INVESTIGATION OF A NOVEL FOLIAR DISEASE ON *MAACKIA AMURENSIS* IN NORTH DAKOTA AND ITS SUBSEQUENT SURVEY

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Title

Investigation of a Novel Foliar Disease on *Maackia amurensis* in North Dakota and Its Subsequent Survey

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

A new, undescribed pathogen was observed on *Maackia amurensis* (Amur maackia or Chinese yellow wood) trees in Fargo, North Dakota, over the course of four growing seasons from 2016 to 2020. Affected trees showed visual signs of necrotic lesions ringed with a yellow halo of various sizes on leaves which began in midsummer and persisted into the fall. Diseased material was collected in October 2020, from which single spore isolations were taken. After a third-party DNA analysis, the causal agent was tentatively identified as an *Alternaria* species. Curious about the full extent of the host and fungal pathogen, a survey was designed and distributed to horticulture industry professionals, botanical gardens, and arboreta in September 2021. The survey results showed where *M. amurensis*, its cultivars, and related species were growing and where similar symptoms were observed in other regions throughout the United States.

ACKNOWLEDGMENTS

Thank you to the U.S. Department of Agriculture (USDA), National Institute of Food and Agriculture, McIntire–Stennis project ND6216 who provided funding for this research project. To Dr. Todd West who helped set me up for future success and was adaptable during an interesting time to be in academia. Thank you to the members of the Woody Plant Improvement Program who helped support and carry out the project: my lab mates Conner Hagemeyer and Maram Milad, and the undergraduate research assistants Sarah Bofferding, Maren Donovan, and Matthew Laliberte. To my graduate committee, Dr. Wenhao Dai and Dr. Jack Rasmussen, thank you for your invaluable expertise and patience with this project. Many thanks and much appreciation to the Secor Lab, Dr. Gary Secor, Viviana Rivera-Varas, and Judith Rengifo, who provided invaluable support and plant pathology expertise. To Dr. David Zlesak for sharing his detached leaf assay protocol. Without the help of many, this project would not have been even half as successful.

DEDICATION

To my family and friends who supported me and cheered me on through this process.

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LIST OF ABBREVIATIONS

NDSU.....North Dakota State University

DEHRA.....Dale E. Herman Research Arboretum

WPIP.....Woody Plant Improvement Program

BCM.....Bacterial Canker of Maackia

SSB.....Slightly Swollen Bark

USDA.....United States Department of Agriculture

DLA.....Detached Leaf Assay

PCR.....Polymerase Chain Reaction

PDA.....Potato Dextrose Agar

DI.....Deionized

DD.....Double Distilled

MS.....Murashige and Skoog

EoTH.....Ethyl Alcohol

LIST OF SYMBOLS

® .....Plant name is a registered trademark

1. EXECUTIVE SUMMARY

Maackia amurensis (Amur maackia or Chinese yellow wood) offers ornamental value to the available plant palette. The tree belongs to the Fabaceae family, which is often in the minority of families in urban plantings. Other trees in this family that are utilized in urban plantings include *Gymnocladus dioicus* (coffeetree), *Gleditsia triacanthos* var. *inermis* (thornless honey locust), *Cladrastis kentukea* (American yellowwood), and *Cercis canadensis* (redbud). Amur maackia has grown comparatively in popularity over the past decade and is being utilized to diversify replacement plantings of previous monocultures.

The tree offers seasonal interest with a show of white flowers in the late spring to early summer, and its peeling copper-colored bark stands out during the winter. Its squat appearance adds a different shape to the landscape, rather than a very tall upright tree such as *Ulmus* (elm). *M. amurensis* currently has no reported disease or pest issues. Over the past five years, *M. amurensis* trees at the North Dakota State University Dale E. Herman Research Arboretum (NDSU DEHRA; Absaraka, ND, USA; Lat. 46.9859, Long. 97.3549)) have shown leaf symptoms prompting concern. Gathering an idea of this potential pathogen is imperative to fully understand how it affects *M. amurensis* and help determine the next steps needed.

2. NORTH DAKOTA STATE UNIVERSITY WOODY PLANT IMPROVEMENT PROGRAM

The Department of Plant Sciences at North Dakota State University (NDSU) houses the Woody Plant Improvement Program (WPIP) where trees and shrubs of ornamental value are studied. The WPIP research program focuses on the selection, evaluation, and introduction of hardy woody plants for the Northern Plains including winter hardiness and tolerances including drought, disease, and high soil pH. This is accomplished through three main goals:

- Evaluate unreleased or released cultivars from the nursery trade to determine usability in the United States Northern Great Plains.
- Select and/or breed new cultivars suitable for the Northern Great Plains.
- Increase plant diversity.

One tree of interest to the WPIP in recent years is *M. amurensis*.

3. LITERATURE REVIEW

3.1. Plant Characteristics

Maackia amurensis Rupr. & Maxim. is a deciduous tree native to Eastern Russia, Northeastern China, Korea, and Japan. Named after Siberian explorer Richard Karlovik Maack, the tree was discovered during the 19th century in the Amur River region between Siberia and China (Missouri Botanical Garden, 2020). *M. amurensis* (Amur maackia or Chinese yellowwood) belongs to the family Fabaceae along with other legume species such as *Gleditsia triacanthos* (honeylocust), *Gymnocladus dioicus* (coffeetree), *Cladrastris kentukea* (American yellowwood), and *Cercis canadensis* (redbud). It is one of a select number of trees which has the ability to fix a small amount of atmospheric nitrogen in the soil with the help of rhizobial bacteria (Batzli et al., 1992). Amur maackia is propagated from softwood cuttings, or, more typically, from seed after a scarification of 24 hours in hot water or an acid soak. An acid soak may consist of 18-M sulfuric acid for one hour followed by a thorough rinse with deionized water (Giridhar et al., 1995).

A few cultivar selections have been made, including 'JFS-Schichtel1' (MaacNificent®) from J. Frank Schmidt (Boring, OR, USA), 'Summertime' introduced by the University of Minnesota (St. Paul, MN, USA), 'Starburst' introduced by Princeton Nurseries (Kingston, NJ, USA), and 'Summerfrost' (Dirr, 2009; The Morton Arboretum, 2020). The trees can be found in USDA cold hardiness zones 4-7 with MaacNificent® and 'Summertime' being touted as able to tolerate colder zone 3 (The Morton Arboretum, 2020).

This slow growing tree offers multi-seasonal interest as it matures. Its copper-colored bark exfoliates to provide winter interest and a show of small creamy, white flowers on a 10 to 15 cm raceme bloom in mid-June to July. Small seed pods 5 to 8 cm long, similar to those of

redbud trees, follow. The leaves emerge olive green in color which fade to a darker greyish green in the summer but fail to put on a spectacular show of color for fall. The odd-pinnately compound leaves sit alternately on branches. Amur maackia offers itself as a good medium tree selection reaching 6 to 9 m in height and as wide if not wider under cultivation, giving it somewhat of a squat appearance. In the wild, trees can reach heights of 14 m.

