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DISTRIBUTION AND MANAGEMENT OF *PHYTOPHTHORA* SPECIES
ON LAVENDER IN THE UNITED STATES

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Plant and Environmental Sciences

by
Daniel Matthew Dlugos
August 2022

Accepted by:
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ABSTRACT

Phytophthora root and crown rot (PRCR) is currently the most important disease on lavender (*Lavandula* spp.) in the United States. The disease was first described on English lavender (*L. angustifolia*) in a Maryland nursery in 1991, with *Phytophthora nicotianae* as the causal agent. Since that time, the disease has been reported on multiple continents and as caused by several species of *Phytophthora*. This study examined the distribution and pathogenicity of *Phytophthora* species on lavender in the United States, requested lavender grower feedback regarding their production and concerns, and examined efficacy of selected management options. Lavender is grown in all regions of the United States, with *L. angustifolia* and *L. ×intermedia* as the most common species planted. In collaboration with the U. S. Lavender Growers Association, samples of diseased lavender plants were collected from growers across the country over a 5-year period, 2015-2019. PRCR was found to be caused primarily by *P. nicotianae*, which was found in each of the 24 states from which PRCR positive samples were obtained, making it also the most widespread of the causal agents. *Phytophthora palmivora* and *P. citrophthora* were the next most abundant species found associated with symptomatic lavender; *P. cinnamomi*, *P. tropicalis*, *P. cryptogea*, *P. sansomeana*, *P. cactorum*, *P. drechsleri*, and *P. megasperma* were recovered from plant samples infrequently. Using Koch's postulates, *P. nicotianae*, *P. palmivora*, and *P. cinnamomi* were documented for the first time as pathogenic on *L. ×intermedia*; *P. cryptogea* and *P. drechsleri* as pathogenic on *L. angustifolia*; *P. nicotianae* as pathogenic on *L. heterophylla*; and *P.*

tropicalis as potentially pathogenic on *L. ×intermedia*. Additionally, first reports of pathogenicity of *P. palmivora* and *P. citrophthora* on *L. angustifolia* in the United States were documented. Some fungicides that target oomycete plant pathogens were shown to effectively manage *P. nicotianae* on *L. angustifolia* under greenhouse conditions. The phosphonate products, containing the active ingredients mono- and di-potassium salts of phosphorous acid or aluminum tris (O-ethyl phosphonate), provided the best protection in repeated trials based on several disease parameters. Attempts to remediate infested field soil using a quaternary ammonia product had some success but did not eradicate the pathogen. This study serves as a foundation for future research on Phytophthora root and crown rot of lavender, the most significant disease affecting this increasingly important specialty crop, in the United States.

DEDICATION

I dedicate this dissertation to my mom and dad, Marge and Jack Dlugos. I am forever appreciative of their love and support and for all the sacrifices they have made for me. There is no way I could have made it this far without their encouragement or them instilling in me a sense of dedication and curiosity and, of course, a love of plants from the time of my earliest memories. Thank you for believing in me!

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I would also like to thank my lab-mates, including lab managers—Lynn Luszcz, Andrew Gitto, and Linus Schmitz; fellow graduate students—Suzette Sharpe, Mastin Greene, and Ray Adcock; and undergraduates—Garner Powell, Bennett Harrelson, Maxwell Sturdivant, and Jennifer Esco. I express my sincere gratitude to Mr. Dick Baker for serving as my companion on sampling trips, Dr. Agudelo for answering my department-related questions, Ms. Meg Williamson for instructing me in diagnostics, and to the entire Plant and Environmental Sciences Department and Clemson University. I

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CHAPTER 1

LITERATURE REVIEW

Information on the Host Plant Lavender—Species of *Lavandula*

Lavender. Lavender (*Lavandula* spp.) is a member of the Lamiaceae/Labiatae or mint family (USDA 2021; Zomlefer 1994)—hereafter referred to as Lamiaceae. The family includes 258 genera and 6,970 species of aromatic herbs, shrubs, and trees (Zomlefer 1994). Species are mostly temperate, predominantly Mediterranean, but also cosmopolitan in distribution range (Zomlefer 1994). Some other notable genera within the family include *Mentha* (peppermint), *Ocimum* (basil), *Origanum* (oregano), and *Hyssopus* (Hyssop) (Zomlefer 1994). Human interest in this family dates back to prehistoric times. Their flowers and foliar scents led to them being currently popular as numerous species and cultivars of ornamental, culinary, and medicinal plants (Devecchi 2006; Naghibi et al. 2005). Lamiaceae species are also popular for plantings in urban areas (Devecchi 2006).

Lavender was documented as early as 370 B.C. (Upson 2002) and was listed as ubiquitous in English gardens by the 1600s (McCoy and Davis 2021). The origin of the name is Latin and derived from *Lavaro*, which means “to clean” (Prusinowska and Śmigiolski 2014). The native range includes the Mediterranean region, Africa, Asia, and the Middle East (Upson 2002). Currently there are 32 known species of *Lavandula* plus several hybrid species (Upson 2002).

Several species of lavender are commercially important. *Lavandula angustifolia* Mill. (previously classified as *L. delphinensis* Jord. ex Billot, *L. officinalis* Chaix, *L. spica* L., and *L. vera* D.C. [Anonymous 1997; Singh et al. 2007]) is English lavender and one of the most

common (McCoy and Davis 2021), hardy (Adam 2006), and expensive (Singh et al. 2007) species of lavender. It occurs naturally in southern Europe, including Italy, France, and Spain, and gains its hardiness from growing in the mountains (Upson 2002). *Lavandula dentata*, known as French lavender (Anonymous 1997; Herring-Murray 2016), has a native range from southern Spain through the Arabian Peninsula (Upson 2002); it is hardy only to about freezing temperatures (Upson 2002). Spanish lavender, *L. stoechas*, is another non-hardy species (Anonymous 1997; Herring-Murray 2016) found throughout the Mediterranean region and now invasive in Australia (Upson 2002). It was used in ancient Greece and Rome (Amidon 2013). *Lavandula latifolia*—commonly known as spike lavender and native to Spain, France, and the Mediterranean (Anonymous 1997; Anonymous 2009; Guenther 1954)—is a lower elevation species (Guenther 1954).

Hybrids of various species also exist. *Lavandula ×intermedia* (synonym *L. ×hybrida*), commonly known as lavandin, is a hybrid between English and spike lavender (Amidon 2013; Anonymous 1997). Hybrids are hardy plants with high oil yields (Guenther 1954; Herring-Murray 2016). Lavandins in France began growing in popularity in the 1950s due to their hardiness, heat tolerance, and longevity and because they can have up to four times the oil production of non-hybrid lavender species (Guenther 1954). However, lavandins have been noted as being less hardy in Ontario, Canada (Anonymous 2012). Lavandin currently is less sought after than non-hybrid lavender even though it possesses the same chemical compounds but in different concentrations (Usano-Alomany et al. 2011). Most lavandins are also able to produce acceptable quality dry flowers (Renaud et al. 2001). Lavandin flowers dry to a color of grey while true lavender flowers dry to blue (Anonymous 2009).

The above-listed species and hybrids—including *L. angustifolia*, *L. latifolia*, *L. stoechas*, *L. dentata*, and *L. ×intermedia*—are among the most common commercial lavender species today (Cavanagh et al. 2004). Differences among them morphologically, chemically, and otherwise exist. That different lavender species have different properties is evidenced in Iran by two native species, which are not utilized commercially, while imported cultivars of popular species are utilized (Naghibi et al. 2005). Different lavender species are known for different therapeutic properties (Cavanagh et al. 2004), and, even among ornamentals, there exist different niches for desired attributes—e.g., height, flower color, time of flowering (Devucci 2006). *Lavandula angustifolia* and *L. ×intermedia* are known for success in the New England states (Adam 2006), and within each of these popular species exists many different cultivars.

Commercial lavender production. Lavender is produced commercially in many regions of the world and for different purposes and products. Obtaining production totals, however, is a challenge. First, lavender is a relatively new crop in commercial production and can fall under various categories such as herb or ornamental plant, making documented crop profiles rare. Secondly, production totals could be from a variety of yields including plants, biomass, or oils. Annual production of lavender oil worldwide was 200 metric tons (Singh et al. 2007; Anonymous 2009) while that of lavandin oil, produced from lavender hybrids, was 1000 metric tons (Anonymous 2009). The top lavender oil producing countries include Bulgaria, England, France, Russia, Ukraine, Moldova, Hungary, Turkey, Romania, nations of the former Yugoslavia, Australia, United States, Canada, South Africa, Tanzania, China, Italy, and Spain (Anonymous 2009).

French production through 1905 occurred mainly from wild plants, but, after World War II, it was more conventionally grown (Guenther 1954). Early on, fields in France were typically

less than 1 hectare in size (Guenther 1954). In the early 1950s, France's annual production of lavender oil went from a peak of 150,000 kilograms to 50,000 kilograms with an additional 300,000 kilograms of lavandin oil produced (Guenther 1954). France now produces 60 to 80 metric tons of lavender oil and 1,100 to 1,150 metric tons of lavandin oil annually (Grebenicharski 2016). Of the 20,000 ha of lavender in France, 16,000 are of lavandin (Grebenicharski 2016). The economic impact of lavender in France is \$30 million, with 30,000 people employed and plants originating from 2,000 farms (Grebenicharski 2016).

France was the leading lavender oil producer in the world until Bulgaria took the lead in production (2006) and export value (2015) (Grebenicharski 2016). Bulgaria's harvest of metric tons of green mass of lavender at 19,000 metric tons in 2015 was a four-fold increase from 2008 (Grebenicharski 2016). Lavender currently is among the most commonly grown organic crops in Bulgaria, constituting about 50% of all organic crops (Grebenicharski 2016). Because of the growing popularity and market for products, many growers starting lavender farms are inexperienced in herb production and sometimes are new to agriculture in general (Grebenicharski 2016). Increases in production areas even led to a lack of seedling availability in which supply could not meet demand in Bulgaria in 2014 and 2015, leading to increased prices (Grebenicharski 2016). France, the U.S., and Germany are among the leading importers of Bulgarian lavender (Grebenicharski 2016).

Lavender production is economically important in many other countries as well. In Spain, four lavender species and lavandin hybrids are grown commercially (Usano-Aleman et al. 2011). Lavender production was reported to be profitable in the Western Himalayas of India (Singh et al. 2007), and Turkish production of lavandin was reported to be 250 hectares and 2.5 tons produced (Kara and Baydar 2013). There were 600 lavender farms in Australia in 2003

(Cavanagh et al. 2004). Of these, older farms were smaller and used for tourism while newer farms were larger at about 200 acres, and most of the farms grew *L. angustifolia* (Cavanagh et al. 2004). Japan has been noted to produce several metric tons per year of *L. angustifolia* while other producers include Argentina, Brazil, and East Africa (Adam 2006). *Lavandula angustifolia* has been noted as being widely grown in Northern Italy (for domestic use), Switzerland, Germany, and the Netherlands (Minuto et al. 2001). The economic impact of lavender production in the U.S. has not been reported. In the United States, California, as of 2006, had 63 lavender farms. The Dungeness Valley on the Olympic Peninsula of Washington is known as the lavender capital of the U.S. and is well known for lavender festivals (Adam 2006). In the coming years, China will likely become one of the largest producers and consumers of lavender oil (Grebenicharski 2016).

Lavender oil. Lavender oil is among the most economically important products of the lavender industry. The oils of the Lamiaceae contain terpenoids, iridoids, phenolic compounds, and flavonoids (Naghibi et al. 2005). Lavender specifically contains linalool, linalyl acetate, 1,8-cineole, b-ocimene, terpin-4-ol, and camphor (Cavanagh et al. 2004). Lavender oil has been studied extensively—with much focus on chemical analysis (Śmigielski et al. 2013) and production by the plants (Biswas et al. 2009). Various methods—including hydro and steam distillation, solvent extraction, and superficial extraction—are utilized for harvesting the oil (Adam 2006). In particular, steam distillation is used to remove oils from flower heads and foliage (Cavanagh et al. 2004).

While oil content in lavandin is greater than that in lavender, lavandin also has a higher camphor content (Renaud et al. 2001); however, it is the lower camphor content that makes lavender oil more desirable (Kara and Baydar 2013). Pure lavender oil is expensive and often

diluted with oil from of *L. latifolia* or a hybrid species, or it even might be diluted with chemicals (Prusinowska and Śmigielski 2014). Oil from lavandin, however, is often mixed with other lavender or essential oils (Adam 2006).

Several factors can further decrease the yield of oil from plant material. Essential oil content declines during storage of flowers (Dušková et al. 2016). Drying of tissues prior to distilling causes a greater than 40% oil yield loss (Śmigielski et al. 2011). Cultivation site (Cavanagh et al. 2004) and even the production system can affect essential oil content yield with lower content reported in organic production systems when compared with conventional production systems in Poland (Seidler-Lozykowska et al. 2014). In terms of quality, flower parts have more aromatic oils than other parts of the plant, and higher quality oils are known to come from plants grown at higher elevations (Anonymous 2009; Cavanagh et al. 2004).

Lavender uses. Plants have been long known as sources of unique compounds—including essential oils, pharmaceuticals, health products, dyes, cosmetics, and plant protection compounds (Lubba and Verpoorte 2011). The species of the Lamiaceae are noted, in particular, for medicinal, aromatic, and ornamental uses (Naghibi et al. 2005). The genus *Lavandula* has had extensive and detailed histories and summaries of its many and varied uses published (Castle and Lis-Balchin 2002; Lis-Balchin 2002a; Lis-Balchin 2002b). Throughout history, lavender has been used for everything from taming tigers to embalming (McCoy and Davis 2021). There are many industrial, cosmetic, and health-based uses (Anonymous 2009). Properties of lavender extracts have been reported as antibacterial and antifungal, carminative, sedative, anti-depressive, and useful for insect bites and burns (Cavanagh et al. 2004). Most of the market for lavender thus lies in the pharmaceutical, agricultural/horticultural, and perfume industries, with much of the latter based in Japan, Europe, and the U.S. (Cavanagh et al. 2004).

As for medicinal uses, the Lamiaceae, in general, have been reported to be used for digestive disorders in Iran (Naghibi et al. 2005). The copious medicinal and pharmacological uses of *Lavandula*, as a genus, have been thoroughly reviewed (Hart and Lis-Balchin 2002; Naghibi et al. 2005), and the psychological impacts of lavender are clearly referenced in literature (Kirk-Smith 2002). Effects include relaxation and sedative effects (Buchbauer 2002) and, when coupled with a patient's medication, can lead to greater successes for the treatment of mild to moderate depression (Akhondzadoh et al. 2003). Oils from *L. angustifolia* and other species have shown the ability to help patients sleep (Wolfe 1996). Other medically related characteristics include the ability to limit severe motion sickness in transport of farm animals (Bradshaw et al 1998), anticancer properties (Buchbauer 2002), and anti-inflammatory properties based on lab animal studies (Hajhashemi et al. 2003).

Lavender also contains chemical compounds that result in antimicrobial capabilities (Deans 2002; Mayaud et al. 2008), and the oil is reported to be bacteriostatic (Cavanagh et al. 2004) or even able to kill harmful bacteria, e.g., *Streptococcus* spp. (Anonymous 2009). The oils are volatile, and this has been reported to inhibit fungus growth and sporulation (Cavanagh et al. 2004). Lavender oils have been said to have the ability to be antimicrobial with no human toxicity (McCoy and Davis 2021). However, an examination of the phytotoxicity or skin irritant effects of lavender species other than *L. angustifolia* is not complete (Cavanagh et al. 2004), and even *L. angustifolia* oil potentially can damage human skin cells (Prashar et al. 2004). These potential antimicrobial properties coupled with a pleasant aroma are responsible for use of lavender extracts in soaps, perfumes, and bath additives dating back to the Middle Ages (Cavanagh et al. 2004; Prusinowska and Śmigielski 2014). The pleasant aroma also has led to a long history in perfume use (Wells and Lis-Balchin 2002) with lavender oil for that purpose

originating in France (Anonymous 2009). Another use for lavender oil is for aromatherapy (Cavanagh et al. 2004; Singh et al. 2007).

The food industry is another major consumer of lavender products. *Lavandula angustifolia* was the most effective essential oil tested for use to prevent stored potatoes from sprouting (Vokou et al. 1993). Flowers, buds, and leaves have been said to be fit for human consumption as flavor additives (Prusinowska and Śmigielski 2014). The oils and buds are used in food preparation and processing —e.g., in ice cream, tea, mustard, and bakery items (Cavanagh et al 2004).

Another very important use of lavender is as an ornamental plant. Many species of *Lavandula* are grown in nurseries and greenhouses for landscape use (Naghibi et al. 2005). They are popular as ornamental plants in South Africa (Anonymous 2009), and one-third of the perennial plants sold in Europe are lavender (Herring-Murray 2016). In Europe, *L. angustifolia* is the top-selling lavender species for ornamental plant purposes (Herring-Murray 2016). Lavender is popular in public areas because of the flowers, low maintenance requirements, tolerance of environmental pollution, and low water needs (Devecchi 2006). Lavender has long been popular as lawn and walkway borders and has increasingly become favored in Italian roundabouts and medians (Devecchi 2006). Green roofs are becoming popular and *L. angustifolia* can be used successfully for this purpose when soils are at least 20 to, preferably, 30 cm deep (Kotsiris et al. 2012). Lavender is a favorite crop grown at entertainment farms (Adam 2006). In Ontario, Canada, lavender is common in the agritourism and ornamental plant industries (Anonymous 2012), and thousands of visitors flock to Canadian lavender festivals each year (Phair 2011). Agritourism is also popular for aromatherapy and recreation purposes

near summer destinations in Bulgaria—such as Sofia, Plovdiv, and the coast of the Black Sea (Grebenicharski 2016).

Lavender cultivation. For commercial production, plants are propagated primarily by vegetative cuttings because seed is often sterile (Adam 2006). A 500-m² nursery can produce 25,000 plants from 30,000 cuttings to establish a 1-ha farm (Singh et al. 2007). In Canada, it takes three years from planting until cuttings are ready to be harvested (Phair 2011). Plants can be harvested for five to thirty years depending on the grower, species, and region—with the middle of that range being more common (Adam 2006; Anonymous 1997; Anonymous 2009; Guenther 1954; McCoy and Davis 2021). Mulching increased lavender height, diameter, and flowering, and polyethylene and transpiring mulches were most effective (Hoeberechts et al. 2004).

Pest and pathogen problems in cultivation. Lavender as a crop has been reported as generally problem-free. Few insect pests have been documented (Adam 2006) and, previously, no serious widespread disease problems were reported (Anonymous 2009). In Canada, *Septoria* leaf spot, caused by *Septoria lavandula*, root rot, and powdery mildew were all observed (Anonymous 2012). In 2006, Adam reported lavender as having only “a few fungal diseases” but with “no known remedies”. In contrast, wild lavender was reported to be disappearing in France by the 1950s, and it was suggested root rots, possibly caused by a fungus, may be involved (Guenther 1954). In fact, it was this root rot that contributed to a lack of commercial expansion early on (Guenther 1954). Much more recently, in the Western Himalayas region of India, plants suffered 16% mortality in part to diseases, and the author recommended the issue be addressed immediately (Singh et al. 2007). In Canada, lavender is described as having a “root mold” that causes mortality (Phair 2011). In Texas, *Rhizoctonia* sp. was reported to be the cause

of a disease that destroyed 90% of a new farm's 'Grosso' and 'Provence' lavender plants (Adam 2006). *Phytophthora nicotianae* causing root rot (Putnam 1991) and *Phytophthora* species causing wilt have also been noted in extension publications (McCoy and Davis 2021).

In Bulgaria, the leading lavender-producing country, reported problems with species of *Septoria*, *Phoma*, *Phomopsis*, *Phytophthora*, *Alternaria*, *Fusarium*, and *Verticillium* (Vasileva 2015). The main cultivars there were all sensitive to fungal pathogens. Across hundreds of hectares and two years, the incidence of plants diseased by fungal pathogens was just over 20% (Vasileva 2015). In France, the second largest producer of lavender, the phytoplasma (*Candidatus Phytoplasma solani*) and mealybugs (*Dysmicoccus multivorus*) led to approximately 50% loss of lavender at harvest and a 45% decline in oil production (Grebenicharski 2016).

Climate and cultivation conditions may make matters worse for diseases. High temperatures and humidity are detrimental for lavender production (Anonymous 2009). Therefore, it is no surprise that the southeastern region of the USA has been called too hot and humid for production of *L. angustifolia* (McCoy and Davis 2021). Moisture was recognized as a problem for lavender as early as 1953 (Guenther 1954). For this reason, drainage of the soil is important and thus lavender is less likely to survive on heavy clay soils (Adam 2006).

When faced with disease problems, lavender growers have the same issues as growers of other minor specialty crops, in that there are few registered options for chemical management (S. N. Jeffers, *personal communication*). Also, as a developing industry, lavender production faces difficulties in that pesticide application rates are not well developed, and tolerances of the various lavender species and cultivars are still being tested (S. N. Jeffers, *personal communication*). Furthermore, the herbal and cosmetic industries are just as concerned with active substance content and yield as with contamination by potential pesticide residues; thus,

organic production has been encouraged in Poland (Seidler-Lozykowska et al. 2014). Organic production is possible for lavender in some localities, such as in the midwestern region of the United States (Renaud et al. 2001), and the process may lead to greater flower yield (Seidler-Lozykowska et al. 2014). Many lavender growers also are interested in using biorational pesticides (e.g., biocontrol products, biofungicides) to avoid using traditional chemical pesticides (S. N. Jeffers, *personal communication*). While lavender continues to be the subject of continued research spanning many disciplines (Lis-Balchin 2002c), the importance of diseases, especially root diseases, make this topic one of the most pressing issues for sustained lavender production in the U.S. and other countries.

Information on *Phytophthora* species as Pathogens of Lavender

The nursery trade and dissemination of plant pathogens. In 2005, there were an estimated 50,000 invasive species in the United States with an annual cost of \$120 billion (Pimentel et al. 2005). The costs due to diseases caused by invasive plant pathogens alone, including damage from and management of, totaled \$21.5 billion per year on crops, \$2 billion per year on landscape plants, and \$2.1 billion per year on forests (Pimentel et al. 2005). These costs do not factor in the loss and endangerment of species or the environmental and biodiversity impacts (Pimentel et al. 2005). It is a big enough problem that the U.S. has been declared to be vulnerable to plant pathogens as biological weapons (Madden and Wheelis 2003). Often, the origin of an invasive pathogen is unknown (Webber 2010), but it is thought that organisms do not cause major problems on host plants in native regions (Hansen 2008). This makes the term “alien invasive species” (Webber and Brasier 2005) particularly applicable for many of these pathogens. Some of these also may be considered “emerging pathogens”, which is a term used

when pathogens, host plants, or both are introduced to a new geographic location, and the relationship becomes increasingly problematic over time (Garbelotto and Pautasso 2012).

