v.9.1.5 (http://www.geneious.com; Kearse et al. 2012). All 10 loci were polymorphic, operationally defined as resulting in at least two alleles, and a minor allele frequency of at least 0.01. PCR assays and fragment analysis were repeated on all 23 *P. betae* isolates to evaluate reproducibility and estimate error rates for all loci (Pompanon et al. 2005). Summary statistics for each locus were calculated in GenAlEx v.6.503 (Peakall and Smouse 2006, 2012) and in the R (R Core Team 2017) package *poppr* v.2.6.1 (Kamvar et al. 2014,

2015) (Table 2.2). PCR products of the 10 markers from four *P. betae* isolates (Pb1, Pb2, Phb18, Phb22) were sequenced using an Applied Biosystems Automated 3730xl DNA Analyzer at the Biotechnology Resource Center, Institute of Biotechnology, Cornell University, Ithaca, NY to ensure they contained the target repeat motif.

Table 2.1 Geographical location and date of collection of *Phoma betae* isolates from Washington (WA) and New York (NY) States, and culture collections used for microsatellite library development for this study

Isolate[a]	Location	Date
CBS 109410 = PD 77/113	Netherlands	-
ICMP 10945	New Zealand	1979
IMI 156653	Warwickshire, England	1968
Pb1	NY	2014
Pb2	Cayuga County, NY	2015
Pb3	Cayuga County, NY	2015
Pb4	Cayuga County, NY	2015
Pb7	Batavia, Genesee County, NY	2015
Pb8	Batavia, Genesee County, NY	2015
Pb9	Batavia, Genesee County, NY	2015
Pb12	Batavia, Genesee County, NY	2015
Phb03	Whidbey Island, Island County, WA	2014
Phb05	Whidbey Island, Island County, WA	2014
Phb10	Whidbey Island, Island County, WA	2014
Phb11	Skagit County, WA	2014
Phb14	Skagit County, WA	2015
Phb16	Skagit County, WA	2015
Phb18	Skagit County, WA	2015
Phb20	Skagit County, WA	2015
Phb22	Skagit County, WA	2015
Phb30	Whidbey Island, Island County, WA	2015
Phb39	Stanwood, Skagit County, WA	2016
Phb40	Stanwood, Skagit County, WA	2016

[a] All non-reference isolates are from table beet. Reference strains were obtained from international culture collections. The host of CBS 109410 = PD 77/113 is listed as *Beta vulgaris*. ICMP 10945 was sourced from a root lesion on *B. vulgaris*. IMI 156653 was sourced from seed of *B. vulgaris*.

The software Multiplex Manager v.1.2 (Holleley and Geerts 2009) assigned the 10 polymorphic markers into two multiplex PCR sets, containing six and four markers, respectively (Table 2.3), with a minimum distance between the loci of 50 bp and a complementary threshold of seven. Multiplex PCR assays were each performed in a 25 µl volume consisting of 8 to 10 ng

template DNA, 1× of the Multiplex PCR 5× Master Mix (New England BioLabs), and 0.1 µM primers. Multiplex PCR assay conditions included an initial denaturation for 2 min at 95°C; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 68°C for 2 min; and a final extension at 68°C for 5 min. Reproducibility of this assay was evaluated by conducting repeat PCR assays and fragment analysis on the same isolates used for marker development. Markers were evaluated for their ability to describe genetic variability of 175 *P. betae* isolates adequately by inspection of a genotype accumulation curve using *poppr*.

Mating type gene discovery and amplification. The genome assembly of *P. betae* was used to create a BLAST database in Geneious. Sequences of *MAT1-1-1* (GenBank Accession AB009451) and *MAT1-2-1* from *Alternaria alternata* (GenBank accession AB005039) were retrieved from the GenBank nucleotide database (Clark et al. 2016) and used in queries against the *P. betae* genome to identify potential orthologs. The identified contig from the *P. betae* genome was analyzed in FGENESH+ (Solovyev 2007) using the sequence of the homologous protein from *P. herbarum* (GenBank accession AAR00940) to find putative ORFs and intron splicing sites.

Only one mating type (*MAT1-2-1*) was detected in the genome of isolate Pb1. Therefore, primers specific to the *MAT1-2-1* ORF (Pb-MAT2-F and Pb-MAT2-R; Table 2.4) were designed using Primer3 to evaluate the five populations of *P. betae* from NY and WA. Individual isolates from which the expected PCR product was not obtained were identified as putative MAT1-1 isolates. PCR assays were each conducted in a total volume of 25 µl, and contained 1× Standard PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.1 µM of each primer, 0.8 U *Taq* polymerase, and 8 to 10 ng of template DNA. The PCR assays included an initial denaturation for 5 min at 95°C; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 68°C for 30 s; and a final extension at 68°C for 5 min.

Additional primers were designed for amplification of the entire *MAT1* locus in putative MAT1-1 isolates (Table 2.4). First, to identify the flanking region of the *MAT1* locus where *MAT1-1* and *MAT1-2* alleles showed high similarity, the *MAT1-2* locus of *P. betae* was aligned with the *MAT1-1* and *MAT1-2* sequences of *Ulocladium botrytis* (GenBank accessions KF533878 and KF533888) using the MAFFT plugin in Geneious. The selection of *U. botrytis* as a template was based on this fungus belonging to the Pleosporaceae and public availability of the entire *MAT1* loci and flanking regions. Primers were designed in the flanking regions to amplify the *MAT1* locus in MAT1-2 and putative MAT1-1 isolates (Table 2.4). Primers (PbMAT-F2 and PbMAT-R3) were used to amplify a ~3,800 bp PCR product targeting the *MAT1* locus of four *P. betae* isolates. PCR assays were each conducted in a total volume of 25 µl and contained 1× Q5 Reaction buffer (New England Biolabs), 200 µM dNTPs, 0.5 µM of each primer, 0.02 U/µl Q5 High-Fidelity DNA polymerase (New England Biolabs), and 8 to 10 ng of template DNA. The PCR conditions included an initial denaturation for 5 min at 98°C; followed by 35 cycles of denaturation at 98°C for 10 s, annealing at 65°C for 30 s, and extension at 72°C for 80 s; and a final extension at 72°C for 5 min. PCR products of the *MAT1* locus were sequenced using the Illumina MiSeq platform at the Center of Computational and Integrative Biology DNA Core Facility at *the* Massachusetts General Hospital, Cambridge, MA.

To identify the *MAT1-1* ORF, the *MAT1* loci of *P. betae* from MAT1-2 and putative MAT1-1 isolates were aligned with the *MAT1-1* sequence of *U. botrytis* (GenBank accession KF533878) and *MAT1-1* and *MAT1-2* sequences of *Stemphylium triglochinicola* (GenBank accession AY335167, which includes the sequences of both mating type genes) in Geneious. The *MAT1-1* region identified from putative MAT1-1 isolates was analyzed in FGENESH+ using the sequence of the homologous protein from *Ulocladium dauci* (GenBank accession AEZ02243) to find the

putative ORFs and intron splicing sites. A pair of primers was designed in the identified flanking regions of the *MAT1-1-1* gene (Table 2.4) using Primer3, and used to amplify the alpha box domain of the *MAT1-1* ORF and *MAT1-1-1* genes in putative MAT1-1 isolates.

Statistics and data analyses. *Measures of genetic diversity*. Clone-corrected datasets were used in analyses when specified and clone-correction of populations was performed in *poppr* using the function clonecorrect. Nei's (1978) measure of allelic diversity, allelic richness, and number of private alleles were estimated in GenAlEx. The number of MLGs, Nei's unbiased gene diversity (Nei 1978), Simpson's complement index (1-λ, measure of genotypic diversity), evenness (E_5), and clonal fraction (1-(MLG/N) were estimated in *poppr*. Significant differences in genotypic diversities for pairwise comparisons of populations was tested in GenoDive v.2.0 (Meirmans and Van Tienderen 2004) using 999 bootstrap replications.

Measures of population structure and differentiation. Analysis of molecular variance (AMOVA) was performed in GenAlEx to determine genetic structure among *P. betae* populations (999 permutations). Pairwise index of population differentiation (Φ_{PT}) (Excoffier et al. 1992) was calculated in GenAlEx (999 randomizations). Jost's measure of population differentiation (D) (Jost 2008) was calculated in the R package *mmod* v.1.3.3 (Winter 2012), with 95% confidence intervals estimated after 1,000 bootstrap simulations. Discriminant analyses of principal components (DAPC) (Jombart et al. 2010) was performed in *adegenet*. A dendrogram based on the unweighted paired group method with arithmetic mean (UPGMA) was developed based on Nei's distance (Nei 1972) in *poppr*.

To test for the underlying population structure without prior assignment criteria of the populations, the non-clone-corrected and clone-corrected datasets were used in three analyses. First, DAPC (Jombart et al. 2010) was performed in *adegenet*. Second, a UPGMA dendrogram

was constructed in *poppr* by grouping individual isolates based on Bruvo's distance (Bruvo et al. 2004). Third, a model based Bayesian clustering method was implemented in STRUCTURE v.2.3.4 (Pritchard et al. 2000). The number of clusters (K) was chosen by calculating ΔK (Evanno et al. 2005) in STRUCTURE HARVESTER v.0.6.94 (Earl and vonHoldt 2012). A graphical output was generated in CLUMPAK v.1.1 (Kopelman et al. 2015).

Tests of linkage equilibrium and recombination. The standardized index of association ($\bar{r}_d$) (Agapow and Burt 2001) was estimated after 1,000 permutations in the package *poppr* to investigate the null hypothesis of random mating. Rejecting this null hypothesis provides evidence for linkage disequilibrium, statistical associations among different loci, which may be associated with asexual reproduction (Agapow and Burt 2001). To investigate the presence of recombination, the proportion of compatible pairs of loci (PrCP) (Estabrook and Landrum 1975) was calculated in Multilocus1.3b (Agapow and Burt 2001) with 999 randomizations. Two loci are considered compatible (PrCP = 1) if they arise from the same phylogenetic position in the absence of homoplasy (parallels, reversals, or convergences) or recombination. Under the assumption that parallels, reversal, or convergences are rare, incompatibility (PrCP < 1) provides evidence of potential exchange of genetic material between genomes and can indicate recombination (Milgroom 2015). The hypothesis of random mating was rejected if less incompatible loci were observed than expected in a randomized population ($P < 0.05$) (Agapow and Burt 2001).

Population screening with mating type markers. Isolates of the five *P. betae* populations were assessed using primers PbMAT1-F3 and PbMAT1-R3 (*MAT1-1* ORF) and PbMAT2-F and PbMAT2-R (*MAT1-2* ORF) in a multiplex PCR assay. Reactions were each conducted in a total volume of 25 µl and contained 1× Standard PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs, 0.1 µM of each primer, 0.8 U *Taq* polymerase, and 8 to 10 ng of template DNA. The PCR conditions

included an initial denaturation for 5 min at 95°C; followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 68°C for 30 s; and completed with a final extension at 68°C for 5 min. Products from 10% of the PCR assays for the *MAT1-1* and *MAT1-2* ORFs were randomly selected from the five populations for sequencing at the Biotechnology Resource Center, Institute of Biotechnology, Cornell University, Ithaca, NY using an Automated 3730xl DNA Analyzer (Applied Biosystems) to ensure they contained the target sequence. PCR products were assessed using gel electrophoresis on a 1% (w/v) agarose gel in Tris-acetate-EDTA amended with 0.001% (v/v) GelRed (Biotium, Inc.). Product size was determined against a 100-bp DNA Ladder (Axygen, Inc.). A Chi-squared (χ^2) goodness-of-fit test (Mangiafico 2015) was performed in the R package *stats* v.3.6.0 (R Core Team 2017) for each population to test if mating type ratios differed significantly from the 1:1 ratio expected in a randomly mating population.

Results

Microsatellite marker development and genotyping. Fragment analysis identified 10 markers to be reproducible (error rate = 0) with only one peak per isolate. The sequences of these loci verified they contained the target repeat motif (GenBank accessions MH823546 to MH823585; Table 2.2). The markers were polymorphic across all 198 isolates (Table 2.3), with an allelic range of 4 to 33 and an average of 11.7 alleles per locus (Table 2.2). Nei's unbiased gene diversity ranged from 0.42 (PbSSR52) to 0.92 (PbSSR0). The genotype accumulation curve showed these markers to describe adequately the genetic variability of *P. betae* (Figure 2.1). Re-genotyping 15% of the *P. betae* isolates revealed no genotyping errors with any of the primer pairs.

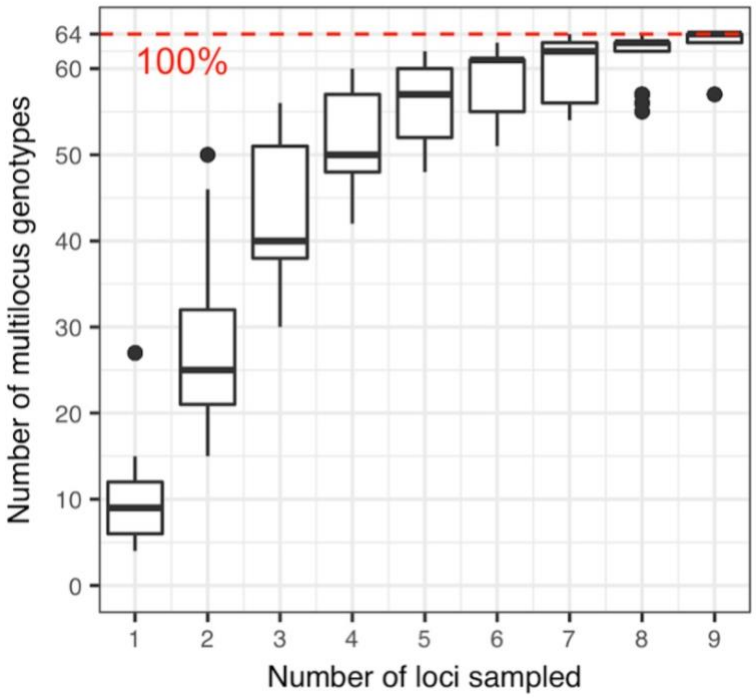

Figure 2.1 Genotype accumulation curve based on 10 microsatellite markers used on 175 *Phoma betae* isolates collected from table beet root crops in New York and table beet seed crops in Washington States to determine the minimum number of microsatellite loci markers needed to distinguish isolates in a population (Kamvar et al. 2014, 2015). To create the distribution for each boxplot, the total number of microsatellite loci markers developed for this study (n) were randomly sampled 1,000 times. The horizontal axis represents the number of microsatellite loci markers randomly sampled without replacement, up to (n – 1) loci. The vertical axis displays the total number of multilocus genotypes observed in 175 *P. betae* isolates. The red line represents 100% of total observed multilocus genotypes.

Table 2.2 Characteristics of microsatellite markers developed for *Phoma betae* based on 198 isolates from New York and Washington States (23 isolates for development of the markers and 175 isolates for genotyping)

Microsatellite locus	Scaffold length[a] (bp)	Repeat motif[b]	Primer pair[c] (5' — 3')	N[d]	H[e]	Accession number[f]
PbSSR0	2,201,606	$(AGT)_{34}$	F: AAATGGGCGGAACACACTAC R: TATTTTGCGCATAGGGAAGG	33	0.92	MH823546 MH823556 MH823566 MH823576
PbSSR4	1,413,381	$(GTT)_{18}$	F: GCAGTCGTCAGCGTCAATTA R: CCGGCACTATCACGTCTTCT	19	0.84	MH823547 MH823557 MH823567 MH823577
PbSSR8	1,307,838	$(GAA)_{24}$	F: CTGGGATTTTGAGGACGAGA R: CATCCTGCAATATGCCCTTT	10	0.68	MH823548 MH823558 MH823568 MH823578
PbSSR24	849,996	$(CAA)_{13}$	F: CAGCGTCACAAGTTCCTTCA R: CCTGTTTGCGTTGACAGAGA	12	0.71	MH823549 MH823559 MH823569 MH823579
PbSSR34	746,462	$(CTTTCA)_{9}$	F: CGTTCCCGGATACAAACTGT R: GATGAGCGGAATAAGGACGA	7	0.62	MH823550 MH823560 MH823570 MH823580
PbSSR36	713,435	$(AAC)_{14}$	F: CGCTGGTGAGAAACATCAGA R: CCATTTTGTGTGTGGTGGAG	11	0.76	MH823551 MH823561 MH823571 MH823581
PbSSR52	483,740	$(GAA)_{11}$	F: GAGCCAACTCAGACGAAACC R: ACCCTGGCATTTATTCATCG	9	0.42	MH823552 MH823562 MH823572 MH823582
PbSSR58	445,932	$(TACA)_{8}$	F: GAGTCGACGACAGGCACATA R: ACCTAACCAATCCGGTAGCC	6	0.76	MH823553 MH823563 MH823573 MH823583
PbSSR78	343,640	$(GGA)_{9}$	F: TCAGGATGTGGAGAGGTTCC R: CAATCCCCTCCTCATGACAC	4	0.55	MH823554 MH823564 MH823574 MH823584
PbSSR80	339,604	$(GAA)_{11}$	F: GACACACTCGCCAAAGTCCT R: TCAAACTCTGCTGCACCAAC	6	0.71	MH823555 MH823565 MH823575 MH823585
Total				11.7	0.7	

[a] Loci were identified in scaffolds from the *de novo* genome assembly of isolate Pb1 (Vaghefi et al. 2019).
[b] Microsatellite motif is indicated in parentheses and copy number (as found in the genome of Pb1) is listed outside parentheses.
[c] F = forward primer, R = reverse primer.
[d] N = number of different alleles.

[e] H = Nei's (1978) Gene Diversity = $\left({}^{N}/_{N-1}\right) \times (1 - \sum p^2)$ where p is the allele frequency at a given locus calculated using *poppr* (Kamvar et al. 2014, 2015).

[f] Genbank accession number; each microsatellite locus was sequenced for four isolates of *P. betae* (Pb1, Pb2, Phb18, Phb22).

Table 2.3 Properties of the microsatellite library used for genotyping *Phoma betae* populations from table beet root fields in New York State and table beet seed fields in Washington State based on 198 isolates (23 isolates used for development of the library and 175 for genotyping)

Microsatellite locus	Fluorescent dye	Multiplex mix[a]	Allele sizes (bp)										
PbSSR0	PET	1	294	303	306	309	312	315	327	345	351	354	360
			363	369	375	381	384	387	390	393	396	399	402
			405	408	414	417	426	456	468	477	483	489	504
PbSSR4	NED	2	395	404	407	410	413	419	422	428	431	434	437
			440	443	458	470	482	491	494	518			
PbSSR8	6-FAM	1	163	166	181	184	199	202	208	226	229	232	
PbSSR24	VIC	1	188	191	194	197	200	203	206	209	212	215	221
			224										
PbSSR34	NED	1	141	147	153	159	165	171	177				
PbSSR36	NED	1	241	244	247	250	256	259	262	265	268	271	274
PbSSR52	NED	2	201	207	210	213	216	219	222	225	228		
PbSSR58	PET	1	104	108	112	116	120	124					
PbSSR78	6-FAM	2	167	170	182	185							
PbSSR80	6-FAM	2	245	248	251	254	266	269					

[a] The 10 microsatellite markers were divided into two multiplex PCR assays, 1 and 2, and contained six and four markers, respectively. Multiplex PCR assays used all primers at a standard concentration of 0.1 µM.

Table 2.4 Characteristics of mating type markers developed for *Phoma betae*

Primer [a]	Sequence (5' to 3')	Target region[b]	PCR product size
PbMAT1-F3	TCTTCAGCATCATATGTCCC	Mating type 1 ORF coding for the conserved alpha box domain	162 bp
PbMAT1-R3	TTCGACAGAGAGATTTCCAG		
PbMAT2-F	TCATCTACCGAGATGCGATG	Mating type 2 ORF coding for the conserved HMG domain	277 bp
PbMAT2-R	CTGGCGCTTCTTCTTCTCTC		
PbMAT-F2	CTCTCTTGGTACACGACTGG	*MAT1* locus	~3,800 bp
PbMAT-R3	GGTGTAAAGCTCGTAAAAGCTAGGTATAG		

[a] Forward primer designated by F; reverse primer designated by R.
[b] Open reading frame (ORF); High mobility group (HMG).

Mating type gene discovery and amplification. Using primers PbMAT-F2 and PbMAT-R3 to amplify the *MAT1* locus in two MAT1-2 isolates and two putative MAT1-1 isolates, both MAT1-2 isolates (Pb1 and Pb2) produced a PCR product of 3,714 bp, and the putative MAT1-1 isolates amplified 3,809 bp (Phb51) and 3,823 bp (CBS 109410) PCR products, respectively (Genbank accessions MH823586, MH823587, MH823588, and MH823589). For both *MAT1-1* and *MAT1-2*, FGENESH+ predicted one protein and two exon sites. A 50 bp intron was identified in the *MAT1-1* ORF (*data not shown*).