3.2. Ornamental Trees as Hosts and Their Economic Implications

The importance of having a diverse plant palette to choose from is imperative for a healthy ecosystem. City foresters and horticulturalists often use the mantra of the 20-10-5 rule of diversity within the canopy. No more than 20% of the urban tree canopy should be made up of a single family, for example *Sapindaceae.* At most 10% of the trees that make up that family should be from the same genus, such as *Acer* (maples). It is recommended that less than 5% of those trees are from the same specific epithet, like the ever-popular *Acer saccharum* (sugar maple). Even if there are multiple cultivars planted, if they all share the same specific epithet, they should be limited to that 5%.

The relationship between plants and the diseases and pests they host is not a new phenomenon. The constant evolution of plant defenses and their antagonistic counterparts is well documented. Because of this, early detection of a potential new disease or pest is imperative for the horticulture and forestry industries.

There are many documented examples of pests or diseases devastating a family of trees. Dutch elm disease (*Ophiostoma novo-ulmi*) on *Ulmus* spp., emerald ash borer (*Agrilus planipennis*) on *Fraxinus* spp. (ash), and fireblight (*Erwinia amylovora*) on plants in the Rosaceae family are just a few examples. These have had devastating effects on the urban forest where monoculture plantings of boulevards and neighborhoods occurred more frequently.

Diseases and insect pressures on ornamental trees present an extra layer of challenge: trees are a long-term investment for the homeowner or the city. Monocultures allow for the swift spread of disease and insect pests. Preventative measures can be costly and become ineffective shortly after the treatment is discontinued. Trunk injections to prevent beetle infestation of emerald ashwood borer can cost between $200 and $500 per tree and are repeated every two years for the rest of the tree's life. Disease and insect pressures have worked their way across the United States, costing billions of dollars in tree maintenance and removal. Not all diseases are fatal, but even those that present a cosmetic challenge can eventually wear down the tree, allowing other pests or diseases to take hold and therefore becoming a secondary cause of death.

Trees are an investment with many benefits. Two measurable types are compensatory and functional benefits. Compensatory benefits can be compared to that of a physical building, like a production factory (Nowak et al., 2002). Functional benefits are similar to the product being produced in the building and the net profit it garners. The US Forest Service estimated trees in the continental United States contributed $2.4 trillion in compensatory benefits contributed to by approximately 3.8 billion trees. It is estimated that the city of Minneapolis, MN, has a net benefit of $15.7 million dollars in functional benefits, which breaks down to $79 per tree annually (McPherson et al., 2005a). Trees in Berkeley, CA, contributed $3.25 million in total annual benefits ($89 per tree) in a study conducted in 2005 (McPherson et al., 2005b). Bismarck, ND, trees garnered just under $1 million in benefits ($56 per tree) (McPherson et al., 2005b). Trees help to trap carbon emissions, reduce the heat island effect of cities, and help to manage stormwater runoff, among other benefits.

Dealing with disease and pest pressures is at the forefront when working with ornamental plants. Breeders, producers, and consumers are worried not only for the health of the plant, but also for how the pathogen or insect is going to cosmetically affect the plant. An ornamental tree is a decades-long investment. A one time or reoccurring issue may be concern enough to cause the removal of a tree or to choose a different tree species or genera altogether.

The green industry was greatly impacted even more recently by the COVID-19 pandemic. People were forced to work from home and limited to the spaces and sizes of gatherings they could go to in the early days of the shutdown. Green spaces became even more important as citizens utilized them as a means of escaping the walls of their living spaces, be it public parks or their own yards. A study out of Texas A&M reported that 64% of Texas Nursery & Landscape Association businesses saw higher sales than the previous year (Marwah et al., 2021). Twenty-two percent of the respondents saw a moderate increase in their landscape tree sales, another 7% saw a significant increase in sales, and 40% were unchanged compared to the past year.

3.3. Currently Reported Disease and Insect Issues of *Maackia amurensis*

The current literature lacks information when it comes to describing any disease or pest issues with *M. amurensis*. Dirr (2009) references the canker found on *M. amurensis* var. *buergeri* but states "…over my entire career I can't remember any insect or disease damage" in respect to the straight species. The Morton Arboretum (2020) and the Missouri Botanical Garden (2020), both similarly state that the plant has no major pest or disease issues.

3.3.1 Bacterial Canker of *Maackia amureniss* var. *buergeri*

M. amurensis var. *buergeri* Schn. is native to the islands of Japan. It is very similar to the straight species with small differences in leaf shape, the apices are obtuse instead of acute, and

the presence of pubescence (Dirr, 2009). A study published in 2000 identified a new bacterial canker which used *M. amurensis* var. *buergeri* as a host. Previously unreported elsewhere, a bacterial member of the *Pseudomonus syringae* strain caused cankers on both branches and trunks of infected trees (Sakamoto et al., 2000). The disease was aptly given the name "bacterial canker of Maackia" (BCM). A second study provided detailed symptoms and signs of the disease. Initial symptoms were observed during late spring to early summer and included irregular longitudinal swellings which would eventually burst and form a whole canker with exposed inner bark (Sakamoto, 1999). Slightly swollen bark (SSB) was observed between the cankers. Upon closer inspection, the SSB areas often appeared water-soaked, and the phloem was enlarged. The canker sections contained areas that appeared water-soaked, dead exposed sapwood, a dead cambial zone, and drying out of the heartwood. The water-soaked appearance was due to large amounts of bacteria oozing out of infected tissues. No other accounts of BCM have been reported in any other region besides the islands of Japan.

3.4. Alternaria Reports on Woody Plants

Several new first reports of *Alternaria* species on woody plants were recently released. *Alternaria alternata* was reported as causing Alternaria Brown Spot on *Aralia elata* (angelica tree) and leaf spot on *Ribes nigrum* (black currant) in China by researchers at Northeast Agriculture University, Harbin, China, (Cheng et al., 2020; Xu et al., 2023). Necrosis of *Thuja occidentalis* (arborvitae) by *A. alternata* in Kazahkstan was reported by researchers at S. Seifullin Kazakh Agrotechnical University, Nur-Sultan, Kazakhstan (Smagulova et al., 2023). All groups reported similar presenting symptoms and fungal colony growth to what was observed by the NDSU WPIP. Equipped with the tentative identification of the causal agent and

the description of the symptoms, the desire to know the potential range of this pathogen was at the forefront.