Plant pathogens are spread by a variety of ways. Many microorganisms, including those regulated in New Zealand, were found on shoes worn by passengers boarding airplanes, and a potentially bigger problem existed with shoes in checked luggage (McNeill et al. 2011). Even loans of plant specimens from museums or herbaria pose a risk of spreading plant pathogens (Brasier 2005). By far, however, live plant material in the global plant trade is the most common means of spreading plant pests and pathogens and presents much risk (Garbelotto and Pautasso 2012; Liebhold et al. 2012). Plant smuggling is included and has likely resulted in pest introductions (Liebhold et al. 2012).

Commercial plant trade and individuals collecting plants pose the greatest risks (Brasier 2008). This is due, at least in part, to methods used in the global plant industry—with the transport of nursery stock to new regions as one of the concerns (Parke and Grünwald 2012). Nursery plants may also undergo potting at various stages, exposing them to pathogens (Parke and Grünwald 2012). Some nurseries even export plants to warmer foreign locations over winter then re-import them, posing an additional risk (Brasier 2008). The greatest threat to nurseries themselves is contamination of the ground, irrigation water, pots, and potting media (Parke and Grünwald 2012).

Plant trade is increasing, and, with that, the potential for pathogen spread. The value of plants imported into the United Kingdom is increasing; the value doubled from £370 to £860 million in the period of 1993 to 2005 (Brasier 2005). In Great Britain between 1970 and 2004, 234 plant pathogens were introduced (Jones and Baker 2007). This figure failed to account for the pathogens that were shipped but were unable to establish. Of these 234 pathogens, 19% were

listed as “important”. While there were many introductions, the numbers of introductions did not seem to be changing, but it is unlikely there will be a decrease as more hybrid pathogen species continue to form and be disseminated (Jones and Baker 2007). Of the 234 introduced pathogens mentioned in the study above (Jones and Baker 2007), 27 were oomycetes and 14 of these were species of *Phytophthora*. More than half of all pathogen introductions were on ornamental plants, which also accounted for half of all *Phytophthora* species introductions (Jones and Baker 2007). These data highlight the risk associated with ornamental plants, which are notorious for moving plant pathogens (Jones and Baker 2007), and often found to be the most common means of pest and pathogen introductions (Migliorini et al. 2015).

***Phytophthora* species as invasive pathogens.** In 2010, Webber listed the most notable invasive plant pathogens, and five of the 13 were species of *Phytophthora*. Numerous species of *Phytophthora*—including *P. nicotianae*, *P. palmivora*, and *P. drechsleri*—were found in potting mixes shipped into Western Australia from other states in that country (Davison et al. 2006). One of the main concerns is that *Phytophthora* species are often found in association with asymptomatic plants (Bienapfl and Balci 2014); these pathogens were found in 70% of asymptomatic nursery plants surveyed in a European study (Migliorini et al. 2015). In a multi-decade study across 23 European nations, *Phytophthora* species were found in over 91% of the 732 nurseries sampled (Jung et al. 2016). Additionally, *P. nicotianae*, like other species in the genus, is often found infecting below-ground parts of plants and infesting soil (Bienapfl and Balci 2014), making early disease detection difficult. Potting media were the primary means of spreading *Phytophthora* species in Maryland nurseries, and often were associated with asymptomatic plants (Bienapfl and Balci 2014). Even soilless media are known to harbor many different species, and transport of potting media is only regulated internationally—leaving states

and regions in the U.S. at risk (Bienapfl and Balci 2014). In a Maryland nursery, eight species of *Phytophthora* were found in water and 13 in media (Bienapfl and Balci 2014). A separate survey of nursery water recirculation system drains, reservoirs, and wells found 12 species, including some previously undescribed species (Themann et al. 2002). However, species found in potting substrates are more likely to be associated with diseased plants than those in water (Bienapfl and Balci 2014).

Nurseries can be hotbeds for species of *Phytophthora* as numerous studies have demonstrated. A four-year survey of Minnesota nurseries found more species of *Phytophthora* than expected, including genetically unique isolates that could not be identified as well as the state's first report of several species, including *P. nicotianae* (Schwingle et al. 2007). In 1988 in Western Australia, eight species of *Phytophthora* were found in nurseries, with *P. drechsleri* accounting for 73% of infections (Hardy and Sivasithampanum 1988). All eight species were even found in one nursery (Hardy and Sivasithampanum 1988). Of the 1,523 samples from 10 Maryland nurseries, 589 isolates yielded 16 species although none were of regulatory significance (Bienapfl and Balci 2014). After introduction on contaminated plants, *Phytophthora* species can become established in nursery soils (Jeffers et al. 2010), which can serve as a source of inoculum for other nursery plants or for dissemination into surrounding natural areas—such as adjacent forests or streams.

Role of nurseries. Nurseries are drastically different than traditional agriculture in that 40 acres may include 500 plant species shipped in a variety of substrates from various localities, with each species having different growth requirements and a different complement of pests and pathogens (Park and Grünwald 2012). In the U.S., 97% of woody plant imports come from Canada although Asia and Oceania are growing sources of incoming material (Liebhold et al.

2012). Most imported woody ornamentals end up in California, Florida, and Ohio, among other states (Liebhold et al. 2012). As invasive pathogens are spread in the nursery trade, new hosts become recognized that otherwise were not known to be susceptible (Osterbauer et al. 2014). Additionally, when exotic plants are relocated to new locations, they are usually susceptible to new microbes (Strange and Scott 2005). When it comes to preventing pathogen spread, it is difficult to balance protection with economics (Brasier 2005). This is especially true in the growing and profitable nursery industry. However, no persons are held accountable to pay for damages done by invasive pathogens (Brasier 2005), and, in general, the largest distribution centers were found to be the most responsible (Nelson and Bone 2015). When attempting to prevent the spread of invasive pathogens, one of the most common and viable options is inspection of nursery stock.

Nursery inspections have a long history and those conducted in New York state in the early part of the 20th century were described by Atwood in 1911. Inspections in New York state began in 1898 with an initial funding of \$10,000 (Atwood 1911). At this time, a very friendly atmosphere was described when thousands of trees were sacrificed by growers who actually requested inspections (Atwood 1911). Additionally, all imported stock required inspection, and cooperation with other states was carried out successfully (Atwood 1911). The limitations of current nursery inspections lie in government funding and lack of symptoms and signs on infested and infected plants (McTavish and Barnett 2014). Inspection station employees are responsible for 43 million plants each year (Liebhold et al. 2012), and a mere 2% of shipments are inspected by the USDA Animal Plant Health Inspection Service (APHIS) (Brasier 2008). In 2009, an estimated 72% of infested plant shipments went undetected (Liebhold et al. 2012).

Phytosanitary regulations are often considered to be ineffective (Roy et al. 2014). Often, these regulations rely on international borders. Moving plants among jurisdictions with varying environments, such as across the European Union, can be just as dangerous as transcontinental movement (Brasier 2008). There are a number of other reasons why nursery inspections may not work. Innocuous, asymptomatic carriers can serve as “Typhoid Marys” (Brasier 2005). Another reason for ineffectiveness is that only roots may be symptomatic, and nursery inspections may easily miss these symptoms. For example, *P. nicotianae*, a root rot pathogen, is frequently isolated from potted plants (Migliorini et al. 2015). In one study, a majority of nursery plants sampled with asymptomatic foliage had symptoms on roots (Migliorini et al. 2015). Simple visual checks may not suffice. For those reasons, inspections are often ineffective against internal or soilborne plant pathogens; plants infected with these pathogens appear asymptomatic in pots because the primary symptoms are on the roots or in the vascular stele (Migliorini et al. 2015). Symptoms often spread to other parts of the plant later on—after leaving the nursery (Webster and Webber 2007). Many long-term root pathogen populations will remain quiescent or as saprobes until adverse conditions arise, stress the plant, and promote infection (Burdon 1993). As an additional problem, unknown pathogens are by default not regulated (Brasier 2008). Many destructive pathogens are likely not yet recognized because they do not cause damage in their natural environments (Brasier 2005). Even among those that are recognized, different mating types of some species of *Phytophthora* may affect certain hosts differently and even differ morphologically (Brasier 1992). In such a case, a mating type may be present and not cause damage, so it is overlooked in inspections, which could allow another more damaging mating type to arrive and become established.

Another challenge to inspections is that fungicides can prevent symptoms and, therefore, mask pathogen presence (Scott et al. 2013). Nursery use of oomycete-specific fungicides without proper sanitation leads to spread of *Phytophthora* species within and from nurseries (Drenth and Guest 2013). For oomycetes, fungicides such as metalaxyl and mefenoxam are actually fungistatic and not fungicidal (Brasier and Jung 2006). Fungistatic chemicals, which inhibit fungus or oomycete activity rather than kill them, can mask symptoms leading to a “Trojan horse”, which is a notorious means of spreading *Phytophthora* species (Brasier 2005). This is not only true of fungicides; root rot of nursery plants also may be suppressed by resident microflora in container mixes until disruption of the system by transport of plants to market and planting in a landscape (Pettitt 2014). For this and the above-mentioned reasons, continuous inspections of nursery stock would be more effective than just pre-shipment inspections (McTavish and Barnett 2014).

There is hope and forward-looking ideas when it comes to dealing with such threats to the nursery industry. States such as Florida have established a successful nursery inspection program that has incentives for growers (Merritt et al. 2012). Furthermore, the Oregon Department of Agriculture began a nursery certification program that will be evaluated for success (Parke and Grünwald 2012). By 2014, it was found that nurseries utilizing grower-assisted inspections had significantly lower incidences of *Phytophthora* species than those using point of shipment inspections (Osterbauer et al. 2014). Records and audits on nurseries have been suggested (McTavish and Barnett 2014). An effective quarantine would involve monitoring of most interconnected growers (Nelson and Bone 2015). Another challenge to inspections is hybrid species of pathogens. Quarantine services need to determine how to evaluate the risk of two common pathogens hybridizing to form a unique species and use

molecular, not morphological, methods to detect them (Brasier 2001). Overall, the most important suggestion is that nursery owners take the same responsibility as restaurant managers to prevent contamination of their products and guarantee consumer safety (Parke and Grünwald 2012).

***Phytophthora* species on lavender.** *Phytophthora* root rots are diseases caused by species of *Phytophthora* that often begin with drought-like symptoms (Agrios 2005). Other symptoms include root necrosis, root and stem discoloration, yellowing, stunting, wilting, and death (Benson and von Broembsen 2009). The problems originally develop in wet, cool to warm soils and later can allow secondary plant pathogens to invade (Agrios 2005). On a susceptible plant, the amount of inoculum, environmental conditions, and size of a root system determine the rate of disease progression (Agrios 2005; Benson and von Broembsen 2009).

Phytophthora nicotianae. *Phytophthora nicotianae* is one of the most studied species of *Phytophthora* (Kamoun et al. 2015). The name *P. nicotianae* and *P. parasitica* are now regarded as synonymous, with *P. nicotianae* the accepted name (Erwin and Ribeiro 1996). However, at one point *P. nicotianae* was preferred by researchers in Europe and Asia while *P. parasitica* was more popular with researchers in the Americas (Hall 1993). *Phytophthora nicotianae* primarily infects dicots and can infect the roots, crowns, fruits, leaves, and flowers of plants (Hall 1993; Taylor et al. 2015). *Phytophthora nicotianae* is largely a root pathogen. However, the same isolate can even cause multiple diseases on multiple parts of a plant (Benson and Jones 1980). The pathogen is known to especially attack fine roots, making up 80% of all infections in a tobacco plant (English and Mitchell 1988; Kosola et al. 1995). Root mass density and sugar ketone concentration explained 86% of monthly soil variation in *P. nicotianae* propagule abundance in citrus grove soil (Duncan et al. 1993). On an annual basis, soil temperature, sugar

concentration, and soil moisture accounted for 76% of the variation in pathogen propagule abundance in soil (Duncan et al. 1993).

Phytophthora species have been associated with lavender production for many years although it is not clear which species of lavender are susceptible to the different species of *Phytophthora*. In some cases, multiple species of *Phytophthora* may be causing disease on one species of lavender, even in a single nursery or greenhouse. The species *Phytophthora nicotianae* was first described in 1896 causing disease on tobacco; it now is known to be pathogenic to plants in 255 genera and 90 families and has a cosmopolitan distribution (Cline et al. 2008; Erwin and Ribeiro 1996).

Phytophthora nicotianae on *Lavandula angustifolia* was first detected on potted plants at a nursery in Maryland in an area recently flooded by rains (Putnam 1991). Plants developed a grey color to the foliage and a blackening of the roots. Vascular discoloration was found in roots and stems, even on otherwise healthy plants. The isolated pathogen produced conspicuously papillate sporangia that were hemispherical at the base, broadly ovoid, and non-caducous. Sexual structures were formed only when paired with known A1 or A2 mating type isolates (Putnam 1991). These morphological characters are consistent with *P. nicotianae* (Erwin and Ribeiro 1996).

Since the first description of *Phytophthora* root rot on English lavender in Maryland, *Phytophthora* species have been found attacking lavender plants in many locations around the world. It is widely believed that *Phytophthora* root rot (PRR) of lavender in Europe is mostly caused by *P. nicotianae* (Faedda et al. 2013). In addition to being the leading cause of PRR, *P. nicotianae* is also listed as the most problematic disease of lavender in Italy and the greatest disease threat to lavender production (Davino et al. 2002; Faedda et al. 2013). Results from an

experiment in Italy showed mortality of lavender (*Lavandula angustifolia*) to be as high as 93% after inoculation with *P. nicotianae* (Minuto et al. 2001). A lower incidence was found in both shaded and clay pots, and smaller pots were found to increase mortality. While the amount of substrate in a pot affected disease development, the type of substrate did not. Substrates in this experiment even reached temperatures as high as 44°C in full sun. Plants in full sun exhibited more severe symptoms as did those with weak roots systems (Minuto et al. 2001). During this experiment, some non-infested soils in the experiment became infested, leading to the hypothesis that the pathogen is easily spread by irrigation and rain splash (Minuto et al. 2001).

Phytophthora nicotianae was first found on *L. angustifolia* in Spain in 2004 (Álvarez et al. 2007). There was 70% incidence of disease in impacted areas, and symptoms included discolored grey foliage, loss of vigor, wilt, and death. Artificial inoculations resulted in 80 to 100% infection of roots, and symptoms were visible 10 weeks after inoculation—followed by mortality two weeks later. The problem also had been occurring on nearby rosemary plants. Of the 10 isolates collected from lavender at this site, five produced chlamydospores, and five had unusual caducous sporangia borne on short pedicels. All isolates formed sexual oospores when paired with the opposite mating type; five isolates were A1 mating type, two were A2, and one isolate was sterile (Álvarez et al. 2007). Chemical treatments did not prevent disease when applied as a drench (Minuto et al. 2001), thus further research on management of this disease was warranted. The importance of *P. nicotianae* on lavender species cannot be ignored, especially as lavender is replacing tobacco, another host of *P. nicotianae*, in formerly heavy production areas in Turkey and other locations (Dervis et al. 2011) and now the southeastern United States.

In Bulgaria, *P. nicotianae* was found on *L. angustifolia* in 2008 and 2009 (Nakova 2011). Hybrid *Phytophthora* species also were found (Vasileva 2015). Symptoms reported on infected lavender plants in Bulgaria were suppressed growth and grey water-soaked lesions at the base of stems; eventually, stems and foliage wilted, leaves turned yellow-grey before dropping, and plants died (Nakova 2011). Damage was reported to increase as plants grew older (Vasileva 2015). Additionally, the pathogen has been reported on lavender in Canada (Westerveld 2015) and Australia (Mammella et al. 2013), on *L. angustifolia* in Greece (Erwin and Ribeiro 1996), Croatia (Jung et al. 2016), and the United Kingdom (Jung et al. 2016), on *L. stoechas* in Italy (Jung et al. 2016), and on hybrid lavender in Croatia (Jung et al. 2016).

***Phytophthora palmivora*.** Described in 1917, *Phytophthora palmivora* is a cosmopolitan pathogen known to have hosts in 160 genera and 60 families (Cline et al. 2008). *Phytophthora palmivora* was first identified on English lavender (*L. angustifolia*) in Sicily, Italy, in 2001 (Davino et al. 2002). Shrubs located in clay loam soil had root rot, and 60% of the 34,000 plants on site had the disease (Davino et al. 2002). The causal agent of the root rot was identified as *P. palmivora* with minimum, optimum, and maximum temperatures at 10, 27, and 35°C, respectively (Davino et al. 2002). The same problem has occurred on *L. angustifolia* in Turkey as well. In this situation, *P. palmivora* was responsible for chlorosis, wilting, and death—with 45% disease incidence on 2-year-old lavender plants (Dervis et al. 2011). Both *P. palmivora* and *P. nicotianae* have been documented to cause root rot simultaneously on individual plants in other plant genera and families—e.g., windmill palms in Italy (Cacciola et al. 2011). Therefore, to find both pathogens simultaneously causing disease on individual lavender plants is not unexpected. This pathogen has also been reported to occur on *L. spica* (= *L. angustifolia*) in Korea (Cho and Shin 2004).

Phytophthora ×pelgrandis. *Phytophthora ×pelgrandis* has been described as common on lavender and possibly spreading worldwide through trade (Faedda et al. 2013). This taxon is a hybrid between *P. nicotianae* and *P. cactorum*, and it has been reported as being as aggressive as *P. nicotianae*. Like *P. nicotianae*, it has as high optimum temperature for growth.

Phytophthora ×pelgrandis is homothallic and produces both amphigynous and paragynous antheridia and non-caducous, papillate sporangia (Faedda et al. 2013; Nirenberg et al. 2009). It is known to infect at least six genera in six families (Cline et al. 2008). A root and collar rot of *L. stoechas* in the ornamental plant industry in Italy was described in 2007, and isolates were collected from diseased plants, saved, and later identified as *P. ×pelgrandis* (Faedda et al. 2013). Symptoms on 4- to 6-month-old potted lavender plants included leaf chlorosis, root and stem necrosis, and wilting. Disease incidence was 30% with 15% mortality in a field of 24,000 plants. *P. ×pelgrandis* also was found in Hungary in 2008 to 2009 on *L. angustifolia* ‘Hidcote’ (Szigethy et al. 2013). In a pathogenicity test, the time from artificial inoculation to symptom expression was approximately 2 months (Szigethy et al. 2013). This pathogen was isolated from lavender and other plant species in the Netherlands in 2000 before it was recognized as a hybrid species (Bonants et al. 2000). According to Faedda et al. (2013), *P. ×pelgrandis* can intensify the root rot problem on lavender caused by *P. nicotianae*. The hybrid pathogen was also reported from *Lavandula* species in the Netherlands (Bonants et al. 2000).

Phytophthora cinnamomi. *Phytophthora cinnamomi* was described in 1922 on a cinnamon tree in Sumatra and has become a cosmopolitan plant pathogen thought to attack approximately 5,000 host plants in at least 266 genera and 90 families (Cline et al. 2008; Erwin and Ribeiro 1996; Hardham and Blackman 2018; Zentmyer 1980). *Phytophthora cinnamomi* was first reported on English lavender (*L. angustifolia*) in Lithuania in 2003 (Orlikowski and

Valjuskaite 2007). Diseases caused by *Botrytis cinerea*, two species of *Fusarium*, and *Sclerotinia sclerotiorum* were also detected on lavender plants although *P. cinnamomi* was the most common pathogen. *Phytophthora cinnamomi* was likely spread on lavender plants by trade and may be a major concern to the lavender industry. *Phytophthora cinnamomi* was subsequently found in the water supplies for lavender nurseries, highlighting the potential danger of contaminated irrigation water. Symptoms included yellowing and then browning of shoots, and disease incidence was reported to be 5% (Orlikowski and Valjuskaite 2007). The pathogen was able to colonize plants at the wide temperature range of 10 to 32.5°C (Orlikowski and Valjuskaite 2007). ‘Blue Dafo’ was the most susceptible cultivar in an inoculation study, in that disease progressed quickly (Orlikowski and Valjuskaite 2007). Additionally, this pathogen was documented by Jung et al. (2016) in the United Kingdom (Farr and Rossman 2021).

Phytophthora citrophthora. *Phytophthora citrophthora* is a cosmopolitan pathogen capable of infecting plants in 88 genera in 51 families (Cline et al. 2008). *Phytophthora citrophthora* as a pathogen on *L. angustifolia* ‘Hidcote’ was detected in Hungary between 2007-2009 and deemed a threat to ornamental production in that country (Józsa et al. 2011). Symptoms included necrotic bark lesions, reduced growth, wilting, and desiccation. Its occurrence was simultaneously documented on other nearby ornamental plants (Józsa et al. 2011). It also was isolated from *L. angustifolia* at a nursery in South Carolina in 2002 (Camacho 2009). This pathogen was documented by Jung et al. (2016) on hybrid lavender in Croatia and *L. stoechas* in the United Kingdom (Farr and Rossman 2021).

Phytophthora tentaculata. *Phytophthora tentaculata* has previously been reported by several authors as occurring on lavender, but this species actually occurred on lavender cotton

(*Santolina chamaecyparissus*), a plant unrelated to lavender (Álvarez et al. 2006). To date, *P. tentaculata* has not been found on *Lavandula* species.

Phytophthora cactorum. *Phytophthora cactorum* is a cosmopolitan pathogen first described in 1886 and capable of infecting plants in 154 genera in 54 families (Cline et al. 2008). This species is known for preferring more temperate climates where it often causes fruit, crown, collar, and root rots on apples and pears (Erwin and Ribeiro 1996). In 2015, between 2,500 and 3,500 *L. angustifolia* plants became symptomatic in the Jiangsu Province, China, and Koch's Postulates were completed (Chen et al. 2017). The pathogen was also documented, by the Ontario Ministry of Agriculture, to occur in lavender plantings in that province (Westerveld 2015).

Phytophthora capsici. *Phytophthora capsici* is a cosmopolitan pathogen described in 1922 and capable of infecting plants in 51 genera in 28 families (Cline et al. 2008). Some of the more important host plants for this species are peppers (*Capsicum* spp.), tomato (*Lycopersicon* spp.), and members of the Cucurbitaceae family (Cline et al. 2008; Granke et al. 2013). There is known to be much host specificity in this species (Erwin and Ribeiro 1996). The only occurrence of this species on lavender is on *L. spica* (synonym *L. angustifolia*) in Korea. (Cho and Shin 2004).

Phytophthora cryptogea. *Phytophthora cryptogea* is a cosmopolitan pathogen described in 1919 and capable of infecting plants in 141 genera in 49 families (Cline et al. 2008).

Phytophthora cryptogea is very similar to *P. drechsleri* both morphologically and genotypically, but these two species usually can be separated by growth at 35°C because *P. drechsleri* can grow at and above this temperature whereas *P. cryptogea* cannot (Chase et al. 2018; Erwin and Ribeiro

1996). A single documentation of this species is listed on hybrid lavender in Croatia by Jung et al. (2016).

Phytophthora drechsleri. *Phytophthora drechsleri* is another cosmopolitan pathogen first described in 1931 and capable of infecting plants in 113 genera in 40 families (Cline et al. 2008). One association with lavender occurred on *L. spica* (synonym *L. angustifolia*) in Korea (Cho and Shin 2004).