Genetic diversity of *P. betae* populations. High genotypic diversity and moderate allelic diversity were quantified in all five *P. betae* populations (Table 2.5). Simpson's complement index (1-λ, a measure of genotypic diversity) ranged from 0.857 (NY1) to 0.924 (WA1). No significant ($P > 0.08$) difference was found in genotypic diversity by pairwise comparisons of populations. Nei's unbiased gene diversity ranged from 0.582 (NY1) to 0.653 (WA1). Across 175 isolates, 64 unique MLGs were found, along with a 63% clonal fraction. WA1 had the smallest clonal fraction (0.429) and the greatest number of unique alleles per locus, with 13 private alleles (alleles not shared with isolates in other populations). NY1 had the largest clonal fraction (0.686). Seventy

isolates had recurrent MLGs. MLG.50 was shared the most frequently and was present in 22 isolates in the WA populations (Figure 2.2). In each population, all loci were polymorphic.

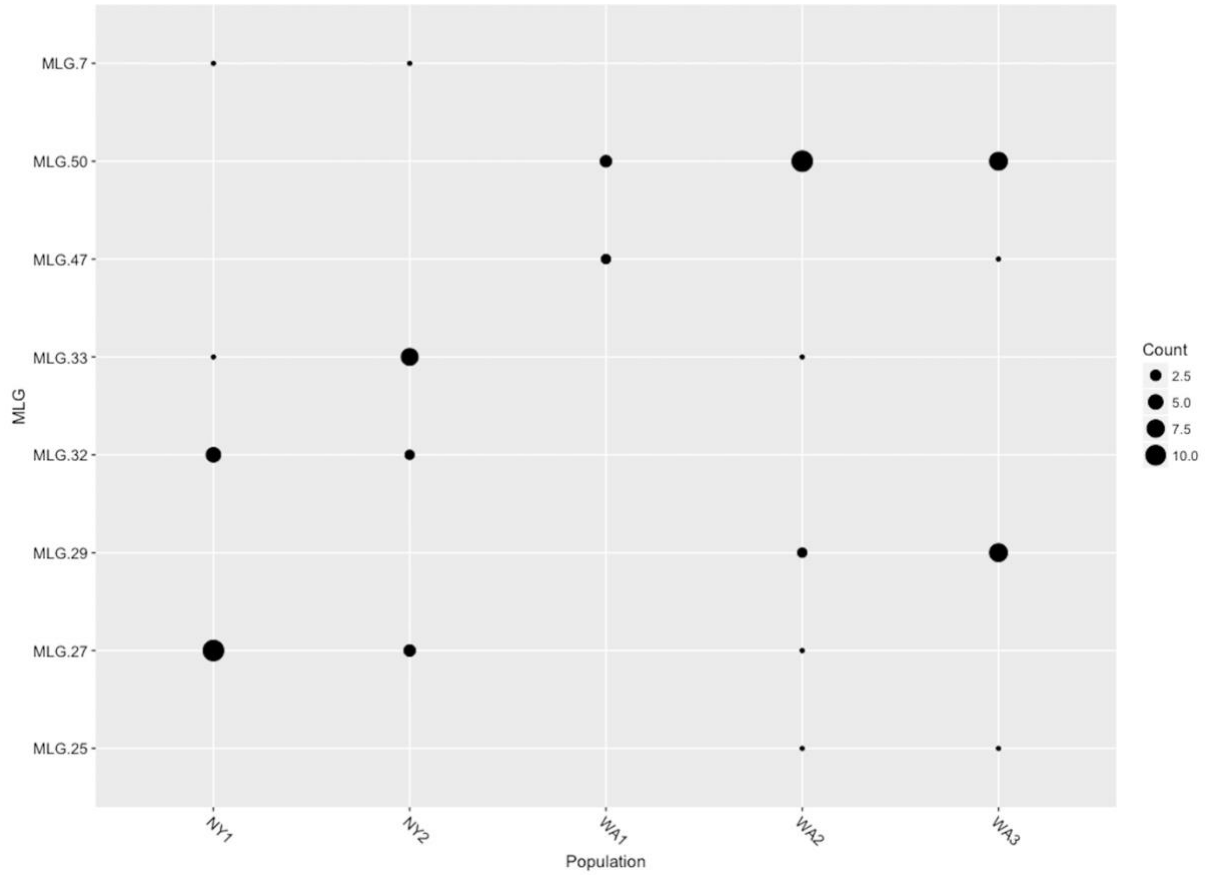

Figure 2.2 Dot plot representing the number and frequency of multilocus genotypes (MLG) shared across populations of *Phoma betae* from New York (NY) and Washington (WA) States. Dot size corresponds to the number of isolates that share specific MLGs. The dot plot was generated with functions mlg.crosspop and qplot in poppr (Kamvar et al. 2014, 2015).

Table 2.5 Indices of genetic diversity for non-clone-corrected data of five *Phoma betae* populations from Washington (WA) and New York (NY) States

Population	N[a]	MLG[b]	1-λ[d]	E.5[e]	H[f]	CF[g]	P_a[h]	N_a[i]
WA1	35	20	0.924	0.646	0.653	0.429	13	5.7
WA2	35	14	0.871	0.652	0.628	0.6	6	5.4
WA3	35	17	0.897	0.656	0.605	0.514	8	5.3
NY1	35	11	0.857	0.735	0.582	0.686	8	4.5
NY2	35	13	0.913	0.820	0.622	0.629	8	4.9
Total	175	64	0.963	0.601	0.69	0.634	-	-

[a] N = Number of isolates.
[b] MLG = Number of multilocus genotypes detected from alleles at 10 microsatellite loci.
[d] 1-λ = Simpson's complement index; $\lambda = \sum p_i^2$ where p is the frequency of the ith genotype in a population (Simpson 1949); values range from 0 (no genotypes are different) to 1 (all genotypes are different) and is corrected for sample size by multiplying by N/(N-1).
[e] E.5 = Evenness is a measure of the uniformity of the distribution of MLGs in a population (Ludwig and Reynolds 1988).
[f] H = Nei's unbiased gene diversity (Nei 1978).
[g] CF= Clonal fraction.
[h] P_a = Number of private alleles (Peakall and Smouse 2006).
[I] N_a = Mean number of alleles per locus.

Genetic differentiation of *P. betae* populations. The majority of genetic diversity detected was among individual isolates within populations. AMOVA of the non-clone-corrected dataset found 87% of the genetic diversity attributed to variation among isolates within populations and 13% among the five populations (Φ_{PT} = 0.127; P = 0.001). After clone-correction, 96% of the observed genetic diversity was partitioned among isolates within populations and 4% among the populations (Φ_{PT} = 0.038; P = 0.004).

Two pairwise indices of differentiation detected low but significant levels of genetic differentiation between populations in the non-clone-corrected and clone-corrected datasets. In the non-clone-corrected dataset, Φ_{PT} values were low, ranging from 0.045 to 0.174, with the largest differentiation detected between WA1 and NY1 populations (0.174; P = 0.001) and the smallest differentiation between NY1 and NY2 populations (0.045; P = 0.009). After clone-correction, WA1 and NY2 populations showed small, although significant, genetic differentiation (0.099; P

= 0.001), but the differentiation between NY1 and NY2 populations, and WA2 and WA3 populations was not significant ($P > 0.05$) (Table 2.6). Jost's D values corroborated these trends. In the non-clone-corrected dataset, Jost's D values ranged from 0.091 to 0.356, with the largest differentiation again detected between WA1 and NY1 populations (0.356; 95% confidence interval = 0.285 to 0.426) and smallest differentiation again detected between NY1 and NY2 populations (0.091; 95% confidence interval = 0.040 to 0.141). After clone-correction, Jost's D values decreased between all populations and trends persisted (Table 2.6).

Table 2.6 Pairwise indices of differentiation (Φ_{PT}[a] and Jost's D[b] values) for clone-corrected data of five *Phoma betae* populations from Washington (WA) and New York (NY) States

	Index of differentiation	
Population pairs[a]	**Φ_{PT} (*P* value)[b]**	**Jost's D (95% CI)[c]**
WA1-WA2	0.005 (0.360)	0.093 (0.006 to 0.180)
WA1-WA3	0.026 (0.063)	0.129 (0.036 to 0.222)
WA1-NY1	**0.062 (0.004)**	0.240 (0.138 to 0.341)
WA1-NY2	**0.099 (0.001)**	0.324 (0.209 to 0.438)
WA2-WA3	0 (0.431)	0.067 (-0.025 to 0.160)
WA2-NY1	0.02 (0.186)	0.145 (0.032 to 0.257)
WA2-NY2	0.033 (0.102)	0.160 (0.052 to 0.268)
WA3-NY1	**0.052 (0.018)**	0.196 (0.091 to 0.301)
WA3-NY2	**0.073 (0.006)**	0.230 (0.124 to 0.336)
NY1-NY2	0 (0.433)	0.066 (-0.038 to 0.169)

[a] WA1, WA2, and WA3 represent three populations of *P. betae* isolates from WA; NY1 and NY2 = two populations of *P. betae* isolates from NY.
[b] Φ_{PT} values (Excoffier et al. 1992) in bold had *P* values < 0.05.
[c] Jost's D values (Winter 2012); 95% confidence interval in parentheses.

Population structure of *P. betae* populations. In the non-clone-corrected and clone-corrected datasets, UPGMA dendrograms based on Nei's genetic distance identified two major clusters of *P. betae* populations distinguished by state (Figure 2.3). DAPC using pre-defined populations also

demonstrated that individuals in each population mostly clustered together by state (Figure 2.4). The first principal component of DAPC (horizontal axis) revealed that WA1 population was genetically more similar to WA2 and WA3 than the NY populations, while the second principal component (vertical axis) separated WA1 population from WA2 and WA3, suggesting population structure within WA (Figure 2.4).

In the clone-corrected dataset, population structure analysis with no *a priori* population assignment using DAPC analysis detected three distinct clusters, which contained 17, 41, and 17 individuals (Figure 2.5). Cluster membership did not necessarily correlate to population assignment (Table 2.7). DAPC clusters two through five are also shown (Figure 2.6). The UPGMA dendrogram based on Bruvo's distance grouped the *P. betae* isolates into two main clades that were not necessarily differentiated by state (Figure 2.7). Bayesian clustering analysis in STRUCTURE also resulted in the detection of three distinct clusters (Figure 2.8), consisting of 38, 19, and 18 individuals (Figure 2.9). Incongruence among DAPC, UPGMA dendrogram, and STRUCTURE clusters was observed (Figure 2.7). For the non-clone-corrected dataset, DAPC analysis was inconclusive as the Bayesian information criterion values consistently decreased with the number of clusters until K reached the total number of MLGs, the UPGMA dendrogram based on Bruvo's distance grouped the *P. betae* isolates into two main clades, and STRUCTURE detected two distinct clusters (*data not shown*).

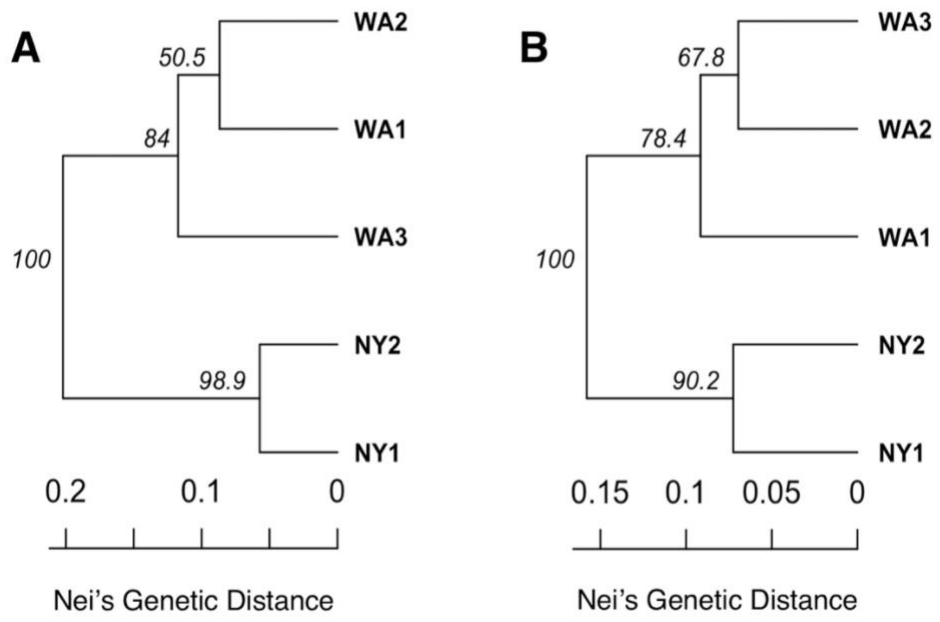

Figure 2.3 Dendrogram of the relationships among **A**, non-clone-corrected and **B**, clone-corrected *Phoma betae* populations from New York (NY1 and NY2) and Washington (WA1 to WA3) States. The dendrogram was constructed using the unweighted pair group method with arithmetic mean based on Nei's (1972) genetic distance. Numbers at branch points indicate the percent occurrence of the cluster to the right of the branch in 1,000 bootstrapped dendrograms.

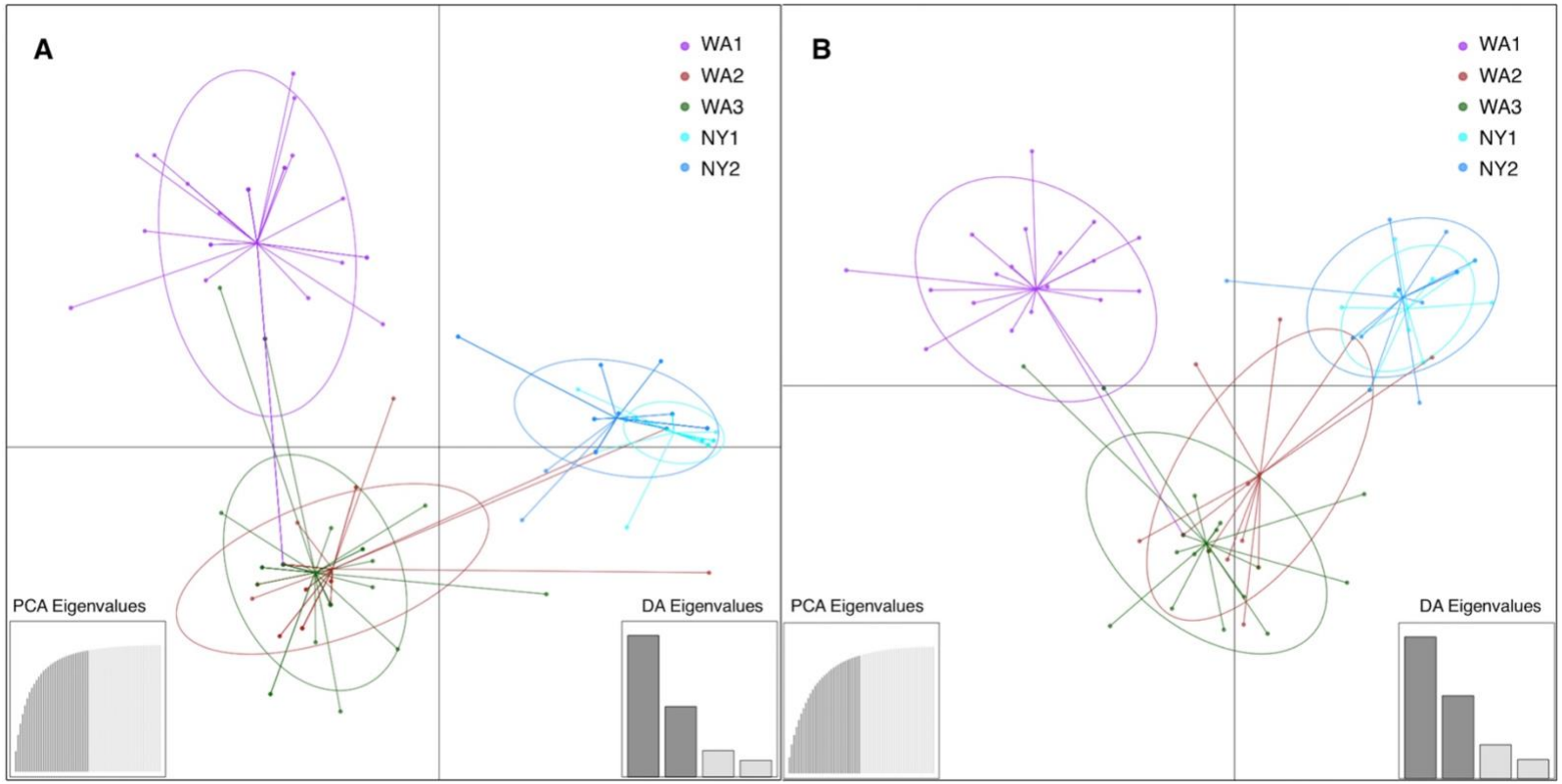

Figure 2.4 Discriminant analysis of principal components (DAPC) for the **A**, non-clone-corrected and **B**, clone-corrected *Phoma betae* populations from New York (NY1 and NY2) and Washington (WA1 to WA3) States. The scatterplots show the first two principal components of the DAPC, and *P. betae* populations are used as prior clusters. Eigenvalues signifying the variance explained by principal component analysis (PCA) and discriminant analysis (DA) indicated the first two principal components adequately explain the genetic structure of the populations. Points represent individual isolates and ellipses represent individual populations.

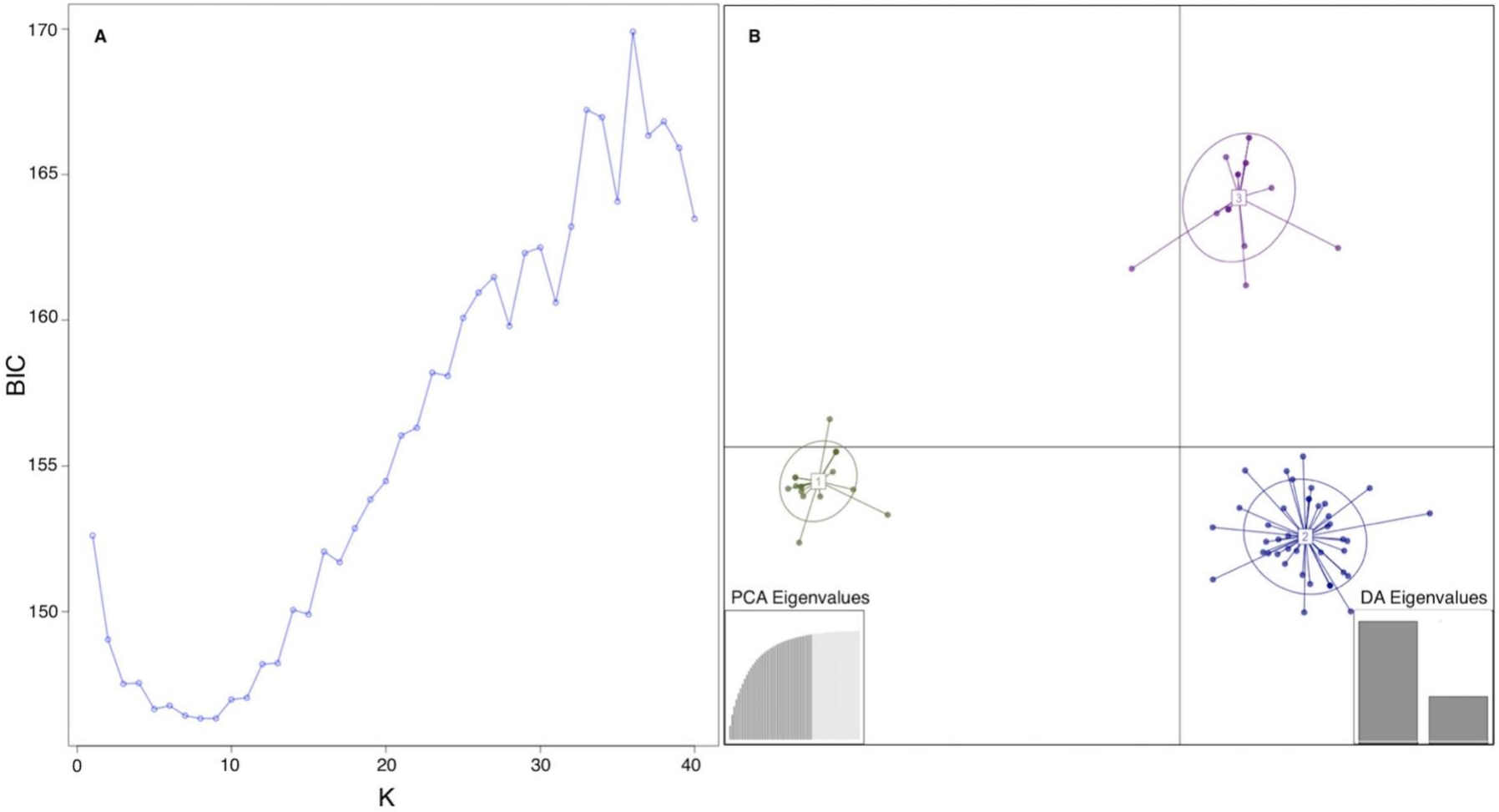

Figure 2.5 Discriminant analysis of principal components (DAPC) for clone-corrected dataset of *Phoma betae* populations from New York and Washington. A, Graph of Bayesian Information Criterion (BIC) values for different number of clusters (K). B, Scatterplot representing the three genetically distinct clusters of *P. betae* isolates as determined by DAPC analysis. Each cluster is distinguished by color (green = cluster 1, blue = cluster 2, purple = cluster 3).