4. ISOLATION OF THE UNKNOWN PATHOGEN

The NDSU DEHRA has a small collection of *M. amurensis* accessions, which includes species and cultivar trees. Over five growing seasons (2016 to 2020), leaf spots were observed on *M. amurensis* trees at the NDSU DEHRA and on the NDSU main campus (Fargo, ND, USA; Lat. 46.8978, Long. 96.8024). With visual inspection, the severity seemed to differ between cultivars, but all shared similar symptoms. Symptoms varied from small, black lesions sporadically spread on the leaf surface to necrosis starting at the leaflet tips or outer margins with yellowing near the edge of the spreading dead tissue (Fig.1 and 2).

Figure 1. Symptoms on *Maackia amurensis* 'JFS-Schichtel1' MaacNificent®
Notes: Photo taken September 12, 2018, by Greg Morgenson at the NDSU DEHRA.

Figure 2. Symptoms on *Maackia amurensis* 'Summertime'
Notes: Photo taken on August 1, 2018, by Greg Morgenson at the NDSU DEHRA.

When isolated and grown on a plate of acidified potato dextrose agar (PDA), the colonies began as grayish-white fuzzy hyphal growth, which grew and turned grayish black with a white outer ring (Fig. 3). When encouraged to sporulate, conidiophores and conidiospores were present and observed under a dissecting microscope (Fig. 4). Upon the initial isolation of the pathogen, DNA isolation using a modified CTAB method was performed (Doyle and Doyle, 1987). The clearest sample of each of the isolates was chosen using the polymerase chain reaction electrophoresis results, and the samples were sent to be further analyzed by McLab (San Francisco, CA). Results came back with a mixture of possible pathogens, but the most prevalent response was a fungal species called *Alternaria*. *Alternaria* species can be found on a wide a wide range of hosts from field crops to woody plants.

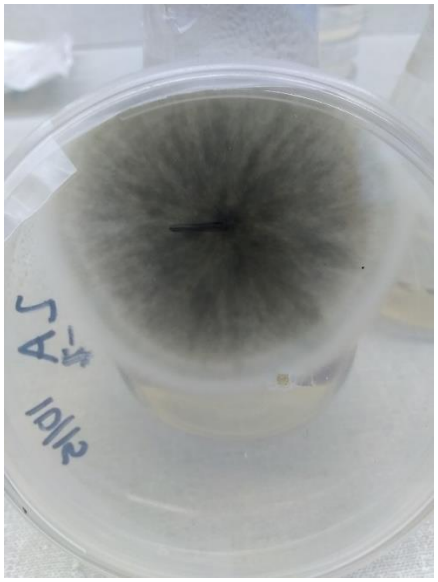

Figure 3. Fungal growth isolated from a necrotic lesion on *Maackia amurensis*
Notes: Fungal growth of what would tentatively be identified as an *Alternaria* species after it had been isolated and cleaned up. Photo taken October 23, 2020, seven days after the isolation was made.

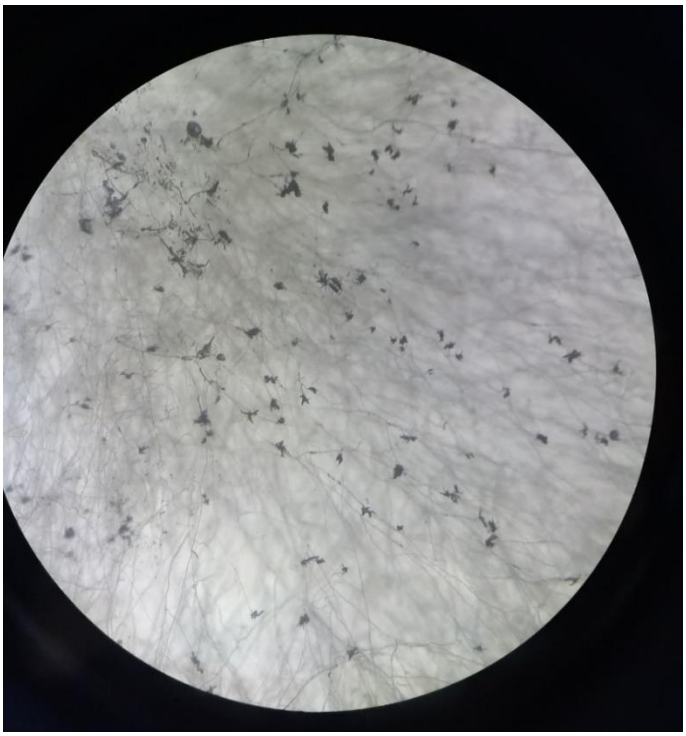

Figure 4. Hyphal growth isolated from a necrotic lesion on *Maackia amurensis*
Notes: Image taken through a dissection microscope of hyphal growth of the isolated fungal pathogen. Photo taken October 15, 2020.

5. SURVEY EVALUATION

It was determined that a survey would be the most effective way to collect data on the extent of the range of this potential new pathogen. Two different surveys were conducted: one for producers/nurserymen and the other for municipalities/botanical gardens/arboreta. They would collect the same information; the only difference would be in collecting ArbNet levels from the municipalities/botanical gardens/arboreta. ArbNet is an arboretum accreditation program run by The Morton Arboretum in partnership with American Public Gardens Association and the Botanic Gardens Conservation International ("The Interactive Community of Arboreta", n.d.). Members of the accreditation program can be accepted at four different levels based on the criteria met (see Appendix A). Historically Amur maackia has been more of a collector's tree and was most likely to be found in a botanical garden or arboretum.

A survey was created using survey software Qualtrics (NDSU Group Decision Center) (see Appendix B and C for the ArbNet and nursery surveys respectively). The survey was sent out in early September 2021 and closed October 31, 2021. The survey was sent to botanic gardens, arboreta, and municipalities directly by ArbNet on the behalf of the NDSU WPIP with a summary giving a brief synopsis of the survey (see Appendix D). State nursery associations were contacted directly and asked for interest in participating in the survey (see Appendix E and F for the inquiry and the ask emails respectively). Again, the associations handled sending the survey out directly to their members. Participation was completely voluntary, and no compensation was offered.

5.1. State Nursery Associations

State nursery associations in UDSA zones 3 – 8 were contacted and asked to help distribute the survey to their state nurseries (see Appendix G). Many of these associations were a

combination of retail, greenhouse, nursery, landscape professionals, city foresters, and state agriculture inspectors. Of the 37 associations contacted, nine agreed to send out the survey either by email or through their online association publication. Respondents were recorded from eight of the nine states. A potential of 4,737 survey links were sent out and 17 participants responded with a participation of 0.003% (Fig. 5).