Phytophthora megasperma. *Phytophthora megasperma* is a cosmopolitan species described in 1931 and generally infecting members of the Fabaceae and other families (Cline et al. 2008). There is a record by Jung (2016) of this species associated with hybrid lavender in Croatia.

Phytophthora plurivora. *Phytophthora plurivora* was previously considered to be a part of the *P. citricola* complex; however, unique morphological, physiological, and molecular characteristics promoted this pathogen to the level of species (Jung and Burgess 2009). There is one report of this species associated with *L. angustifolia* and one report associated with *L. stoechas*, both in the United Kingdom as reported by Jung et al. (2016).

Unidentified *Phytophthora* species. According to the U.S. National Fungus Collections Database, there have been other reported instances of unidentified *Phytophthora* species found associated with *Lavandula* species (Farr and Rossman 2021). These instances include isolates associated with hybrid lavender and *L. angustifolia* in Croatia, as reported by Jung et al. (2016), as well as isolates associated with *L. angustifolia* in Greece and California (Farr and Rossman 2021).

Information on the Pathogens—Species of *Phytophthora*

Biology of *Phytophthora* species: An overview. *Phytophthora* species are in the Kingdom Chromista (Agrios 2005) and fall within the Stramenopila, which are fungus-like microorganisms (Strange and Scott 2005). Oomycetes, including *Phytophthora* species, are members of the Phylum Oomycota, and are believed to have originated in the ocean (Beakes et al. 2012). The organisms are characterized by no cell walls, and by mycelia containing cellulose and glucans (Agrios 2005). There is a lack of chitin, and very little sterol is found in cell membranes (Strange and Scott 2005). Both chitin and sterols are common in true fungi. Oomycetes typically form mycelia that is non-septate and coenocytic (Webster and Webber 2007). Vegetative structures of oomycetes are predominantly diploid (Brasier 1992; Strange and Scott 2005; Webster and Webber 2007). Sporangia, zoospores, and thick-walled chlamydospores represent asexual spore stages (Agrios 2005). Sporangia, which are produced on sporangiophores, can germinate directly to form germ tubes or indirectly by producing biflagellate zoospores (Agrios 2005; Erwin and Ribeiro 1996; Strange and Scott 2005). Sexual reproduction occurs when an oogonium is fertilized by an antheridium to produce an oospore, another thick-walled spore capable of long-term survival (Erwin and Ribeiro 1996). Oospores can germinate to produce sporangia or hyphae (Webster and Webber 2007).

In the present day, many oomycetes are common as pathogens on plants and animals while some are saprophytes (Lévesque 2011). Overall, the majority of oomycetes are pathogenic to plants (Thines and Kamoun 2010). The oomycetes consist of three orders: Saprolegniales (water molds), Albuginales (white rusts), and Peronosporales (Piepenbring 2015). Peronosporales contains most of the important plant pathogens—including species of *Phytophthora*, *Pythium*, *Phytophthora*, and the causal agents of downy mildews.

The genus *Phytophthora* is in the order Peronosporales (Ivors and Moorman 2014), and the family Peronosporaceae (Thines 2013). Three separate evolutionary events led oomycetes to plant parasitism (Thines and Kamoun 2010). There has been evolution from opportunistic or saprotrophic *Pythium* to hemi-biotrophic *Phytophthora*, to the biotrophic causal agents of downy mildew (Brasier and Hansen 1992; Thines and Kamoun 2010). This suggests that *Phytophthora* species are more recently derived than *Pythium* species (Brasier and Hansen 1992) while the downy mildews are the most advanced or evolved members of the Peronosporales (Scott et al. 2013). Species of *Phytophthora* are primarily pathogenic while species of *Pythium* are predominantly saprotrophic (Webster and Weber 2007). *Phytophthora* species are biotrophic early in the infection process but then kill cells and feed off them as necrotrophs placing the *Phytophthora* species between obligate biotrophs and necrotrophs (Thines 2013). Some species of *Phytophthora* are similar to downy mildew species with aerial dispersal of sporangia that can germinate directly (Brasier and Hansen 1992). Both *Phytophthora* and *Pythium* species can undergo polyploidy (Brasier 1992). The separation or distinction between the species causing downy mildew and *Phytophthora* species may need to be reexamined as some species of grass parasites have unique characteristics of each group (Thines 2009).

Phytophthora species are among the most devastating plant pathogens within the oomycetes, with potato late blight and sudden oak death as just two examples of the major diseases caused by the plant pathogens in this genus of (Solomon et al. 2015). In fact, it was *P. infestans*, the causal agent of potato late blight, which led to the Irish Potato Famine, that gave rise to modern plant pathology (Agiros 2005; Andrivon 1995). Still, 10% of the plant pathology literature is about *P. infestans*, a species which continues to pose threats to agriculture today (Webster and Webber 2007). Late blight of potatoes and tomatoes is only one of the many

significant and potentially devastating *Phytophthora*-caused diseases. In 1876, the first species in the genus, *P. infestans*, was described with additional species described in the years that followed: 11 species by 1917, 21 species by 1931, 41 species by 1963, 50 species by 1976, and, in 1996, there were 54 documented species of *Phytophthora* (Brasier 2009). By December 2007, there were 86 species and six hybrids known, with half of the species found in the United States (Cline et al. 2008). As of 2012, there were 116 species documented (Kroon et al. 2012). Currently, there are 182 species described and several genotypically unique taxa waiting to be fully described (Abad et al. 2019).

Of special note is that 70 species were recorded after the year 2000, while less than 60 were documented before 2000 (Scott et al. 2013). At least 16 of the new species of *Phytophthora* since 2000 have been found associated with ornamental crops, and many other species were recovered and described from natural ecosystems (Perez-Sierra and Jung 2013). There has been an undeniable increase in newly described species reported in recent years (Lévesque 2011), and molecular tools and increased surveys of nurseries and forests may be among the reasons for such a rapid increase in species descriptions since 2000 (Brasier 2009). The importance of *Phytophthora* species as plant pathogens is evidenced by their rise in research presented (Lévesque 2011). However, still, more research is needed to identify undetected or undescribed species of *Phytophthora* before they become problematic (Brasier and Hansen 1992). It is estimated that between 200 and 600 species of *Phytophthora* exist worldwide (Brasier 2009). Of the known species in 2013, 41 were listed as agricultural pathogens, 31 as agricultural and natural areas pathogens, 41 as natural areas pathogens, and the impact of eight species was not known (Scott et al. 2013).

While many new species are being identified, the original native ranges of many species of *Phytophthora* have not been determined although it is believed a number of species originated in Asia. *Phytophthora nicotianae*, one of the first in the genus to be described (Ribeiro 2013), was found on native subtropical vegetation in Nepal during a search for the origin of the genus *Phytophthora* (Vannini et al. 2009). Human colonization of the new world and the back-and-forth shipment of live plants may have caused much of the spread of *Phytophthora* species (Scott et al. 2013). Globalization and human activities of today continue to spread species of *Phytophthora* by frequent and rapid movement of plant material (Scott et al. 2013; Webster and Weber 2007). Currently, species of *Phytophthora* are found on all continents except Antarctica (Scott et al 2013). The United States, Australia, United Kingdom, France, and Germany top the list for number of *Phytophthora* species reported by country, but this is possibly because of the scientific funding in those locations (Scott et al. 2013). *Phytophthora* species have become common today in forest soils of the eastern United States (Balci et al. 2007). Many species of *Phytophthora* are climate specialized and even climate limited (Brasier and Hansen 1992), so temperature may play a role in species distribution and survival, as *P. cinnamomi* is not found north of 40° latitude in North America (Balci et al. 2007).

Sporangia are one of the asexual structures produced by species of *Phytophthora*, usually produced within 3 to 5 days after infection (Ludowici et al. 2013) and are borne on sporangiophores (Erwin and Ribeiro 1996; Webster and Weber 2007). In the genus *Phytophthora*, as in some genera of downy mildew oomycetes, sporangia are capable of both direct, by a germ tube, and indirect, by zoospore release, germination (van West et al. 2003; Webster and Weber 2007). For soilborne species of *Phytophthora* undergoing indirect germination, zoospores will exit sporangia while the entire sporangium is still attached to the

sporangiophore (Webster and Weber 2007). Motile zoospores then navigate through films of water in soil pores to find host plants (van West et al. 2003).

Chlamydospores are another asexual structure that can survive in soil for years (Erwin and Ribeiro 1996; Webster and Weber 2007). They are thick-walled spores, which can be present in plant tissue or soil, and are important for the persistence and survival of some species of *Phytophthora* (Erwin and Ribeiro 1996; Ludowici et al. 2013). In the case of *P. cinnamomi*, chlamydospores are present throughout the year and, in winter, allow the pathogen to survive colder temperatures than it otherwise would, even free in the soil; this was the case when *P. cinnamomi* was found associated with Fraser fir trees in western North Carolina (Kenerley and Bruck 1983).

Approximately 50% of the species of *Phytophthora* are homothallic and form oospores in single-isolate culture (Brasier 1992; Ivors and Moorman 2014). In contrast, heterothallic species require the pairing of two isolates with opposite mating types (A1 and A2) for oospore production (Erwin and Ribeiro 1996; Ivors and Moorman 2014). Therefore, oospore formation can be either homothallic or heterothallic and involves the union of gametangia—an antheridium and an oogonium (Erwin and Ribeiro 1996). Attachment of the antheridium to the oogonium can be paragynous or amphigynous (Erwin and Ribeiro 1996; Webster and Weber 2007). Oospores have thick walls for protection and energy storage, enabling survival for many years—usually in soil (Webster and Weber 2007). Oospore formation also allows for sexual recombination between isolates, allowing for adaptation and evolution (Erwin and Ribeiro 1996). When conditions are suitable for growth, oospores germinate to produce one or more germ tubes and form hyphae or sporangia, depending on the environmental conditions (van West et al. 2003).

Propagules of *Phytophthora* species are easily disseminated, particularly by other organisms or movement of soil and contaminated plants. Some propagules of *Phytophthora* species have been shown to be able to be passed through gnats, flies, and snails while remaining viable (Hyder et al. 2009). Oospores of *P. plurivora* were viable upon excretion from the invasive slug *Arion vulgaris* (Telfor et al. 2015). When *P. cinnamomi* was present in plant material ingested by feral pigs, the pathogen had reduced viability but remained infective and capable of infecting and killing plants even after a duration of 7 days in the digestive tract (Li et al. 2014). The spread of soilborne members of this genus was demonstrated in Hawaii, where *P. cinnamomi* was capable of being spread in soil on boots, vehicle tires, and hoofs of feral pigs (Kliejunas and Ko 1976).

***Phytophthora nicotianae*: Host specialization and genetic diversity.** Isolates of *Phytophthora* species can be classified by morphology, physiology, pathology, and biology (Hall 1993), in addition to genetically. Host specialization by *P. nicotianae* was found to occur more often in intensive cropping systems (Biasi et al. 2016). Differences in pathogenicity do exist within *P. nicotianae*, but a single isolate can be pathogenic to multiple hosts (Apple 1957). Genetically, isolates may vary. One study looked at several isolates of *P. nicotianae* from a small sample size on *Lavandula* species in Italy and demonstrated they were like other isolates from ornamental species in that they had high genetic diversity and were able to be divided into distinct groups (Biasi et al. 2016). Eight of these isolates were of the A1 mating type and 14 were of A2. The genotypes of *P. nicotianae* found on lavender were also found on other ornamental plants (Biasi et al. 2016).

Mammella et al. (2013) conducted an analysis of 96 isolates of *P. nicotianae* from various hosts and locations with four mitochondrial and three nuclear loci. The results suggested

that infected plant material is spreading pathogen isolates. Ornamental plants were likely the means of dissemination worldwide, with isolates nearly identical genetically in both Florida and Germany. Some isolate diversity indicated sexual reproduction. Occurrence in and dissemination from nurseries may contribute to *P. nicotianae* evolution by allowing recombination. Mammella et al. (2013) hypothesized that crops that need specialty cultivation result in asexually produced heterozygous clones thus expanding their host specificity. Field crop isolates had positive inbreeding coefficients while ornamentals had negative inbreeding coefficients likely due to plant transport placing genetically different isolates in close proximity. The methods of mitochondrial and nuclear sequences used can help determine the origin, reproduction, and introductions of populations of *P. nicotianae*.

Molecular detection, identification, and characterization of *Phytophthora* species.

Molecular methodologies can be used for a variety of purposes including identification, detection, and characterization (Milgroom 2015). Oomycetes have larger genome sizes than true fungi (Judelson and Blanco 2005). DNA extraction should occur prior to extensive culturing due to possible changes (Lamour 2013). When using Blast analysis for identification, small sequences could give a false match (Martin 2013). Also, identifying some species, such as *P. drechsleri*, can be a challenge using molecular techniques (Martin 2013). In addition to identification, molecular tools can be used to help detect a pathogen or identify the primary source of inoculum (Kageyama 2015). Polymerase chain reaction (PCR) can only detect medium to high levels of infection while nested PCR is up to 1000 times more sensitive for detection (Grote et al. 2002). When using a qPCR technique, no difference in success was found between symptomatic and asymptomatic plants or plants vs. soil for isolation (Migliorini et al. 2015). However, nested PCR was listed as being just as good as microscopic visual techniques

at detecting *P. nicotianae* (Grote et al. 2002). Gel-based methods, mitochondrial haplotypes and diagnostic markers are all used for detection of *Phytophthora* species in samples (Martin 2013).

Overall, molecular markers and phenotypes together are good for describing populations (Lamour 2013). Mitochondrial DNA characterization is objective, and highly reproducible results are obtained (Mammella et al. 2011). Fatty acid methyl ester analysis and amplified fragment length polymorphisms have been used to characterize a population of *P. cinnamomi* isolates (Duan et al. 2008), and microsatellites were used to characterize populations of *P. nicotianae* (Biasi et al. 2015).

Surveys of nurseries for *Phytophthora* species. Many surveys of nurseries and greenhouses have been conducted to detect and characterize the species of *Phytophthora* present. Two of the more commonly recorded characteristics of isolates include mating type, for those species that are heterothallic, and mefenoxam sensitivity. Determining the mating type is important to know the potential for genetic variability and oospore production (Olson and Benson 2011). Knowing the mefenoxam sensitivity of isolates is important to determine fungicide efficacy and resistance issues (Olson and Benson 2011). Both of these characters have direct implications for management.

The results of nursery surveys show just what a problem *Phytophthora* species are within the ornamental plant trade worldwide. A survey of greenhouses in the Netherlands found 21 species of *Phytophthora* present (Man in't Veld et al. 1998). In Georgia, 17 wholesale nurseries were sampled and found to have *P. nicotianae* (constituting 30% of all isolates detected), *P. pini*, *P. undulata*, *P. cinnamomi*, *P. citrophthora*, *P. palmivora*, *P. drechsleri*, and *P. cryptogea* (Williams-Woodward and Demott 2014). In Tennessee nurseries, a survey of ericaceous plants found *P. citrophthora* was most abundant at 38% (26 isolates) while only seven isolates of *P.*

nicotianae and one isolate of *P. palmivora* were recovered. The isolates of *P. citrophthora* detected were very diverse (Donahoo and Lamour 2008). A 2001 to 2002 survey of 41 symptomatic floriculture plant species from 29 sites in North Carolina yielded 483 isolates from eight crops and seven locations with *P. nicotianae* most prevalent (Hwang and Benson 2005). A 2007 to 2008 survey in that same state collected 1,228 samples from 39 plant species at 25 wholesale sites for a total of 163 isolates; again *P. nicotianae* was most abundant (59% of isolates) while *P. drechsleri* constituted 23% of isolates (Olson and Benson 2011). In Florida, ornamental plants submitted to the plant problems clinics in Apopka and Homestead found *P. nicotianae* (73%) and *P. palmivora* (19%) as the two most recovered species of *Phytophthora* (Patel et al. 2016). In a study of southeastern horticultural operations, of the 488 isolates recovered from plants, the only species recovered from all six states sampled was *P. nicotianae*, which represented 27% of isolates (Olson et al. 2013). Other abundant species included *P. cinnamomi* (23%) from four states, *P. palmivora* (11%) from three states, and *P. drechsleri* (3%) from three states (Olson et al. 2013).

In the above cited surveys, there were many isolates recovered that were resistant to mefenoxam—one of the most frequently used fungicides on ornamental crops. In Georgia, all isolates of *P. nicotianae* were sensitive to mefenoxam, but three of the sites had mefenoxam insensitive *P. palmivora* populations (Williams-Woodward and DeMott 2014). In the 2001 to 2002 study in North Carolina, half of all 483 isolates of *P. cryptogea*, *P. nicotianae*, and *P. palmivora* were mefenoxam sensitive at 1 µg/ml, with the remainder insensitive or intermediate (Hwang and Benson 2005). In the 2007 to 2008 survey in North Carolina, 66% of isolates, including 68% of *P. nicotianae* isolates, were found to be resistant to mefenoxam at 1 µg a.i./ml. When tested at 100 µg a.i./ml, 58% of *P. nicotianae* isolates were resistant and another 10%

were intermediate in resistant (Olson and Benson 2011). In Florida, some isolates of *Phytophthora* species were insensitive to mefenoxam including three isolates of *P. nicotianae* (Patel et al. 2016).

In the North Carolina survey, all *P. nicotianae* and *P. palmivora* isolates from a given site were of the same mating type (Hwang and Benson 2005). The later study within that state, however, found both mating types of *P. nicotianae* (80% A1 and 20% A2), while only finding one mating type (A1) of *P. drechsleri* (Olson and Benson 2011). Multiple nurseries had both mating types of *P. nicotianae*, but the mating types were not in close proximity to each other, thus preventing mating and sexual recombination. When combined in the lab, isolates on opposite mating type produced oospores (Olson and Benson 2011). In Florida, most of the heterothallic species recovered within the state had isolates of both A1 and A2 mating types represented (Patel et al. 2016).

Even though it is clear from the surveys of ornamental plants that two of the most commonly occurring pathogens are *P. palmivora* and *P. nicotianae*, there is no evidence of them hybridizing. This is most likely due to their divergence into separate genotypic clades (Patel et al. 2016). Also, the incidence of *Phytophthora* diseases in North Carolina floriculture is increasing, with an increase of mefenoxam resistant isolates of *P. nicotianae* (Olson and Benson 2011). Both *P. nicotianae* and *P. palmivora* showed much genetic variation (Patel et al. 2016). The great diversity of these species suggests that isolates were introduced into trade multiple times (Olson and Benson 2011; Patel et al. 2016).

The environment and disease development. The environment has a major impact on pathogen growth and survival and, therefore, on disease development. Temperatures are important for infection, spore production, and pathogen survival. Colonization of tobacco plant

material in tissue culture by *P. nicotianae* was impacted by temperature, inoculum concentration, and morphology of tissue cultures (Helgeson et al. 1972), and temperature specifically correlated with incidence of black shank (Jacobi et al. 1983). Tissues would be colonized at 15 to 32°C with increases in rate of colonization as temperature increased (Helgeson et al. 1972). There are also temperature ranges—which include minimum, optimum, and maximum temperatures—for survival of oospores and chlamydospores, sporangium production, zoospore production and release, and oospore production (Brasier 1969; Drenth et al. 1995; Erwin and Ribeiro 1996; Kaosiri et al. 1980; McIntosh 1972; Zentmyer et al. 1979).

Temperatures are known to play a role in survival with upper and lower limits reported for many species, and this influences the natural survival range of these pathogens. For example, *P. cinnamomi* typically is not found north of 40° North latitude in the U.S. because of limitations from colder temperatures (Balci et al. 2007). *Phytophthora nicotianae* prefers warmer temperatures and tends to be more common in warmer regions (Erwin and Ribeiro 1996; Hall 1993); therefore, it is expected to be favored by climate change (Kamoun et al. 2015). However, winter temperatures in Kentucky had no adverse effect on *P. nicotianae* in the soil (Flowers and Hendrix 1974). Its ability to maintain a heavy presence in the upper soil throughout winter in central Kentucky demonstrated its potential to overwinter—which depends on the magnitude and duration of cold temperatures (Flowers and Hendrix 1972). The temperature range for *P. nicotianae* growth is from 10 to 35°C, with an optimal growth temperature of 27.5°C (Erwin and Ribeiro 1996). More work needs to be conducted to determine the temperature tolerances for *P. nicotianae* in natural environments and where this easily transportable pathogen can survive winters.

Soil moisture is another important environmental factor affecting growth and survival. First, the amount of available soil moisture can have an impact on the host plant. Both drought and flooding are stresses that can predispose host plants to root rot (Blaker and MacDonald 1981). Increased soil moisture can lead to increased mortality of hosts (Ferrin and Mitchell 1986). Even just the rapid changing of soil moisture levels could impart stress, with susceptible cultivars more likely to experience mortality during many wet and dry cycles (Ferrin and Mitchell 1986). Saturated conditions caused root cracks in alfalfa, which led to amino acids and sugars leaching out and serving as chemical attractants to zoospores of *P. megasperma* (Kuan and Erwin 1980). Similarly for tobacco hosts, drought periods may decrease a cultivar's resistance by adding stress (Ferrin and Mitchell 1986).

In addition to the impact on the host, soil moisture is known to impact *Phytophthora* species and disease development. For *P. nicotianae* on tobacco, a single 30-min saturation period was all that was needed for zoospore release (Shew 1983). In general, soil matric potential significantly affected disease on tobacco seedlings caused by *P. nicotianae* (Shew 1983), and disease caused by *P. cinnamomi* was less severe in dryer soils (Sterne et al. 1977). Soil water content is also important for oospore survival, sporangium production, and zoospore release. For *P. nicotianae*, sporangium production and zoospore release on rhododendron was optimal under flooded conditions (Kuske and Benson 1983). Further, rainfall could aid in dispersal of sporangia through splashing (Granke et al. 2009).

While moist to saturated conditions are conducive to disease, it is possible for soils to be too wet for survival of propagules of some *Phytophthora* species. *P. cinnamomi* had minimal survival in soil under submerged conditions for 12 months (Hwang and Ko 1978). Soil moisture extremes were detrimental to survival of mycelium of *P. palmivora* whereas this species could

survive more than two years under appropriate conditions (Turner 1965). The same goes for survival in dry soils. In drier soils with matric potentials of -0.25 bar, mycelium of *P. cinnamomi* was not as capable of causing disease, and chlamydospore germination and germ tube development was also hindered in such situations (Sterne et al. 1977). Environmental factors such as pH, temperature, oxygen levels, and microbial interactions can impact survival ability of *Phytophthora* species in water (Ivors and Moorman 2014).

Disease management principles for phytophthora root and crown rot. Management of plant diseases, including those caused by *Phytophthora* species, can be summed up by a four-part plan of exclusion of the pathogen, avoidance of conducive environmental conditions, eradication of the pathogen, and protection of the plant (Agrios 2005; Jarvis 1992; and Ludowici et al. 2013). These principles are grounded in the knowledge that plant disease development is always based on three factors—a susceptible host, a virulent pathogen, and a conducive environment (Agrios 2005; Erwin and Ribeiro 1996), commonly referred to as the disease triangle. The above mentioned four management principles are attempts to prevent the three disease development factors from coming together simultaneously.