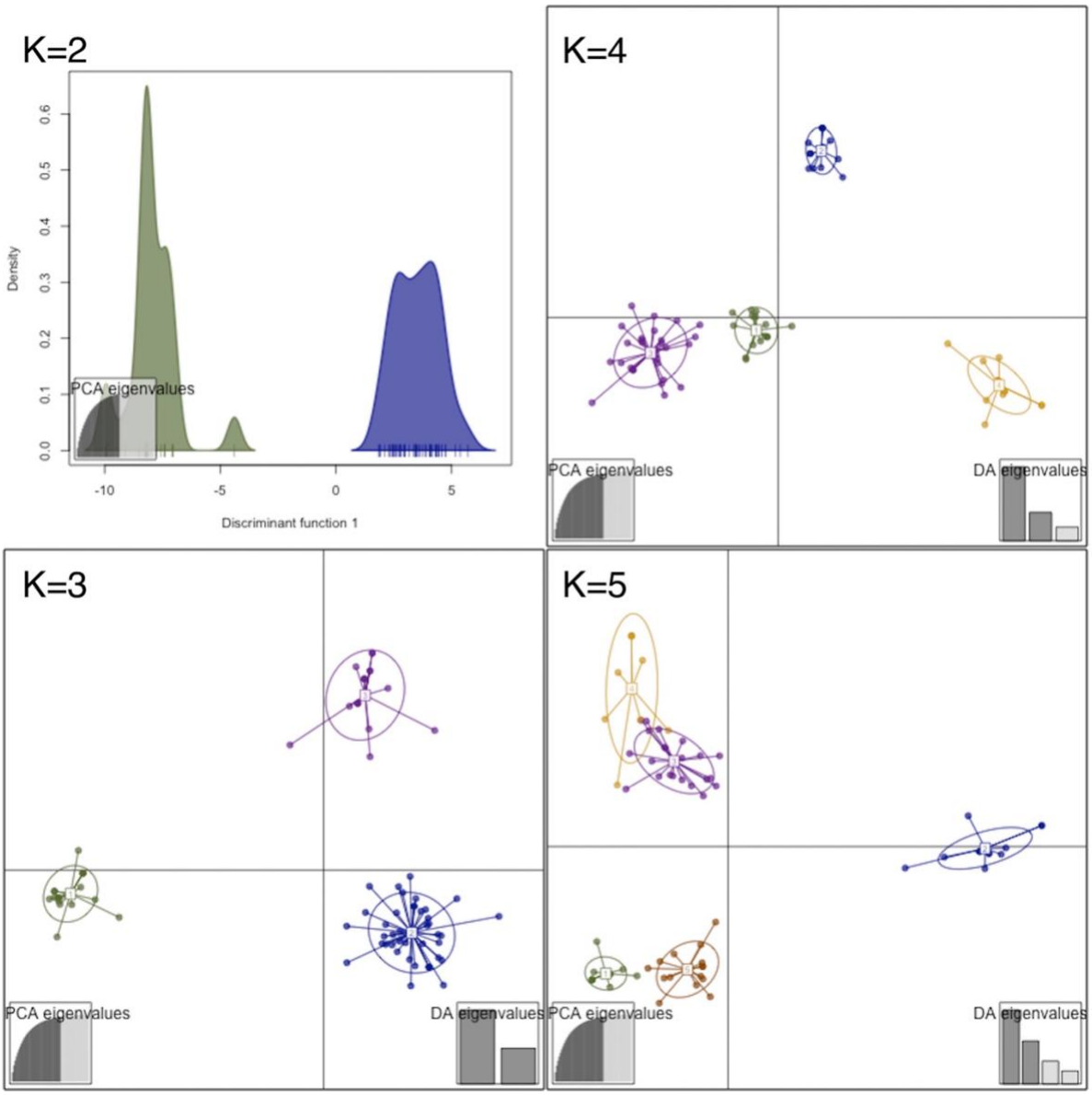

Figure 2.6 Discriminant analysis of principal components (DAPC) for clone-corrected dataset of *Phoma betae* populations from New York and Washington. Scatterplots representing the two through five genetically distinct clusters of *P. betae* isolates as determined by DAPC analysis. Each cluster is distinguished by color and cluster number.

Table 2.7 Assignment of isolates from five *Phoma betae* populations in the clone-corrected dataset to specific clusters as specified by discriminant analysis of principal components (DAPC) using k-means clustering for cluster values two through five

	***Phoma betae* populations[a]**					
K=2						
Cluster	**WA1**	**WA2**	**WA3**	**NY1**	**NY2**	**Total**
Cluster 1	2[b]	4	4	5	8	23
Cluster 2	18	10	13	6	5	52
K=3						
Cluster 1	2	4	4	3	4	17
Cluster 2	16	6	8	6	5	41
Cluster 3	2	4	5	2	4	17
K=4						
Cluster 1	3	4	6	0	1	14
Cluster 2	13	6	6	6	4	35
Cluster 3	4	3	5	2	3	17
Cluster 4	0	1	0	3	5	9
K=5						
Cluster 1	9	5	5	1	3	23
Cluster 2	2	4	5	0	0	11
Cluster 3	1	1	2	4	1	9
Cluster 4	7	1	5	2	1	16
Cluster 5	1	3	0	4	8	16

[a] WA1, WA2, and WA3 represent three populations of *P. betae* isolates from WA; NY1 and NY2 = two populations of *P. betae* isolates from NY.

[b] Number of individuals in a population that belong to each cluster specified by DAPC analysis (Jombart et al. 2010).

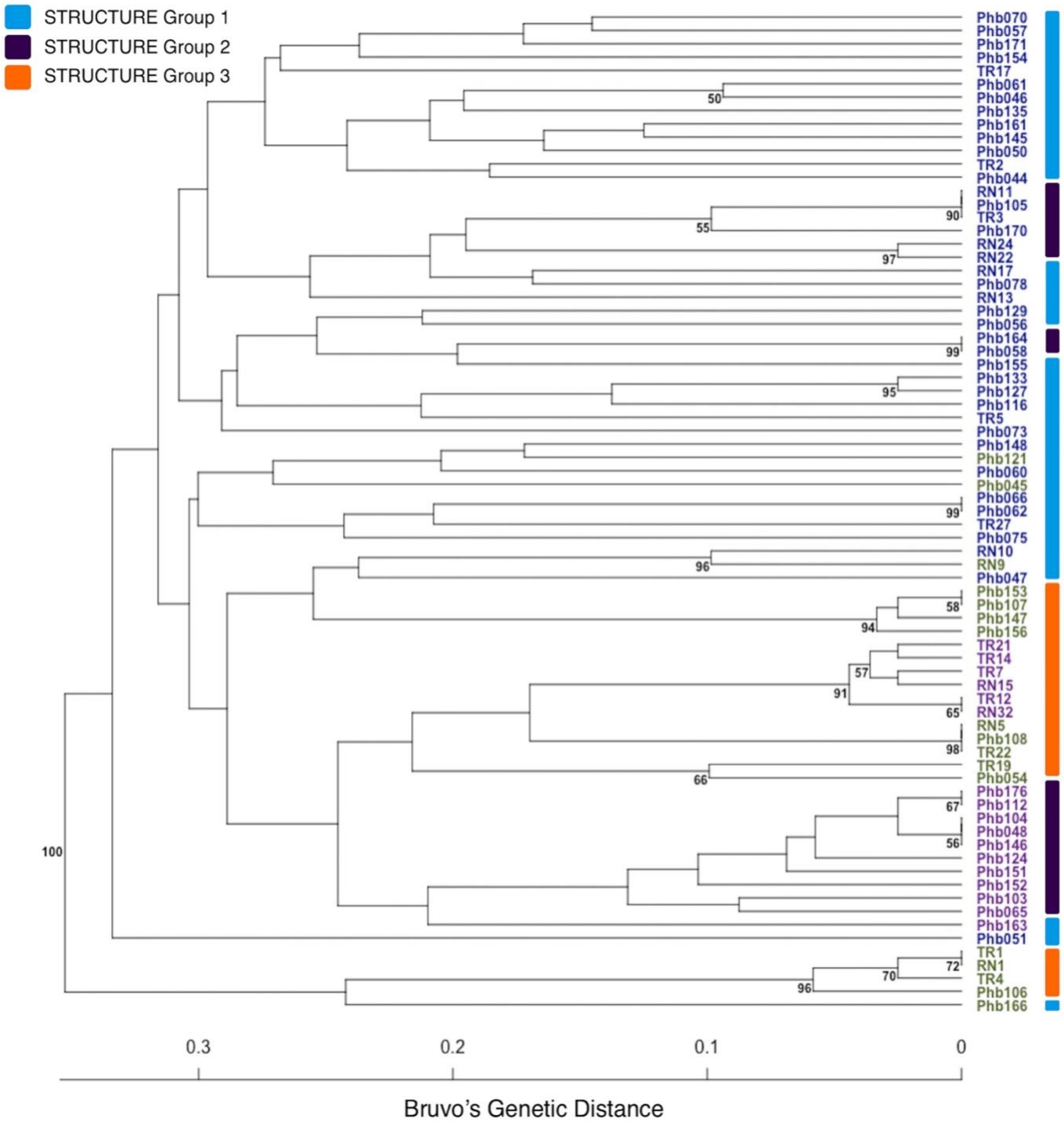

Figure 2.7 A dendrogram based on the unweighted pair group method with arithmetic mean for clone-corrected data of *Phoma betae* isolates from New York (TR and RN) and Washington (Phb) States based on Bruvo's distance (Bruvo et al. 2004). Bootstrap support >50 is shown. Isolate color corresponds to discriminant analysis of principal components (DAPC) grouping color (green = cluster 1, blue = cluster 2, purple = cluster 3) (Jombart et al. 2010). The color of the bar next to each isolate indicates the STRUCTURE grouping (Pritchard et al. 2000).

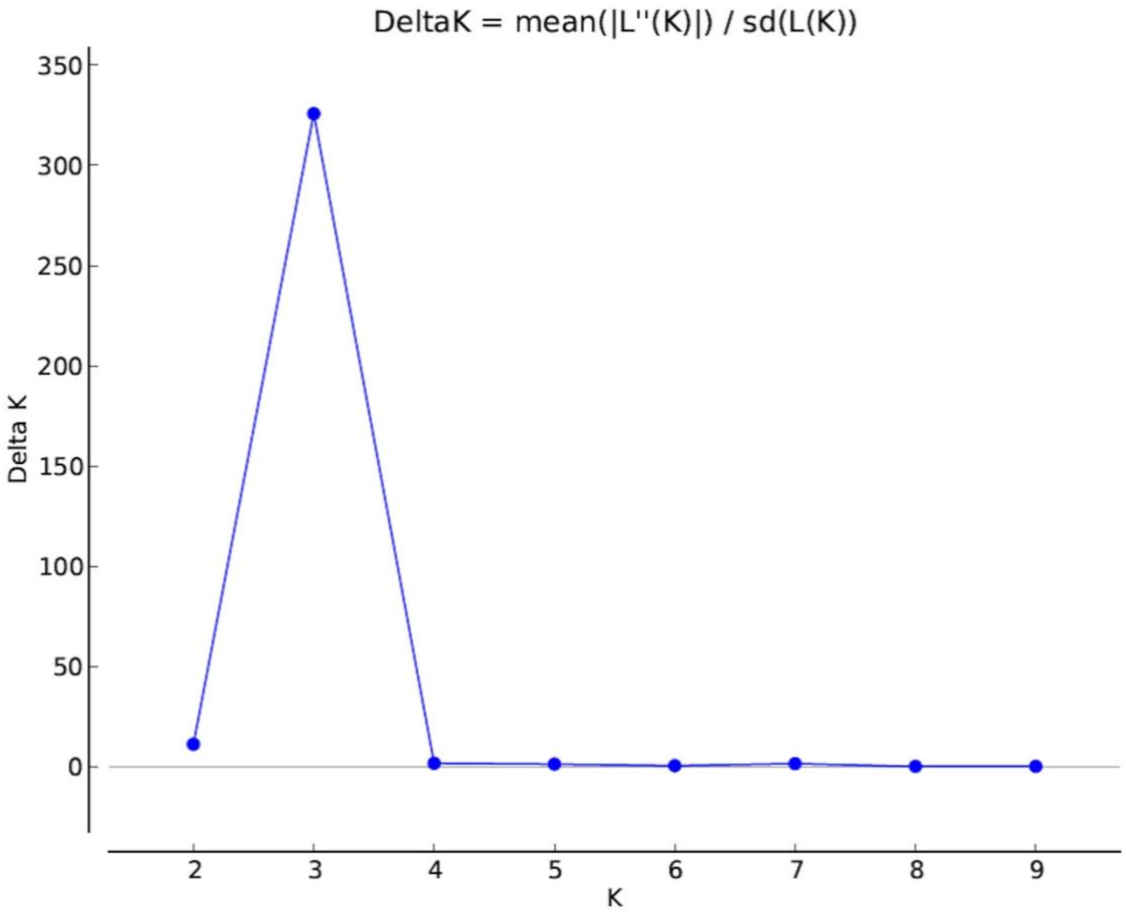

Figure 2.8 Determination of delta K values to find the optimal K (number of clusters) in STRUCTURE analysis (Pritchard et al. 2000) of *P. betae* populations based on the method where delta K = mean (|L''(K)|) / sd(L(K)) (Evanno et al. 2005).

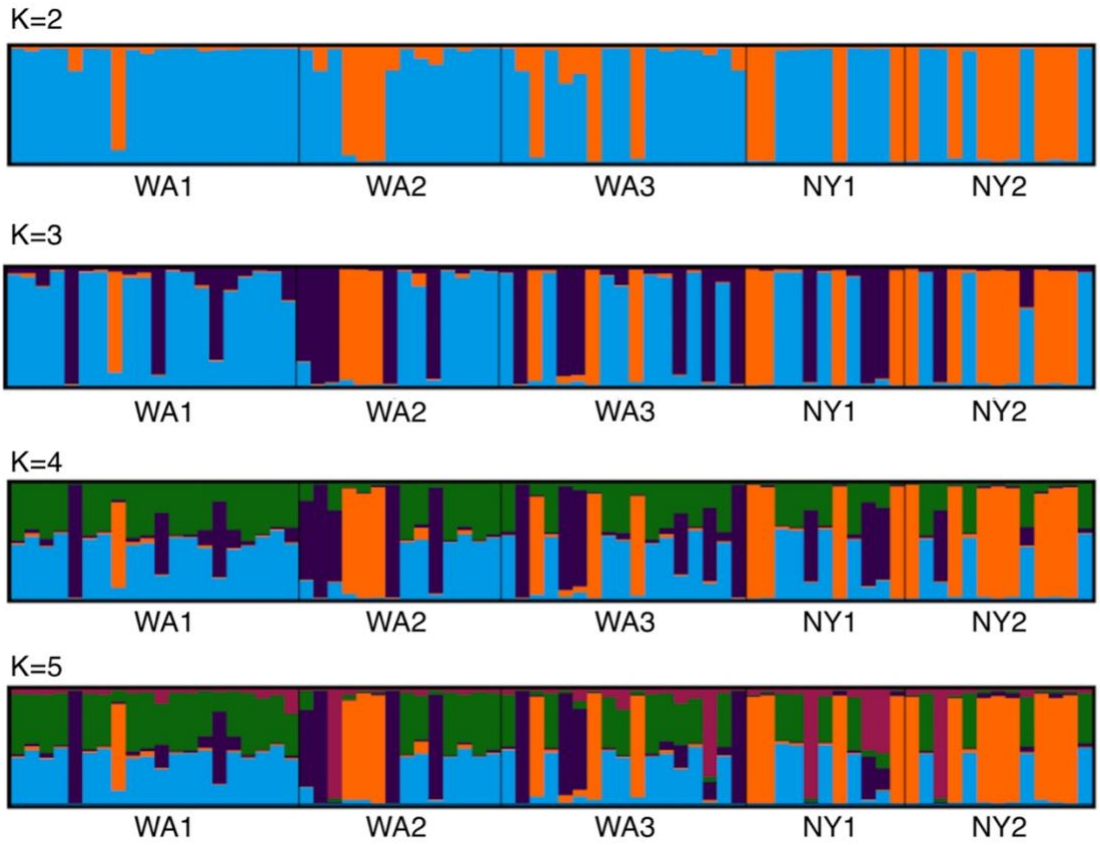

Figure 2.9 Population structure of clone-corrected data of *Phoma betae* populations from New York (NY) and Washington (WA) States as determined by STRUCTURE (Pritchard et al. 2000). STRUCTURE bar plots depicting two through five clusters in the *P. betae* populations from NY (NY1 and NY2) and WA (WA1 to WA3). Each vertical bar represents an individual isolate. Clusters are distinguished by colors, and color differences within a vertical bar designate membership in multiple clusters. The bar height of each color is proportional to cluster membership (Kopelman et al. 2015).

Linkage disequilibrium of *P. betae* populations. For the non-clone-corrected dataset, the calculated standardized index of association ($\bar{r}_d$) resulted in rejection of the null hypothesis of random mating for all five populations ($P < 0.001$). In the clone-corrected dataset, the calculated $\bar{r}_d$ also resulted in rejection of the null hypothesis of random mating for all populations ($P < 0.001$) except WA1 ($P = 0.064$) (Table 2.8). PrCP values were significant ($P < 0.045$) for all populations except WA1 ($P = 0.06$), providing further evidence to reject the null hypothesis of random mating among isolates in all but the WA1 population. PrCP ranged from 0.089 (WA1) to 0.733 (NY2). In WA1, 91% pairs of loci were incompatible (Table 2.8). The three clusters detected by STRUCTURE rejected the null hypothesis of random mating in linkage disequilibrium analyses. The calculated $\bar{r}_d$ for the three clusters ranged from 0.016 (Cluster 1) to 0.215 (Cluster 2) ($P < 0.04$). PrCP values were significant ($P < 0.001$) for all clusters except Cluster 1 ($P = 1$) providing further evidence to reject the null hypothesis of random mating among isolates in the genetically distinct clusters.

Mating type ratios. Primers designed to amplify the Alpha-box region of *MAT1-1-1* in *P. betae* isolates produced a product of 162 bp (GenBank accessions MH823590 to MH823599). Primers designed to amplify the HMG DNA-binding domain of *MAT1-2-1* in *P. betae* isolates produced a product of 277 bp (GenBank accessions MH823600 to MH823606). In three of the five populations (WA2, NY1, and NY2), ratios of MAT1-1 and MAT1-2 isolates did not differ significantly from 1:1 ($P > 0.05$) (Table 2.8), suggesting panmixis for these populations. In populations WA1 and WA3, the ratio of MAT1-1 and MAT1-2 isolates did deviate significantly from 1:1 ($P < 0.004$).

Table 2.8 Standardized index of association, proportion of compatible pairs of loci, and mating type frequencies (MAT1-1 and MAT1-2) in the clone-corrected populations of *Phoma betae* from table beet root crops in New York (NY1 and NY2) and table beet seed crops in Washington (WA1 to WA3) States

Population	$\bar{r}_d$[a]	PrCP[b]		MAT1-1	MAT1-2	χ^{2c}
		Observed	*P* value			
WA1	0.0209 (0.064)	0.089	0.060	26	9	**8.26**
WA2	0.1012 (<0.001)	0.578	0.001	21	14	1.40
WA3	0.0654 (0.002)	0.378	0.002	30	5	**17.86**
NY1	0.1638 (<0.001)	0.667	0.045	13	22	2.31
NY2	0.1997 (<0.001)	0.733	< 0.001	15	20	0.71

[a] $\bar{r}_d$ = Standardized index of association (*P* values in parentheses) (Agapow and Burt 2001).
[b] PrCP = Proportion of phylogenetically comparable pairs of loci, where Observed = the observed value. *P* values < 0.05 were considered significant (Agapow and Burt 2001).
[c] Chi-square (χ^2) value based on a 1:1 ratio and one degree of freedom. Mating-type frequencies with significant deviation from this ratio ($P < 0.05$) indicated in bold.