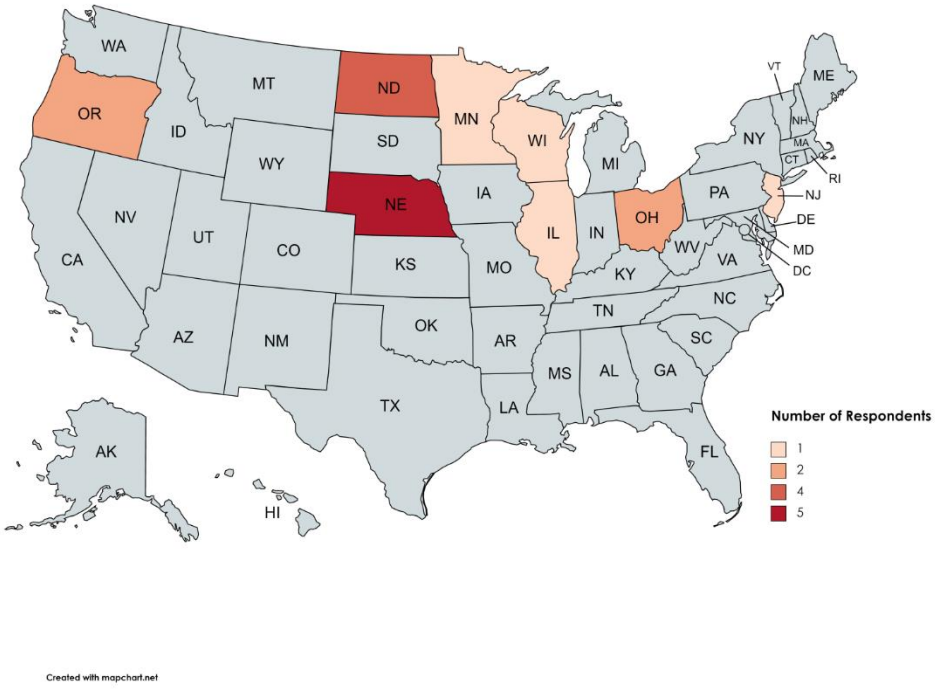

Figure 5. Nursery survey responses per state

Of the 17 respondents, six reported having *M. amurensis* or its related species or cultivars in cultivation (Fig. 6). It was most common for the straight species of *M. amurensis* to be observed in cultivation, with four respondents saying they had it. ‘JFS-Schichtel1’ (MaacNificent®) was reported by three, ‘Starburst’ and ‘Summertime’ were each only reported by a single respondent. ‘Summerfrost’, *M. amurensis* var. *buergeri*, and *M. chinensis* were not reported as being in cultivation by the respondents at the time. Of those who responded “Yes” to

having *M. amurensis* or related species or cultivars, only three said they had seen necrotic spots on the leaves fitting the description provided. The respondents were located in Ohio, Nebraska, and North Dakota. All the symptoms were observed solely on *M. amurensis.*

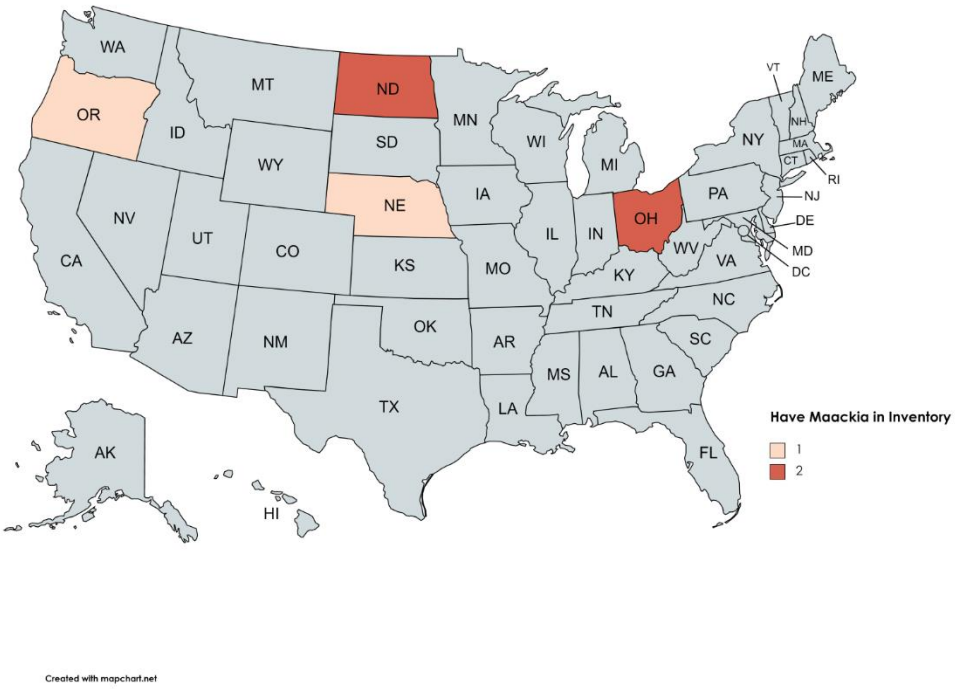

Figure 6. Nurseries with *Maackia* species or cultivars in the inventory

5.2. Public Institutions

Arboreta surveys were distributed by AbrNet, an accreditation system provided through the Morton Arboretum along with partner institutions. A total of 331 institutions spanning the whole United States, including the District of Columbia, covering USDA zones 3 – 13 received the survey link. Thirty-seven of the 331 institutions responded for 11.18% participation (Fig. 7). Of those who responded, 12 institutions had *M. amurensis*, its cultivars, or related species present in their collection covering 10 different states (Fig. 8).