The first management principle is exclusion of the pathogen. Exclusion, as applied to ornamental plantings of lavender can be achieved predominantly by quarantines, inspections, and testing to achieve only pathogen-free propagating material for the site (Agrios 2005; Jarvis 1992; Ludowici et al. 2013). For *P. nicotianae*, this can be difficult to achieve, as its soilborne nature and latency periods make it difficult to detect and contain (Ludowici et al. 2013).

As pathogen detection in nursery stock can be difficult, the principle of avoidance should always be practiced. This relies on avoiding conditions favorable and conducive to disease development; so, for *Phytophthora* root and crown rot on lavender caused by *P. nicotianae*, this

primarily involves avoiding warmer climates and excess soil moisture (Agrios 2005; Ludowici et al. 2013). For *Phytophthora* species, the most critical environmental condition to avoid is excess soil moisture, but other factors include hardpan soil layers, drought stress, excess fertilization, soil salinity, increased soil pH, and foliar wetness (Erwin and Ribeiro 1996). The principle of avoidance is clearly more easily achieved in controlled environment settings, such as greenhouses and nurseries, where conditions are more easily manipulated (Jarvis 1992).

After pathogens are introduced and established into a cultivation setting, eradication of the pathogen is the ultimate goal, if at all possible. Eradication is the elimination of pathogen inoculum from all plants, soils, and supplies (Jarvis 1992; Ludowici et al. 2013). Sanitation practices are an important part of this step (Ludowici et al. 2013). Other methods include pasteurization, sterilization, solarization, and fumigation of soils, as well as chemical soil drenches and addition of suppressive soils (Agrios 2005; Jarvis 1992).

The fourth principle, and often the first applied by growers, is the protection of existing plant material. The most common action for management of *Phytophthora* species is that of applying conventional chemical pesticides (Agrios 2005; Erwin and Ribeiro 1996; Jarvis 1992). Biological control products also have potential efficacy (Erwin and Ribeiro 1996; Jarvis 1992; Ludowici et al. 2013).

For chemical inputs, many different compounds of various sources and toxicities have been used. Copper algicides have been shown to be lethal to zoospores, sporangia, and chlamydospores of *Phytophthora* species (Meadows et al. 2011). Chemical activators of disease resistance have given some protection against certain *Phytophthora* species (Matheron and Porchas 2002). However, by far, chemical fungicides have been the most popular choice for management of root and crown rots caused by *Phytophthora* species.

Not all chemical fungicides used on oomycetes kill the organism and, therefore, some are considered fungistatic and not fungicidal (Linderman and Davis 2008; Olson et al. 2013). The effectiveness of fungicides in some host-pathogen relationships does not equally translate to all (Linderman and Davis 2008) and being aware of fungicide resistance issues is important for effective disease management (Olson and Benson 2011; Olson et al. 2013). It is also necessary to determine the best method of application, should the label allow more than one.

There are a large number of products on the market from various classes of fungicides labeled for diseases caused by *Phytophthora* species. One of the most popular compounds for management of oomycetes, including species of *Phytophthora*, is the chemical mefenoxam, known by the common trade names such as Subdue Maxx and Ridomil Gold (Agrios 2005; Herman et al. 2019). The precursor to mefenoxam was metalaxyl, which is a mixture of chemical isomers. Subdue Maxx was effective at suppressing disease by *P. ramorum*, *P. citricola*, and *P. nicotianae* but not *P. citrophthora* on detached leaves of rhododendron and lilac (Linderman and Davis 2008). While the products are useful in trying to prevent disease, sometimes they only delay the onset of disease. Subdue Maxx, for instance, was able to delay mortality of Fraser fir trees by *P. cinnamomi* in North Carolina by up to three seasons (Benson et al. 2006).

Mefenoxam products are capable of working relatively quickly. A soil drench of Ridomil provided protection of seven-leaf tomato plants in 1-liter pots within an hour of allowing translocation from roots to leaves at 25°C (Cohen et al. 1979). Ridomil was still protective of tomato plants if applied within 2 days of inoculation (Cohen et al. 1979). On tobacco, treating plants with mefenoxam 24 h after inoculation in vitro was less effective (Staub and Young 1980). When treated 48 h after inoculation, the roots became symptomatic but disease was

stalled (Staub and Young 1980). Mefenoxam products are extremely long lasting as well. In one study, the effectiveness of Subdue Maxx lasted approximately 6 weeks, but it still inhibited infection by an isolate of *P. ramorum* at 8 weeks after application (Linderman and Davis 2008).

Recently, another new compound has become popular and shown great promise at managing species of *Phytophthora*. Oxathiapiprolin was very effective against *P. nicotianae* on tobacco (Ji et al. 2014). Sporangium production was the most sensitive stage of the *P. nicotianae* lifecycle to oxathiapiprolin (Bittner and Mila 2016). Isolates of *Phytophthora* species from ornamental plants insensitive to mefenoxam were sensitive to oxathiapiprolin, suggesting a different mode of action and no cross-resistance (Bittner and Mila 2016). Care must be exercised, however, due to the possibility of resistance developing to oxathiapiprolin itself because, in one study, a single isolate of *P. nicotianae* from tobacco showed some insensitivity to oxathiapiprolin in vitro, which the authors believed to be a variant capable of growth at a higher concentration (Bittner and Mila 2016).

The phosphonate/phosphite fungicides. A class of fungicides, which has been studied extensively, is the phosphonates (also known as phosphites). Phosphonates have long held popularity as both fungicides and fertilizers. Before discussing them, it is important to describe the various forms of these phosphorous containing compounds and how they are produced. First, phosphorous acid, a solid substance, is dissolved in water to form phosphonic acid, a strong acid. To reduce the acidity, the acid can be mixed with a salt, such as potassium hydroxide, or ethanol, forming salts of phosphorous acid (known as phosphite) and ethyl phosphonate, respectively. Collectively they are often referred to as phosphonate compounds (Landschoot 2016). These compounds have been successful in managing oomycetes in some pathosystems. There are also

some concerns regarding the use of phosphonates—including phytotoxicity, microflora changes, resistance, and consumption by humans (McDonald et al. 2001).

While phosphonates are often labeled as fertilizers, this use has been controversial. Phosphonates were tested as early as the 1930s but were found not to be suitable as fertilizers, and only delayed enhanced growth was observed (Guest and Grant 1991; Landschoot 2016). However, after a number of years, phosphonate products came to the market labeled as fertilizers. Research clearly states that phosphonates are not a suitable source of phosphorous for plants (McDonald et al. 2001; Ratjen and Gerendás 2009). Any increases in leaf tissue nutrient content are due to the increased concentration in what are now smaller tissues because of less growth (Ratjen and Gerendás 2009). Phosphates, the phosphorous source able to be utilized by plants, have different chemical structure that allow for binding; therefore, phosphonates cannot be utilized for necessary processes such as production of ATP (McDonald et al. 2001). Phosphonates are well known to accumulate in plant tissue and are unable to be metabolized by the plant (Guest and Grant 1991; McDonald et al. 2001). This has been noted for a long time due to inferences made regarding the persistence of these compounds in plant tissues—e.g., up to 8 weeks in avocado and 7 months in pineapple (Ouimette and Coffey 1989; Rohrbach and Schenck 1985; Smillie et al. 1989).

In addition to a lack of nutritional benefit, there are also concerns that phosphonates may have phytotoxic effects on plants. At low phosphorous concentration, phosphonates can be phytotoxic resulting in decreased growth and necrosis across a wide variety of plant species (Carswell et al. 1996; McDonald et al. 2001; Ratjen and Gerendás 2009). However, it is unlikely that phosphonates will cause problems unless phosphorous is limited to begin with (Carswell et al. 1996). Growth of *Brassica nigra* seedlings was significantly lower when fertilized with 1.5

mM phosphonate in phosphorous limited seedlings whereas 10 mM was required to suppress growth in non-phosphorous limited seedlings (Carswell et al. 1996).

The one particularly notable and well documented use of phosphonates in agriculture is use as a fungicide, particularly against a wide range of oomycete plant pathogens. Applications of both foliar sprays and soil drenches were found to yield similar control of root rot caused by *P. cinnamomi* (Fenn and Coffey 1984). The downward translocation of the chemicals within plant tissue could protect roots within 24 h of foliar treatments (Rohrbach and Schenck 1985). It has been noted that phosphonates do not have the same efficacy in all pathosystems (Guest and Grant 1991). They are particularly effective where there is some level of cultivar resistance (Guest and Grant 1991).

The fate of phosphonate products depends on where these products end up—in plants, soil, or the environment. Phosphonates are truly systemic because they can be transported in both the xylem and phloem (Guest and Grant 1991; Ouimette and Coffey 1989; Ouimette and Coffey 1990). This allows rapid downward translocation from shoots to roots in less than 24 h after foliar applications (Rohrbach and Schenck 1985). Once in plant tissues, the phosphonates last a long time, even up to 7 months (Ouimette and Coffey 1989; Rohrbach and Schenck 1985). In contrast, the chemicals did not persist for more than 4 to 6 weeks in the soil around potted *Persea americana* plants (Ouimette and Coffey 1989). Bacteria are known to oxidize both phosphonates and phosphates, which is the source that they need for metabolism for energy (McDonald et al. 2001). However, when treating *P. cinnamomi* on avocado and pepper corn seedlings, soil application of phosphonate at recommended rates did not impact the numbers of bacteria and fungi in the rhizosphere (Wongwathanarat and Sivasithamparam 1991). In fact, one of the benefits of phosphonates is that there is no direct negative impact on true fungi (Fenn and

Coffey 1987) or other organisms in soil antagonistic to *P. cinnamomi* (Wongwathanarat and Sivasithamparam 1991). The long-term impact on soil microflora is unknown because only a few organisms directly utilize phosphonates, which could lead to potential shifts in soil microflora over prolonged use of the chemicals at a given site (McDonald et al. 2001).

There are speculated to be a number of physiological impacts on plants from phosphonate treatments. Regarding the potential beneficial physiological responses, it has been hotly debated whether physiological responses occur in plants to stimulate host defenses (Guest and Grant 1991) with some studies making this claim (Rouhier et al. 1993). One study found no direct fungitoxicity in vitro to isolates of 25 *Pythium* species while the same isolates were controlled in vivo, suggesting that host effects were the reason for control (Sanders et al. 1983). The exact mechanisms, if any, are still currently unknown. Foliar applications of potassium phosphite also appear to increase fruit per tree in citrus production. The author did not speculate how that effect occurred (Lovatt 1999). Phosphonates have been reported to increase turf quality, but without explanation (Landschoot 2016).

On the opposite end of the spectrum, some negative physiological impacts have been suggested, specifically including a decrease in root growth. Both Aliette (a phosphonate-type product) and Ridomil were found to have negatively impacted root and shoot growth in onion (Sukarno et al. 1993). In *Eucalyptus marginata*, phosphite caused decreased root growth for five days, after which time the rate increased but remained below that of non-phosphonate treated plants (Jackson et al. 2000). When *Brassica nigra* was phosphate limited, adding phosphonate at a concentration of 10 mM caused 80% reductions in fresh weight of roots as opposed to the non-phosphonate-added plants (Carswell et al. 1996)

In general, the phosphonates work by: 1) acting as fungistats and slowing fungus growth and prohibiting sporulation; which also allows 2) host defenses time to function efficiently and overcome the pathogen; and 3) altering pathogen metabolism, which makes host defenses more effective (Guest and Grant 1991).

Phosphonate fungicides work against oomycetes by multiple mode of action sites (Guest and Grant 1991). The first case is the phosphonate interactions with phosphorous metabolism in *Phytophthora* species causing accumulations of polyphosphate and pyrophosphate, something metalaxyl did not do (Guest and Grant 1991; McDonald et al. 2001; Niere et al. 1994; Niere et al. 1990). As such, the authors propose this interaction with phosphorous metabolism as the primary site of phosphonate inhibition (Niere et al. 1994). In the second site, phosphonate ions compete with phosphate for transporters potentially blocking phosphate transport (McDonald et al. 2001). Phosphite uptake by *Phytophthora* species was reported to be lower when phosphate was abundant (Fenn and Coffey 1984; Smillie et al. 1989). In the third case, phosphonates inhibit enzymes in both glycolytic and oxidative pentose-phosphate pathways (McDonald et al. 2001), likely by competing with phosphate for binding sites (Stehmann and Grant 2000).

A potential fourth means by which phosphonates act against species of *Phytophthora* is in causing plants to create a more antimicrobial environment (Guest and Grant 1991; McDonald et al. 2001; Smillie et al. 1989). It was suggested that at low concentrations, the role of phosphonates is mainly in interacting with the pathogen in a way that stimulates host defenses. At high concentrations, the action is on direct inhibition of the pathogen prior to host-pathogen interaction (Jackson et al. 2000).

Fungicide resistance. Fungicide resistance can pose a serious problem for management of some diseases, and this applies to fungicides active against the oomycetes. Fungicide

resistance refers to naturally occurring individuals in the pathogen population that develop resistance to a specific fungicide active ingredient (a.i.) due to selection pressure from overuse of the a.i., and this causes a reduction in disease management (Gisi et al. 2000). The initial problems with fungicide resistance were all specific to active ingredients with single-site modes of action—such as benzimidazoles, dicarboximides, and phenylamides (Russell 1995). To combat this, fungicides that were high risk would be added into a fungicide rotation cycle or tank mixed with other fungicides. Mixing fungicides when one is “at risk” or even if both are “at risk” should reduce selection pressure for resistance development (van der Bosch 2014). Nine classes of fungicides have resistance issues for ornamental crops in the United States, and the presence is often unreported in publications for ornamental crops (Garzon et al. 2019). However, 11 of the 23 classes of fungicides registered for ornamental plants have resistance in other agricultural systems, but not in ornamental plant systems (Garzon et al. 2019).

Phenylamides are long lasting, preventative, and highly mobile fungicides that rank at the top of sales for oomycete disease management; however, the importance is declining due to resistance issues (Herman et al. 2019). They are used as seed treatments (46% of use), soil treatments (38% of use), and foliar treatments (16% of use) (Herman et al. 2019). Foliar applications to manage airborne oomycetes are especially linked to resistance issues (Herman et al. 2019).

Metalaxyl, the precursor to mefenoxam, affects nucleoside incorporation into nucleic acids, thus impacting DNA and RNA synthesis, particularly ribosomal RNA (rRNA) synthesis in oomycetes (Fisher and Hayes 1984; Herman et al. 2019). Mefenoxam did not affect zoospore release (Staub and Young 1980). It has been speculated that the impact on mycelium growth and sporangium production, but not on zoospore release or germination, is due to the many

ribosomes present (Herman et al. 2019). For *P. nicotianae*, chlamydospore germination was reduced by 53%, but zoospore germination was not. The effects were reversible, however. In one study, after treatment with metalaxyl, washing mycelia with water could remove 80% of metalaxyl and decreased the fungicidal effect (Fisher and Hayes 1984).

For chemical management of *P. nicotianae*, drenches with metalaxyl prevented the buildup of pathogens in Madagascar periwinkle (Ferrin and Rohde 1992); however, metalaxyl insensitive isolates of *P. nicotianae* have been found on this host in southern California (Ferrin and Kabashima 1991). Metalaxyl resistance is also present in ornamental plant populations of *P. nicotianae* present in South Carolina (Duan and Jeffers 2001). Between 1995 and 2000, 59 isolates were recovered in which 24 were A1 and 35 were A2; 50 isolates were sensitive at 1 mg/L, but nine were insensitive up to 500 or 1000 mg/L. All the insensitive isolates were of the A2 mating type (Duan and Jeffers 2001). Drenches with fosetyl Al, a phosphonate fungicide, had some impact on *P. nicotianae* populations on Madagascar periwinkle (Ferrin and Rhode 1992).

Metalaxyl and the other phenylamides provide one of the most well-known instances of fungicide resistance. Resistance first appeared 2 to 3 years after the introduction of metalaxyl (Herman et al. 2019). In regard to the closely related fungicide mefenoxam, studies have shown increases in fungicide resistance among oomycetes in recent years, particularly with *P. nicotianae* on ornamental plants (Herman et al. 2019). When metalaxyl was applied, it was taken up at the same rate by both sensitive and insensitive isolates of *P. infestans* (Fisher and Hayes 1984).

In the field, insensitive isolates to a specific fungicide are selected by pressure from growers treating their plants with fungicides in the same chemical class. Continual soil drenches

of mefenoxam imposed a selection pressure that encouraged reproduction of insensitive isolates in tobacco fields in North Carolina (Shew 1985). For *P. capsici* in South Carolina cucurbit fields, prior mefenoxam applications were a good predictor of sensitivity level of isolates from that field (Keinath 2007). In a survey of the North Carolina floriculture industry, locations with mefenoxam resistance reported prior use of mefenoxam (Olson and Benson 2011). A survey of *P. nicotianae* isolates in Florida citrus nurseries found a lack of metalaxyl resistant isolates in fields incorporating methyl bromide fumigation as part of their program (Timmer et al. 1998). This is likely due to the elimination of most propagules after each season. Most importantly is that once these isolates have been selected, they are dispersed through movement of infected or infested plant material. It was shown that extensive metalaxyl use in citrus nurseries led to dissemination of metalaxyl-resistant *P. nicotianae* isolates on trees and into groves, infecting both new and established plantings (Timmer 1998).

No reduction in fitness or pathogenicity were reported for multiple species of *Phytophthora* that became resistant to fungicides (Bruin and Edington 1981; Lucas et al. 1990). For *P. nicotianae*, isolates remained strongly competitive and, regardless of initial proportions of mefenoxam resistant isolates, could outcompete sensitive isolates within six life cycles on lupin plants (Hu et al. 2008; Kadish and Cohen 1988; Timmer et al. 1998). Isolates of *P. erythroseptica* resistant to mefenoxam had no fitness differences compared to isolates sensitive to mefenoxam, either in the absence of selective pressure or under a phosphorous acid fungicide treatment program (Chapara et al. 2011). Proportions of phenylamide resistant isolates have even been shown to sometimes increase once present, even after no phenylamide applications (Herman et al. 2019). There were no differences in growth and fitness between sensitive and insensitive isolates in the absence of metalaxyl (Fisher and Hayes 1984), so the problems with

resistant isolates may continue to persist once resistance develops. In fact, many Florida citrus groves still had high populations of metalaxyl resistant isolates after 2.5 years without metalaxyl or mefenoxam use (Timmer et al. 1998).

With the number of pathogens transported on ornamental plants, there has been much research to examine the populations of *Phytophthora* species on these plants. It has been said insensitivity to mefenoxam in ornamental horticulture is not a major cause for concern because most isolates are sensitive to mefenoxam 30 years later (Olson et al. 2013). In a survey of ornamental horticulture operations in the southeastern U.S., 7% of all isolates of *Phytophthora* species from multiple sources and 6% of all isolates from ornamental plants were insensitive while 4% of isolates were of intermediate sensitivity (Olson et al. 2013). Out of the 27 species found in southeastern horticultural operations, *P. nicotianae* was the most abundant, and constituted 78% of all insensitive isolates found (Olson et al. 2013). Of the insensitive *P. nicotianae* isolates, 67% of them were from herbaceous annual plants (Olson et al. 2013). The authors further hypothesized that mefenoxam insensitive isolates on perennials may have come from nearby annuals in those nurseries (Olson et al. 2013).

In another study, 66% of *Phytophthora* species isolates and 68% of *P. nicotianae* isolates were resistant to mefenoxam at 1 μg a.i./ml (Olson and Benson 2011) while 58% and 10% of *P. nicotianae* isolates were resistant and intermediately sensitive at 100 μg a.i./ml (Olson and Benson 2011). This is similar to results from other studies that found resistant isolates grow well at 100 $\mu\text{g}/\text{ml}$ while 1 $\mu\text{g}/\text{ml}$ is inhibitory to sensitive isolates of *P. nicotianae* in Florida citrus nurseries (Timmer et al. 1998). To prevent resistance, applications of different classes of chemicals could be applied together. Once resistance occurs, there is the possibility of cross resistance developing. Fosetyl Al is usually effective at managing metalaxyl resistant isolates of

P. nicotianae within Florida citrus groves (Timmer et al. 1998). However, there does exist a potential for selection of oomycetes for resistance against phosphonate fungicides. Less sensitive mutants have been produced in the laboratory in several instances (Guest and Grant 1991; Sanders et al. 1990). *Bremia lactucae*, the lettuce downy mildew pathogen, isolates in California were found insensitive to fosetyl-Al after prolonged use of that chemical in the field (Brown et al. 2004). Isolates of *P. cinnamomi* from avocado orchards with extensive phosphite use were more likely to colonize phosphite treated tissues in a lab study suggesting decreased sensitivity (Dobrowolski et al. 2008). In France, an isolate of *P. cinnamomi* was found to have decreased sensitivity to fosetyl-Al (Fenn and Coffey 1987). Much more recently, a study found that almost 90% of sampled *P. nicotianae* isolates causing citrus brown rot in California had developed resistance to potassium phosphite (Hao et al. 2021).

Compared to the well-known chemical mefenoxam, there is less of a risk of reduced sensitivity with phosphonate compounds. All phenylamide fungicides are thought to act by the same mode of action, RNA synthesis inhibition (Morton and Urech 1988). The first reports of resistance to metalaxyl occurred just two years after its release on the commercial market. These problems were prevented, however, in regions or on crops which used a prepack mixture of multiple chemicals with different modes of action (Morton and Urech 1988). The phosphonates, with multiple modes of action, have less chance of selecting for resistance since there would need to be multiple mutations for a species of *Phytophthora* to survive the application (Landschoot 2016). Unfortunately, the isolates with resistance to phenylamides and/or phosphonates do not appear impaired compared to wild type isolates (Lucas et al. 1990).

Soil infestation and remediation. One of the problems of planting infected or infested plants is contamination of the soil at the field site. This can be exacerbated by dissemination

through and persistence in the planting field. However, a random occurrence of *P. nicotianae* in fields suggests a lack of spread down rows in tobacco production (Campbell et al. 1984).

Typically, disease in fields is most severe near the source of primary inoculum (Neher and Duniway 1992). Still, even small resident soil populations can become large upon re-introduction of susceptible host plants. In one instance, a soil population of *P. nicotianae* of 0.75 propagules per gram (ppg) of soil was present when tobacco was planted, and it increased to 250 ppg during the growing season; however, the population decreased again once the plants died (Kannwischer and Mitchell 1978).