Discussion

To the best of our knowledge, this is the first study to investigate the population biology of *P. betae* from table beet root and seed fields in the United States. Robust microsatellite and mating type markers were developed as a tool to genotype *P. betae* populations. Microsatellite markers were validated on 175 isolates and demonstrated the ability to capture sufficiently the genetic diversity exhibited by *P. betae* populations from two states, NY and WA. High genotypic diversity and moderate allelic diversity were detected in *P. betae* populations from NY and WA. The relationships between these populations offer insights into the spread and biology of *P. betae* across the United States.

P. betae populations had significant but low genetic differentiation among the five locations sampled (three in WA and two in NY). Despite the overall limited differentiation among the five populations, the two *P. betae* populations from NY were differentiated from the three WA populations. Based on DAPC analysis using pre-defined populations, those within each state clustered closer together than those between states. Six of eight shared MLGs were only shared

among populations in the same state. The remaining two shared MLGs (MLG.27 and MLG.33) were among the WA2, NY1, and NY2 populations. The UPGMA dendrogram based on Nei's (1972) distance also divided the populations into two main groups based on states. After clone-correction, genetic differentiation decreased although general genetic differentiation trends persisted and differentiation between the NY populations was no longer significant. Taken together, these results suggested the presence of two populations of *P. betae*, one in WA and one in NY, and the dominant role of an external source of inoculum, such as windblown ascospores, contributing to homogeneity between populations within each state. Further studies designed to analyze isolation by distance of *P. betae* populations may be warranted.

Alternatively, *P. betae* has been reported multiple times to enter fields externally through infected beet seed (Edson 1915; Herr 1971; Leach and MacDonald 1976; Mangan 1971). Infected plants that do not succumb to damping-off from seedborne *P. betae* can persist and be a source of inoculum for development of Phoma leaf spot and/or root rot and decay in storage (Bugbee and Soine 1974; Edson 1915). Edson (1915) estimated that up to 75% of seedlings infected with *P. betae* can survive under favorable environmental conditions. Moreover, Bugbee (1982) estimated *P. betae* can remain latent for up to 100 days in storage before causing root rot. Despite the fact that much of the seed planted in NY originates from the maritime Pacific Northwest United States (western WA and OR), and distinct differentiation was observed between *P. betae* populations from NY and WA, greater understanding of the mycoflora of table beet seed planted in NY would be valuable.

Furthermore, while this study of *P. betae* in NY and WA did not directly assess the impact of seedborne inoculum of *P. betae* in disease outbreaks, population biology studies investigating the contribution of isolates from infected seed to field outbreaks would be useful. For example, the

mark-release-recapture strategy has been used to investigate the importance of various inoculum sources contributing to different diseases (Bennett et al. 2007; Muzhinji et al. 2018; Zhan and McDonald 2013). Bennett et al. (2007) marked winter wheat seed by infecting it with two groups of genetically distinct isolates of *P. nodorum*, released the isolates by planting infected seed in the field, and recaptured them by sampling throughout the growing seasons in Georgia and NY. In that study, seed-transmitted genotypes accounted for over half of all recovered isolates, however, the high frequency of novel genotypes suggested that other inoculum sources may also be important in initiating disease outbreaks (Bennett et al. 2007).

Results of this study indicated that inoculum originating from outside table beet fields may be important; however, within field sources of inoculum cannot be disregarded as *P. betae* has alternative hosts beyond subspecies of *B. vulgaris*. In addition to sugar beet, table beet, and Swiss chard, *P. betae* has been reported to infect *Aloe vera*, *Spinacia oleracea* (spinach), *C. album*, and *Avena sativa* (oat) (Avasthi et al. 2012; Bassimba et al. 2014; Bugbee and Soine 1974). *P. betae* has also been reported as an endophyte on *Anisomeles malabarica* (catmint) and *Ginkgo biloba* (maiden hair tree) (Jayanthi et al. 2014; Kumaran et al. 2012). Further studies evaluating local weeds and crops found in typical NY and WA rotations are hence warranted.

The limited but significant genetic differentiation observed across populations of *P. betae* in NY and WA in this study corroborates the finding of the greatest genetic diversity occurring within populations rather than among populations. Structuring of the *P. betae* populations among each field suggests that local inoculum source(s) may also play a role within each field surveyed. Studies of *P. betae* populations in a field over time may be useful to investigate hypotheses concerning sources of inoculum for initiating disease outbreaks (Grünwald et al. 2017; Milgroom 2015). For example, Knight et al. (2018) sampled *C. beticola* populations in three table beet fields over a two-

year period in NY and found populations to be genetically distinct each year, suggesting that primary inoculum was originating from external sources in some instances.

Microsatellite markers and mating type markers were used to explore the reproductive strategy of *P. betae* populations in this study. All populations appeared to experience a mixed reproductive mode of sexual and asexual reproduction, showing evidence of both random mating and clonality. Signs of random mating consisted of evidence for recombination including high genotypic diversity, and incongruence among the STRUCTURE, DAPC, and UPGMA dendrogram clusters. Similar findings have been described for *C. beticola* populations on table beet (Vaghefi et al. 2017b). Recombination may have a direct role in generating genotypic diversity (Diao et al. 2015; Liang et al. 2009; Vaghefi et al. 2017b). The mating type ratios in three of the five *P. betae* populations examined in this study were not significantly different from a 1:1 ratio, providing additional support for random mating.

Evidence for clonality in the five populations of *P. betae* sampled from NY and WA included moderate clonal fractions (0.429 to 0.686) and linkage disequilibrium. Besides a lack of recombination, linkage disequilibrium may result from selection, linkage, population admixture, and random genetic drift (Milgroom 2015). After clone-correction, standardized index of association values did not differ significantly from zero in the WA1 population, resulting in failure to reject the null hypothesis of random mating. For the remaining four populations, the null hypothesis of random mating was rejected. PrCP values also rejected the null hypothesis of random mating in all populations except for WA1. Tests of random mating conducted on the three major clusters detected by STRUCTURE analysis also were found to reject the null hypothesis of random mating, confirming that the detected linkage disequilibrium was not a function of population structure. This provides evidence for mixed modes of reproduction in *P. betae* populations. To

explain genetic diversity observed in populations, other population biology studies on fungal pathogens have postulated fungi predominantly undergo asexual reproduction with occasional recombination (Burt et al. 1996; Romero Luna et al. 2017; Taylor et al. 1999; Vaghefi et al. 2017b).

Maintaining both sexual and asexual reproduction may enable *P. betae* to adapt readily to different hosts and environments (Calo et al. 2013). When interpreting the results of this study, it is important to emphasize that population genetic analyses can only provide indirect evidence for sexual reproduction and recombination. Recombination may allow organisms to adapt to new environments and purge deleterious mutations (Zeyl and Belle 1997). This study found high genotypic diversity among five populations of *P. betae* from two states, indicating the potential for the fungus to adapt to selection pressures such as predominant use of fungicides with single-site modes of action (Milgroom 1996). Recombination, random mating tests, and molecular-based evidence of sex (presence of both mating types) also suggest the occurrence of a sexual cycle (Milgroom 2015). Evidence of sexual reproduction within populations of *P. betae*, i.e., the presence of pseudothecia, has been observed in the United Kingdom and midwestern United States (Bugbee 1979; Cornford 1958), but not in NY. The production of pseudothecia can have a significant impact on survival and dispersal of the fungus, and has previously been observed in sugar beet infested debris (Duffus and Ruppel 1995). Bugbee (1979) also observed pseudothecia on inoculated sugar beet stem pieces in North Dakota and plant debris following harvest of sugar beet seed crops in Oregon. Pathogenicity testing with sugar beet revealed infections arising from *P. betae* ascospores to result in more severe root rot than those initiated from conidia (Bugbee 1979). Observation and depiction of temporal dynamics of pseudothecia production in infested plant material in fields will enhance understanding of the biology of this pathosystem under local conditions.

This study developed microsatellite and mating type markers for *P. betae* that can be used to characterize populations of *P. betae* in future studies. The high genotypic diversity observed in all populations suggested that larger population sample size may be useful in future assessments. Evidence for the presence of two populations distinguished by state of origin, NY and WA, suggested that inoculum of *P. betae* disease outbreaks in these regions may originate predominantly from an external source, such as ascospores or seeds, and that dispersal of inoculum between states is not frequent. Despite evidence for external inoculum sources, the structure observed in individual populations provided evidence that local inoculum sources such as infected alternative hosts or soilborne inoculum associated with infested crop debris cannot be discounted. Further assessment of inoculum sources of *P. betae* causing disease outbreaks is warranted. Investigation of *P. betae* reproductive strategies in this study provided evidence for a mixed reproductive mode occurring in all five populations. Direct observation of pseudothecia, as an indication of sexual reproduction in the field, would also be instrumental to a comprehensive understanding of the role of overwintering inoculum and dispersal of *P. betae* in outbreaks.

REFERENCES

Abawi, G. S., Crossier, D. C., Cobb, A. C., and Becker, R. F. 1986. Root rot of table beets in New York State. NY Food Life Sci. Bul. No. 115.

Agapow, P.-M., and Burt, A. 2001. Indices of multilocus linkage disequilibrium. Mol. Ecol. Notes 1:101-102.

Ariyawansa, H. A., Thambugala, K. M., Manamgoda, D. S., Jayawardena, R., Camporesi, E., Boonmee, S., Wanasinghe, D. N., Phookamsak, R., Hongsanan, S., Singtripop, C., Chukeatirote, E., Kang, J.-C., Jones, E. B. G., and Hyde, K. D. 2015. Towards a natural classification and backbone tree for *Pleosporaceae*. Fung. Div. 71:85-139.

Avasthi, S., Gautam, A. K., and Bhadauria, R. 2013. First report of *Phoma betae* on *Aloe vera* in India. Arch. Phytopathol. Plant Prot. 46:1508-1511.

Aveskamp, M. M., Verkley, G. J. M, de Gruyter, J., Murace, M. A., Perelló, A., and Woudenberg, J. H. C. 2009. DNA phylogeny reveals polyphyly of *Phoma* section *Peyronellaea* and multiple taxonomic novelties. Mycologia 101:363–382.

Aveskamp, M. M., de Gruyter, J., Woudenberg, J. H. C., Verkley, G. J. M., and Crous, P. W. 2010. Highlights of the *Didymellaceae*: A polyphasic approach to characterize *Phoma* and related Pleosporalean genera. Stud. Mycol. 65:1-60.

Banke, S., and McDonald, B. A. 2005. Migration patterns among global populations of the pathogenic fungus *Mycosphaerella graminicola*. Mol. Ecol. 14:1881-1896.

Bassimba, D. D. M., Mira, J. L., and Vicent, A. 2014. First report of leaf spot of spinach caused by *Pleospora betae* in Spain. Plant Dis. 98:1583.

Bennett, R. S. Milgroom, M. G., Sainudiin, R., Cunfer, B. M., and Bergstrom, B. C. 2007. Relative contribution of seed-transmitted inoculum to foliar populations of *Phaeosphaeria nodorum*. Phytopathology 97:584-591.

Benson, G. 1999. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 27:573–580.

Bruford, M. W., and Wayne, R. K. 1993. Microsatellites and their application to population genetic studies. Curr. Opin. Genet. Dev. 3:939-943.

Bruvo, R., Michiels, N. K., D'Souza, T. G., and Schulenburg, H. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. Mol. Ecol. 13:2101–2106.

Bugbee, W. M. 1979. *Phoma bjoerlingii* in the USA. Phytopathology 69:277-278.

Bugbee, W. M. 1982. Storage rot of sugar beet. Plant Dis. 66:871-873.

Bugbee, W. M., and Soine, O. C. 1974. Survival of *Phoma betae* in soil. Phytopathology 64:1258-1260.

Bugbee, W. M., and Cole, D. F. 1981. The effect of seed infected with *Phoma betae* and sucrose yield of stored sugar beet. Phytopathology 71:357-359.

Burt, A., Carter, D. A., Koenig, G. L., White, T. J., and Taylor, J. W. 1996. Molecular markers reveal cryptic sex in the human pathogen *Coccidioides immitis*. Proc. Natl. Acad. Sci. U.S.A. 93:770-773.

Calo, S., Billmyre, R. B., and Heitman, J. 2013. Generators of phenotypic diversity in the evolution of pathogenic microorganisms. PLoS Pathogens 9:e1003181.

Carbone, I., and Kohn, L. M. 2004. Inferring process from pattern in fungal population genetics. Pages 29-58 in: Fungal Genomics, Applied Mycology and Biotechnology, D. K. Arora, and G. G. Khachatourians, eds. Elsevier Science, Amsterdam, The Netherlands.

Clark, K., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., and Sayers, E. W. 2016. GenBank. Nucleic Acids Res. 44:D67-D72.

Coppin, E., Debuchy, R., Arnaise, S., and Picard, M. 1997. Mating types and sexual development in filamentous ascomycetes. Microbiol. Mol. Biol. Rev. 61:411-428.

Cornford, C. E. 1958. Perfect state of *Phoma betae* found in England. Plant Pathol. 7:113-114.

de Gruyter, J., Woudenberg, J. H. C., Aveskamp. M. M., Verkley, G. J. M., Groenewald, J. Z., and Crous, P. W. 2013. Redisposition of *Phoma*-like anamorphs in *Pleosporales*. Stud. Mycol. 75:1-36.

Debuchy, R., and Turgeon, B. G. 2006. Mating-type structure, evolution, and function in Euascomycetes. Pages 293-323 In: The Mycota Vol. I. U. Kues and R. Fischer, eds. Springer-Verlag, Berlin, Germany.

Diao, Y., Zhang, C., Xu, J., Lin, D., Lui, L., Mtung'e, O. G., and Lui, X. 2015. Genetic differentiation and recombination among geographic populations of the fungal population *Colletotrichum truncatum* from chili peppers in China. Evol. Appl. 8:108-118.

du Toit, L. J. 2007. Crop profile for table beet seed in Washington. U.S Dept. Ag. Pest Management Centers. Online. <https://ipmdata.ipmcenters.org/documents/cropprofiles/WAbeetseed2007.pdf> Accessed 4 January 2018.

du Toit, L. J. 2017. *Phoma betae* in table beet and Swiss chard seed crops in Washington. Pages 27-31 in: Northwest Agricultural Research Foundation and Affiliated Organizations: 2017 Research Proposals and 2016 Research Reports, Mount Vernon, WA.

Duffus, J. E., and Ruppel, E. G. 1995. Major fungal diseases. Pages 369-393 in: The Sugar Beet Crop. D. A. Cooke and R. K. Scott, eds. Chapman & Hall, London, United Kingdom.

Earl, D. A., and vonHoldt, B. M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4:359-361.

Edson, H. A. 1915. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. J. Agric. Res. 4:135-168.

Ellegren, H. 2004. Microsatellites: simple sequences with complex evolution. Nat. Rev. Gen. 5:435-445.

Estabrook, G., and Landrum, L. 1975. A simple test for the possible simultaneous evolutionary divergence of two amino acid positions. Taxon 24:609-613.

Estoup, A., and Guillemaud, T. 2010. Reconstructing the routes of invasion using genetic data: why, how and so what? Mol. Ecol. 19:4113-4130.

Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. 14:2611-2620.

Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Goss, E. M., Tabima, J. F., Cooke, D. E. L., Restrepo, S. Fry, W. E., Forbes, G. A., Fieland, V. J. Cardenas, M., and Grünwald, N. J. 2014. The Irish potato famine pathogen *Phytophthora infestans* originated in central Mexico rather than the Andes. Proc. Natl. Acad. Sci. U.S.A. 111:8791-8796.

Grünwald, N. J., and Goss, E. M. 2011. Evolution and population genetics of exotic and re-emerging pathogens: novel tools and approaches. Annu. Rev. Phytopathol. 49:249-267.

Grünwald, N. J., Garbelotto, M., Goss, E. M., Heungens, K., and Prospero, S. 2012. Emergence of the sudden oak death pathogen *Phytophthora ramorum*. Trends Microbiol. 20:131-138.

Grünwald, N. J., Everhart, S. E., Knaus, B. J., and Kamvar, Z. N. 2017. Best practices for population genetic analyses. Phytopathology 107:1000-1010.

Harveson, R. M., Hanson, L. E., and Hein, G. L. 2009. Compendium of Beet Diseases and Pests, 2nd Edition. APS Press, St. Paul, MN.

Herr, L. J. 1971. Hot water treatment for elimination of seed-borne *Phoma betae* and other microbial contaminants from sugar beet seed. J. Amer. Soc. Sugar Beet Technol. 16:568-574.

Holleley, C. E., and Geerts, P. G. 2009. Multiplex Manager 1.0: a cross-platform computer program that plans and optimizes multiplex PCR. BioTechniques 46:511-517.

International Seed Testing Association (ISTA). 1982. Beet damping-off, blackleg. *Beta vulgaris*, *Phoma betae*. Working Sheet No. 49. ISTA Handbook on Seed Health Testing, ISTA, Zurich, Switzerland.

Jayanthi, G., Karthikeyan, K., and Muthumary, J. 2014. Pervasiveness of endophytic fungal diversity in *Anisomeles malabarica* from Aliyar, Western Ghats, South India. Mycosphere 5:830-840.

Jeffers, S. 2015. PROTOCOL 07-11.1: V8 agar (V8A) or broth. Pages 1-2 in: Laboratory Protocols for *Phytophthora* Species, J. Steve, ed. APS Press, St. Paul, MN.

Jombart, T. 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405.

Jombart, T., Devillard, S., and Balloux, F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94.

Jost, L. 2008. GST and its relatives do not measure differentiation. Mol. Ecol. 17:4015-4026.

Kamvar, Z. N., Tabima, J. F., and Grünwald, N. J. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281.

Kamvar, Z. N., Brooks, J. C., and Grünwald, N. J. 2015. Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Front Genet. 6:208.

Knight, N. L, Vaghefi, V. Hansen, Z. R., Kikkert, J. R., and Pethybridge, S. J. 2018. Temporal genetic differentiation of *Cercospora beticola* populations in New York table beet fields. Plant Dis. 102:2074-2082.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P., and Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., and Mayrose, I. 2015. CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Res. 15:1179-1191.

Kumaran, R. S., Choi, Y. K., Lee, S., Jeon, H. J., Jung, H., and Kim, H. J. 2012. Isolation of taxol, an anticancer drug produced by the endophytic fungus, *Phoma betae*. Afr. J. Biotechnol. 11:950-960.

Leach, L. D., and MacDonald, L. D. 1976. Seed-borne *Phoma betae* as influenced by area of sugarbeet production, seed processing and fungicidal seed treatments. J. Amer. Soc. Sugar Beet Technol. 19:4-15.

Li, H. X., and Brewer, M. T. 2016. Spatial genetic structure and population dynamics of gummy stem blight fungi within and among watermelon fields in the southeastern United States. Phytopathology 106:900-908.

Liang, J. F., Xu, J., and Yang, Z. L. 2009. Divergence, dispersal and recombination in *Lepiota cristata* from China. Fung. Div. 38:105-124.

Ludwig, J. A., and Reynolds, J. F. 1988. Statistical Ecology: a Primer on Methods and Computing. John Wiley & Sons, New York.

Mangiafico, S. S. 2015. An R companion for the handbook of biological statistics, version 1.09 i. Rutgers Cooperative Extension, New Brunswick, New Jersey.

Mangan, A. 1971. New method for the detection of *Pleospora bjoerlingii* infection of sugar beet seed. Trans. Br. Mycol. Soc. 57:169-172.

Maude, R. B., Vizor, A. S., and Shuring, C. G. 1969. The control of fungal seed-borne diseases by means of a thiram seed soak. Ann. Appl. Biol. 64:245-257.

McDonald, B. A. 2015. How can research on pathogen population biology suggest disease management strategies? The example of barley scald (*Rynchosporium commune*). Plant Pathol. 64:1005-1013.

McDonald, B. A., and Mundt, C. C. 2016. How knowledge of pathogen population biology informs management of *Septoria tritici* blotch. Phytopathology 106:948-955.

McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349-379.

Meirmans, P. G., and Van Tienderen, P. H. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4:792-794.

Miller, P. M. 1955. V-8 juice agar as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.

Milgroom, M. G. 1996. Recombination and the multilocus structure of fungal populations. Annu. Rev. Phytopathol. 34:457-477.

Milgroom, M. G. 2001. The synthesis of genetics and epidemiology: Contributions of population biology in plant pathology. J. Plant Pathol. 83:57-62.

Milgroom, M. G., del M Jiménez-Gasco, M., García, C. O., Drott, M. T., and Jiménez-Díaz, R. M. 2014. Recombination between clonal lineages of the asexual fungus *Verticillium dahliae* detected by genotyping by sequencing. PloS One 9:e106740.

Milgroom, M. G. 2015. Population biology of plant pathogens: genetics, ecology, and evolution. APS Press, St. Paul, MN.

Monte, E., and García-Acha, I. 1988. Vegetative and reproductive structures of *Phoma betae in vitro*. Trans. Br. Mycol. Soc. 90:233-245.