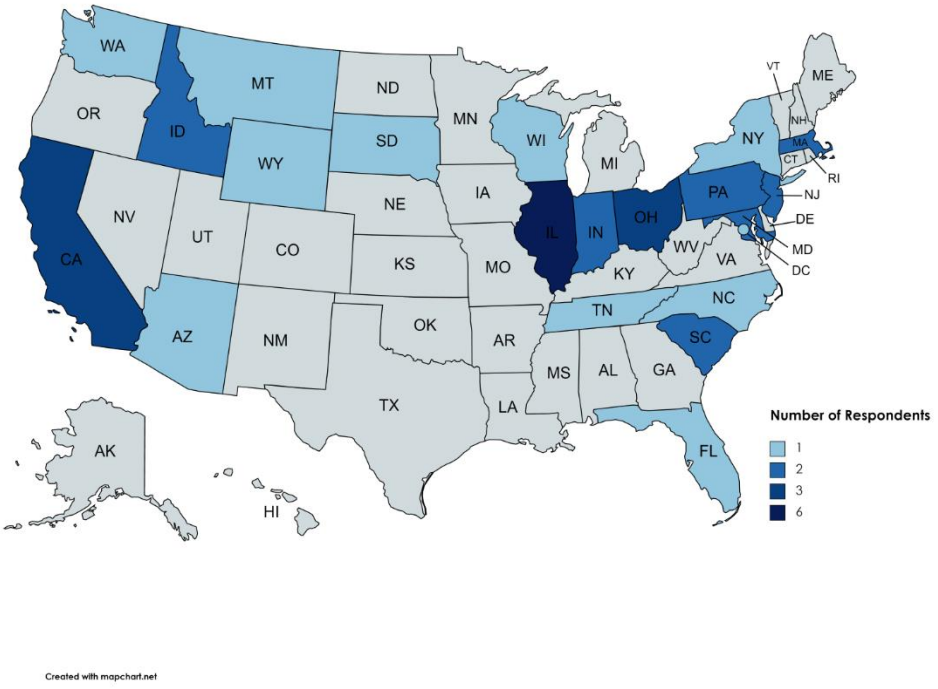

Figure 7. ArbNet responses per state

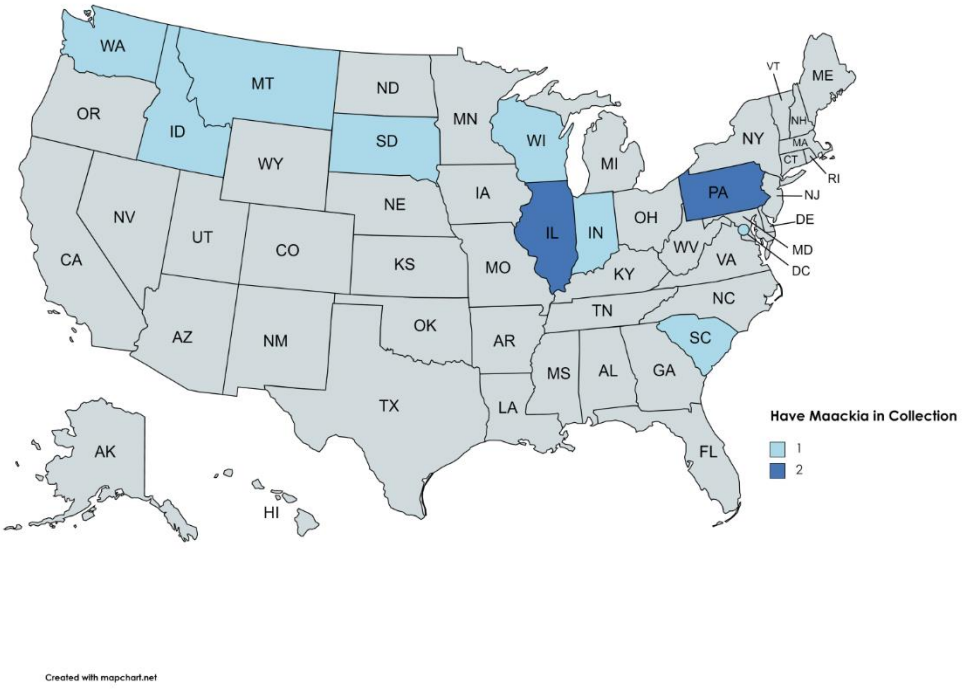

Figure 8. States with ArbNet members growing *Maackia* species or cultivars

Of the 37 respondents, 12 reported having *M. amurensis* or its related species or cultivars in their collections. *M. amurensis* was the most commonly reported, being held by 6 institutions. ‘JFS-Schichtel1’ (MaacNificent®) and ‘Summertime’ were each held by 2 institutions. *M. amurensis* var. *buergeri*, *M. chinensis*, and ‘Starburst’ were each reported to be held at only one site. ‘Summerfrost’ was not in any collection. Foliar lesions were observed on MaacNificent® in Illinois, on the straight species in Idaho, and on the straight species as well as ‘Summertime’ in Wisconsin.

6. DETACHED LEAF ASSAY

6.1. Researching Existing Protocols

6.1.1. Black Spot on Roses

Dr. David Zlesak (professor of horticulture at the University of Wisconsin River Falls) specializes in plant pathology, plant breeding, and plant propagation, among other specialties. He has an ongoing project working with identifying resistant cultivars and varieties of roses to black spot. He has developed a lab protocol for a detached leaf assay (DLA) of roses. His protocol provided a starting point to look at when developing a detached leaf assay protocol for *M. amurensis*.

> …the leaves are harvested at a young, but fully expanded size for consistency as age can matter at least in relative degree of susceptibility. We wash the leaves in 70% alcohol for a few seconds gently running them and then rinsing them a few times in distilled water and then blot them dry. We use 48-ounce food containers with lids and then select a size [of] Bounty paper towel sheets moistened with [approximately] 30mL distilled water. Diseased leaves are steep in distilled water for a bit and shaken around to dislodge spores. We use a hemocytometer to get the concentration between 30,000 – 80,000 spores per milliliter and put the droplets on the leaves (with sterile pipettes). After two days we come back and blot them dry with paper towels, so bacteria don't come in as a secondary infection. We then watch disease development for 2 weeks to mark which are resistant and which are susceptible. The alcohol wash helps the droplets not roll off as bad.
>
> If you use a mist bottle you can see individual infections better and can measure lesion size at a common point in time using the same controls for reference to get an idea of some aspect of horizontal/field resistance relatively. The mist bottle makes smaller droplets, but they tend to ounce off the leaves and are challenging but would be a better way if you are looking at horizontal resistance too. Perhaps you can pipette small droplets too with a pipetter (2-5 uL??) if they don't dry up too fast.
>
> We leave the lights on in the lab so the leaves stay green and in better shape through the process. (D. Zlesak, personal communication, March 29, 2021)

6.1.2. Fungal Diseases on Potato

Dr. Gary Secor (professor of plant pathology at NDSU) works closely with potato and sugar beet diseases found in the Northern Great Plains region. The Secor Lab assisted with the initial isolation process in the fall of 2020. This included the initial isolation, cleaning the initial

isolations to a single pathogen per petri dish, sporulation, and DNA extraction through PCR. Working with potato pathogen in the hopes of identifying resistance, the lab often uses DLA. Through spoken conversations in the winter of 2020 through the spring of 2021, sugar beet and potato assay protocols were shared and compared for successful protocols when working with herbaceous material. Their protocol is to use Magenta boxes (Thermo Fisher Scientific, Hampton, NH) with a square of Oasis® floral foam (Oasis® Floral Products, Kent, OH) or similar type plant foam which retains moisture and keeps the leaflets moist. The high levels of humidity in the Magenta box helps to create a conducive environment for the pathogen around the leaf for the fungal pathogen. They also cut their leaf segments to the desired size underwater, as they have observed this helped the leaf last longer. In conversation, it was also suggested that we find a way to have a barrier between the paper towel and the leaf itself to prevent faster desiccation. It was determined a plastic mesh would be utilized for the barrier. Pieces of the plastic mesh (Loops & Threads; Michaels Stores, Irving, TX) were cut from the 34 cm x 59 cm sheets to fit into the 10 cm x 18 cm plastic container (Lid C64DLR, Container C48DER; ClearPack®, Dart Container Corp., Mason, MI, USA). It was also suggested that a nutrient-based solution be compared to distilled water to see if it would affect the longevity of the leaf material.