In terms of spread of *Phytophthora* species in soil, both laterally and vertically, there are conflicting data. In relation to depth, the pathogen concentration declined within 7.5 cm of the soil surface and was not found more than 15 cm below the soil surface (Flowers and Hendrix 1972). Very few propagules of *P. nicotianae* contaminated soil 15 to 45 cm away from tobacco plants that were heavily infected (Flowers and Hendrix 1972). On the contrary, fruit infections of tomato, pepper, and squash were higher downstream, which suggested that *P. nicotianae* and *P. capsici* can pool after flowing in surface water (Café Filho and Duniway 1995). *Phytophthora nicotianae*, along with *P. capsici*, were found capable of spreading 70 m from a source in irrigation water furrows when watered on 14-day cycles (Café Filho and Duniway 1995). In a separate study of tomato fields, *P. nicotianae* was able to spread 68 m downstream, and the disease was reported 50 m downstream; the pathogen population, in this instance, actually increased with downstream distance suggesting an accumulation of zoospores (Neher and Duniway 1992). Although there is the case for movement over distance in fields, in the work by Neher and Duniway (1992), propagules that spread downstream yielded relatively minimal disease.

Once soils are infested with propagules of species of *Phytophthora*, different approaches may be used to remediate a field site. One of these approaches is by applying surfactants, a group of chemicals known to affect the membranes of microorganisms (Hultberg and Alsanius 2014). Regarding species of *Phytophthora*, surfactants are particularly effective on zoospores, which lack cell walls and only have a protective membrane that is easily ruptured (Hultberg and Alsanius 2014; Stanghellini 1997). One such group of surfactants is a cationic class known as the quaternary ammonia compounds (Hultberg and Alsanius 2014), which are commonly used for sanitation in the nursery and greenhouse industries (e.g., KleenGrow, Green-Shield II, and SA-20).

Another method of remediating contaminated soil is solarization, which effectively raises the soil temperature. Solarization uses the sun to heat soil covered with plastic to high temperatures over extended periods to kill soilborne organisms (Agrios 2005). For example, solarization raised the temperature in the upper 5 cm of soil to 52°C while soil in the control treatment was merely 37°C (Agrios 2005). For *P. nicotianae*, soil solarization leading to soil temperatures of 44°C for a 5-h duration yielded a decrease to less than one propagule of *P. nicotianae* per gram of soil (Coelho et al. 2001). Even at that low level, infection would still eventually occur. The *P. nicotianae* population decreased to the point of no infection of seedlings after 15 days of 47°C for 3 h (Coelho et al. 2001). It is thought that soil moisture levels, in addition to temperature, plays a role in sterilizing soil. Saturated soils and higher temperatures made solarization more effective (Coelho et al. 2001). The solarization process has been used effectively to kill residual populations of *Phytophthora* species in empty nursery pots (Suslow and Kosta 2016). In the field, there is potential to combine solarization with other

management methods that have shown some efficacy, such as organic amendments or biofumigation.

Project Proposal

The purpose of this study is to examine the disease *Phytophthora* root and crown rot of lavender, which was identified as an emerging problem in 2015. The etiology of the disease will be examined by sampling lavender plants and associated soils from nurseries and farms across the United States and maintaining active cultures of recovered isolates in the lab; these isolates will be identified and further characterized to understand the breadth of the pathogen population associated with lavender. To further assess and quantify the impact of this problem on the lavender industry in the United States, a survey to growers will be distributed. This survey data, in ArcGIS, can then be mapped along with the occurrence of the different species of *Phytophthora* to help determine the geographic distribution of the problem.

Secondly, for any new host-pathogen interactions or first reports of a species of *Phytophthora* on a species of lavender in the United States, Koch's postulates will be used to confirm pathogenicity on the species of lavender from which the pathogen species was originally isolated. To provide growers potential management strategies, fungicides will be evaluated on lavender plants in greenhouse trials, using products currently labeled for use on greenhouse and/or field grown herbs. To assist growers who already planted infested or infected plants in their fields and have contaminated soil with *Phytophthora* species, a soil remediation experiment will be conducted using a quaternary ammonia product. Efficacy of this product against *P. nicotianae*, which has been widely distributed on lavender plants, will be tested in both controlled greenhouse trials as well as at a current lavender farm in Greenville County, South

Carolina. The results from this study will contribute to the knowledge of an emerging disease problem and will be directly reported to growers through webinars, meetings, and publications.

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CHAPTER 2
DISTRIBUTION AND CHARACTERIZATION OF *PHYTOPHTHORA* SPECIES
ON LAVENDER IN THE UNITED STATES AND AN OVERVIEW
OF THE U.S. LAVENDER INDUSTRY

Abstract

What is now known as *Phytophthora* root and crown rot on lavender (PRCR) was first reported in a Maryland nursery in 1991 on English lavender and was caused by *P. nicotianae*. Since that time, the pathogen has been reported in numerous lavender-producing regions of the world and on different species of lavender. In addition, other species of *Phytophthora*, including *P. palmivora* and *P. cinnamomi*, have been found to cause PRCR. The objective of this study was to sample symptomatic lavender plants from across the United States to identify the species of *Phytophthora* causing PRCR in the U.S. Pathogen recovery was accomplished using direct isolation from root and root crown tissue on PARPH-V8 selective medium whenever possible and by using a standard baiting bioassay of field soil, container mix, and root wash debris. All isolates initially were observed for morphological features and colony patterns on PARPH-V8 because *P. nicotianae* isolates could be recognized on this medium and segregated. Thirty representative isolates of *P. nicotianae* and nearly all isolates of other species of *Phytophthora* were identified by DNA sequencing. Molecular identifications were confirmed by examining standard morphological features—including sporangium type, mating system, and antheridium attachment. All isolates were also tested for mefenoxam sensitivity using a standard assay. A total of 389 isolates were examined and characterized from samples sent from 24 states, and 10 species of *Phytophthora* were identified. *P. nicotianae* was the most common species recovered

(346 isolates = 89%), and it was distributed widely across the U.S.—in all states submitting Phytophthora-positive samples. Some isolates of both *P. nicotianae* and *P. citrophthora* showed mefenoxam resistance. In coordinating with growers across the country to submit samples, a formal survey of lavender production was sent to growers to quantify some aspects of lavender production in the U.S. and the impact and threat of PRCR on this rapidly expanding specialty crop industry. The survey showed a profitable and rapidly expanding industry and identified concern about PRCR among lavender growers.

Introduction

Lavender (*Lavandula* spp.) is a member of the Lamiaceae/Labiatae or mint family, a family of nearly 7,000 species of aromatic herbs, shrubs, and trees—including such other genera as *Mentha* (peppermint), *Ocimum* (basil), *Origanum* (oregano), and *Hyssopus* (hyssop) (USDA 2021; Zomlefer 1994). Human interest in lavender dates back to prehistoric times, and the flowers and foliar scents of these plants have kept them popular, even today, as numerous species of ornamental, culinary, and medicinal plants are cultivated worldwide (Devecchi 2006; Naghibi et al. 2005). Of the 32 known species and hybrids of *Lavandula* (Upson 2002), several are commercially important today. English lavender, *L. angustifolia*, is the most popular while Spanish lavender (*L. stoechas*), French lavender (*L. dentata*), and spike lavender (*L. latifolia*) are also grown commercially (Anonymous 1997; Herring-Murray 2016; McCoy and Davis 2021). *Lavandula* × *intermedia* is a hybrid of English and spike lavenders, commonly known as lavandin, and is currently one of the most widely grown forms of lavender (Amidon 2013; Anonymous 1997).

The first report of *Phytophthora* species causing disease on lavender was root rot on *L. angustifolia* caused by *P. nicotianae* in a Maryland nursery (Putnam 1991). Diseased plants exhibited several symptoms: a grey color to the foliage, blackened and rotted roots, and vascular discoloration in the roots and stems (Putnam 1991). Additional observations of decay in the root crown of symptomatic plants led to the disease being renamed *Phytophthora* root and crown rot of lavender (PRCR) (Jeffers et al. 2016). Since the initial report, PRCR caused by *P. nicotianae* has become very widespread, is listed as the most problematic disease of lavender in Italy, and has been called the greatest threat to lavender production (Davino et al. 2002; Faedda et al. 2013).

Other species of *Phytophthora* have since been found to cause PRCR on multiple species of lavender. *P. palmivora* was reported as pathogenic to lavender in Italy (Davino et al. 2002) and Turkey (Dervis et al. 2011) and was found associated with lavender in South Korea (Cho and Shin 2004) and Croatia (Jung et al. 2016). *P. cinnamomi* was reported as pathogenic to lavender in Lithuania (Orlikowski and Valjuskaite 2007) and reported to be associated with lavender in several other European countries (Jung et al. 2016). *P. citrophthora* was pathogenic to lavender in Hungary (Jozsa et al. 2011) and associated with diseased lavender plants in South Carolina (Camacho 2009), Pennsylvania (Molnar et al. 2020), Croatia, Italy, and the United Kingdom (Jung et al. 2016). *P. cactorum* was shown to be pathogenic in China (Chen et al. 2017) and reported on lavender in Ontario, Canada (Westerveld 2015) and the United Kingdom (Jung et al. 2016). A hybrid species, *Phytophthora* ×*pelgrandis* has been found on lavender in several countries in Europe including Italy (Faedda et al. 2013), Hungary (Szigethy et al. 2013), and the Netherlands (Bonants et al. 2000) and has been described as spreading worldwide through trade (Faedda et al. 2013). Other species of *Phytophthora* found in association with

symptomatic lavender plants include *P. tropicalis* and *P. parvispora* in Pennsylvania (Molnar et al. 2020) and *P. cryptogea*, *P. hibernalis*, *P. kernoviae*, *P. megasperma*, and *P. plurivora* in Europe (Jung et al. 2016). *P. capsici* and *P. drechsleri* were reported on lavender in South Korea (Cho and Shin 2004) and unidentified species of *Phytophthora* were reported on lavender in Greece and California (Farr and Rossman 2022) and Europe (Jung et al. 2016).

Lavender is commercially produced for various purposes in many regions of the world, but obtaining up-to-date production totals, acreage, and values can be a challenge. Lavender is a relatively new crop in terms of commercial production and can fall under various categories—e.g., specialty crops, herbs, and ornamental plants—making documented crop profiles rare. Also, production totals can be reported in various units—including plants, biomass, or oils. Although production trends of lavender are not well documented, the two leading producers of lavender oil are Bulgaria and France (Grebenicharski 2016). However, in the United States, the economic impact of lavender production has not been reported.

The objective of this study was to identify the species of *Phytophthora* causing PRCR on lavender and to determine the impacts of PRCR, if any, on lavender production in the United States. Specifically, the study had three objectives: To collect samples of lavender plants with symptoms of PRCR from across the country and identify and characterize the species of *Phytophthora* present; to determine the geographic distribution of *Phytophthora* species on *Lavandula* species in the U.S.; and to conduct a preliminary survey of the U.S. lavender industry to determine future research needs and the impact PRCR has had on production and industry expansion.

Materials and Methods

Sample collection and pathogen isolation. Collecting symptomatic plants from lavender growers across the United States was accomplished by coordinating with the United States Lavender Growers Association (USLGA) and diagnosticians. Beginning in 2015, USLGA members were encouraged to submit samples of symptomatic or suspect lavender plants to the Clemson University Plant and Pest Diagnostic Clinic (PPDC) or to the S. N. Jeffers laboratory at Clemson University for diagnosis. Lavender samples also were requested from diagnostic labs in other states. Samples submitted to the PPDC were diagnosed, and then isolates were sent to the Jeffers lab for identification and storage. Many submissions consisted of plants received or planted by growers during the current growing season or within the last year; these plants had been obtained from commercial nurseries. When available, information on the nurseries that provided the infected plants was requested, but those data are not reported here. For this study, samples obtained between 2015 and 2019 were used.

Direct isolation from diseased plants was accomplished by placing symptomatic root and root crown tissue onto PARPH-V8 selective medium (Jeffers 2015a). Isolation plates were held at 20°C in the dark for 5 to 7 days and observed for the characteristic mycelium of *Phytophthora* species. Pathogens also were recovered from diseased plants using a standard baiting bioassay (Ferguson and Jeffers 1996) to bait field soil or soilless container mix accompanying plant samples or rhizosphere soil and debris washed from the roots. To bait soil and container mix, 100 ml of substrate was mixed with 200 ml of reverse-osmosis (RO) water in a 400-ml plastic container, and rhododendron and camellia leaf pieces (~5 ml in diameter) were floated on the surface. Containers were held at room temperature (22 to 24°C) for 3 days, and then *Phytophthora* species were isolated from bait pieces as described above. Rhizosphere soil and

debris were collected by placing roots of a suspect plant in a beaker (500 ml to 1 liter depending on root mass) and covering the roots with RO water. Roots were vigorously agitated in the water to remove associated soil and debris and then soaked for 10 to 20 min. Roots were removed from the beaker, and the suspension was baited as described above. Putative isolates of *Phytophthora* species were subcultured on fresh PARPH-V8 medium. Eventually, isolates were placed in long term storage by growing each isolate on 5 ml of 5% clarified V8 juice agar (cV8A; Jeffers 2015b) in an 8-ml screw-cap glass vial or by placing five plugs (3 to 4 ml in diameter) from 10% cV8A colonized by an isolate into 5 ml of sterile RO water in an 8-ml screw-cap glass vial. Vials were stored in the dark at 15°C. All isolates used in these experiments were received and used under current USDA-APHIS PPQ 526 permits and are maintained in a permanent culture collection in the S. N. Jeffers laboratory at Clemson University.

Morphological characters. All isolates were characterized morphologically by examining standard taxonomic features—including colony and mycelium morphologies, sporangia and sporangiophores, and oogonia and antheridia if present—and comparing these to published descriptions (Erwin and Ribeiro 1996; Gallegly and Hong 2008; https://idtools.org/id/phytophthora/factsheet_index.php). Colony and mycelium morphologies were examined on 100-mm-diameter plates of PARPH-V8 that had been incubated at 25°C in the dark for 5 to 10 days. Colony morphology was examined macroscopically because some species of *Phytophthora* produce unique colony patterns on this medium (S. N. Jeffers, *personal observation*)—including *P. nicotianae*, which typically produces a stoloniferous pattern. Mycelium on PARPH-V8 was examined microscopically (20 to 70×) for sexual and asexual structures, which are known to be produced on this isolation medium (Ferguson and Jeffers

1999). Isolates initially were grouped based on similar colony patterns and structures produced on PARPH-V8.

To produce sporangia, isolates were grown on 10% cV8A in the dark at 25°C for 3 to 5 days, and then three replicate plugs (~3 mm in diameter) with actively growing mycelium of each isolate taken from the edge of the colony were transferred to one well of a 24-well culture plate (Cellstar®; Greiner Bio-One North America Inc., Monroe, NC). Each well was filled with just enough 1.5% non-sterile soil extract solution (Jeffers 2015c) to cover the plugs. Culture plates were placed under fluorescent light for 24 h and then observed microscopically for sporangia and sporangiophores (20 to 70×). The type of papillae and method of proliferation were of primary interest as key characters (Erwin and Ribeiro 1996). Sporangium production was conducted twice for each isolate.

If oospores were observed on PARPH-V8, isolates were determined to be homothallic. Isolates that did not produce oospores on PARPH-V8 medium were assumed to be heterothallic or sterile. To determine if isolates were heterothallic or were sterile and to observe how antheridia attached to oogonia, each isolate was paired with isolates of known, A1 or A2, mating type. Isolates first were grown on 10% cV8A in 60-mm-diameter plastic plates in the dark at 25°C for 5 to 7 days. Then, each isolate was paired with known A1 and A2 isolates on super V8 agar (sV8A; Jeffers 2015d) in 48-well culture plates (Cellstar®; Greiner Bio-One North America Inc.). Known isolates were selected from a species of *Phytophthora* with morphological characters similar to those of the unknown isolate being tested. Each well contained 0.5 ml of sV8A, one 1-mm-diameter plug of the test isolate, and one 1-mm-diameter plug of a known isolate. Each pairing was replicated three times, so each test isolate was placed in six wells—paired in three wells with a known A1 isolate and paired in three wells with a known A2 isolate.

Culture plates were placed in the dark at 25°C for 1 to 2 weeks. Oospores were examined microscopically (100 to 400×) for morphological features. If oospores were produced when paired with a known A1 isolate, the test isolate was judged to be A2; likewise, if oospores were produced when paired with the known A2 isolate, the test isolate was judged to be A1. Isolates that did not produce oospores in paired cultures were judged to be sterile. This oospore production experiment was conducted twice for each isolate.

Molecular identification. Thirty representative isolates of *P. nicotianae*, including any with atypical morphological characters, and all isolates morphologically identified as not being *P. nicotianae* were identified using standard molecular methods (<https://idtools.org/id/phytophthora/molecular.php>; <http://phytophthora-id.org/index.html>). DNA was extracted from the mycelium of each isolate and ITS, *cox1*, and/or *cox2* loci were sequenced by Sanger Sequencing. DNA was extracted using one of two methods. A boiling extraction method was used in which a small amount of aerial mycelium was placed in 400 µl of sterile TE buffer in a 1.5-ml centrifuge tube (F. N. Martin, *personal communication*). The mixture was boiled in a water bath for 10 min, cooled on ice for 5 min, and then centrifuged to spin down the pellet. When using the boiling method, we were unable to achieve PCR results for the *cox* genes and had occasional quality issues with PCR of the ITS region. A second method utilized a DNeasy Plant Mini Kit (QIAGEN Sciences, Germantown, MD). In this method, a 5-mm plug was removed from an actively growing isolate on 10% cV8A, placed in a sterile 60-mm petri plate, and covered with approximately 10 ml of 10% cV8 broth (cV8B; Jeffers 2015b). The plugs were allowed to grow for 2 to 3 days at 25°C in the dark. Once a mycelium mat formed in the broth, the mat was collected using filter paper, a Buchner filter, and a vacuum flask while rinsing the isolate with RO water.

The rinsed mat was placed in a bead beater tube along with 200 μ l of 0.5-mm beads, a 5-mm bead, 400 μ l of buffer AP1 and 4 μ l of RNase A; this mixture was homogenized for 1.5 min. The tubes were held in a water bath at 65°C for 10 min and inverted twice during that time. Each tube then received 130 μ l of buffer P3, was vortexed, and placed on ice for 5 min. Tubes were centrifuged for 5 min at 13,500 rpm. The lysate was pipetted into a purple QIAshredder spin column that was inside a 2-ml collection tube, and the tube was centrifuged for 2 min at 13,500 rpm. The flow-through was transferred into a 1.5-ml tube, and 650 μ l of buffer AW1 was added. The solution was mixed with a pipet, and 650 μ l of the mixture was transferred into a white DNeasy mini spin column that was inside a 2-ml collection tube, and the tube was centrifuged for 1 min at 8,000 rpm. The flow through discarded, and this process was repeated for the entire mixture volume. The spin column was extracted from the tube, placed into a new 2-ml collection tube, and 500 μ l of buffer AW2 was added; then, the tube was centrifuged for 1 min at 8,000 rpm with the flow-through discarded. It was repeated with another 500 μ l of buffer and centrifuged at 13,500 rpm for 2 min. The spin column was removed and transferred to a new 2-ml tube, and 100 μ l of buffer AE was added for elution from the spin column. After incubating at room temperature for 5 min, the spin column within a tube was centrifuged for 1 min at 8,000 rpm, then the spin column was discarded, and the extracted DNA was contained in the tube. DNA from both extraction methods was held at -20°C until used in a polymerase chain reaction (PCR).

PCR reaction volumes included 10.04 μ l of sterile molecular water, 4 μ l of 5 \times PCR Platinum II Taq buffer, 4 μ l of 1 mM dNTPs, 25 μ M of each primer, and 0.16 μ l of Platinum II Taq polymerase. A 1- μ l volume of DNA template then was added to the master mix. The PCR cycle was performed in a T1 Thermocycler (Biometra[®], Göttingen, Germany). The cycles were

the same for all three genetic regions. Step 1: 94°C for 2 min, Step 2: 60°C for 15 sec, Step 3: 68°C for 40 sec, and Step 4: 94°C for 15 sec; then Steps 2 to 4 were repeated for 35 cycles, with a final extension at 68°C for 1 min (Step 5) followed by Step 6: 4°C and pause. To clean PCR products, 2 µl of ExoSAP-IT (Affymetrix, Inc., Cleveland, OH) was mixed with 5 µl of PCR product in a PCR tube, and the mixture was incubated at 37°C for 15 min, inactivated by heat at 80°C for 15 min, and then stored at -20 °C.

ITS regions 1 and 2, which had a ~800-bp amplicon length, were amplified using genus specific primers ITS6 (5' -GAA GGT GAA GTC GTA ACA AGG -3') (Cooke et al. 2000) and ITS4 (5' -TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990). The *cox1* region, which had a ~700-bp amplicon length, was amplified using Oomcox1-levup (5' -TCA WCW MGA TGG CTT TTT TCA AC-3') and Oomcox1-levlo (5' -CYT CHG GRT GWC CRA AAA ACC AAA-3') (Choi et al. 2015). The *cox2* region, which had a ~600-bp amplicon length, was amplified by *cox2-F* (5' -GGC AAA TGG GTT TTC AAG ATC C-3') and *cox2-rc4* (5' -TGA TTW AYN CCA CAA ATT TCR CTA CAT TG-3') (Choi et al. 2015). Purified DNA was sequenced at the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University in New Haven, CT. Sequences were paired, trimmed, and blasted using Geneious Prime (Dotmatrix, Boston, MA).

Mefenoxam sensitivity. All isolates were tested for sensitivity to the fungicide mefenoxam at 100 ppm using a standard assay and 48-well culture plates (Olson et al. 2013). Culture plates had six rows by eight columns and were prepared such that the upper three rows contained 5% cV8A amended with 100 ppm mefenoxam (Subdue Maxx, Syngenta Crop Protection, Greensboro, NC) and the lower three rows contained non-amended 5% cV8A. One isolate occupied each column, so each isolate was replicated in three wells of amended medium

and three wells of non-amended medium. Two independent cultures of each isolate were tested. Six 1-mm-diameter plugs were taken from the leading edge of a colony growing on 5% cV8A, and one plug was placed in each of the six wells in a column. Culture plates were incubated in the dark at 25°C for 3 days. Wells were then observed both macroscopically and microscopically (20 to 70×) and rated on a 0 to 5 scale (Olson et al. 2013): 0 = no growth; 1 = few hyphae growing from the plug but visible only microscopically; 2 = hyphae growing uniformly around plug but visible only microscopically; 3 = hyphae growing uniformly around plug and just visible macroscopically; 4 = hyphae visible macroscopically but not completely covering the agar surface in a well; 5 = agar surface in a well was completely covered by mycelium. Plate means were calculated for each isolate, and the overall mean of the two plates was used to determine sensitivity of each isolate. Scores of 0 to 2.99 were rated as mefenoxam sensitive, 3.00 to 3.99 were rated as intermediately sensitive, and 4.00 to 5.00 were rated as mefenoxam resistant.

Survey of lavender growers. A grower survey titled *A Survey of Lavender Production in the United States* (Appendix 1) was approved on 07 Feb 2019 by the Clemson University Office of Research Compliance as Exempt under category 2 in accordance with federal regulations 45 CFR 46.104(d), IRB number IRB2019-024. The survey was generated and conducted by Survey123, a feature of ArcGIS Online software (Esri, Redlands, CA). The completely electronic survey was distributed in cooperation with the USLGA to all of its members. In addition, the survey was sent to lavender growers who had submitted samples and were not members of the USLGA. Questions pertained to farm size, value of and markets for lavender and lavender products, current and potential management practices, and the impact of PRCR on their business. Replies were collected from Mar through Nov 2019. Submissions

were deidentified up to the state level to protect grower information. Descriptive statistics were generated for quantitative data, and qualitative responses were compiled into graphs.