Muzhinji, N., Woodhall, J. W., Truter, M., and van der Waals, J. E. 2018. Relative contribution of seed tuber- and soilborne inoculum to potato disease development and changes in the population genetic structure of *Rhizoctonia solani* AG 3-PT under field conditions in South Africa. Plant Dis. 102:60-66.

Nei, M. 1972. Genetic distance between populations. Amer. Naturalist 106:283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Peakall, R., and Smouse, P. E. 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288-295.

Peakall, R., and Smouse, P. E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539.

Pethybridge, S. J., Vaghefi, N., and Kikkert, J. R. 2017. Horticultural characteristics and susceptibility of table beet cultivars to Cercospora leaf spot in New York. HortTechnol. 27:530-538.

Pethybridge, S. J., Kikkert, J. R., Hanson, L. E., and Nelson, S. C. 2018. Challenges and prospects for building resilient disease management strategies and tactics for the New York table beet industry. Agronomy 8:112.

Pompanon, F., Bonin, A., Bellemain, E., and Taberlet, P. 2005. Genotyping errors: causes, consequences and solutions. Nat. Rev. Genet. 6:847-859.

Pool, V. W., and McKay, M. B. 1915. *Phoma betae* on the leaves of sugar beet. J. Agric. Res. 4:169-177.

Pritchard, J. K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Romero Luna, M. P., Aime, M. C., Chilvers, M. I., and Wise, K. A. 2017. Genetic diversity of *Stenocarpella maydis* in the major corn production areas of the United States. Plant Dis. 101:2020-2026.

R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Short, D. P. G., Gurung, S., Hu, X., Inderbitzin, P., and Suubarao, K. V. 2014. Maintenance of sex-related genes and the co-occurrence of both mating types in *Verticillium dahliae*. PloS One 9:e112145.

Simpson, E. H. 1949. Measurement of diversity. Nature 163:688.

Solovyev, V. V. 2007. Statistical approaches in Eukaryotic gene prediction. Pages 97-159 in: Handbook of Statistical Genetics. D. J. Balding, M. Bishop, and C. Cannings, eds. John Wiley & Sons, Ltd., West Sussex, United Kingdom.

Stivers, L. 2001. Crop profile: beets in New York. U.S. Dept. of Pest Management Centers. Online. http://pmep.cce.cornell.edu/fqpa/crop-profiles/beet.html. Accessed 4 January 2018.

Taylor, J. W., Jacobson, D. J., and Fisher, M. C. 1999. The evolution of asexual fungi: reproduction, speciation and classification. Annu. Rev. Phytopathol. 37:197-246.

United States Department of Agriculture National Agricultural Statistics Service. 2012. USDA-NASS Quick Stats. Online. http://www.nass.usda.gov/Quick_Stats/. Accessed 20 April 2018.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., and Rozen, S. G. 2012. Primer3--new capabilities and interfaces. Nucleic Acids Res. 40:e115.

Vaghefi, N., Kikkert, J. R., Bolton, M. D., Hanson, L. E., Secor, G. A., and Pethybridge, S. J. 2017a. *De novo* genome assembly of *Cercospora beticola* for microsatellite marker development and validation. Fung. Ecol. 26:125-134.

Vaghefi, N., Nelson, S. C., Kikkert, J. R., and Pethybridge, S. J. 2017b. Genetic structure of *Cercospora beticola* populations on *Beta vulgaris* in New York and Hawaii. Sci. Rep. 7:1726.

Vaghefi, N., Silva, A., Koenick, L. B., and Pethybridge, S. J. 2019. Genome resource for *Neocamarosporium betae* (syn. *Pleospora betae*), the cause of Phoma leaf spot and root rot on *Beta vulgaris*. MPMI. (In Press) https://doi.org/10.1094/MPMI-12-18-0334-A.

Winter, D. J. 2012. MMOD: an R library for the calculation of population differentiation statistics. Mol. Ecol. Resour. 12:1158-1160.

Zeyl, C., and Bell, G. 1997. The advantage of sex in evolving yeast populations. Nature 388:465-468.

Zhan, J., Kema, G. H. J., Waalwijk, C., and McDonald, B. A. 2002. Distribution of mating type alleles in the wheat pathogen *Mycosphaerella graminicola* over spatial scales from lesions to continents. Fung. Genet. Biol. 36:128-136.

Zhan, J., and McDonald, B. A. 2013. Field-based experimental evolution of three cereal pathogens using a mark-release-recapture strategy. Plant Pathol. 62:106-114.

CHAPTER 3

PHOMA LEAF SPOT SUSCEPTIBILITY AND HORTICULTURAL CHARACTERISTICS OF TABLE BEET CULTIVARS IN NEW YORK*

Abstract

Phoma leaf spot (PLS) caused by *Phoma betae* (syn. *Neocamarosporium betae*) is an important fungal disease affecting table beet (*Beta vulgaris* L. subsp. *vulgaris*) production in New York (NY). PLS lesions on the foliage can lead to rejection in fresh market sales, and reduce leaf integrity, which can disrupt mechanized harvesting. Eight popular table beet cultivars were assessed for susceptibility to PLS using *P. betae* isolates representative of the NY population in two mist chamber trials and two small plot, replicated field trials in Geneva and Freeville, NY. No significant differences were found among cultivars in disease severity and epidemic progress (as measured by area under the disease progress stairs) in both mist chamber trials. In the field trials, there were significant differences in PLS incidence, severity, and epidemic progress and horticultural characteristics among cultivars. Non-red table beet cultivars (Avalanche, Boldor, and Chioggia Guardsmark) were less susceptible to PLS than red cultivars (Falcon, Merlin, Rhonda, Red Ace, and Ruby Queen). In both field trials, significant differences in the fresh weight of roots and dry weight of foliage were detected between cultivars at harvest (86 DAP in Freeville and 91 DAP in Geneva). Falcon had significantly higher root weight than Boldor, and Ruby Queen produced significantly more foliage than Boldor. Information on the performance of these cultivars provides locally valuable information for cultivar selection in a broad range of markets.

*Parts of this chapter were included in Koenick, L. B., Kikkert, J. R., and Pethybridge, S. J. 2019. Phoma leaf spot susceptibility and horticultural characteristics of table beet cultivars in New York. Plant Health Progress (Accepted March 28, 2019).

Introduction

New York (NY) is currently the second largest producing state of table beet (*Beta vulgaris* L. subsp. *vulgaris*) in the United States, representing 26% of that grown in 2012 (USDA 2012). Recently, there has been an expansion in table beet production due to an increased awareness by consumers of the potential health benefits of consumption (Clifford et al. 2017; Navazio et al. 2010; Pethybridge et al. 2018). Table beets are grown in NY for processing into cans and jars, fresh market sales of roots and/or foliage, and use in value-added products such as juices and powders (Pethybridge et al. 2018). For processing, root size, shape, texture, uniformity, flavor, color, and quality are important cultivar characteristics (Goldman and Navazio 2003; Kikkert et al. 2010; Pethybridge et al. 2018; USDA 2017). Root and foliage characteristics are also important for fresh market sales, yet can be more diverse in color and appearance to appeal to a broader consumer base.

Disease management is critical to ensuring profitability and productivity of table beet production (Abawi et al. 1986; Kikkert et al. 2010; Pethybridge et al. 2018; Shah and Stivers-Young 2004). One of the most important foliar diseases is Phoma leaf spot (PLS), caused by the fungus, *Phoma betae* (syn. *Neocamarosporium betae*) (Ariyawansa et al. 2015), which can cause loss of green leaf area, deleteriously affecting root yield and quality (Pool and McKay 1915). On foliage, PLS symptoms are tan-brown lesions with light brown centers and dark concentric rings (Figure 3.1) (Harveson et al. 2009; Pool and McKay 1915). At crop emergence, *P. betae* can cause pre-and post-emergence damping-off, which can negatively affect crop stands (Edson 1915; Pethybridge et al. 2018). On roots, *P. betae* can cause Phoma root rot which manifests as dry, black necrotic lesions usually beginning at the crown (Harveson et al. 2009). Latent infection of roots can lead to substantial losses from decay in storage (Bugbee 1982; Pethybridge et al. 2018). In

NY, surveys detected PLS in 35 of 60 table beet fields in 2017 and 2018. Moreover, the prevalence of PLS was higher in fields grown using organic production practices (74%) than conventional techniques (49%) (Appendix).

Phoma betae is commonly introduced to fields through infected seed (Edson 1915; Herr 1971; Leach and MacDonald 1976; Mangan 1971), and can overwinter in soil on plant debris for up to 26 months (Bugbee and Soine 1974). During the cropping season, *P. betae* undergoes asexual reproduction producing pycnidia and the resulting conidia can be dispersed short distances via water splash (Harveson et al. 2009; Monte and Garcia-Acha 1988a). Although the sexual form has not been observed in NY (Pethybridge, *unpublished data*), *P. betae* is capable of sexual reproduction producing pseudothecia and ascospores that can be disseminated long distances via wind (Bugbee 1979). Koenick et al. (2019) found *P. betae* to be heterothallic and molecular evidence for NY populations of *P. betae* to use a mixed reproductive mode throughout the growing season. Genetic differentiation and population structure among NY populations and those from Washington State supported within field and external inoculum sources to influence PLS epidemics (Koenick et al. 2019). Quantifying the temporal progression of a disease can also enhance our understanding of the relative importance of different inoculum sources and the various options, such as cultivar susceptibility, for improving disease management strategies (Jeger 2004; Madden et al. 2007; Vanderplank 1963).

Disease management in conventional table beet production is often reliant upon multiple applications of fungicides (Pethybridge et al. 2018; Vaghefi et al. 2016). If best management guidelines are not used, reliance on fungicides may not be sustainable due to the potential for development of resistance to those with single-site modes of action (Brent and Hollomon 2007). For organic table beet production, the inability to use conventional fungicides makes cultural

strategies, such as crop rotation, field design manipulation, and cultivar selection, critical for effective disease management (Shah and Stivers-Young 2004). These strategies are also beneficial to ensure horticultural characteristics, such as root size and shape, meet market requirements (Clark et al. 1967; Kikkert et al. 2010). The processing industry dictates acceptable table beet root size and shape to enable fit into cans and jars (USDA 2017) resulting in the use of only a few cultivars. For the fresh market, there is more flexibility in cultivar selection, and hence there is potential to select cultivars that are less susceptible to disease. Variation in the susceptibility of selected table beet cultivars has been quantified for Cercospora leaf spot caused by *Cercospora beticola* (Pethybridge et al. 2017a) and other diseases of sugar beet (Smith and Martin 1978). In organic production systems, the selection of cultivars with enhanced disease resistance may also reduce the use of copper, lessening build-up in soil and the potential for plant damage from phytotoxicity resulting from interactions at high temperatures (Pethybridge et al. 2017a; 2017b). To the best of our knowledge, no information on the susceptibility of table beet cultivars to PLS is available. The objectives of this study were to evaluate PLS susceptibility of popular table beet cultivars; and characterize important horticultural characteristics under NY growing conditions.

Materials and Methods

The susceptibility of eight table beet cultivars (Avalanche, Boldor, Chioggia Guardsmark, Falcon, Merlin, Rhonda, Red Ace, and Ruby Queen) to infection by *P. betae* was assessed in replicated mist chamber experiments in 2018/2019 and two small plot, replicated field trials in 2018. Cultivars were selected based on popularity of use in NY small, mixed vegetable farms, and to encompass a broad range of end-uses and root characteristics (Table 3.1). Commercial seed lots were used and seed treatments are listed in Table 3.1. Notably, Boldor seeds were untreated,

Rhonda seeds had a proprietary organic film coat, while seeds of other cultivars were fungicide-treated.

Mist chamber trials. Two trials took place in a centrally controlled mist chamber facility (Cornell AgriTech at The New York State Experiment Station, Geneva, NY) with 12 h of light at 820 lux (110 W fluorescent mercury bulbs, Philips, Andover, MA) and a temperature of 20 ± 4°C. Relative humidity was maintained at >90% for 4 h per day.

All plants were grown in the greenhouse from seed in 15.2 cm pots (HC Companies, Middlefield, OH) in a potting mix containing Canadian sphagnum peat moss, perlite, vermiculite, and dolomitic limestone (Sunshine® Mix #8/ Fafard®-2; Sun Gro Horticulture, Agawam, MA). The same randomized block design was used for both trials to allocate the spatial positions of plants in the mist chamber including five replications of each cultivar. All plants used in the experiments were placed in the mist chamber for 7 to 9 days prior to inoculation to remove any that may already be infected with *P. betae* through seedborne inoculum (Edson 1915; Herr 1971; Mangan 1971).

P. betae isolates collected in NY from diseased table beet leaves [RN1, RN20, TR1, and TR30] were used to prepare conidial inoculum to inoculate seven or eight-week-old plants with 6 to 11 true leaves. No information is available on differences in virulence and aggressiveness among *P. betae* isolates in the NY population. However, the high genetic diversity typically observed in *P. betae* populations suggests that differences in virulence may be likely (Koenick et al. 2019), and hence a mixed inoculum of representative isolates was preferred for these trials. Inoculum was prepared by removing the isolates from long-term storage (-20°C) and sub-culturing onto clarified V8 (CV8) juice [10% (v/v) CV8 juice (Campbell's Soup Co., Camden, NJ), 0.5% (w/v) $CaCO_3$] agar (Jeffers 2015; Miller 1955). After 5 to 7 days growth, each plate was subcultured onto four

new CV8 plates and incubated for 14 days in a 12 h photoperiod at room temperature to induce sporulation. Sterile distilled water (5 ml) was added to each plate and a scalpel was used to dislodge pycnidia into a falcon tube with 20 ml sterile distilled water. The falcon tube was vigorously vortexed and the solution was passed through two layers of sterile muslin cloth into a new suspension. A hemocytometer was used to quantify conidial concentration in the resulting suspension. The conidial suspension was diluted to approximately 1×10^5 spores per ml for inoculations and 0.1% (v/v) polysorbate 20 (Tween-20; Agdia Inc., Elkhart, IN) added before application to plants using a hand-held spray bottle (All Purpose Sprayer; The Bottle Crew; Farmington Mills, MI). Each plant received approximately 10 ml of inoculum. To quantify germination efficiency, 100 µl of the suspension was spread over the surface of three 2% water agar (Difco™ Agar; Becton, Dickinson and Company, Sparks, MD) plates. After 24 h, germination percentage was calculated for 100 conidia per plate. Germination was defined as the germ tube being twice the length of the conidium. Germination efficiency was 85% and 95% for trials one and two, respectively. An additional five plants of each cultivar served as noninoculated controls and received an equal volume of sterile distilled water + 0.1% (v/v) polysorbate 20.

Following inoculation, plants were individually placed in clear, plastic bags for 2 days to increase relative humidity and promote infection. For the next 7 days, plants remained in the mist chamber and the mist was applied for 2 h each day. Plants were monitored over the next 17 days for PLS symptoms.

PLS incidence and severity were evaluated on eight occasions at 2 to 3 day intervals (0, 6, 8, 10, 12, 14, 16, and 17 days post-inoculation; DPI). Incidence was defined as the number of leaves with at least one PLS lesion, divided by the total number of leaves on each plant × 100. Severity

was rated on three arbitrarily selected leaves per plant and defined as the percentage of symptomatic area relative to total leaf area and averaged per plant.

After the final evaluation, foliage from each plant was clipped at the juncture of petiole and stem. On one diseased leaf per plant (five leaves per cultivar), the presence of pycnidia was confirmed at 63× magnification (Olympus SZX10 Zoom Stereo Microscope, Tokyo, Japan). Pycnidia from 12 diseased leaves were excised and placed on CV8 agar to complete Koch's postulates. Re-isolation frequency (%) was calculated by dividing the total number of isolations conducted by the number of isolations that resulted in *P. betae*. Identity was confirmed by observation of colony morphology on CV8 agar, and the presence of pycnidia and conidia typical of *P. betae* (olivaceous to olivaceous-black pilose pycnidia containing unicellular hyaline conidia with dimensions in the published range; Boerema et al. 2004; Monte and García-Acha 1988b; Monte et al. 1989).

Field trials. Two field trials were conducted in 2018 to further evaluate the susceptibility of table beet cultivars to PLS and quantify horticultural characteristics under NY growing conditions. One field trial was at the Homer C. Thompson Research Farm (Freeville, NY; 42.52 N, 76.34 W) and the other at the Research North farm of Cornell AgriTech at The New York State Agricultural Experiment Station (Geneva, NY; 42.87 N, 77.03 W). The soil type at Freeville was a Howard gravel loam. The soil type at Geneva was a Lima silt loam.

Each trial was planted using an identical randomized block design and each cultivar was replicated five times. Each cultivar was planted with the same seed used for the mist chamber trials (Table 3.1). In Freeville, a plot consisted of four rows (38 cm between individual rows and 4.6 m long). There were four unplanted buffer rows between plots and a ~1.5 m unplanted alley between plots within rows, which were cultivated for weed control at regular intervals. In Geneva, a plot

consisted of two rows (76.2 cm between individual rows), each 4.6 m long. Two buffer rows of Ruby Queen separated plots among rows and a ~1.5 m unplanted alley between plots within rows.

The trial at Freeville was planted on June 12, 2018 with a Monosem vacuum planter (Monosem, Edwardsville, KS) at an in-row planting density of 56.1 seeds/m. The trial at Geneva was planted on June 21, 2018 with a Jang JP1 clean seeder (Jang Automation Co., Cheongju, South Korea) at an in-row plant density of 30.4 seeds/m.

In Freeville, 560.43 kg/ha of 20N-10P-10K fertilizer (Phelps Supply, Phelps, NY) was broadcast applied and shallow incorporated prior to planting and S-metolachlor herbicide (Dual Magnum; Syngenta Corp., Greensboro, NC) applied at 0.78 L/ha on the same day as planting. At 51 days after planting (DAP), an application of spinosad insecticide (0.58 L/ha; Entrust SC; Dow AgroSciences, Indianapolis, IN) with an adjuvant (0.04% v/v; Induce; Helena Agri-Enterprises, Collierville, TN) was made for leaf miner control. An application of 22.7 kg/ha ammonium nitrate (Phelps Supply, Phelps, NY) was side-dressed by hand at 49 DAP.

In Geneva, 10N-5P-10K + Boron at 2.2 kg/ha (Phelps Supply, Phelps, NY) was broadcast at 336.26 kg/ha prior to planting and banded at 392.3 kg/ha during planting. The herbicide S-metolachlor (0.78 L/ha) was applied on the same day as planting. Both trials were irrigated as needed to maintain optimal plant growth and disease development.

Table 3.1 Characteristics of cultivars evaluated for susceptibility to Phoma leaf spot caused by *Phoma betae* in replicated field trials in New York in 2018

Cultivar	Color	Root Shape	Primary Use	Time to Maturity (Days)[a]	Seed Company[b] (City, State)	Seed Treatment
Avalanche	White	Globe	Fresh market	55	Harris Seeds (Rochester, NY)	Mefenoxam, Thiram, Fludioxonil
Boldor	Yellow	Globe	Fresh market	55	Johnny's Seeds (Fairfield, ME)	Untreated
Chioggia Guardsmark	Pink and white-striped	Globe	Fresh market	55	Sakata (Morgan Hill, CA)	Mefenoxam, Thiram, Fludioxonil
Falcon	Red	Blocky	Fresh market	80 to 85	Sakata (Morgan Hill, CA)	Mefenoxam, Thiram
Merlin	Red	Globe	Fresh market	48	Harris Seeds (Rochester, NY)	Mefenoxam, Thiram
Red Ace	Red	Globe	Processing and fresh market	50	Harris Seeds (Rochester, NY)	Mefenoxam, Thiram
Rhonda	Red	Globe	Fresh market	65 to 70	Bejo (Geneva, NY)	Organic seed treatment
Ruby Queen	Red	Globe	Processing	55	Seneca Foods (Geneva, NY)	Thiram

[a] From direct seeding to harvest.
[b] Company is where seeds were obtained and are not necessarily the breeder of the cultivar.

Inoculation with P. betae. The entire trial area was inoculated at 42 DAP (Geneva) and 56 DAP (Freeville) when plants had 10 to 15 true leaves. Inoculum was prepared using the same protocol as described for the mist chamber experiments and the same four *P. betae* isolates. For each trial, 6 L of inoculum (+ 0.1% v/v polysorbate-20) was applied within 5 h of preparation. The inoculum was applied with a backpack sprayer (DB Sprayer Professional; Fountainhead Group, New York Mills, NY) at a rate of 8.2 ml/m with a walking speed of 1.67 s/m. The concentration of the inoculum was 7×10^4 conidia/ml (Geneva) and 4×10^4 conidia/ml (Freeville). Germination

efficiency of the conidia was quantified as described above for the mist chamber trials and was 98% and 97% for the Geneva and Freeville trials, respectively.