6.1.3. Woody Plant Tissue Culture

Pulling from the NDSU WPIP lab disinfestation protocols for tissue culture, it was determined to trial disinfesting the leaf material with a 10% bleach concentration for five minutes and rinsing the material off after. Murashige and Skoog (MS) is a commonly used nutrient base in tissue culture. The base liquid solutions are combined with sugar and agar, resulting in a jelly-like tissue culture medium after heating. By leaving out the agar and the heat,

the remainder of the nutrients would still be in the solution but the medium would not solidify. It was determined that MS would be the nutrient solution that would be trialed against deionized (DI) water.

6.2. Initial Trial

Utilizing information from all three labs, an initial factorial experiment was designed. The experiment would compare the six different isolates from 2020 and a DI water control, disinfestation method, container style, solution used to keep the material moist, pipetting on the spore concentration versus using a spray bottle, and leaflet versus complete leaf performance. The spores used for inoculation were grown and sporulated on petri dishes containing PDA. A target concentration of 50,000 spores per milliliter was calculated for each spore solution. A total of 560 samples between two simultaneous runs were observed from June 26, 2021, through July 15, 2021.

While there were no definitive infections from the reinoculations during the summer of 2021, measurable observances in the performances of the different factors were observed. When looking at the quality of the leaves at the completion of the run, the full leaf typically held up better than the single leaflet. The full leaf was typically much greener and showed less signs of overall desiccation. The material on the distilled water had a lot less introduced fungal or bacterial growth on them compared to the samples on the MS solution. This could be explained by the addition of extra free nutrients available for the leaf and potentially present competitive pathogens to utilize.

There seemed to be no noticeable difference between the humidity levels and how the fungus performed in each of the containers. Both containers received additional moisture midway through the run to ensure humidity was present around the leaf surface at a favorable

level for the fungus. Since it was observed that the complete leaf performed better than the leaflet, the clam shell was chosen for the lab protocol.

It is still undetermined if the spray bottle or the pipette worked better for applying the inoculant. Further investigation is needed to determine the effect of the spore size on the application method. There were also no noticeable differences between the 10% bleach and 70% ethyl alcohol disinfestation methods.

6.3. Creation and Trialing of the Lab Protocol

A detached leaf assay protocol for *M. amurensis* was created for the NDSU WPIP from the observations of the 2021 trial. (Appendix H)

Leaf samples from the same trees on campus were collected again in the fall of 2021. Infected leaves were also collected from *M. amurensis* 'Summertime' from the NDSU DEHRA. During a fall trip to the Minnesota Landscape Arboretum (Chaska, MN, USA), similar symptoms were observed on *M. amurensis* trees in the collection as had been seen in North Dakota. Leaf samples were collected from *M. amurensis* 6070974B, *M. amurensis* 670974H, and *M.* amurensis 'Summertime'. Isolations from these three new samples were initiated. All six isolations were initiated on November 2, 2021, and visually compared for similarities. They all showed similar growing patterns to the initial isolations, which had been identified through ncBlast as potentially being *Alternaria*.

A second trial of the DLA was run in August 2022. This time infected leaves were submerged in distilled water and tween20 to dislodge the spores. The target was for 50,000 spores per milliliter was not attained, but the minimum of 30,000 spores was reached. Once again, no noticeable infections were observed.

7. DISCUSSION

There were several challenges which arose throughout the process of this project. As shown by the survey, survey participation was low, and from responses, *M. amurensis* is not widely grown in large quantities, which made acquiring material for greenhouse trials challenging. Plants needed to be able to fit in the greenhouse, and most of the stock available at the time of searching in 2020-2021 were well over 3 to 4 m tall or of a significant caliper already. The COVID-19 pandemic put a lot of stress on many of the markets, and the green industry was no different. It was almost the opposite effect; many places were selling out of the stock they had on hand well before they historically had or were facing production setbacks limiting their stock. As a result, we were unable to acquire plants of the same age to hold in an isolated greenhouse to be able to run a resistance screening with.

Amur maackia is slow growing from seed; therefore, it would not have been feasible to have plants of size ready in the time frame of this study. The age of the material was also a concern, as it was and still is unknown if the symptoms present differently based on the age of tree or if the juvenile or more mature leaf material is susceptible to begin with. All the stock at the NDSU DEHRA would be considered mature where the disease was first observed. Further work is needed to see if there is a difference in the presentation of the disease based on age.

Clean material was hard to acquire for these reasons. To run a detached leaf assay, fresh material was needed. By the time the symptoms appeared and were isolated, the threshold on the clean material was gone. A proper run of the detached leaf assay protocol is still needed to prove that the initially identified Alternaria fungus is what is truly causing the symptoms. While Koch's postulate was not completed, a streamlined process and lab protocol was created, shared

with the WPIP lab, and lab members were trained. What initially was over 500 units in the initial trial run was narrowed down, therefore approximately 40 samples are needed for the next trial.

The low participation in the survey was another challenge. While we do have a better idea of where *M. amurensis* and its related species can be found, it is an incomplete snapshot. The disease symptoms were observed on trees at the Minnesota Landscape Arboretum by members of the WPIP lab on November 10, 2021. Through conversation, similar symptoms were seen on *Maackia* trees in the city of Seattle, Washington, in 2022. Further research into the full extent of the pathogen range is needed, as well as continued work solidifying the identification of the causal agent.

From casual observances in the field, there seems to be differing levels of susceptibility and resistance between the cultivars and the straight species of *M. amurensis*. Further research into disease screening is needed. This will help guide future management decisions surrounding the relationship between the causal agent and host tree. It will also help with future breeding and selection efforts of *Maackia*.

While the survey helped to provide an initial snapshot of where *M. amurensis* may be being grown, it is incomplete. As the survey was completely voluntary, some grower and collections of the tree may have been unintentionally missed. The low response percentage by the industry professionals could be attributed to the large mix of member demographics. A landscaping or turf company was not the intentional demographic for the survey, but they were included in many associations. Another opportunity where current growers may have been missed was during the initial ask. Some states where Amur maackia may have been on the edge of tolerating did not consent to participating, often citing that the zones did not match for where Amur maackia would grow. States were contacted regardless of the extent of the zones so long

as any USDA plant hardiness zone between 3 and 8 were present. A more comprehensive survey of where *M. amurensis* is being grown is still needed.

Sending a survey out multiple times during the growing season would also give a more reliable picture of when and where the fungal spots are appearing. The original survey was sent out a single time in the fall.

This study is a good starting point. There is a better understanding of the scope of where *M, amurensis* is being grown and where the fungal pathogen has been observed. *M. amurensis* is an important ornamental tree, but further research is needed to understand the developing relationship between it and the *Alternaria* fungal pathogen.