Results

Sample collection and pathogen isolation. Between 2015 and 2019, a total of 571 isolates of *Phytophthora* species were obtained from lavender samples that were submitted by growers in 24 states. A small number of samples or isolates were obtained from academic and state government sources, some of which were received or collected prior to 2015. Specifically, this included 20 isolates obtained from Cornell University, Purdue University, Ohio State University, the Pennsylvania Department of Agriculture, and the Clemson University PPDC. Over the course of this study, some isolates were not able to be revived from storage, some became contaminated, a few grew too slowly to be tested, and some were from redundant samples. Therefore, a total of 389 isolates was used for this study (Table 2.1).

Lavender samples received for diagnosis consisted of four species of *Lavandula* as well as lavender plants with the species not identified (Table 2.1). However, the vast majority of samples were the two most common commercial species of lavender—English lavender and hybrid lavender. Consequently, the largest numbers of isolates were recovered from *L. ×intermedia*, hybrid lavender—201 isolates, and *L. angustifolia*, English lavender—145 isolates. Only one sample each of sweet lavender (*L. heterophylla*) and Spanish lavender (*L. stoechas*) were received and resulted in three isolates. A total of 24 isolates came from lavender samples that were not identified to species (Table 2.1).

Morphological characters. Morphological characters of isolates initially were used to separate isolates into tentative species or morphological groups. Isolates were grouped based on

type of papillae on sporangia, antheridium attachment to an oogonium, and mating system (Table 2.1). Although considerable efforts were made to identify mating type of each heterothallic isolate, some discrepancies and inconsistencies were detected in the data, so this information is not included here. Isolates of *P. nicotianae* were the most frequently encountered on lavender samples and were relatively easy to recognize based on colony growth patterns on PARPH-V8. In fact, we noticed that the *P. nicotianae* isolates produced three unique colony growth patterns: Aerial, distinctly stoloniferous, and uniformly stoloniferous (Figure 2.1). Aerial colonies also had stoloniferous hyphae embedded in the medium. Of 330 isolates of *P. nicotianae* that were examined for colony growth, 63 (19.1%) had an aerial colony pattern, 207 (62.7%) has a distinctly stoloniferous pattern, and 60 (18.2%) had a uniformly stoloniferous pattern. Distinctly stoloniferous was the most common colony pattern on PARPH-V8.

Species identification. Once isolates were separated into tentative species or morphological groups, 71 isolates were identified by DNA sequencing (Table 2.1). Using a combination of the ITS regions and *cox1* and/or *cox2* genes, isolates were sequenced and matched by blast analyses of greater than 99% similarity to sequences of confirmed isolates in the *Phytophthora*-ID (ver. 2.0) database (<http://phytophthora-id.org/>). Isolates recovered from lavender samples included 10 species of *Phytophthora* based on DNA sequencing, and morphological characters of these isolates were consistent with these species identifications (Table 2.1). *P. nicotianae* was recovered most frequently and accounted for 88.9% (346/389) of the isolates used in this study. This species was recovered from samples in 24 states with many of the isolates originating in South Carolina, California, and Pennsylvania (Table 2.1, Figure 2.2). The other two most frequently recovered species were *P. palmivora* (14/389 isolates = 3.6%) and *P. citrophthora* (11/389 isolates = 2.8%); both species were recovered from plant

samples sent from four states (Table 2.1). The other seven species were recovered infrequently and were only found in samples from one or two states each (Table 2.1). Five isolates of *P. cinnamomi* were recovered from young ‘Phenomenal’ hybrid lavender plants at a new lavender farm in South Carolina, and one isolate was recovered from field soil at this farm (Table 2.1). Other isolates of *P. cinnamomi* not included in the study were recovered from adjacent fields at the farm, which suggested the source of *P. cinnamomi* inoculum at this location was naturally infested soil.

Mefenoxam Sensitivity. A total of 383 isolates were tested for sensitivity to mefenoxam (Table 2.1). Of these, a total of 332 isolates (86.7%) were found to be sensitive to the fungicide, and 14 isolates (3.7%) were considered to be intermediately sensitive—including 11 isolates of *P. nicotianae*, two isolates of *P. citrophthora*, and one isolate of *P. tropicalis* (Table 2.1). A total of 37 isolates were found to be mefenoxam resistant in the standard laboratory assay, with growth in mefenoxam-amended agar wells the same as in non-amended control wells. Of the resistant isolates, 32 were *P. nicotianae* and five were *P. citrophthora*. For *P. nicotianae*, that accounted for less than 10% of isolates tested whereas it was nearly 50% of the *P. citrophthora* isolates tested.

Lavender grower survey. In early 2019, a survey of lavender growers was sent to all members of the USLGA across its six growing regions in the country (Figure 2.3) as well as to a small number of lavender growers that were not associated with the USLGA. The survey remained open for much of the calendar year, and we received 94 submissions from growers across 30 U.S. States (Figure 2.4). The greatest numbers of surveys were submitted from growers in Pennsylvania, Michigan, Kansas, Colorado, and the three states on the west coast (Figure 2.4). One grower who responded was no longer growing lavender and, therefore, was

excluded from analyses—leaving a final sample size of 93. With a rough estimate of 500 members in the USLGA at that time, the survey received nearly a 20% response rate, which is very good and indicative of the growers' interest in the survey. Lavender growers responded to several quantitative questions about their growing operations and the results from these questions are reported in Table 2.2. Lavender was shown to be a profitable crop with the mean annual income across all USLGA regions calculated to be \$35,809, but the range went from \$0 to \$400,000. Profits came from relatively small operations. The average size of growers' lavender fields by region ranged from 0.5 to 4.9 ha with a national range of 0.1 to 56.7 ha. Growers reported a total of 140.6 ha of lavender across all USLGA regions. The lavender industry in the U.S is relatively young. The average time most lavender growers have been growing this crop was between 3 and 9 years, but some growers in the northwest region had been growing lavender for 37 years while some in the northeast have been growing lavender for 29 years. The youngest segment of the industry was in the southeast region where growers reported growing lavender for only 1 to 6 years or an average of 3.3 years.

With lavender production being an expanding industry across the U.S., one question asked what species of lavender are being planted. Across all six regions, both *L. angustifolia* and *L. ×intermedia* were the dominant species being planted (Figure 2.5). The popularity of *L. stoechas* in the south-central region may have to do with the heat tolerance of this species. The occurrence of *L. stoechas* and *L. dentata* in the northwest, northeast, and southeast are noteworthy and may reflect growers looking to diversify. Other species of lavender reported included *L. ×chaytoarea*, *L. ×gingins*, *L. var buchi*, *L. viridis*, and *L. multifida*. The intended market for lavender impacts the potential management strategies used. All six regions had the five major markets represented, while non-commercial uses and “other” were not listed in every

region (Figure 2.6). Most of the growers considered their farms to be managed by conventional production practices, closely followed by organic and certified naturally grown (Figure 2.7). Within the “other” category of production practices, growers responded that their farms were managed by forms of organic or natural, chemical-free production but were not currently certified.

If another crop had previously been grown on the land currently growing lavender, then it could impact the occurrence of pests and pathogens affecting the crop now or in the future. For example, a former peach orchard in South Carolina was planted with lavender decades after the peach trees were removed and potentially left populations of root-knot nematodes in the soil that were then found to colonize lavender roots (Oliveira et al. 2022). However, the majority of growers were growing lavender on fields that had not been cultivated recently (Figure 2.8). Of all surveyed growers, barely a third of them had submitted samples to Clemson University for disease diagnosis (Figure 2.9A) while 16% had sent samples to other labs, public or private (Figure 2.9B). Among the other labs receiving multiple samples were the University of Kentucky and Texas A&M University. *Phytophthora* root and crown rot had been confirmed in 21.5% of growers’ fields, with another 3.2% declining to answer (Figure 2.9C). Only a small minority of growers had pests or pathogen problems other than PRCR in their fields, with the most frequent of these problems reported as root rots caused by *Pythium* species and *Fusarium* species and grasshopper damage (Figure 2.9D). Other minor problems reported were anthracnose stem blight, unknown fungi, fire ants, root circling, environmental problems, and alfalfa mosaic virus.

With the emerging problem of PRCR on lavender, a question was asked to determine the current management practices used on lavender fields for any pest or pathogen. A surprising

number of growers reported they did not use any pest management practices, and this was roughly the same number of growers who reported using conventional practices (Figure 2.10). The number of growers using resistant cultivars or biocontrol strategies were few, probably because there is a lack of published information on these topics. A very large number of growers declined to answer this question, and only 20.4% would be ready to use conventional pesticides and another 37.6% would consider using this management option (Figure 2.11). The lavender industry is growing. Currently, most growers reported they were looking to expand their operations (Figure 2.12A), and over half of these growers said the threat of PRCR has influenced their business (Figure 2.12B). Almost 80% of growers stated they were strongly or somewhat confident in their sources of lavender plants (Figure 2.12C).

Discussion

Based on isolation results from lavender samples received from growers in 24 states over a 5-year period, 2015 to 2019, it is apparent that PRCR is a serious problem on lavender, and the problem is expanding along with the U.S. lavender industry that this disease is affecting. It is a challenging problem involving 10 species of *Phytophthora* and at least four species of *Lavandula*. The list of *Phytophthora* species includes those commonly found in ornamental plant nurseries—i.e., *P. nicotianae*, *P. cinnamomi*, *P. citrophthora*, *P. cryptogea*, *P. drechsleri*, *P. palmivora*, and *P. tropicalis* (Chase et al. 2018; Donahoo and Lamour 2008; Hwang and Benson 2005; Olson and Benson 2011; and Williams-Woodward and Demott 2014). Of these, *P. nicotianae* was the most frequently isolated species from lavender plants with PRCR symptoms, and it was the most widespread species, having been found in samples from every state that had PRCR detected—24 in all. In comparison to other reported problems in lavender

production, PRCR is by far the most important one in the U.S. Of the four species of lavender diagnosed with PRCR, most of the isolates of *Phytophthora* species recovered in this study came from plant samples that were cultivars of hybrid lavender (*L. ×intermedia*) and English lavender (*L. angustifolia*), which makes sense because these are the two most popular species of lavender being grown in the U.S. based on our survey.

Over the course of this study, it became apparent that infected or infested plants are being distributed by nurseries. This is based on the number of samples that were plants growers had recently purchased or planted and the wide distribution of *P. nicotianae* on lavender samples, a pathogen usually found in warm climates and attacking warm-season crops—e.g., citrus, tobacco, annual vinca, tomatoes, and peppers (Erwin and Ribeiro 1996; Farr and Rossman 2022). However, grower confidence in their plant suppliers was high, but among those surveyed that reported purchases from three major lavender-growing nurseries—two in the northwest and one in the northeast regions—confidence declined (data not shown). It was also these three nurseries that were listed as the source of plants received from many of the growers who submitted diseased plant samples to our labs for diagnoses. While these three nurseries did not submit samples or surveys, 28 nurseries did complete the survey. Of these, 11 nurseries claimed to have sent plant samples to our labs, and five nurseries sent samples to other labs. Seven of the nurseries stated PRCR was diagnosed on samples from their nurseries (data not shown). This demonstrates that some nurseries take the PRCR problem seriously, and these nurseries recognize that infected plants are part of the problem. One nursery owner wrote in the survey: “I would like to clear our nursery and also offer my services to those dealing with Phytophthora. I would be willing to send plants in to get tested and I am willing to help fight this however I can. Thank you for your work!”

Upon characterizing the isolates, it is clear that most of the species on lavender are heterothallic and both mating types were common in the overall population (data not shown), sometimes even on a single sample. Isolates had varying levels of sensitivity to mefenoxam although most were sensitive to this important and widely used fungicide. Both of these characteristics are concerns when considering disease management options, and both would be expected to be exacerbated by nursery spread of the pathogens. Having both mating types present in one location could lead to greater genetic variability through recombination, which could enhance the risk of fungicide resistance, promote increased virulence, or allow production of long-lived oospores (Erwin and Ribeiro 1996; Olson and Benson 2011). Mefenoxam resistance limits the chemical options available for disease management (Erwin and Ribeiro 1996; Olson et al. 2013). In this study, we identified 37 resistant isolates, with 32 being *P. nicotianae* and five being *P. citrophthora*. Interestingly, 26 of the 32 resistant *P. nicotianae* isolates were obtained from lavender growers from four different states and included hosts that were numerous cultivars of both *L. angustifolia* and *L. ×intermedia*. However, all resistant isolates were traced back to plants purchased from one nursery in the northwest region. In addition, there were 14 isolates—11 *P. nicotianae*, two *P. citrophthora*, and one *P. tropicalis*—that were determined to be intermediately sensitive (i.e., intermediately resistant). Ten of the 14 isolates came from plants for which the nursery source was known. We determined that eight isolates of *P. nicotianae* and the one isolate of *P. tropicalis* came from one nursery in the northeast region. These isolates with reduced sensitivity to mefenoxam were distributed with plants shipped to three different states on two different species of lavender. One of the resistant *P. nicotianae* isolates also came from this nursery. Therefore, it is important to note that

mefenoxam resistant or intermediately sensitive isolates were being shipped with lavender plants to multiple states.

In summary, *Phytophthora* root and crown rot poses a serious threat to the lavender industry in the U.S. It currently is the most important problem affecting lavender plants in the field. Although management options exist, growers appear reluctant to apply traditional chemicals to manage the disease due to the herbal and holistic nature of this crop. One grower responded in the survey: “Would only think about conventional fungicides if we are in the midst of losing our entire crop.” For that reason, exclusion should be the primary disease management principle utilized, and all plants and fields to be planted should be screened by trained diagnosticians before planting (Agrios 2005; Jarvis 1992; Ludowici et al. 2013). Some growers are even responding to the PRCR epidemic by producing their own lavender plants, with one such grower responding in the survey, “I never wanted to propagate but fear of *Phytophthora* affecting my farm is highly motivating.”

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Table 2.1. Identification and geographic distribution of 10 species of *Phytophthora* isolated from lavender (*Lavandula* spp.) plant samples in the United States^a

Species	Isolates (no.)	Criteria for species identification					Mefenoxam sensitivity (no.) ^d			States ^e	Original lavender hosts: no. of isolates ^f
		DNA sequencing ^b		Morphological characters ^c			S	I	R		
		Total	%	Sporangia	Antheridium	Mating					
<i>P. nicotianae</i> ^g	346	30	8.7	P	Amphigynous	Heterothallic	297	11	32	24 states: see Fig. 2	<i>L. angustifolia</i> : 125 <i>L. ×intermedia</i> : 181 <i>L. stoechas</i> : 2 <i>L. heterophylla</i> : 1 <i>L. species</i> : 22
<i>P. palmivora</i>	14	13	92.9	P	Amphigynous	Heterothallic	14	0	0	NJ, PA, SC, TN	<i>L. angustifolia</i> : 7 <i>L. ×intermedia</i> : 7
<i>P. citrophthora</i>	11	10	90.1	SP / P	Amphigynous	Sterile	4	2	5	CO, NY, PA, SC	<i>L. angustifolia</i> : 10 <i>L. ×intermedia</i> : 1
<i>P. cinnamomi</i>	6	6	100	NP	Amphigynous	Heterothallic	6	0	0	SC	<i>L. ×intermedia</i> : 5 ^h
<i>P. tropicalis</i>	4	4	100	P	Amphigynous	Heterothallic	3	1	0	PA, TN	<i>L. angustifolia</i> : 1 <i>L. ×intermedia</i> : 1 <i>L. species</i> : 2
<i>P. cryptogea</i>	3	3	100	NP	Amphigynous	Heterothallic	3	0	0	CO	<i>L. ×intermedia</i> : 3
<i>P. sansomeana</i>	2	2	100	NP	Amphigynous	Homothallic	2	0	0	TN	<i>L. ×intermedia</i> : 2

<i>P. cactorum</i>	1	1	100	P	Paragynous	Homothallic	1	0	0	KS	<i>L. ×intermedia</i> : 1
<i>P. drechsleri</i>	1	1	100	NP	Amphigynous	Heterothallic	1	0	0	IN	<i>L. angustifolia</i> : 1
<i>P. megasperma</i>	1	1	100	NP	Paragynous	Homothallic	1	0	0	OH	<i>L. angustifolia</i> : 1
Isolate totals	389	71	18.3				332	14	37	24	373

^a Plant samples or isolates were received by the S. N. Jeffers lab or the Plant and Pest Diagnostic Clinic at Clemson University between 2015 and 2019; a few samples and isolates were recovered prior to 2015.

^b Number of isolates and the percentage of the total for each species that were identified by sequencing the ITS region and the *cox1* or *cox2* genes.

^c Morphological characters used to corroborate species identifications: Sporangia were papillate (P), semi-papillate (SP), or non-papillate (NP), antheridium attachment on oogonia, and the mating system.

^d Numbers of isolates determined to be sensitive (S), intermediately sensitive (I), or resistant (R) to the fungicide mefenoxam using a standard assay.

^e States in which each species of *Phytophthora* was isolated from a lavender sample.

^f Numbers of isolates of each species of *Phytophthora* isolated from each lavender host species.

^g Isolate totals for *P. nicotianae* in the different column categories are not the same because information for some isolates was not available.

^h One isolate of *P. cinnamomi* was recovered from field soil at a lavender farm in South Carolina.

Table 2.2. Summary of three quantitative questions and their answers in a survey sent to U.S. lavender growers in 2019 regarding lavender production.

Question 1: Estimated total area of lavender being grown per lavender grower (ha)

USLGA region ^a	Received (no.)	Responses (no.)	Standard					Total
			Mean	deviation	Minimum	Maximum	Median	
All	93	91	1.5	6.4	0.1	56.7	0.4	140.6
Northeast	31	30	1.3	4.4	0.1	24.3	0.4	37.6
Southeast	8	8	0.7	0.4	0.4	1.3	0.5	5.6
North Central	20	19	0.7	0.4	0.4	2.0	0.8	14.1
South Central	6	6	0.5	0.3	0.1	0.8	0.4	2.7
Northwest	13	13	0.6	0.7	0.1	2.8	0.4	7.2
Southwest	15	15	4.9	14.4	0.1	56.7	0.4	73.4

Question 2: Period of time your land has been used for lavender production (years)

All	93	92	6.0	6.7	0	37	3	552.5
Northeast	31	30	4.6	6.5	0.0	29.0	2.8	136.5
Southeast	8	8	3.3	1.5	1.0	6.0	3.0	26.0
North Central	20	20	6.9	6.0	1.0	19.0	4.0	138.0
South Central	6	6	6.3	5.0	2.5	14.0	3.5	37.5
Northwest	13	13	9.4	9.7	2.0	37.0	7.0	122.0
Southwest	15	15	6.2	6.6	1.0	25.0	3.0	92.5

Question 3: Anticipated annual income from lavender production (US \$)

All	93	77	35,809.05	69,923.72	0.00	400,000.00	10,000.00	2,757,297.00
Northeast	31	23	26,323.70	82,284.46	0.00	400,000.00	5,000.00	605,445.00
Southeast	8	8	48,625.00	62,206.88	2,000.00	180,000.00	22,500.00	389,000.00
North Central	20	17	39,888.35	61,846.40	2.00	250,000.00	15,000.00	678,102.00
South Central	6	5	50,600.00	84,197.98	3,000.00	200,000.00	15,000.00	253,000.00
Northwest	13	11	26,954.55	43,792.95	500.00	150,000.00	10,000.00	296,500.00
Southwest	15	13	41,173.08	82,063.58	0.00	300,000.00	8,000.00	535,250.00

^a The six regions of the United States recognized by the United States Lavender Growers Association (USLGA).

Figure 2.1. Isolates of *Phytophthora nicotianae* recovered from *Lavandula* species exhibited three unique colony patterns when grown on PARPH-V8 selective medium (from left to right): Uniformly stoloniferous (isolate 19-0687), distinctly stoloniferous (isolate 163s), and aerial (isolate 132).



Figure 2.2. Distribution of *Phytophthora nicotianae* on lavender in the United States based on 337 plant samples and nine isolates received at Clemson University, primarily between 2015 to 2019. Samples and isolates were sent from 24 states (diamond symbol), and the size of the symbol represents the relative number of samples received from each state.



Figure 2.3. Map of the United States showing the six lavender-production regions recognized by the U.S. Lavender Growers Association (USLGA; uslavender.org); a seventh region includes international members; used with USLGA permission.

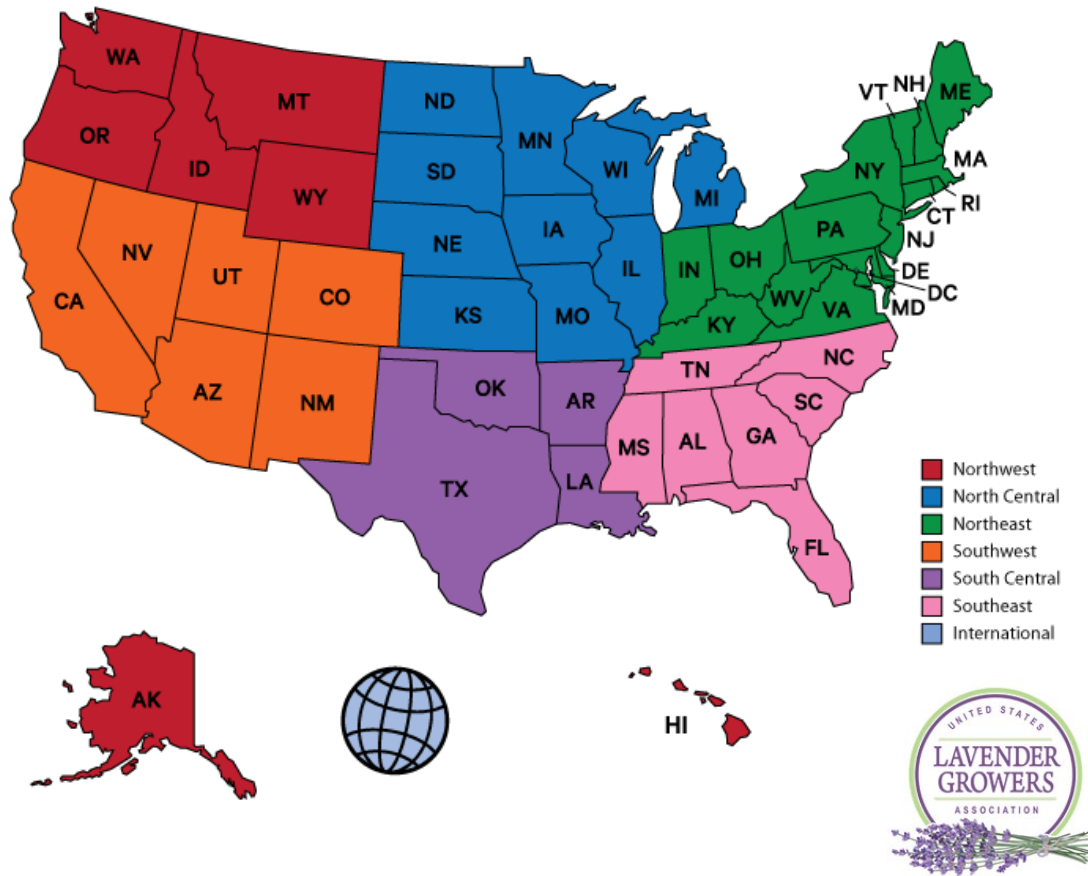


Figure 2.4. Map of the United States showing the location of growers who answered a survey about lavender production in the U.S. The size of each circle indicates the relative number of survey responses from that state.