Disease evaluations. In-row plant density, PLS incidence and severity was evaluated in each plot. In-row plant density counts (number of plants/m) were conducted at 28 DAP at both locations following stabilization of plant stands. PLS incidence and severity was assessed on ten arbitrarily selected leaves from each of two rows (20 leaves per plot). In Freeville, leaves were only selected from the middle two rows of each plot. Disease assessments were conducted at least six times throughout the growing season. In Freeville, assessments were made at 0 (Aug 3), 8 (Aug 15), 12 (Aug 19), 15 (Aug 22), 22 (Aug 29), and 28 (Sept 5) DPI. In Geneva, assessments were made at 0 (Aug 2), 8 (Aug 8), 13 (Aug 15), 16 (Aug 18), 20 (Aug 22), 26 (Aug 28), 32 (Sept 4), 39 (Sept 11), and 46 (Sept 18) DPI. Disease assessments were recorded on a tablet using the Android application Field Book (Rife and Poland 2014).

Yield and other horticultural characteristics. Trials were harvested by hand at 86 DAP (Freeville) and 91 DAP (Geneva). Plants were removed from two 0.5-m transects within each plot and separated into foliage and roots. The fresh weight of foliage (kg) was recorded in the field. A subsample of the foliage was weighed and dried at 37.5°C for four days to calculate percentage of dry matter. The roots from each plot were counted and weighed (kg). A subsample of up to 20 roots were randomly selected from each plot for measurement of root shoulder diameter (mm) with calipers. When less than 20 roots were present in the sample for each plot, the diameter of all roots were measured.

Weather. On-site average monthly temperature and precipitation data for both field locations was obtained from the Northeast Regional Climate Center (NRCC; http://climod2.nrcc.cornell.edu/). Data at each location were compared to the long-term averages

(LTA) (1981 to 2010) (Northeast Regional Climate Center 2018). Growing degree days (GDD_{10}) calculated with a base of 10°C were also obtained from the NRCC and compared to long term averages.

Data analysis. All statistical analyses were performed in R statistical software v.3.5.1 (R Core Team 2017). Disease severity at each evaluation were used to calculate the area under the disease progress stairs (AUDPS) as a measure of epidemic progression (Simko and Piepho 2012). First, the area under the disease progress curve (AUDPC) was calculated in the R package *agricolae* v.1.2-8 (De Mendiburu 2017) and then converted to AUDPS using the formula AUDPS = AUDPC $+ [\frac{y_1 + y_n}{2} \times \frac{D}{n-1}]$ (Simko and Piepho 2012).

Effect of cultivar. For both the mist chamber trials and field trials, the R package *lme4* v.1.18 (Bates et al. 2015) was used to create mixed models with table beet cultivar as a fixed effect and replication as a random effect. The effect of cultivar on PLS incidence, severity, and AUDPS was evaluated through analysis of variance (ANOVA). To check assumptions of normality and homoscedasticity, quantile-quantile plots (Atkinson 1987), histograms with a normality curve overlaid, and residual plots were evaluated visually in the R packages *car* v.3.0-2 (Fox and Weisberg 2011), *rcompanion* v.2.0.0 (Mangiafico 2018), and *ggplot2* v.3.1.0 (Wickham 2016), respectively. When necessary, the Shapiro-Wilk's test (Shapiro and Wilk 1965) was conducted in the R package *stats* v.3.6.0 (R Core Team 2017) as another formal test of normality. Outliers were checked with Cook's distance (Cook and Weisberg 1982) in *stats*. Transformations were made as needed. Data for root number in the Geneva field trial benefited from a log transformation, and AUDPS (Freeville field trial) required a square root transformation to remove skew and meet ANOVA assumptions.

Data from the two independent mist chamber trials were analyzed together as initial the assumptions of equal variance and homogeneity were satisfied. Equal variance for all variables was assessed using Levene's test (Fox 2015) in *car*. For disease severity, no significant differences among means between trials were found except for Rhonda ($P = 0.013$) using the using the Tukey-Kramer honestly significant difference method (Miller 1981) in the R package *emmeans* v.1.3.0 (Lenth et al. 2018). For AUDPS, no significant differences between means across trials were found. There was not a significant interaction among cultivar and trial for disease severity, AUDPS, or incidence. Trial was significant for incidence in the combined dataset.

In the field trial data, the effect of plant density as a covariate on disease and yield component response variables was assessed using ANOVA and included in models when significant ($P < 0.05$). Significant differences ($P = 0.05$) in the means between cultivars in each trial were identified using the Tukey-Kramer honestly significant difference method (Miller 1981) in *emmeans*. Data for each field trial was analyzed separately.

Temporal disease progress. Temporal analysis of epidemics in the field trials was conducted using PROC REG (SAS v.9.4 (SAS Institute Inc., Cary, NC) using data collected from Rhonda and Falcon cultivars. Disease progression was fit to exponential, monomolecular, logistic, and Gompertz models for each field trial. Disease epidemics may be classified as monocyclic (one infection cycle per crop cycle) or polycyclic (multiple infection cycles per crop cycle) (Vanderplank 1963; Madden et al. 2007). A monomolecular model is usually the best fit of a monocyclic epidemic, whereas Gompertz and logistic models are usually best descriptors of a polycyclic epidemic (Madden et al. 2007). Goodness of each model fit was determined by examining residual plots for normality, randomness and constant variance, and by viewing the following fitness statistics for linearity: *F*-statistic, coefficient of determination (R^2), the root mean

square error (RMSE), and coefficient of variation (CV). Comparisons between models were conducted by calculating the back-transformed fitted (R^{*2}) severity values of the original. Within each cultivar, fit of the selected model was summarized using the R^{*2}, RMSE, CV, and slope and intercept values.

Results

Mist chamber trials. No significant differences in PLS incidence were identified among cultivars (Table 3.2). ANOVA found a significant difference among cultivars for disease severity; however, pairwise comparisons between cultivars were not significant after correcting for multiple comparisons. Significant differences were observed among cultivars for AUDPS. Chioggia Guardsmark and Merlin had significantly lower AUDPS values than Boldor (Table 3.2). No PLS symptoms were detected on control plants.

Table 3.2 Effect of table beet cultivar on Phoma leaf spot incidence, severity and disease progress following inoculation with selected *Phoma betae* isolates. Means represent a dataset following combining of two mist chamber trials.

Cultivar	Incidence (%)	Disease Severity (%)[x]	AUDPS[y]
Avalanche	29 a[z]	35.4 a	167.9 ab
Boldor	29 a	53.2 a	473.8 b
Chioggia Guardsmark	33 a	20 a	140.6 a
Falcon	36 a	28.4 a	200.1 ab
Merlin	37 a	23.3 a	161.1 a
Red Ace	38 a	55 a	405.4 ab
Rhonda	41 a	40.3 a	257.1 ab
Ruby Queen	42 a	39.9 a	326.4 ab
HSD	32	52.91	312.58
F =	0.903	2.4	3.19
P =	0.509	0.03	0.006

[x] Severity is the percentage of the leaf affected by PLS.
[y] Area Under the Disease Progress Stairs (Simko and Piepho 2012).
[z] Values given are the means of ten replicates. Means followed by the same letter in a column are not statistically different using the Tukey-Kramer honestly significant difference ($P \leq 0.05$).

Field trials. Symptoms of PLS were typical tan-brown lesions with dark concentric rings of pycnidia and similar across table beet cultivars (Figure 3.1). Significant differences in PLS susceptibility and horticultural characteristics were observed among cultivars at both locations. Cultivar had a significant effect on in-row plant density at both locations (Table 3.3) and was a significant covariate for root diameter and average root weight at Geneva, but did not influence response variables at Freeville.

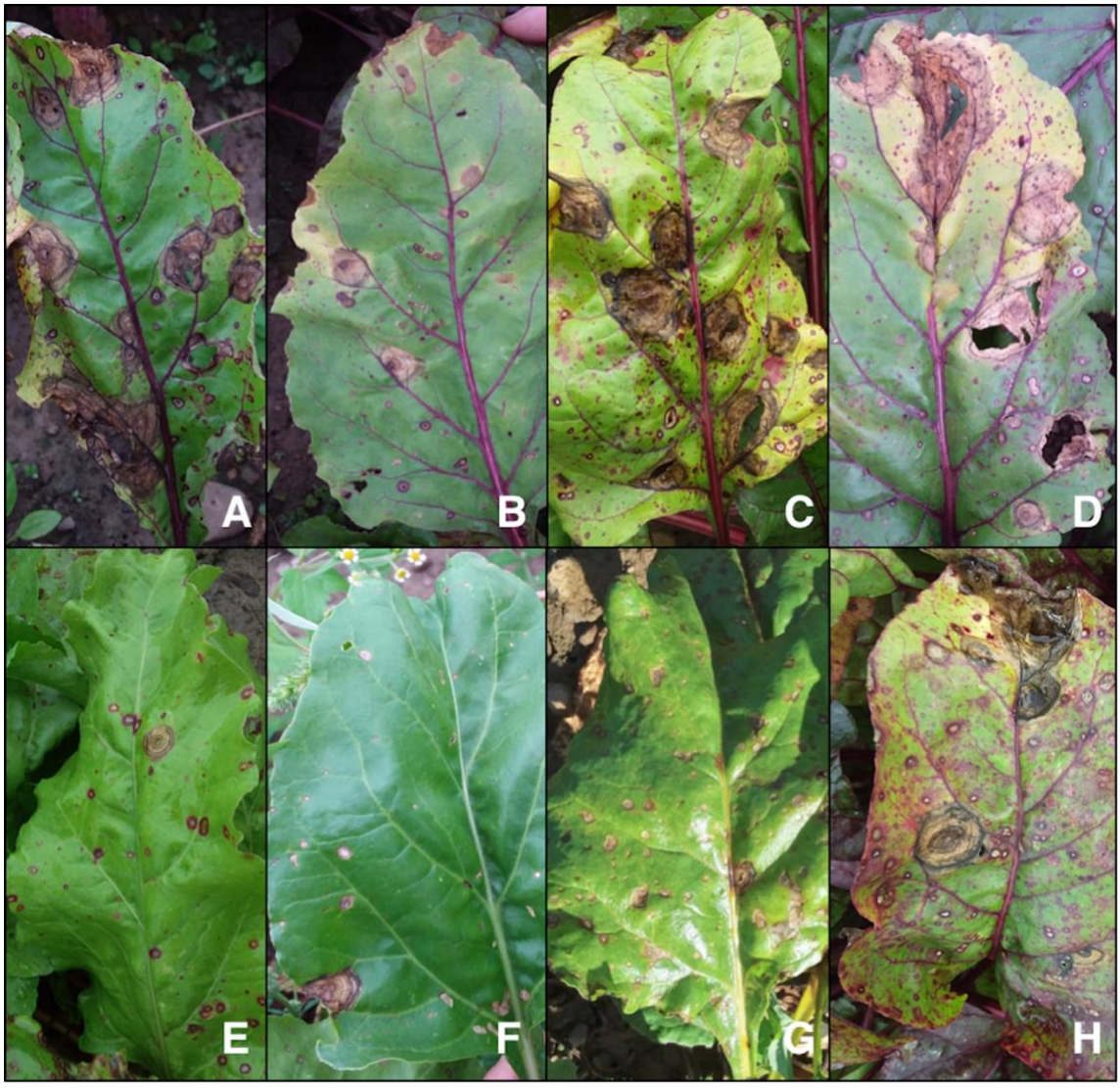

Figure 3.1 Phoma leaf spot symptoms caused by *Phoma betae* on different table beet cultivars (**A**, Rhonda; **B**, Red Ace; **C**, Merlin; **D**, Falcon; **E**, Chioggia Guardsmark; **F**, Avalanche; **G**, Boldor; and **H**, Ruby Queen) within two replicated field trials conducted in New York in 2018.

Differences in PLS incidence, severity, and progression were also observed among cultivars. Non-red cultivars had significantly lower PLS incidence than red cultivars (Table 3.3). At Geneva, on average, PLS incidence was 47.3% and 86% in non-red and red cultivars, respectively, at the final assessment. In Freeville, PLS incidence was lowest in Chioggia Guardsmark and Boldor with an average of 29.5%. PLS incidence was significantly higher in red than non-red cultivars with an average of 77.2% (Table 3.3).

PLS severity was lowest in Chioggia Guardsmark in both trials (7.9% and 5% in Geneva and Freeville, respectively at 91 DAP and 86 DAP). In contrast, PLS severity was highest in Rhonda in both trials, and significantly different from all cultivars except Falcon in the Geneva trial. PLS severity in Rhonda was 342 and 862% higher than in Chioggia Guardsmark in the Geneva and Freeville trials, respectively. Of the red cultivars, final PLS severity was not significantly different between Red Ace, Falcon, Merlin, and Ruby Queen in both trials (Table 3.3). PLS severity in Red Ace was 48.5% and 123.7% less than in Rhonda at Geneva and Freeville, respectively. Similar trends in PLS susceptibility were observed among non-red and red cultivars when evaluating AUDPS (Figure 3.2; Table 3.3). AUDPS was significantly lower in non-red cultivars than red cultivars in the Geneva trial. Chioggia Guardsmark and Boldor had the lowest AUDPS values at Freeville and AUDPS values were not significantly different among Falcon, Merlin, Red Ace, and Ruby Queen in both trials (Table 3.3). Rhonda had the highest AUDPS values in both trials. Temporal disease progress in Rhonda and Falcon was best fit to a Gompertz model (Table 3.4).

Table 3.3 Effect of table beet cultivar on plant density, and the incidence and severity of Phoma leaf spot caused by *Phoma betae* at harvest, and epidemic progress in two field trials at Geneva and Freeville, New York in 2018

	Plant Density[w]		Incidence (%)[x]		Disease Severity (%)[x]		AUDPS[y]	
Cultivar	**Geneva**	**Freeville**	**Geneva**	**Freeville**	**Geneva**	**Freeville**	**Geneva**	**Freeville**
Avalanche	13.9 ab[z]	27.5 ab	56 a	48 b	15.3 b	14.6 ab	352.7 b	15.2 (233.3) b
Boldor	8.5 a	14.7 a	42 a	28 a	10.9 ab	5.2 a	264.6 ab	7.7 (60.0) a
Chioggia Guardsmark	21.9 c	35.3 b	44 a	31 a	7.9 a	5.0 a	168.7 a	8.7 (76.9) a
Falcon	17.5 bc	25.9 ab	87 b	78 cd	29.2 cd	30.7 c	560.2 c	20.9 (445.3) c
Merlin	16.3 bc	21.1 ab	87 b	76 cd	27.0 c	30.1 c	599.5 c	20.7 (427.8) c
Red Ace	12.2 ab	18.4 a	82 b	65 c	23.5 c	21.6 bc	554.1 c	17.8 (318.4) bc
Rhonda	14.6 ab	17 a	90 b	88 d	34.9 d	48.1 d	786.1 d	27.0 (732.4) d
Ruby Queen	39.4 d	29.9 ab	84 b	79 cd	26.9 c	27.8 c	613.4 c	19.3 (375.4) c
HSD	7.3	16.3	16	15	7.11	10.61	139.52	3.58
F =	41.54	4.65	37.96	56.90	41.65	45.66	49.07	74.48
P =	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[w] In-row plant density (number of plants/m) at 28 days after planting.

[x] Incidence is the number of leaves with at least one PLS lesion / total number of leaves × 100; severity is the percentage of the leaf affected by PLS relative to the total leaf area.

[y] Area Under the Disease Progress Stairs (Simko and Piepho 2012), non-transformed values in parentheses.

[z] Values given are the means of five replicates. Means followed by the same letter in a column are not statistically different using the Tukey-Kramer honestly significant difference ($P \leq 0.05$).

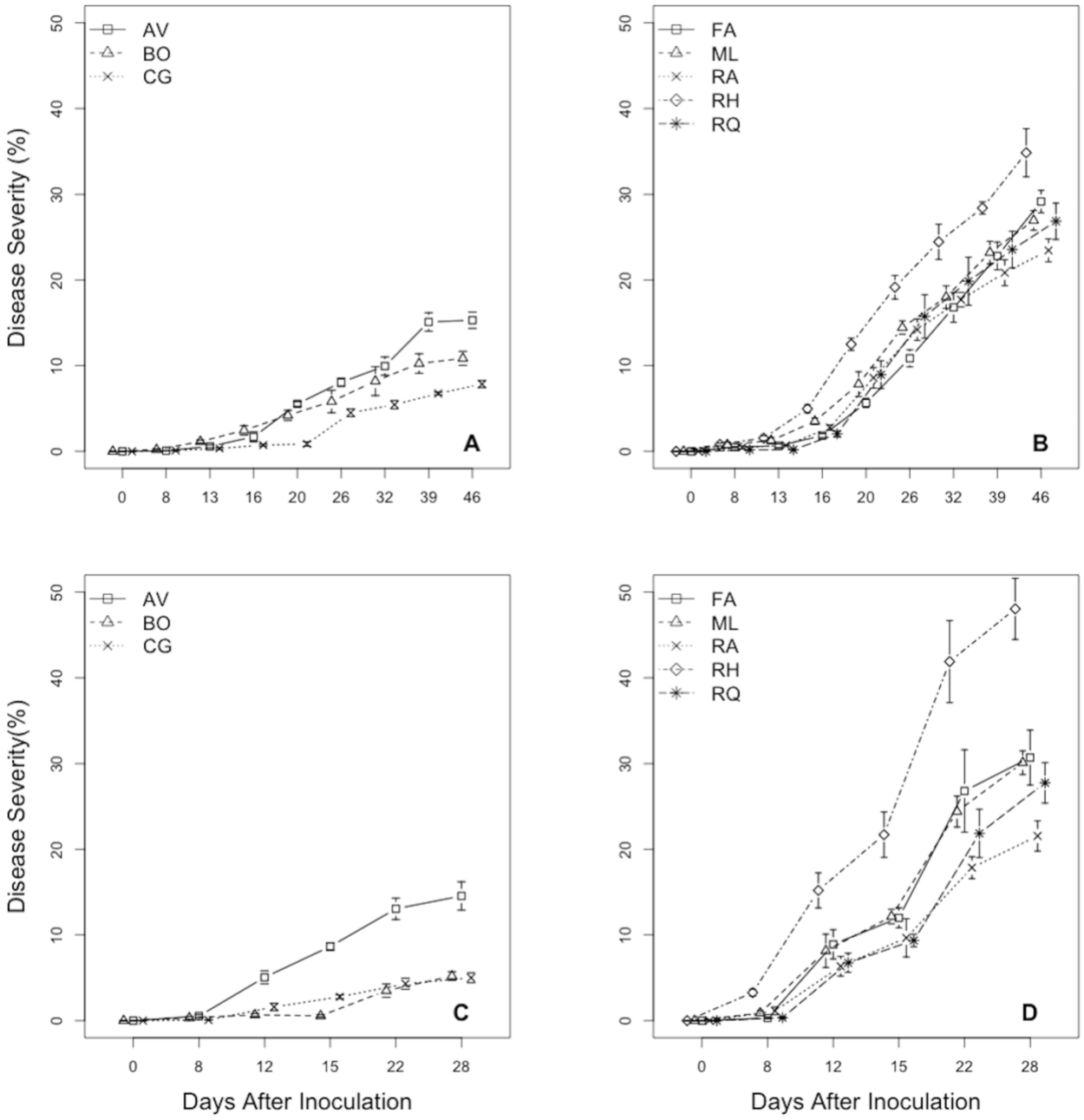

Figure 3.2 Disease progress curves depicting Phoma leaf spot epidemics caused by *Phoma betae* for **A/C,** non-red table beet cultivars [Avalanche (AV), Boldor (BO), Chioggia Guardsmark (CG)] and **B/D,** red table beet cultivars [Falcon (FA), Merlin (ML), Red Ace (RA), Rhonda (RH), and Ruby Queen (RQ)] in **A/B,** Geneva and **C/D,** Freeville field trials in New York in 2018.

Table 3.4 Temporal disease progression of Phoma leaf spot epidemics caused by *Phoma betae* in table beet cultivars Rhonda and Falcon at Freeville and Geneva, New York in 2018

Cultivar, Field	Best Fit[a]	Slope	Intercept	R^{*2}[b]	RMSE[c]	*F*-statistic	*P* =	CV (%)[d]
Rhonda, Geneva	Gompertz	0.041	-1.78	0.92	0.18	65.85	0.0002	-23.71
Rhonda, Freeville	Gompertz	0.075	-1.64	0.96	0.19	45.61	0.0066	-48.36
Falcon, Geneva	Gompertz	0.041	-2.0	0.97	0.11	162.53	< 0.0001	-11.83
Falcon, Freeville	Gompertz	0.072	-1.99	0.89	37.95	15.82	0.0284	-37.95

[a] Best fit from comparisons between the exponential, monomolecular, logistic, and Gompertz models.
[b] Back-transformed R^{*2}.
[c] Root Mean Square Error.
[d] Coefficient of variation.