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APPENDIX A. ARBNET ACCREDITATION LEVELS

	LEVEL I	LEVEL II	LEVEL III	LEVEL IV
Arboretum plan	■	■	■	■
Organizational or governance group	■	■	■	■
Labeled tree and woody plant taxa				
25+	■			
100+		■		
500+			■	■
Staff or volunteer support				
Volunteer or paid	■			
Paid management		■	■	■
Curator			■	■
Scientific or conservation staff				■
Public dimension				
Public access and at least one event per year	■	■	■	■
Enhanced public and educational programs		■	■	■
Substantial educational programming			■	■
Collections policy		■	■	■
Collaboration with other arboreta			■	■
Collections data sharing with networked collections			■	■
Agenda for tree science, planting, and conservation			■	■
Collections conservation				■
Conservation role in Global Trees Campaign				■

Table pulled from *The Interactive Community of Arboreta* (n.d.)

APPENDIX B. ARBNET SURVEY

Default Question Block

General Information

Institution Name

Email

City

State

USDA Hardiness Zone

ArbNet Accreditation Level

- ○ Level I
- ○ Level II
- ○ Level III
- ○ Level IV

- ○ Not yet accredited

Do you currently have *Maackia amurensis* (Amur maackia or Chinese yellowwood) or related species in your collections? Related species include *Maackia amurensis* var. *buergeri* or *Maackia chinensis*.

- ○ Yes
- ○ No

Which cultivars are in the collection? Please select a response for each cultivar or species.

	Yes	No
Maackia amurensis (straight species)	○	○
Maackia amurensis 'JFS-Schichtell' (MaacNificent)	○	○
Maackia amurensis 'Starburst'	○	○
Maackia amurensis 'Summertime'	○	○
Maackia amurensis 'Summerfrost'	○	○
Maackia amurensis var. buergeri	○	○

	Yes	No
Maackia chinensis	○	○

Have you observed foliar lesions on *Maackia* trees in production? Symptoms include small necrotic spots/lesions with a yellow halo that may coalesce as time goes on. Disease symptoms typically appear in July or August, when being observed in North Dakota.

○ Yes

○ No

If you have observed foliar disease symptoms on trees, which one(s) specifically? Please select a response for each cultivar or species.

	Yes	No
Maackia amurensis (straight species)	○	○
Maackia amurensis 'JFS-Schichtell' (MaacNificent)	○	○
Maackia amurensis 'Starburst'	○	○
Maackia amurensis 'Summertime'	○	○

	Yes	No
Maackia amurensis 'Summerfrost'	◯	◯
Maackia amurensis var. buergeri	◯	◯
Maackia chinensis	◯	◯

Questions or Comments

Powered by Qualtrics

APPENDIX C. NURSERY SURVEY

Default Question Block

General Information

Company Name

Email

City

State

USDA Hardiness Zone

Do you currently have *Maackia amurensis* (Amur maackia or Chinese yellowwood) or related species in production? Related species include *Maackia amurensis* var. *buergeri* or *Maackia chinensis*.

○ Yes

○ No

What cultivars are currently in production? Please select a response for each cultivar or species.

	Yes	No
Maackia amurensis (straight species)	○	○
Maackia amurensis 'JFS-Schichtell' (MaacNificent)	○	○
Maackia amurensis 'Starburst'	○	○
Maackia amurensis 'Summertime'	○	○
Maackia amurensis 'Summerfrost'	○	○
Maackia amurensis var. buergeri	○	○
Maackia chinensis	○	○

Have you observed foliar lesions on *Maackia* trees in production? Symptoms include small necrotic spots/lesions with a yellow halo that may coalesce as time goes on. Disease symptoms typically appear in July or

August, when being observed in North Dakota.

○ Yes

○ No

If you have observed foliar disease symptoms on trees, which one(s) specifically? Please select a response for

each cultivar or species.

	Yes	No
Maackia amurensis (straight species)	○	○
Maackia amurensis 'JFS-Schichitell' (MaacNificent)	○	○
Maackia amurensis 'Starburst'	○	○
Maackia amurensis 'Summertime'	○	○
Maackia amurensis 'Summerfrost'	○	○
Maackia amurensis var. buergeri	○	○
Maackia chinensis	○	○

Questions or Comments

Powered by Qualtrics

APPENDIX D. 200 WORD SURVEY SYNOPSIS

North Dakota State University is investigating a potential new foliar disease on *Maackia amurensis* (Amur maackia or Chinese yellow wood) through a short survey. Disease symptoms caused by what we have tentatively identified as an Alternaria species of fungi include small necrotic lesions that are ringed by a yellow halo which grow in number and merge as the summer goes on. The survey consists of 6 questions and will take about five minutes of your time to fill out plus the time it takes to look for disease symptoms in the field. Your contribution to this study will help us to better understand the range of where *Maackia amurensis* and its cultivars are being grown throughout the United States, as well as where foliar disease symptoms are appearing. Please follow the link to participate in the survey. Thank you very much for your time and participation, it is greatly appreciated. Even if you do not currently have *Maackia amurensis,* its cultivars, or related species in production, please still consider filling out the survey as that is valuable information as well. If you have any questions or concerns, feel free to reach me at sarah.steffen@ndsu.edu or at ***-***-****. We will be in touch with the results of the survey in a couple of months. Survey closes Oct. 31, 2021.

APPENDIX E. INQUIRY EMAIL TO THE ASSOCIATIONS

Good afternoon,

My name is Sarah Steffen, and I am a graduate student at North Dakota State University. I am reaching out to you today in the hopes that your association can help me out with answering a research question through a survey. We would like to have you send a link with a short survey out to your association members on our behalf. We are curious to know if there are growers in your state who are producing *Maackia amurensis* who have noticed a foliar pathogen in recent years. The survey would consist of six questions simply inquiring if growers have seen leaf spots and results would be shared back with them. Thank you for your time and I look forward to hearing your response. I can be reached by email, or my phone number is ***-***-****, and I would be happy to field your questions.

Best regards,

APPENDIX F. SUMMARY AND REQUEST FOR HELP EMAIL

Hello,

My name is Sarah Steffen, and I am a graduate student at North Dakota State University pursuing my Master of Science in Horticulture. I am conducting a research project looking at the extent of a potential new foliar disease on *Maackia amurensis*. *Maackia amurensis* grows in zones 3 - 7, hence why you are being contacted. I am collecting data through a survey which consists of 6 questions and will take about five minutes of your time to fill out plus the time it takes to look for disease symptoms in the field. Your contribution to this study will help us better understand the range of where *Maackia amurensis,* its cultivars, and related species are being grown throughout the United States, as well as where foliar disease symptoms are appearing.

Currently there are no reported disease or pest issues on this tree. Understanding how this fungal pathogen is affecting trees is important as *Maackia* is being more commonly planted. Disease symptoms caused by what we have tentatively identified as an Alternaria species of fungi include small necrotic lesions ringed by a yellow halo which grow in number and coalesce as the summer goes on. Symptoms in Fargo, North Dakota, and our Dale E. Herman Research Farm near Absaraka, North Dakota, have appeared in July and worsen through the rest of the late summer into the fall. Please refer to the attached images to see a visual of what we have observed.