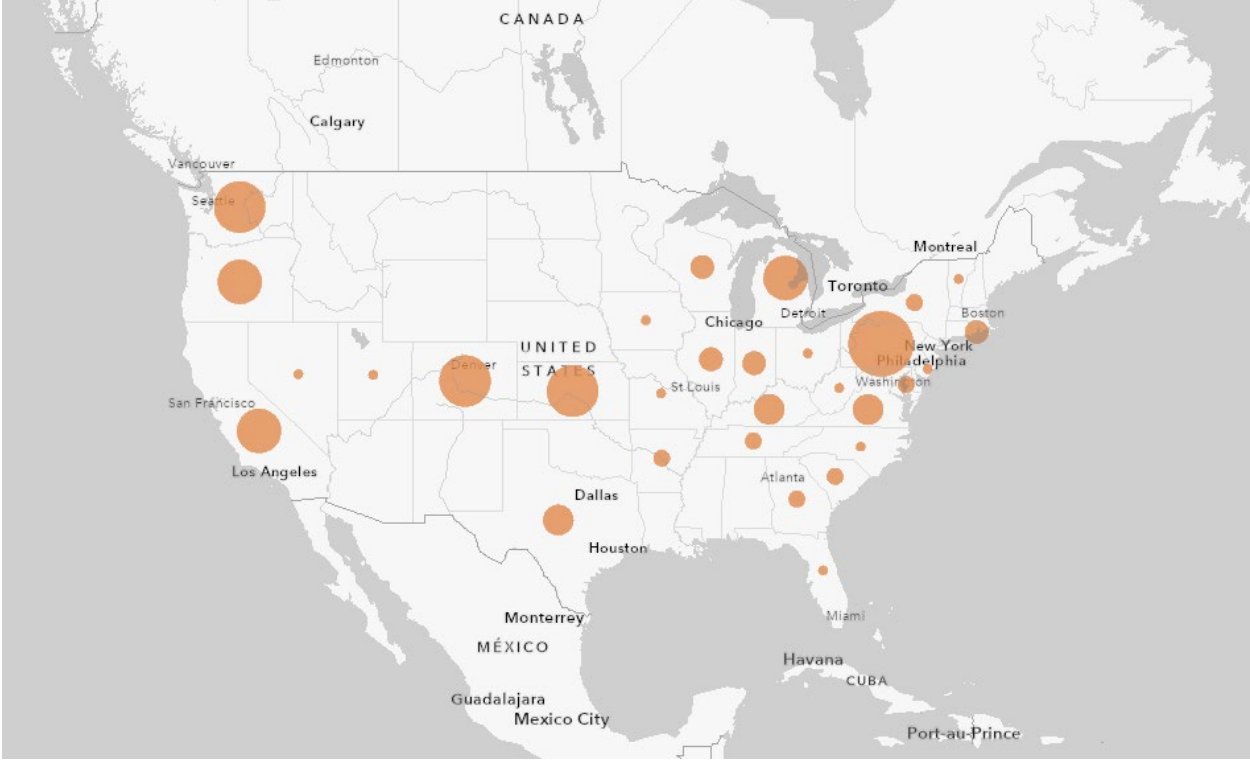


Figure 2.5. The numbers of growers in each USLGA* region growing each of five common species of lavender (*Lavandula*). Data are based on 93 responses to a lavender grower survey in 2019; *USLGA = US lavender Growers Association.

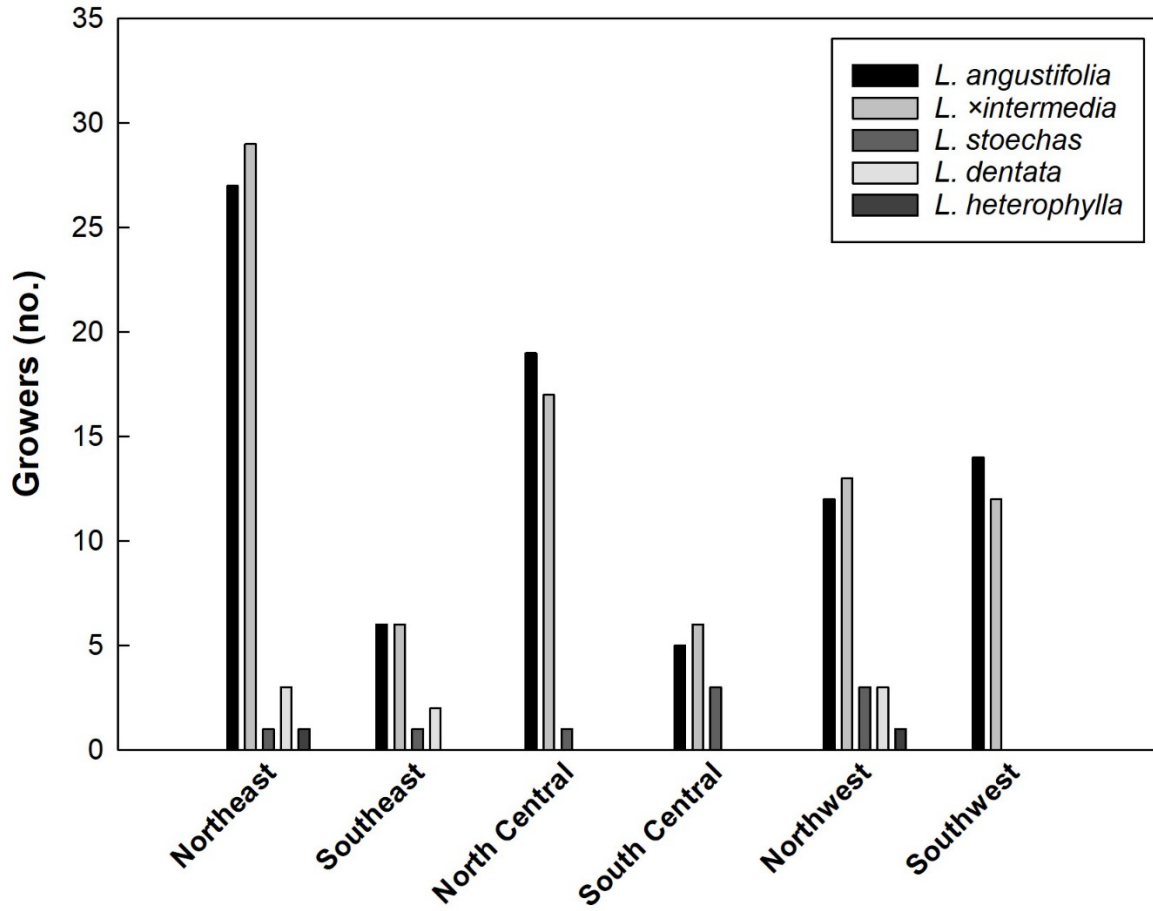


Figure 2.6. Intended markets for lavender produced in the six USLGA* regions of the United States. Data are based on 93 responses to a lavender grower survey in 2019; *USLGA = US lavender Growers Association.

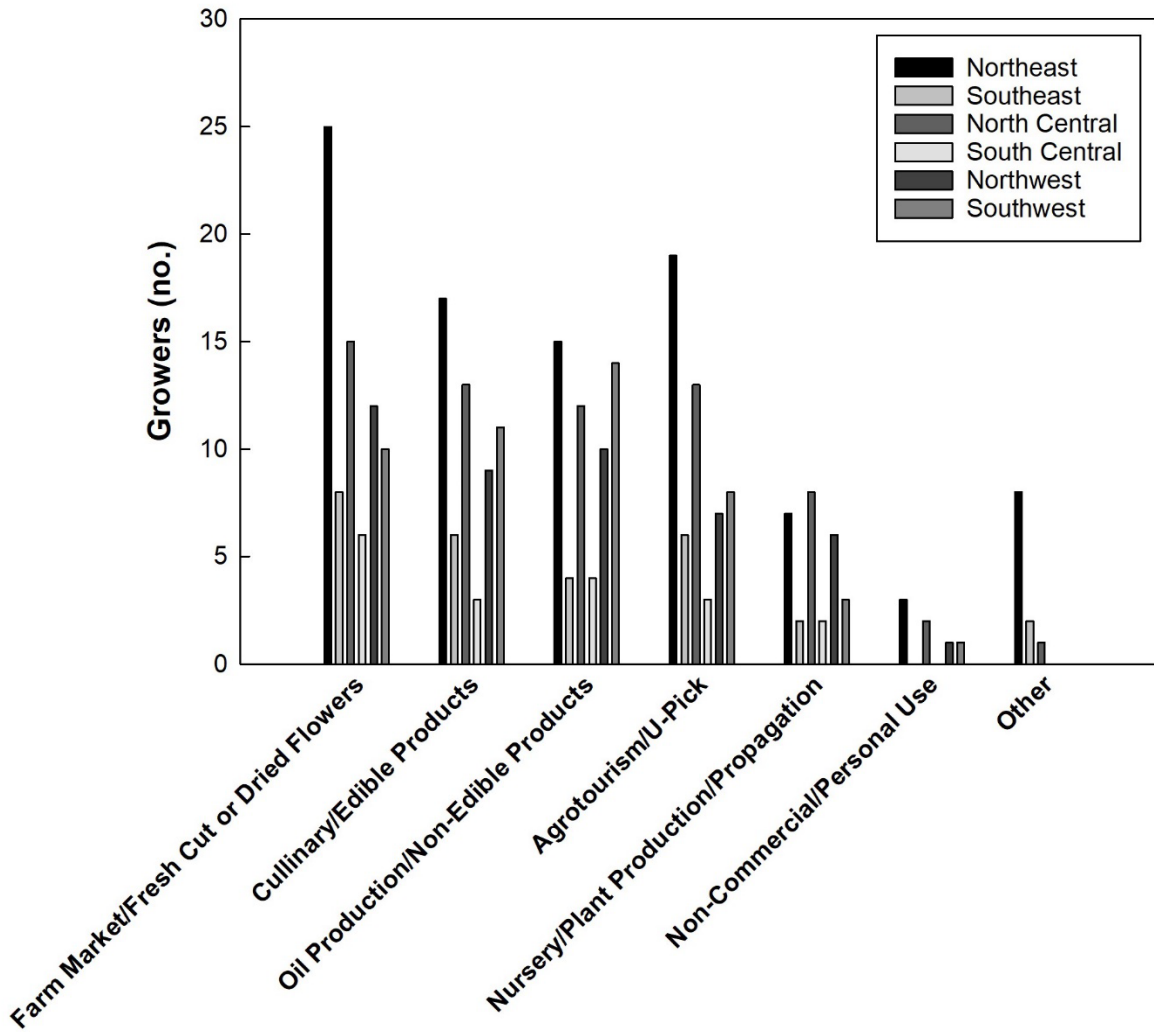


Figure 2.7. The numbers of growers using four different production methods to grow lavender in the United States. Data are based on 93 responses to a lavender grower survey in 2019.

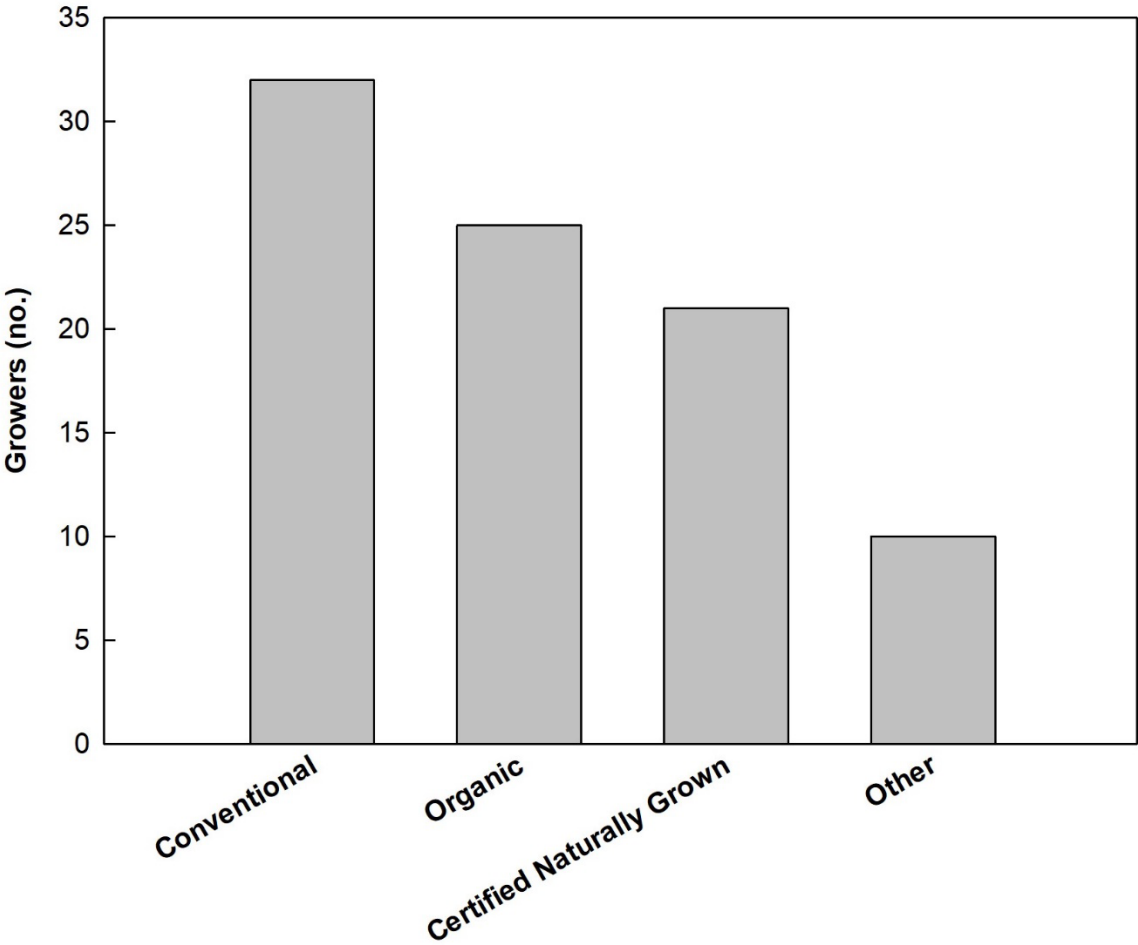


Figure 2.8. Relative proportions and numbers of U.S. lavender growers reporting that another crop previously had been grown on their farm before lavender was planted. Data are based on 93 responses to a lavender grower survey in 2019.

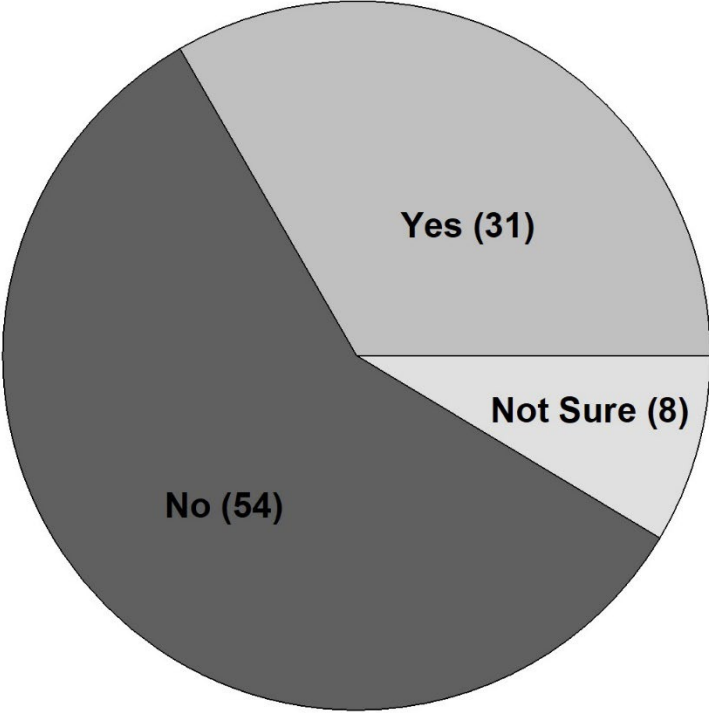


Figure 2.9. Relative proportions and numbers of U.S. lavender growers who responded “yes” or “no” to four questions: A) Did you send lavender samples to the Plant and Pest Diagnostic Clinic or to the S. N. Jeffers lab at Clemson University; B) Were lavender samples sent to other diagnostic labs, either public or private; C) Has Phytophthora root and crown rot on lavender been documented in your lavender production areas; and D) Were other pests, pathogens, or problems documented in your lavender production areas? Data are based on 93 responses to a lavender grower survey in 2019; NR: no response.

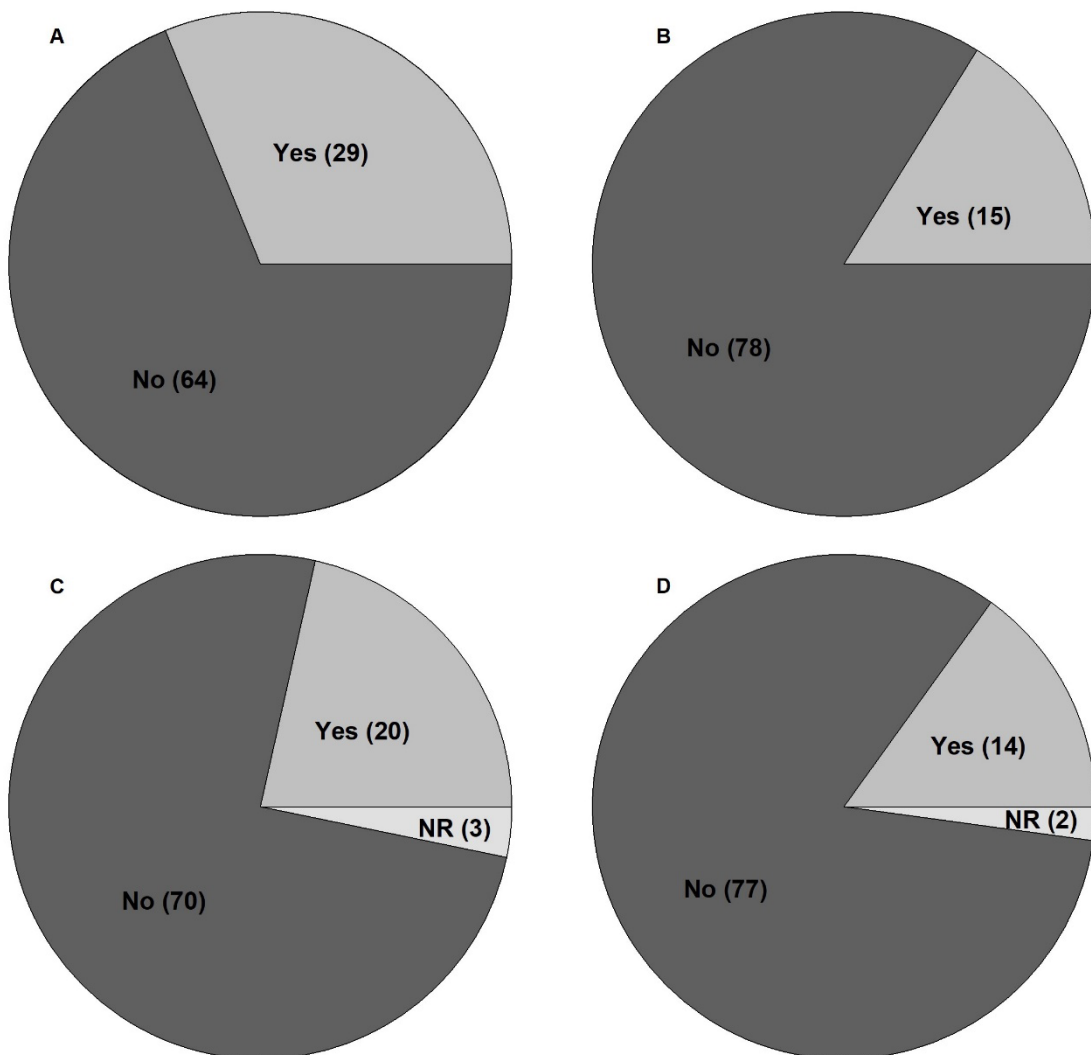


Figure 2.10. The numbers of U.S. lavender growers using different strategies to manage pests and diseases at their lavender farms. About 50% of the growers did not respond to this question.

Data are based on 93 responses to a lavender grower survey in 2019.

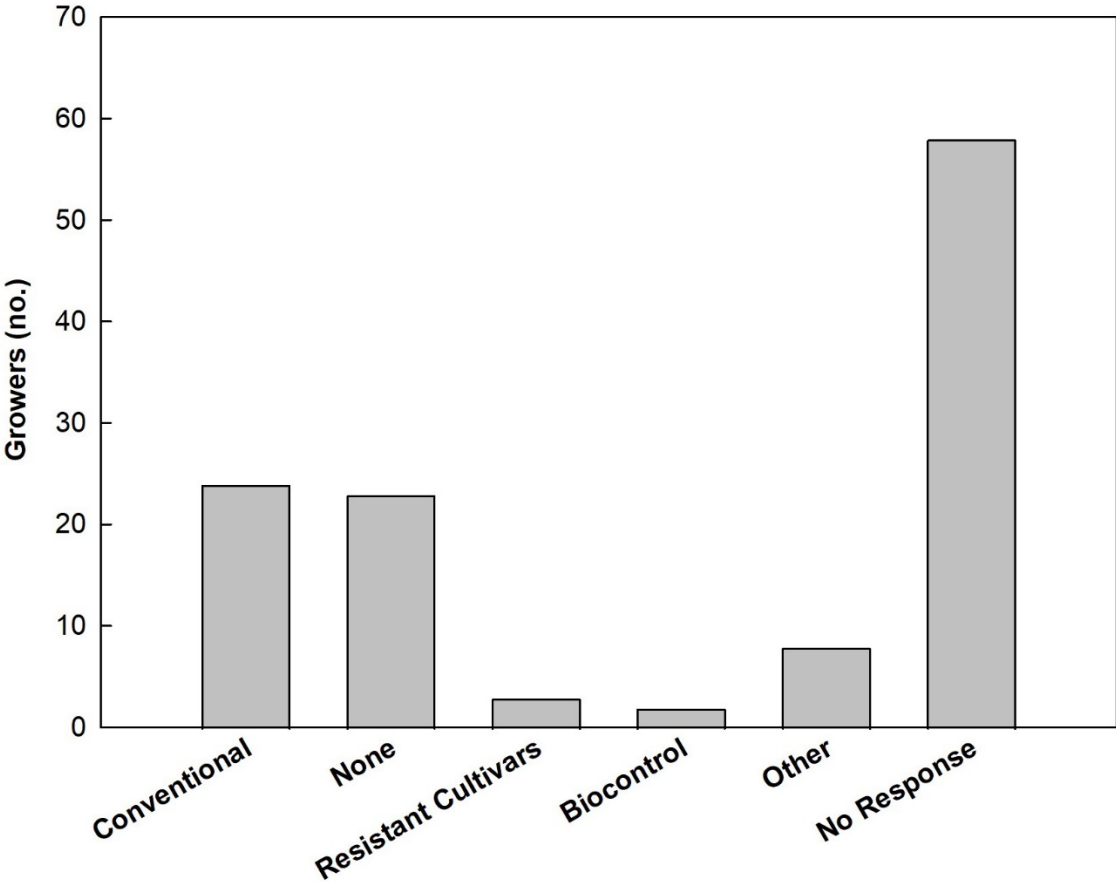


Figure 2.11. Willingness of U.S. lavender growers to apply conventional pesticides to their lavender crops. Data are relative proportions and numbers of growers (total = 93) who responded to a lavender grower survey in 2019; NR: no response.