Significant differences were found among cultivars for all root characteristics at Geneva (Table 3.5). Ruby Queen produced significantly more roots than other cultivars. Of the red cultivars, Ruby Queen produced 151, 153, 259, 230% more roots than Falcon, Merlin, Red Ace, and Rhonda, respectively. Root number was not significantly different between Avalanche, Boldor, Red Ace, and Rhonda (Table 3.5). At Geneva, Ruby Queen and Chioggia Guardsmark produced smaller roots. Root diameter was not significantly different between Avalanche, Boldor, Falcon, Red Ace, and Rhonda but were significantly larger than Ruby Queen. Root weight was significantly higher in Falcon than Ruby Queen in Geneva, and Boldor at both locations. For example, Falcon produced 100 and 107% more root biomass than Boldor at Geneva and Freeville, respectively. There were no significant differences in root number, root diameter, and average root weight among cultivars at Freeville (Table 3.5).

Cultivar also had a significant effect on the dry weight of foliage in both trials (Table 3.5). For example, Boldor produced significantly less foliage than Chioggia Guardsmark and Ruby Queen. At Freeville, Boldor produced significantly less foliage than other cultivars except for Rhonda. The dry weight of foliage was not significantly different between Chioggia Guardsmark, Falcon,

Merlin, Red, Ace, Rhonda, and Ruby Queen at Freeville; and Avalanche, Chioggia Guardsmark, Falcon, Merlin, Rhonda, and Ruby Queen at Geneva (Table 3.5).

Table 3.5 Horticultural characteristics of table beet cultivars at harvest[y] in two field trials inoculated with *Phoma betae* in Geneva and Freeville, New York in 2018

	No. Roots/m		Root Diameter (mm)		Fresh Root Weight (kg/m)		Average Root Weight (g)		Dry Foliage Weight (g)	
Cultivar	**Geneva**	**Freeville**	**Geneva**	**Freeville**	**Geneva**	**Freeville**	**Geneva**	**Freeville**	**Geneva**	**Freeville**
Avalanche	2.71 (15.6) a[z]	33.3 a	69.1 cd	35.7 a	3.7 bc	1.3 abc	245.4 cd	41.2 a	165.2 ab	197.1 c
Boldor	2.5 (12) a	20.1 a	66.9 cd	39.8 a	2.2 a	0.9 a	187.1 bc	54.6 a	101.0 a	67.6 a
Chioggia Guardsmark	3.4 (30.6) bc	24.1 a	53.9 ab	36.2 a	2.9 ab	1.0 ab	97.9 ab	41.2 a	196.5 b	152.4 bc
Falcon	2.9 (19.8) ab	27.7 a	64.6 bcd	42.7 a	4.4 c	1.8 c	229.4 cd	64.2 a	157.6 ab	128.0 b
Merlin	3.0 (19.6) ab	28.9 a	60.8 bc	41.8 a	3.4 bc	1.4 abc	183.9 bc	55.3 a	151.6 ab	133.8 b
Red Ace	2.6 (13.8) a	30.4 a	73.1 d	40.0 a	4.0 bc	1.2 ab	305.0 d	44.3 a	109.7 a	130.8 b
Rhonda	2.7 (15.8) a	33.9 a	71.3 cd	41.0 a	3.9 bc	1.5 bc	269.4 cd	51.3 a	136.1 ab	98.8 ab
Ruby Queen	3.9 (49.6) c	30.3 a	47.2 a	38.0 a	2.9 ab	1.5 bc	60.2 a	47.5 a	197.1 b	133.5 b
HSD	0.53	17.19	12.16	10.97	1.21	0.56	108.07	33.85	80.95	57.96
F =	18.3	1.56	2.76	0.79	7.64	5.99	5.22	1.19	4.52	9.31
P =	< 0.001	0.19036	0.026	0.600	< 0.001	< 0.001	< 0.001	0.341	0.002	< 0.001

[y] Trials were harvested at 91 and 86 days after planting in Geneva and Freeville, respectively. Non-transformed values in parentheses.

[z] Values given are the means of five replicates harvested from a one meter transect in each plot. Means followed by the same letter in a column are not statistically different using the Tukey-Kramer honestly significant difference ($P \leq 0.05$).

Weather. Weather during the growing seasons in Geneva and Freeville was similar to the LTA. At Geneva, the average minimum and maximum temperatures were: 12.9°C and 23.5°C (June); 16.7°C and 27.8°C (July); 17.4°C and 26.1°C (August); and 13.2°C and 22.8°C (September). At Freeville, the average minimum and maximum temperatures were: 11.3°C and 23.2°C (June); 15.2°C and 27.8°C (July); 16.6°C and 25.9°C (August); and 12.9°C and 22.8°C (September). Temperatures were less than 10% different than the LTA in both locations with the only exceptions being minimum temperatures in August and September. In both locations, August and September minimum temperatures were up to 12% higher compared to the LTA. The average monthly rainfall was 6.9 cm (June), 8.1 cm (July), 17 cm (August), and 8.7 cm (September) at Geneva; and 4.9 cm (June), 17.7 cm (July), 11.3 cm (August), 13.1 cm (September) at Freeville. The cropping season was wetter with a 14% and 15% increase in total precipitation between June through September compared to the LTA in Geneva and Freeville, respectively. A total of 2,246 GDD_{10} accumulated during the growing season in Geneva (LTA = 2,052) and 2,111 GDD_{10} accumulated in Freeville (LTA = 1,880).

Discussion

Maintaining foliar health is essential for profitable table beet production to minimize crop losses through facilitation of mechanized harvesting and ensuring high quality for direct sales (Pethybridge et al. 2018). Strict quality standards make selection of cultivars with varying susceptibility to PLS and other diseases an important tool for disease management. To the best of our knowledge, this is the first study to evaluate the susceptibility of eight popular table beet cultivars to PLS. In the mist chamber trials, there were no significant differences among cultivars in disease severity and incidence after correcting for multiple comparisons. ANOVA found a

significant difference among cultivars for disease severity. In future research, this could be mitigated by reducing the number of cultivars tested or increasing sample size (Keselman 1976). Merlin and Chioggia Guardsmark were found to have significantly lower epidemic progress than Boldor. Despite limited differences among cultivars in susceptibility to PLS observed in the mist chambers, there were significant differences in the field.

Differences in PLS incidence, severity, and epidemic progress among mist chamber and field trials may be explained by variation in experimental design (trial duration and sample size), plant age, environmental conditions, and agronomic factors. The final evaluation was conducted at 17 DPI in the mist chamber trials, whereas in the field trials, final evaluations were made at 46 and 28 DPI in Geneva and Freeville, respectively. In the mist chamber, individual plants were inoculated versus a population of plants within plots in the field trials. Increased epidemic duration and larger sample sizes may have contributed to inconsistent results between the mist chamber and field trials. Moreover, in the mist chamber trials, plants were inoculated with *P. betae* at 8 to 12 true leaves. In contrast, plants were inoculated at the 10 to 15 true leaf stage in field trials (42 and 56 DAP at Geneva and Freeville, respectively). Age has been reported as a factor in the susceptibility of sugar beet to PLS with older leaves being more susceptible (Pool and McKay 1915).

Mist chamber conditions provided optimal conditions for pathogen infection and disease development for PLS. In the field trials, environmental conditions were inherently more variable and plants were subjected to additional abiotic and biotic stresses. Abiotic stresses include diverse weather and edaphic conditions. Biotic stresses included pest damage and other diseases. For example, moderate damage from leaf miner was incurred at Freeville and required an insecticide

application. Despite the leaf miner damage at Freeville, similar trends in cultivar susceptibility to PLS were found to those at Geneva.

Minor differences in PLS severity and epidemic progress between field trials can be attributed to field design. Due to machinery differences, row spacing within plots at Freeville was half that of Geneva. Row spacing can affect final yields, root size, and disease incidence (Kikkert et al. 2010; Shah and Stivers-Young 2004). A survey conducted between 1998 and 2000 found that narrower row spacing increased the incidence of root decay in table beets (Shah and Stivers-Young 2004). Moreover, in general, positive correlations between plant density and disease incidence have been well characterized across many pathosystems (e.g. Burdon and Chilvers 1982). Trade-offs between yield components and disease are important to quantify as they may form part of an integrated and durable strategy for disease management in table beet.

In both field trials, non-red cultivars (Avalanche, Boldor, Chioggia Guardsmark) were less susceptible to PLS than red cultivars (Red Ace, Ruby Queen, Merlin, Rhonda, Falcon). Betalains, a class of alkaloid pigments, are responsible for color variation in table beet (Goldman and Navazio 2008). Betalains include red-violet betacyanin and yellow betaxanthin pigments, and convey multiple advantages for plant growth and defense (Georgiev et al. 2008). For example, betalains have been reported as important for attracting pollinators and animals for seed dispersal, and mitigating the effects of abiotic stress (Georgiev et al. 2008; Polturak and Aharoni 2018; Stintzing and Carle 2004). The function of betalains in response to biotic stress is less described in the literature; however, the few studies available identify betalain production as beneficial for defense against fungal and bacterial pathogens (Polturak and Aharoni 2018). Tobacco plants producing red betalain pigments exhibited increased resistance towards the gray mold pathogen, *Botrytis cinerea* (Polturak et al. 2017) and it has been suggested betalains evolved in plants for their antifungal

properties (Brockington et al. 2011). Further studies are warranted to elucidate the genetic basis of resistance and any associations with betalain production in response to specific pathogens, such as *P. betae*.

Temporal progress of PLS epidemics in Rhonda and Falcon were best fit to a Gompertz model, suggestive of a polycyclic disease. Similar temporal patterns have been depicted for comparable pathosystems including ray blight of pyrethrum caused by *Phoma ligulicola* (syn. *Stagonosporopsis tanaceti*; Pethybridge et al. 2005) and disease caused by *Corynespora cassiicola* on cotton (Bowen et al. 2018). Foundational knowledge of the biology of *P. betae* also supports a polycyclic epidemic in table beet (Harveson et al. 2009). Polycyclic diseases can be managed by reducing the rate (*dy/dt*) of disease progression (Madden et al. 2007). Selection of table beet cultivars that are less susceptible to PLS or other in-season control tactics (i.e., fungicides) are important tools to achieve this goal.

This study also found that in-row plant density significantly differed among cultivars and can affect table beet root characteristics, including root diameter and weight. This finding confirms previous studies that higher plant density is positively correlated with root number, but negatively correlated with root size and weight (Benjamin and Bell 1985; Kikkert et al. 2010; Mack 1979). At Geneva, Ruby Queen produced the highest number of roots per row meter, however Ruby Queen also produced roots with the smallest diameters and lower masses on average than other cultivars. Most yield components evaluated were similar among non-red and red cultivars. In Geneva, root number did not significantly differ between Avalanche (white), Boldor (yellow), Red Ace (red), and Rhonda (red). Additionally, root diameter did not significantly differ between Avalanche (white), Boldor (yellow), Falcon (red), Red Ace (red), and Rhonda (red). In Freeville, there were no significant differences among cultivars in root diameter and individual root weight.

Cultivars that were less susceptible to PLS did not necessarily have higher yields. In both trials, there were no significant differences in foliage among six table beet cultivars, including red and non-red cultivars. While Boldor was less susceptible to PLS, root and foliage biomass was significantly less compared to other cultivars at both locations. Additional research to disentangle relationships between PLS and crop loss is essential to fully understand the effects of this disease on productivity and profitability. Cultivar evaluation in other locations would also be beneficial, as disease susceptibility and horticultural characteristics may vary among environmental conditions and pathogen isolates used.

This study quantified differences among eight table beet cultivars in PLS susceptibility and horticultural characteristics in NY. No significant differences were detected among cultivars in PLS severity and epidemic progress in the mist chamber trials under optimal environmental conditions for disease. In the field trials, non-red cultivars were less susceptible than red cultivars to PLS. Of the red cultivars in this study, Rhonda was most susceptible to PLS. The PLS epidemic in Falcon and Rhonda were best described by Gompertz models typical of a polycyclic epidemic. Further studies are needed to elucidate associations between specific betalains and their role in susceptibility to important fungal pathogens. To design a comprehensive disease management program for PLS, information on efficacy of conventional and OMRI-approved fungicides is also essential.

REFERENCES

Abawi, G. S., Crosier, D. C., Cobb, A. C., and Becker, R. F. 1986. Root rot of table beets in New York State. NY Food Life Sci. Bul. No. 115.

Ariyawansa, H. A., Thambugala, K. M., Manamgoda, D. S., Jayawardena, R., Camporesi, E., Boonmee, S., Wanasinghe, D. N., Phookamsak, R., Hongsanan, S., Singtripop, C., Chukeatirote, E., Kang, J.-C., Jones, E. B. G., and Hyde, K. D. 2015. Towards a natural classification and backbone tree for *Pleosporaceae*. Fung. Div. 71:85-139.

Atkinson, A. C. 1987. Plots, Transformations, and Regression: An Introduction to Graphical Methods of Diagnostic Regression Analysis. Oxford University Press, Oxford, NY.

Bates, D., Mächler, M., Bolker, B., and Walker, S. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1-48.

Benjamin, L. R., and Bell, N. 1985. The influence of seed type and plant density on variation in plant size of red beet (*Beta vulgaris* L.) crops. J. Agric. Sci. 105:563-571.

Boerema, G. H., Gruyter, J. de, Noordeloos, M. E., and Hamers, M. E. C. 2004. *Phoma* Identification Manual. Differentiation of Specific and Infra-Specific Taxa in Culture. CABI Publishing, Wallingford, UK.

Bowen, K. L., Hagan, A. K., Pegues, M., Jones, J., and Miller, H. B. 2018. Epidemics and yield losses due to *Corynespora cassiicola* on cotton. Plant Dis. 102:2494-2499.

Brent, K. J., and Hollomon, D. W. 2007. Fungicide Resistance in Crop Pathogens: How Can It Be Managed? FRAC Monograph No. 1 (2nd Revised Edition). Fungicide Resistance Action Committee, Bristol, UK.

Brockington, S. F., Walker, R. H., Glover, B. J., Soltis, P. S., and Soltis, D. E. 2011. Complex pigment evolution in the Caryophyllales. New Phytol. 190:854-864.

Bugbee, W. M. 1979. *Pleospora bjoerlingii* in the USA. Phytopathology 69:277-278.

Bugbee, W. M. 1982. Storage rot of sugar beet. Plant Dis. 66:871-873.

Bugbee, W. M., and Soine, O. C. 1974. Survival of *Phoma betae* in soil. Phytopathology 64:1258-1260.

Burdon, J. J., and Chilvers, G. A. 1982. Host density as a factor in plant disease ecology. Annu. Rev. Phytopathol. 20:143-166.

Clark, B. E., Peck, N. H., Becker, R. F., and Kline, D. B. 1967. Predicting field stands of table beets. New York State Agr. Expt. Sta., Cornell Univ., NY Seed Res. Circ. 2:15.

Clifford, T., Constantinou, C. M., Keane, K. M., West, D. J., Howatson, G., and Stevenson, E. J. 2017. The plasma bioavailability of nitrate and betanin from *Beta vulgaris rubra* in humans. Eur. J. Nutr. 56:1245-1254.

Cook, R. D., and Weisberg, S. 1982. Residuals and Influence in Regression. Chapman and Hall. New York, NY.

De Mendiburu, F. 2017. agricolae: statistical procedures for agricultural research. Available at: https://CRAN.R-project.org/package=agricolae.

Edson, H. A. 1915. Seedling diseases of sugar beets and their relation to root-rot and crown-rot. J. Agric. Res. 4:135-168.

Fox, J. 2015. Applied Regression Analysis and Generalized Linear Models, 3rd Edition. SAGE Publications, Inc. Los Angeles, CA.

Fox, J., and Weisberg, S. 2011. An R companion to applied regression. 2nd ed. SAGE Publications, Inc. Thousand Oaks, CA.

Georgiev, V., Ilieva, M., Bley, T., and Pavlov, A. 2008. Betalain production in plant *in vitro* systems. Acta Physiol. Plant. 30:581-593.

Goldman, I. L., and Navazio, J. P. 2003. History and breeding of table beet in the United States. Plant Breed. Rev. 22:357-388.

Goldman, I. L., and Navazio, J. P. 2008. Table Beet. In *Vegetables I: Asteraceae, Brassicaceae, Chenopodicaceae, and Cucurbitaceae*, Handbook of Plant Breeding, eds. Jaime Prohens and Fernando Nuez. New York, NY: Springer New York, p. 219-238.

Harveson, R. M., Hanson, L. E., and Hein, G. L. 2009. Compendium of Beet Diseases and Pests, 2nd Edition. APS Press, St. Paul, MN.

Herr, L. J. 1971. Hot water treatment for elimination of seed-borne *Phoma betae* and other microbial contaminants from sugar beet seed. J. Amer. Soc. Sugar Beet Technol. 16:568-574.

Jeffers, S. 2015. PROTOCOL 07-11.1: V8 agar (V8A) or broth. In Laboratory Protocols for *Phytophthora* Species, Protocols, ed. Steve Jeffers. APS Press, St. Paul, MN.

Jeger, M. J. 2004. Analysis of disease progress as a basis for evaluating disease management practices. Annu. Rev. Phytopathol. 42:61-82.

Keselman, H. J. 1976. A power investigation of the Tukey multiple comparison statistic. Educational and Psychological Measurement 36:97-104.

Kikkert, J. R., Reiners, S., and Gugino, B. K. 2010. Row width, population density, and harvest date effects on marketable yield of table beet. HortTechnol. 20:560-567.

Koenick, L. B., Vaghefi, N., Knight, N. L., du Toit, L. J., and Pethybridge, S. J. 2019. Genetic diversity and differentiation in *Phoma betae* populations on table beet in New York and Washington States. Plant Dis. Available at: https://apsjournals.apsnet.org/doi/abs/10.1094/PDIS-09-18-1675-RE.

Leach, L. D., and MacDonald, J. D. 1976. Seed-borne *Phoma betae* as influenced by area of sugar beet production, seed processing and fungicidal seed treatments. J. Amer. Soc. Sugar Beet Technol. 19:4-15.

Lenth, R., Singmann, H., Love, J., Buerkner, P., and Herve, M. 2018. emmeans: estimated marginal means, aka least-squares means. Available at: https://CRAN.R-project.org/package=emmeans.

Mack, H. J. 1979. Effects of row spacings, fertilizers, and harvest dates on table beets. J. Amer. Soc. Hort. Sci. 104:717-720.

Madden, L. V., Hughes, G., and Van den Bosch, F. 2007. The Study of Plant Disease Epidemics. APS Press, St. Paul, MN.

Mangan, A. 1971. A new method for the detection of *Pleospora bjoerlingii* infection of sugar beet seed. Trans. Br. Mycol. Soc. 57:169-172.

Mangiafico, S. 2018. rcompanion: Functions to support extension education program evaluation. Available at: https://CRAN.R-project.org/package=rcompanion.

Miller, P. 1955. V-8 juice agar as a general-purpose medium for fungal and bacteria. Phytopathology 45:461-462.

Miller, R. G. J. 1981. Simultaneous Statistical Inference. 2nd ed. Springer-Verlag. New York, NY.

Monte, E., and García-Acha, I. 1988a. Germination of conidia in *Phoma betae*. Trans. Br. Mycol. Soc. 91:133-139.

Monte, E., and García-Acha, I. 1988b. Vegetative and reproductive structures of *Phoma betae in vitro*. Trans. Br. Mycol. Soc. 90:233-245.

Monte, E., Martin, P. M., and García-Acha, I. 1989. Pycnidial development in *Phoma betae*. Mycol. Res. 92:369-372.

Navazio, J., Colley, M., and Zyskowski, J. 2010. Principles and practices of organic beet seed production in the Pacific Northwest. Org. Seed Alliance. Available at: https://seedalliance.org/publications/principles-practices-organic-beet-seed-production-pacific-northwest/.

Northeast Regional Climate Center. 2018. CLIMOD 2. CLIMOD 2 Mon. Summ. Data. Available at: http://climod2.nrcc.cornell.edu/.

Pethybridge, S. J., Esker, P., Hay, F., Wilson, C., and Nutter, F. W. 2005. Spatiotemporal description of epidemics caused by *Phoma ligulicola* in Tasmanian pyrethrum fields. Phytopathology 95:648-658.

Pethybridge, S. J., Vaghefi, N., and Kikkert, J. R. 2017a. Horticultural characteristics and susceptibility of table beet cultivars to Cercospora leaf spot in New York. HortTechnol. 27:530-538.

Pethybridge, S. J., Vaghefi, N., and Kikkert, J. R. 2017b. Management of Cercospora leaf spot in conventional and organic table beet production. Plant Dis. 101:1642-1651.

Pethybridge, S., Kikkert, J., Hanson, L., and Nelson, S. 2018. Challenges and prospects for building resilient disease management strategies and tactics for the New York table beet industry. Agronomy 8:112.

Polturak, G., and Aharoni, A. 2018. "La Vie en Rose": biosynthesis, sources, and applications of betalain pigments. Mol. Plant 11:7-22.