I am reaching out to you today in the hopes that your association can help with answering this research question through a short survey. We would like to have a link to this survey sent out to your association members on our behalf. I can send you a preview of the survey questions if you would like to see them before sending it out. Results will be shared upon the completion of this survey in the coming months. If this is something that you would be able to send out to members, I will send an email containing the information about the survey and a link to the survey to you. Thank you for your time and I look forward to hearing your response. I can be reached by email, or my phone number is ***-***-****, and I am happy to answer any questions.

Best regards,
Sarah Steffen

APPENDIX G. STATE NURSERY & LANDSCAPE ASSOCIATIONS CONTACTED

State	Association	Zone[1]	Participated
AL	Alabama Nursery & Landscape Association	7a-9a	No
AR	Arkansas Green Industry Association	6b-8a	No
AZ	Arizona Nursery Association	4b-10b	No
CA	Plant California Alliance	5a-11a	No
CO	Colorado Nursery & Landscape Association	3a-6a	No
CT	Connecticut Nursery & Landscape Association	5b-7a	No
CT, MA, ME, NH, RI, VT	New England Nursery Association	3b-7a	No
DE	Delaware Nursery & Landscape Association	7a-7b	No
GA	Georgia Green Industry Association	6a-9a	No
IA	Iowa Nursery & Landscape Association	4b-6a	No
ID	Idaho Nursery & Landscape Association	3b-7b	No
IL	Ornamental Growers Association of Illinois	5a-7a	No
IL	Illinois Green Industry	5a-7a	Yes
IN	Indiana Nursery and Landscape Association	5b-6b	No
KY	Kentucky Landscape and Nursery Association	6a-7a	No
MA	Massachusetts Nursery and Landscape Association	5a-7b	No
MD	Maryland Nursery, Landscape, and Greenhouse Association	5b-8a	No
MI	Michigan Nursery and Landscape Association	4a-6b	No
MN	Minnesota Nursery & Landscape Association	3a-5a	Yes
MO	Missouri Green Industry Alliance	5b-7b	No
MS	Mississippi Nursery and Landscape Association	7b-9a	No
MT	Montana Nursery & Landscape Association	3a-6a	No

NC	North Carolina Nursery & Landscape Association	5b-8b	No
ND	North Dakota Nursery, Greenhouse, and Landscape Association	3a-4b	Yes
NE	Nebraska Nursery and Landscape Association	4a-5b	Yes
NJ	New Jersey Nursery and Landscape Association	6a-7b	Yes
NM	New Mexico Chapter of Colorado Nursery and Greenhouse Association	4b-9a	No
NV	Nevada Nursery & Landscape Association	4a-10a	No
OH	Ohio Nursery & Landscape Association	5b-6b	Yes
OK	Oklahoma Nursery & Landscape Association	6a-8a	No
OR	Oregon Association of Nurseries	4b-9b	Yes
RI	Rhode Island Nursery & Landscape Association	5b-7a	No
SD	South Dakota Nursery & Landscape Association	3b-5b	No
TN	Tennessee Nursery & Landscape Association	5b-8a	No
TX	Texas Nursery & Landscape Association	6b-10a	No
UT	Utah Nursery and Landscape Association	4a-9a	No
VA	Virginia Nursery & Landscape Association	5a-8a	No
VT	Vermont Nursery and Landscape Association Green Works	3b-5b	No
WA	Washington State Nursery & Landscape Association	4a-9a	No
WI	Wisconsin Nursery & Landscape Association	3b-5b	Yes
WV	West Virginia Nursery and Landscape Association	5a-7a	Yes
WY	Wyoming Groundskeepers & Growers Association	3a-6a	No

[1]Zones based on the USDA Plant Hardiness Map (2012)

APPENDIX H. NDSU WPIP LAB DETACHED LEAF ASSAY PROTOCOL

Detached Leaf Assay Procedure Using Spores Directly from Leaves

Last Modified: August 2022

Spore Suspension

Materials: diseased leaves, spray bottle, DD water, tween20

The spore suspension can be prepared the night before and stored at 5°C.

1. Using DD water from the carboy, add 100 mL to a container with a lid. Place collected infected leaves into the container. Add a couple drops of tween20 and gently agitative for 5 minutes.
2. Using a clean pipette, place 5 uL of the spore suspension on the hemocytometer plate and cover with the coverslip. Place the slip on the microscope.
3. Count the spores on the four corners which contain the 16 squares as well as the middle square, recording the number of spores from each quadrant. Repeat 3-6 times and then calculate the average number of spores.

	A	**B**	**C**	**D**	**E**	**Sum**
1						
2						
3						
4						
5						
6						
					Total	
					Average	

4. Calculate the number of spores in the current concentration:
    a. Average # of spores x constant = concentration of suspension
        i. Constant = 2,000
5. Determine the number of spores needed for the final concentration. The desired final concentration should be between 30,000 – 80,000 spores.
    a. $C_1V_1 = C_2V_2$
6. Make up the final solution and place into spray bottle.

Clam Shell Preparation

Materials: Plastic containers, paper towels, DI water

1. Place a single paper towel on the bottom of the container.
2. Place plastic mesh screen on top of paper towel.
3. Add 30 mL of DI water.

Inoculation of Leaves with Spray Bottle

Materials: Prepared spore suspension and containers, DI water, Bleach, tween20, paper towels

1. Disinfest freshly collected leaves in a solution of 70% ethyl alcohol (EoTH). Place the leaves in the EoTH for a few seconds and gently rub the leaves. Remove the leaves and rinse with DI water and pat dry with paper towels.
2. Place full leaves (not individual leaflets) into prepared containers. Spray the leaves with the spore suspension, making sure to see visible droplets (2-3 squeezes).

3. Close the lids and set aside in a well-lit area for 10-15 days, checking frequently for the beginning of symptoms.

Inoculation of Leaves with Pipette

Materials: Prepared spore suspension and containers, DI water, Bleach, tween20, paper towels

1. Disinfest freshly collected leaves in a solution of 70% EoTH. Place the leaves in the EoTH for a few seconds and gently rub the leaves. Remove the leaves and rinse with DI water and pat dry with paper towels.
2. Place full leaves (not individual leaflets) into prepared containers. Pipette a 5 uL drop of spore suspension directly into the middle of the leaf.
3. Close the lids and set aside in a well-lit area for 10-15 days, checking frequently for the beginning of symptoms.

Disease Rating Scale

0 = no symptoms
1 = lesions accounting for 1 to < 25% of the leaf
2 = lesions accounting for 26 to < 50% of the leaf
3 = lesions accounting for 51 to < 75% of the leaf
4 = lesions accounting for 76 to < 100% of the leaf