Polturak, G., Grossman, N., Vela-Corcia, D., Dong, Y., Nudel, A., Pliner, M., et al. 2017. Engineered gray mold resistance, antioxidant capacity, and pigmentation in betalain-producing crops and ornamentals. Proc. Natl. Acad. Sci. 114:9062-9067.

Pool, V. W., and McKay, M. B. 1915. *Phoma betae* on the leaves of sugar beet. J. Agric. Res. 4:169-177.

R Core Team. 2017. R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.R-project.org/.

Rife, T. W., and Poland, J. A. 2014. Field Book: an open-source application for field data collection on Android. Crop Sci. 54:1624-1627.

Shah, D. A., and Stivers-Young, L. 2004. Factors associated with decay in table beets identified by a statistical analysis of grower and processor records. HortTechnol. 14:25-29.

Shapiro, S. S., and Wilk, M. B. 1965. An analysis of variance test for normality (Complete Samples). Biometrika 52:591-611.

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

Smith, G. A., and Martin, S. S. 1978. Differential response of sugarbeet cultivars to Cercospora leaf spot disease. Crop Sci. 18:39-42.

Stintzing, F. C., and Carle, R. 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends Food Sci. Technol. 15:19-38.

U.S. Department of Agriculture (USDA). 2017. United States Standards for Grades of Canned Beets. Federal Register 81 FR 93572.

U.S. Department of Agriculture (USDA) - National Agricultural Statistics Service (NASS). 2012. 2012 Census of Agriculture. Available at: https://www.nass.usda.gov/Publications/AgCensus/2012/Full_Report/Volume_1,_Chapter_1_US/.

Vaghefi, N., Hay, F. S., Kikkert, J. R., and Pethybridge, S. J. 2016. Genotypic diversity and resistance to azoxystrobin of *Cercospora beticola* on processing table beet in New York. Plant Dis. 100:1466-1473.

Vanderplank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press. New York, NY.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. 2nd Edition. Springer-Verlag. New York, NY.

CHAPTER 4

CONCLUSION

This thesis investigated the population biology of the fungal pathogen, *Phoma betae*, in table beet and evaluated management options for Phoma leaf spot (PLS). Population biology analyses investigated the relative influence of external and internal inoculum sources for PLS epidemics and reproductive biology of *P. betae* in New York (NY) and Washington States (WA). Mist chamber and field trials assessed the susceptibility of table beet cultivars to PLS and their horticultural characteristics under NY growing conditions.

Ten reproducible and polymorphic microsatellite markers were developed from a *de novo* genome assembly of *P. betae* for use in population biology analyses. Five populations of *P. betae* (two from NY and three from WA) were genotyped using the markers developed. Markers revealed high genotypic and moderate allelic diversity in *P. betae* populations. This genetic diversity indicates the potential of *P. betae* to overcome selection pressures such as single-site mode of action fungicides, and the benefits of using larger sample sizes in future studies. Larger sample sizes may assist in improved resolution of diversity within populations. Analyses identified small, yet significant differentiation among populations. Greater differentiation was noted between populations of different states versus populations within the same state, suggestive of an external inoculum source and its potential for homogenizing populations within states. Furthermore, inoculum dispersal between states may not be frequent. However, more variation in diversity was quantified within populations rather than among them, indicating that within field inoculum sources cannot be discounted. For a more comprehensive examination of the role of inoculum

sources in epidemics, designed experiments are required. To investigate the likelihood of local inoculum sources, sampling over consecutive years in the same fields may be helpful to evaluate temporal changes in *P. betae* populations. To assess the role of seedborne inoculum, mark-release-recapture experiments may also be informative.

This thesis research also made significant strides in describing the reproductive biology of *P. betae*. Microsatellite markers found evidence for populations to be in linkage disequilibrium. While linkage disequilibrium can be caused by selection, linkage, population admixture, and random genetic drift, asexual reproduction cannot be discounted. Investigation of the *de novo* genome assembly found *P. betae* to heterothallic with the discovery of the *MAT1-1* and *MAT1-2* loci in separate isolates. Mating type markers were developed and used to investigate the reproductive strategy of *P. betae*. Three of five populations had a 1:1 mating type ratio indicating panmixis, and the remainder significantly deviated from the 1:1 mating type ratio suggestive of clonality. Populations showed evidence for a mixed reproductive mode, likely involving asexual and sexual reproduction throughout the growing season. Future studies should aim to directly examine diseased plant material in the field for the presence of pseudothecia to confirm the presence of functional mating type genes. The presence of a functioning teleomorph would have substantial implications for survival and dispersal of *P. betae*, evolution of populations, and the durability of management strategies.

Mist chamber and field trials were conducted to assess the susceptibility of eight locally popular table beet cultivars to PLS. In the mist chamber trials, no significant differences among cultivars were detected in PLS incidence and severity, whereas significant differences among cultivars was detected for AUDPS. In the mist chamber trials, epidemic progress was higher in Boldor than Merlin and Chioggia Guardsmark. In the field trials, differences were observed among

eight table beet cultivars in PLS susceptibility and horticultural characteristics. Non-red cultivars were less susceptible to PLS than red cultivars. Of the red cultivars, Rhonda was the most susceptible to PLS. Cultivars that were more susceptible to PLS, such as Falcon and Ruby Queen, produced some of the highest yields. Temporal analysis of PLS disease progression found PLS epidemics on Falcon and Rhonda best fit a Gompertz model, providing quantitative evidence of a polycyclic epidemic.

Further studies are needed to investigate potential interactions between other biotic stresses, such as insects and other pathogens in PLS epidemics; to elucidate any associations between secondary metabolites (betalains and other pigments) and susceptibility to fungal pathogens; and to use genomic-based tools to explore the epidemiology of diseases caused by *P. betae*. Evaluating efficacy of conventional and OMRI-approved products for PLS control and continuing to assess susceptibility of new table beet cultivars as they become available are also complementary research goals of high priority to NY table beet growers.

APPENDIX

FIELD SURVEYS OF PHOMA LEAF SPOT IN NEW YORK

Field surveys were conducted in the summers of 2017 and 2018 to estimate the intensity of Phoma leaf spot (PLS) in NY table beet production. Fields were representative of broad acre processing and fresh market production in western and central NY, and in small, diversified farms across NY. Fields were scouted once in the middle of the growing season. Six arbitrarily selected table beet leaves at one foot intervals along linear transects extending up to 50 feet were assessed. Prevalence was defined as [(the number of fields containing PLS / total number of fields evaluated) × 100]. In each field, disease incidence was quantified and defined as [(the number of leaves with at least one PLS lesion / total number of leaves evaluated) × 100] within a field. Data on disease severity was collected from 29 fields. Disease severity was visually estimated and defined as the percentage of the leaf surface affected by PLS.

In total, surveys were conducted on 60 farms and fields (Table A.1) around NY (Figure A.1). The author collected survey data in approximately half of the fields visited (fields surveyed by S. J. Pethybridge are indicated with an asterisk in Table A.1). On some farms, multiple fields were visited (37 processing fields and 23 fields for the fresh market) (Table A.1). PLS was found in 35 of 60 fields surveyed in the two-year period. PLS was found in 17 of 23 fields (74%) grown for the fresh market and 18 of 37 fields (49%) for processing (Figure A.2). In fields with PLS, average incidence was up to 31%. Average PLS severity ranged from 1.4 to 15%.

Table A.1 Table beet fields visited in 2017 and 2018 to quantify the prevalence, incidence, severity of Phoma leaf spot caused by *Phoma betae*

Field/Farm Number	Year	Grower	Production System	Location (Town, County)
1	2017	Pleasant Valley Farm	Organic	Argyle, Washington County
2	2017	Pleasant Valley Farm	Organic	Argyle, Washington County
3	2017	Denison Farm	Organic	Schaghticoke, Rensselaer County
4	2017	Kirby Farm	Organic	Albion, Orleans County
5	2017	Tangle Root Farm	Organic	Essex, Essex County
6	2018	Tangle Root Farm	Organic	Essex, Essex County
7	2017	Juniper Hill Farm	Organic	Wadhams, Essex County
8	2017	Fledging Crow Farm	Organic	Keeseville, Clinton and Essex Counties
9*	2017	Fellenz Farm	Organic	Phelps, Ontario County
10	2017	Muddy Fingers Farm	Organic	Hector, Schuyler County
11	2018	Muddy Fingers Farm	Organic	Hector, Schuyler County
12*	2017	MY-T-Acres	Conventional	Batavia, Genesee County
13	2017	Kludt: Carr Rd Field	Conventional	Kendall, Orleans County
14	2017	Kludt: Billy Klicks Field	Conventional	Kendall, Orleans County
15	2017	Kludt: Chet Wolf Field	Conventional	Kendall, Orleans County
16*	2017	Kludt: Tytlers North Field	Conventional	Kendall, Orleans County
17*	2017	Kludt: Tytlers South Field	Conventional	Kendall, Orleans County
18*	2017	MY-T-Acres: State Street Field	Conventional	Batavia, Genesee County
19*	2017	MY-T-Acres: Front Near Road Field	Conventional	Batavia, Genesee County
20*	2017	MY-T-Acres: Back Under Wire Left Field	Conventional	Batavia, Genesee County
21*	2017	MY-T-Acres: Back Under Wire Right Field	Conventional	Batavia, Genesee County
22*	2017	MY-T-Acres: Strouts Road Field	Conventional	Batavia, Genesee County
23*	2017	L-Brooke: Bank Street Road Field	Conventional	Batavia, Genesee County
24*	2017	L-Brooke: Townline & Drum Road Field	Conventional	Batavia, Genesee County
25*	2017	L-Brooke: Cockram Road Field	Conventional	Batavia, Genesee County
26*	2017	L-Brooke: Townhill & Byron Road Field	Conventional	Batavia, Genesee County
27*	2017	L-Brooke: Route 98 Field	Conventional	Elba, Genesee County
28*	2017	L-Brooke: Cockram & W Bergen Field	Conventional	Bergen, Genesee County
29	2018	Thorpe's Organic Family Farm	Organic	East Aurora, Erie County
30	2019	Thorpe's Organic Family Farm	Organic	East Aurora, Erie County
31	2018	Juniper Hill Farm	Organic	Wadhams, Essex County
32	2018	Tangleroot Farm	Organic	Essex, Essex County

33	2018	Tangleroot Farm	Organic	Essex, Essex County
34	2018	Fledging Crow Vegetables	Organic	Keeseville, Clinton and Essex Counties
35	2018	Full and By Farm	Organic	Essex, Essex County
36	2018	Full and By Farm	Organic	Essex, Essex County
37	2018	J&A Farm	Organic	Goshen, Orange County
38	2018	R & G Produce	Conventional	Goshen, Orange County
39	2018	S & SO Produce Farms	Conventional	Goshen, Orange County
40	2018	Dagele Brothers Farm	Conventional	Florida, Orange County
41	2018	Dagele Brothers Farm	Conventional	Florida, Orange County
42	2018	Morgiewicz Produce	Conventional	Goshen, Orange County
43	2018	Roxbury Farm	Organic	Kinderhook, Columbia County
44	2018	Denison Farm	Organic	Schaghticoke, Rensselaer County
45	2019	Denison Farm	Organic	Schaghticoke, Rensselaer County
46*	2018	Love Beets Batavia	Conventional	Batavia, Genesee County
47*	2018	Highgrove Field 1	Conventional	Geneseo, Livingston County
48*	2018	Highgrove Field 2	Conventional	Geneseo, Livingston County
49*	2018	Highgrove Field 3	Conventional	Geneseo, Livingston County
50*	2018	Kludt Field 1	Conventional	Kendall, Orleans County
51*	2018	Kludt Field 5	Conventional	Kendall, Orleans County
52*	2018	Kludt Field 6	Conventional	Kendall, Orleans County
53*	2018	L-Brooke Field 14	Conventional	Batavia, Genesee County
54*	2018	L-Brooke Field 15	Conventional	Oakfield, Genesee County
55*	2018	L-Brooke Field 16	Conventional	Batavia, Genesee County
56*	2018	Merimac Field 1	Conventional	Mount Morris, Livingston County
57*	2018	MY-T-Acres Field 1	Conventional	Stafford, Genesee County
58*	2018	MY-T-Acres Field 2	Conventional	Batavia, Genesee County
59*	2018	MY-T-Acres Field 3	Conventional	Avon, Livingston County
60*	2018	MY-T-Acres Field 13	Conventional	Lyndonville, Orleans County

*Other lab personnel (S. J. Pethybridge) conducted disease survey.

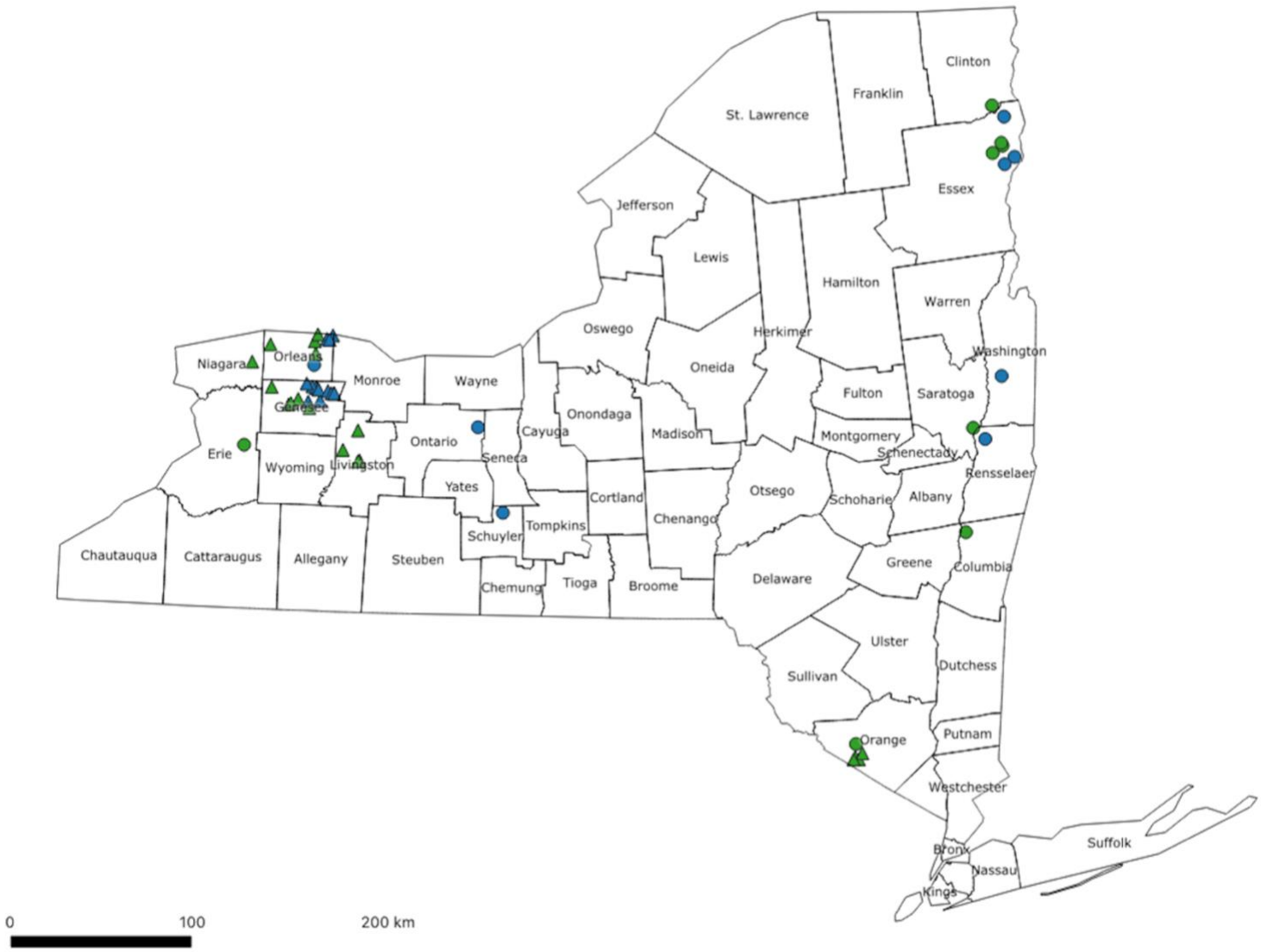

Figure A.1 Field survey locations for Phoma leaf spot intensity in New York table beet production. Green symbols depict fields visited in 2017 and blue symbols indicate fields visited in 2018. Circles indicate organic systems and triangles indicate conventional systems.

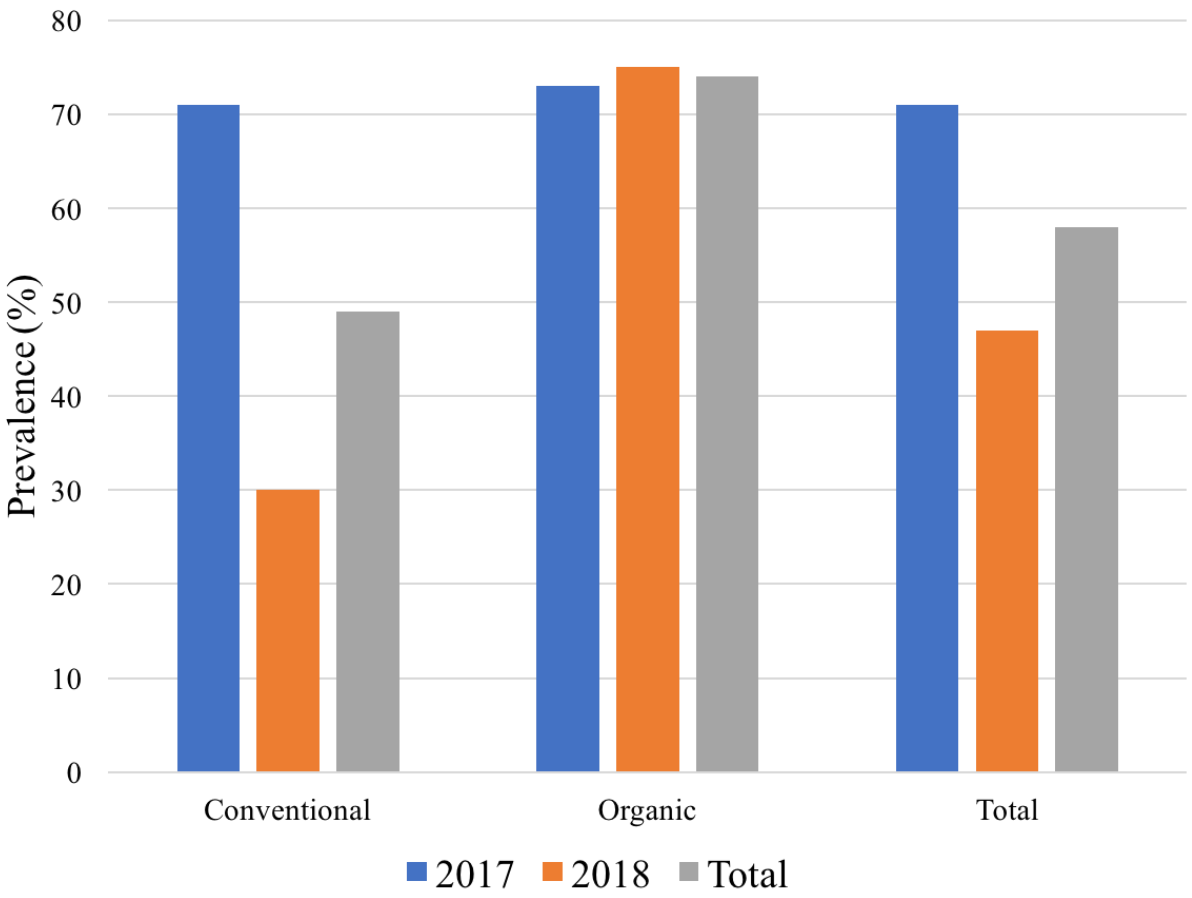

Figure A.2 Prevalence of Phoma leaf spot caused by *Phoma betae* in table beet production systems in New York in 2017 and 2018.

Table A.2 Minimum, maximum, and average disease incidence and severity of fields surveyed in 2017 and 2018 for Phoma leaf spot caused by *Phoma betae* in organic and conventional table beet fields in New York

		Incidence (%)[a]			Severity (%)[b]		
System	Year	Minimum	Maximum	Average	Minimum	Maximum	Average
Organic	2017	0	31.1	5.8	0	10.5	4
Conventional	2017	0	4	1.1	1.5	1.5	1.5
Organic	2018	0	6.7	2.4	0	15	5
Conventional	2018	0	3.6	0.3	0	12.1	4.6

[a] Incidence was defined as [(the number of leaves with at least one PLS lesion / total number of leaves evaluated) × 100] within a field.

[b] Severity was defined as the percentage of the leaf surface affected by necrosis caused by PLS.