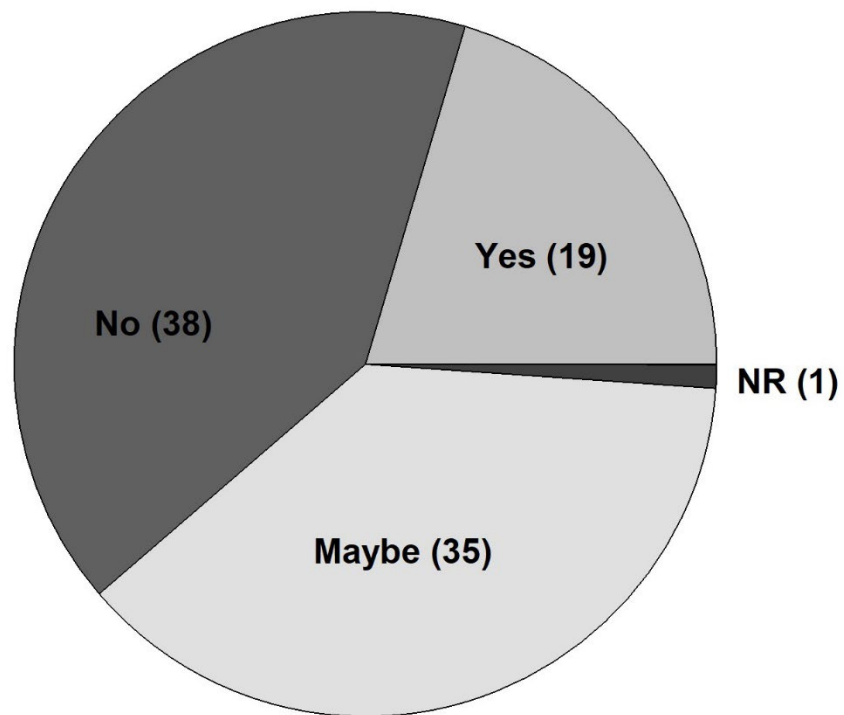
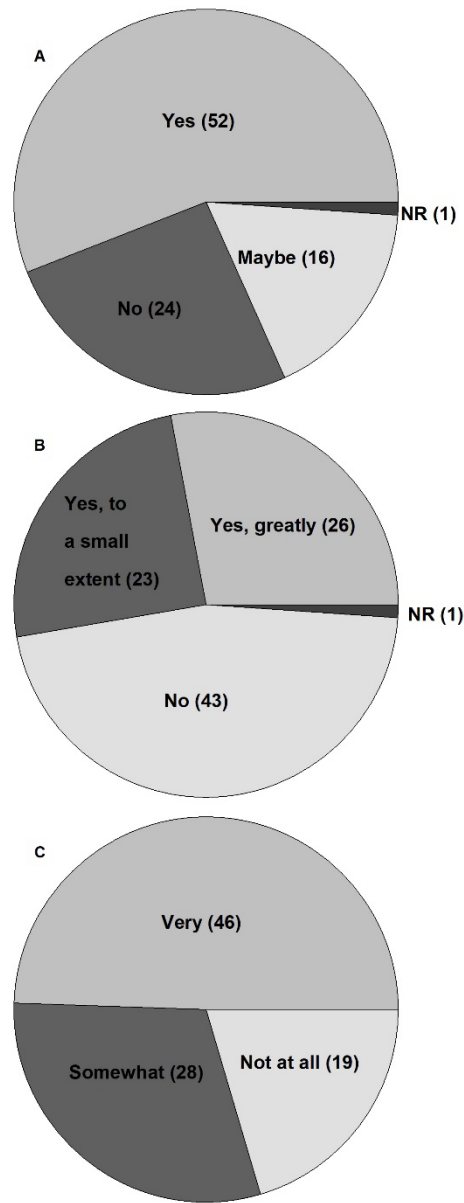


Figure 2.12. Relative proportions and numbers of U.S. lavender growers who responded to these three questions: A) Are you looking to expand your lavender production; B) Has the threat of Phytophthora root and crown rot on lavender influenced your lavender business; and C) How confident are you in your lavender plant sources? Data are based on 93 responses to a lavender grower survey in 2019; NR: no response.



CHAPTER 3
PHYTOPHTHORA ROOT AND CROWN ROT OF LAVENDER:
PATHOGENICITY OF EIGHT SPECIES OF *PHYTOPHTHORA*
TO THREE SPECIES OF *LAVANDULA*

Abstract

Phytophthora root and crown rot (PRCR) has become a major threat to the lavender industry worldwide. Isolations from symptomatic plants between 2015 and 2019 revealed a number of potential causal agents in the United States. This study used Koch's Postulates to evaluate pathogenicity of eight species of *Phytophthora* to three species of *Lavandula* in six experiments. Each experiment consisted of two independent trials. Only host-pathogen combinations that occurred in the field were evaluated. All isolates used in these experiments were recovered from diseased lavender plants or, for one isolate, soil associated with a diseased plant sent to our lab or the Clemson University Plant and Pest Diagnostic Clinic for diagnosis. Experiments were conducted over 3 years, 2017 to 2019, in a research greenhouse under relatively uniform environmental conditions following a standard protocol. Plants were evaluated weekly for foliage symptom severity, and, at the end of each trial, plants were scored for final foliage symptom severity, area under the disease progress curve was calculated, total plant mass was weighed, and pathogen isolation from roots was attempted. These studies successfully demonstrated for the first time pathogenicity of *P. nicotianae*, *P. palmivora*, and *P. cinnamomi* to hybrid lavender (*L. ×intermedia*), *P. nicotianae* to sweet lavender (*L. heterophylla*), and *P. cryptogea* and *P. drechsleri* to English lavender (*L. angustifolia*). A soil isolate of *P. tropicalis* was shown to be potentially pathogenic to *L. ×intermedia*. In addition,

results documented for the first time in the United States pathogenicity of *P. palmivora* and *P. citrophthora* to *L. angustifolia*. Results from this study expand the list of *Phytophthora* species causing PRCR on lavender (*Lavandula* species) in the United States and elsewhere. Preliminary reports have been published (Dlugos and Jeffers 2018, 2019, 2021).

Introduction

Lavender (*Lavandula* spp.) is a commercially important genus in the Lamiaceae/Labiatae family (USDA 2021; Zomlefer 1994). As with many other members of the family, species of *Lavandula* are popular for ornamental, culinary, and medical uses (Devecchi 2006; Naghibi et al. 2005). Reports of lavender occurred as early as 370 B.C. (Upson 2002) and, in its extensive written history, lavender has reported uses ranging from embalming to the taming of tigers (McCoy and Davis 2021).

Currently, there are 32 known species of lavender plus hybrids (Upson 2002), with several of them being commercially important. *Lavandula angustifolia* Mill. (previously classified as *L. delphinensis* Jord. ex Billot, *L. officinalis* Chaix, *L. spica* L., and *L. vera* D.C. [Anonymous 1997; Singh et al. 2007]) is commonly referred to as English lavender and is one of the most common and desirable lavender species (McCoy and Davis 2021; Singh et al. 2007). The natural range for this species is southern Europe, including Italy and Spain (Upson 2002). *Lavandula stoechas*, Spanish lavender, is found throughout the Mediterranean region and has become invasive in Australia (Upson 2002). Spike lavender, *L. latifolia*, is a lower altitude species native to Spain, France, and the Mediterranean region (Anonymous 1997; Anonymous 2009; Guenther 1954). A cross between *L. angustifolia* and *L. latifolia* resulted in the hybrid lavender *L. ×intermedia* (synonym *L. ×hybrida*), commonly known as lavandin (Amidon 2013;

Anonymous 1997). Lavandin cultivars have been steadily growing in popularity since the 1950s due to their hardiness, heat tolerance, longevity, and high oil yields—with up to four times the oil production compared to non-hybrid lavender species (Guenther 1954; Herring-Murray 2016).

The first report of a *Phytophthora* disease on lavender was root rot caused by *P. nicotianae* on *L. angustifolia* in a Maryland nursery (Putnam 1991). Symptoms included grey colored foliage, blackened roots, and vascular discoloration in roots and stems (Putnam 1991). In preliminary studies in our lab, symptoms of decay in the root crown led to this disease being renamed *Phytophthora* root and crown rot (PRCR) of lavender (Jeffers et al. 2016). In Europe, *P. nicotianae* became the leading cause of PRCR, and it is considered the most problematic disease of lavender in Italy and the greatest threat to lavender production (Davino et al. 2002; Faedda et al. 2013). *P. nicotianae* has also been reported to cause PRCR on *L. angustifolia* in Spain (Álvarez et al. 2007) and Bulgaria (Nakova 2011). It was found associated with *L. angustifolia*, *L. stoechas*, *L. ×hybrida*, and *Lavandula* sp. in nurseries and horticultural plantings in several European countries (Jung et al. 2016), and it was associated with diseased lavender plants in Canada (Westerveld 2015) and Australia (Mammella et al. 2013).

Other species of *Phytophthora* cause or are also associated with PRCR on lavender as well. *Phytophthora palmivora* was first reported as a pathogen of *L. angustifolia* in Sicily, Italy (Davino et al. 2002) and later in Turkey (Dervis et al. 2011); this pathogen also was reported on *L. spica* in South Korea (Cho and Shin 2004) and associated with lavender plants in Croatia and Italy (Jung et al. 2016). *Phytophthora cinnamomi* was found to be pathogenic on *L. angustifolia* in Lithuania (Orlikowski and Valjuskaite 2007) and associated with *L. angustifolia* in three European countries (Jung et al. 2016). *Phytophthora citrophthora* was isolated from and pathogenic to *L. angustifolia* ‘Hidcote’ in Hungary (Jozsa et al. 2011). It also was isolated from

L. angustifolia in South Carolina (Camacho 2009) and Pennsylvania (Molnar et al. 2020) and was reported to be associated with several lavender species in Croatia, Italy, and the United Kingdom (Jung et al. 2016).

A large planting of symptomatic *L. angustifolia* was found to be infected with *P. cactorum* in Jiangsu Province, China (Chen et al. 2017), and this species also has been isolated from dead and dying lavender plants in Ontario, Canada (Westerveld 2015) and associated with *Lavandula* sp. in the United Kingdom (Jung et al. 2016). A hybrid between *P. nicotianae* and *P. cactorum*, *P. ×pelgrandis*, was found on *L. stoechas* in Italy and was described as common on lavender and spreading worldwide through trade (Faedda et al. 2013). This hybrid pathogen was also found on *L. angustifolia* ‘Hidcote’ in Hungary (Szigethy et al. 2013) and was reported on lavender in the Netherlands before it was recognized as a hybrid species (Bonants et al. 2000).

Several species of *Phytophthora* have only been reported as associated with various species of *Lavandula* during nursery surveys and summaries of diagnostic efforts. Molnar et al. (2020) reported *P. tropicalis* (three isolates) and *P. parvispora* (one isolate) were isolated from diseased lavender plant specimens submitted to the Pennsylvania Department of Agriculture for diagnosis. Jung et al. (2016) reported limited incidences of *P. cryptogea*, *P. hibernalis*, *P. kernoviae*, *P. megasperma*, *P. plurivora*, and unidentified species of *Phytophthora* associated with several species of lavender in Europe. Cho and Shin (2004) reported *P. capsici* and *P. drechsleri* on *L. spica* in South Korea. Farr and Rossman (2022) list two instances of unidentified species of *Phytophthora* associated with *Lavandula* species in Greece and California.

Since 2015, our lab at Clemson University and the Clemson University Plant and Pest Diagnostic Clinic have collaborated with the United States Lavender Association (USLGA) to

receive and diagnose samples of lavender plants with PRCR symptoms from growers across the United States. Isolations from these plants and associated soil and container mix have produced a large collection of isolates of *Phytophthora* species. For this study, isolates collected from 2015 to 2019 were identified and characterized by host-pathogen relationships. The objective of the study was to identify new lavender hosts of species of *Phytophthora* and to use Koch's Postulates to determine pathogenicity and document new host-pathogen occurrences in the United States and worldwide.

Materials and Methods

The greenhouse and plants for inoculation. In total, 12 pathogenicity experiments were conducted and identified by sequential numbers, and six of these produced meaningful results. For each experiment, two independent trials were conducted between 2017 and 2019 in a research greenhouse at Clemson University in Clemson, SC (Table 3.1). Experiments were designed to test pathogenicity of nine species of *Phytophthora* on four species of *Lavandula*, to document first reports of these pathogenic relationships, either in the USA or worldwide. The successful pathogenicity experiments involved eight species of *Phytophthora* and three species of *Lavandula* (Table 3.2). Trial durations varied from 9 to 18 weeks among experiments, but trial durations within an experiment were kept nearly identical for analysis purposes (Table 3.1). Experiments were run as long as necessary to allow for adequate symptom development. During the trials of these experiments, environmental conditions in the greenhouse, which were controlled and measured by a central computerized system (Argus Controls, Conviron, Winnipeg, Manitoba, Canada), were relatively uniform based on measurements taken every 15 min (Table 3.1). Across all experiments, mean temperatures ranged from 21 to 24°C and mean

relative humidity measurements ranged from 50 to 78%. A 16-h photoperiod was maintained throughout all experiments with artificial lighting coming on when outside light energy was below 350 W/m².

Lavender plants for the experiments were obtained from several commercial producers based on needs for the experiment and plant availability. Species, and if possible, cultivars were selected to match the species and cultivar of symptomatic plants from which isolates originally were recovered (Table 3.2): *L. ×intermedia* cultivars Grosso and Phenomenal were used in Experiment 1 (Ball Horticultural Co., West Chicago, IL), *L. angustifolia* ‘Hidcote’ was used in Experiments 2 and 5 (Ball Horticultural Co.), *L. heterophylla* potted plants were used in Experiment 3 (Mountain Valley Growers, Squaw Valley, CA); *L. angustifolia* ‘Hidcote’ was used for Experiment 7 (Creek Hill Nursery, Leola, PA), and *L. angustifolia* ‘Phenomenal’ was used for Experiment 11 (Creek Hill Nursery). All plants except those of *L. heterophylla*, were received as rooted cuttings (i.e., plugs) in 72-cell trays (83 ml/cell); *L. heterophylla* plants were received in 7.6-cm-diameter pots. To confirm that plugs and plants were not contaminated with *Phytophthora* species prior to use, subsamples of the plugs and plants used in all experiments were tested for the presence of *Phytophthora* species by several methods—including isolation from roots; baiting of root clippings, rootwash debris, and root-associated container mix; and visual inspection for PRCR symptoms. *Phytophthora* species were not detected on any of the plants used in these experiments. Individual plugs were transplanted into 1.3-liter pots (15 cm in diameter); each pot contained 1 liter of a soilless peat- and bark-based container mix (Fafard 3B; Sun Gro Horticulture, Agawam, MA). For the *L. heterophylla* plants in Experiment 3, these plants were transplanted into 300 ml of container mix in 400-ml pots (9 cm in diameter). Plants were placed in the greenhouse, watered overhead by hand as needed, and fertilized weekly with a

fertilizer solution delivering 100 ppm of nitrogen (PowerPak 20-20-20 [N-P-K] Soluble Fertilizer with Minor Elements; Southern Agricultural Insecticides, Inc., Hendersonville, NC).

Isolates and identification. The isolates used in this study originated in nine different states (Table 3.2). Most isolates were obtained from plant samples exhibiting typical PRCR symptoms submitted for diagnosis to the Clemson Plant and Pest Diagnostic Clinic or our lab at Clemson University between 2015 and 2018 (Table 3.2). Pathogens were isolated by plating root or root-crown tissue on PARPH-V8 selective medium (Jeffers 2015a). However, one isolate, 15-1194.D, was recovered from soil around the roots of a plant with PRCR symptoms using a standard baiting bioassay. Two isolates (PIN1 and Purdue 15-927A) were recovered from diseased lavender plants at diagnostic labs in New York and Indiana, respectively. All the isolates tested in these experiments were received and used under USDA-APHIS PPQ 526 permits and are maintained in a permanent culture collection in the S. N. Jeffers laboratory at Clemson University.

To verify species identification of isolates used in pathogenicity experiments, isolates were identified using standard molecular methods (<https://idtools.org/id/phytophthora/molecular.php>; <http://phytophthora-id.org/index.html>). DNA was extracted from mycelium of each isolate, and ITS, *cox1*, and/or *cox2* loci were sequenced by Sanger Sequencing. DNA was extracted using one of two methods. A boiling extraction method was used in which mycelium was placed in 500 μ l of sterile TE buffer in a 400- μ l microfuge tube (F. N. Martin, *personal communication*). The mixture was boiled in a water bath for 10 min, cooled on ice, and then centrifuged to spin down the pellet. A second method utilized a DNeasy Plant Mini Kit (QIAGEN Sciences, Germantown, MD). Purified DNA was sequenced by the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University in New

Haven, CT. Sequences were paired, trimmed, and blasted using Geneious Prime (Dotmatics, Boston, MA). Isolate identities were further validated by examining morphological characters—colony and mycelium morphologies, sporangia and sporangiophores, and oogonia and antheridia if present—and comparing these to published descriptions (Erwin and Ribeiro 1996; https://idtools.org/id/phytophthora/factsheet_index.php).

Inoculum preparation and treatments. For each experiment, isolates used were ones originally isolated from the host species being inoculated. Inoculum for each trial was prepared independently by growing each isolate on sterile vermiculite moistened with 10% V8 juice broth (2:1, v:v) in glass bottles (Jeffers 2015b; Roiger and Jeffers 1991). Bottles were placed in the dark at 25°C for 2 weeks, so each isolate could thoroughly colonize the vermiculate in a bottle. After 10 to 12 days of incubation, a small aliquot (1 to 2 ml) of vermiculite from each bottle was spread on a plate of 10% clarified V8 juice agar (Jeffers 2015b) to ensure purity and uniform colonization.

For each trial of each experiment, plants in inoculated and non-inoculated treatments were arranged in a completely randomized design on a greenhouse bench. Each plant was inoculated by spreading approximately 5 to 12 ml of inoculum on the surface of each pot, and then the inoculum was mixed by hand into the upper 1 cm of the container mix. A 1-cm layer of fresh container mix was added to each pot to cover the inoculum, and all pots were gently watered to incorporate the inoculum and prevent desiccation. Specifically, 7 ml of inoculum was applied to plants in Experiment 1, but the amount was increased to 10 to 12 ml in all subsequent experiments, except Experiment 3, to increase infection and improve disease development. For the smaller pots used in Experiments 3, only 5 ml of inoculum was added to each pot. Plants in all non-inoculated control treatments received no inoculum. After inoculation, plastic saucers

(14 cm diameter, 3.5 cm deep) were placed under all pots, and plants were watered from the bottom, by adding water to the plastic saucers, for the remainder of the experiment. This was done to keep the container mix in each pot near field capacity throughout the trial, which promoted disease development and minimized splashing of infested container mix and pathogen propagules among pots. Replication of treatments varied among experiments based on availability of plants. Numbers of replicate plants per treatment in a trial were 12 for Experiment 1 (i.e., six of each of two cultivars); seven for Experiment 2; four for Experiments 3, 5, and 7; and six for Experiment 11. Replicate numbers were doubled when trials of an experiment were combined for analysis.

Data collection and analysis. Each trial was run for a period of 9 to 18 weeks post-inoculation (Table 3.1), and then four disease parameters were evaluated. Plants were evaluated weekly for foliage symptom severity, which was scored on a 0 to 5 scale based on the percentage of foliage showing symptoms of gray discoloration, wilting, or necrosis: 0 (0% of foliage symptomatic; no symptoms), 1 (1 to 10%), 2 (11 to 50%), 3 (51 to 90%), 4 (91 to 99%), and 5 (100% of foliage symptomatic; mortality). For all analyses, weekly and final foliage symptom severity scores were converted to the midpoint of each range—e.g., a score of 1 (1 to 10%) was converted to 5.5%. Area under the disease progress curve (AUDPC) was calculated based on weekly and final foliage evaluations using the method reported by Shaner and Finney (1977). At the end of each trial, plants were harvested independently, roots were gently washed free of container mix and blotted dry, and fresh plant masses were weighed. Non-inoculated plants were always harvested before inoculated plants to prevent cross contamination. To document infection on inoculated plants and a lack of infection on non-inoculated control plants—i.e., to fulfill Koch's Postulates, root tissues were assayed for the presence of the pathogens. Five root

bundles from each plant were embedded in PARPH-V8 selective medium (Jeffers 2015a) to isolate the pathogen. Root bundles were composed of five to ten segments, approximately 1 to 2 cm in length, of fibrous feeder roots. Isolation plates were held at 25°C in the dark for 7 days and examined regularly for typical hyphae of the species of *Phytophthora* used in a given experiment.

Data for all experiments were analyzed with JMP Pro, ver. 14, statistical software (Cary, NC). The data initially were examined using analysis of variance (ANOVA) along with Levene's and Shapiro-Wilk's tests for variance and normality assumptions. When necessary, data were transformed before analyses or subjected to non-parametric analyses. However, results from analyses using data transformations and non-parametric tests were consistent with those from standard analyses, so parametric analyses of non-transformed data were used for all experiments. Experiment 1 required a three-way ANOVA (with treatment, cultivar, and trial as factors) while the remainder of the experiments were analyzed with two-way ANOVAs (with treatment and trial as factors). Trials of each of the two experiments were analyzed together with blocking by trial as a factor. When a significant ($P < 0.05$) trial effect occurred, data for individual trials were not reported separately as this did not affect evaluation of inoculum treatments. When there was a significant treatment by trial interaction for any of the disease parameters in an experiment, a separate ANOVA was conducted for each trial and means from each trial are reported. However, when treatment by trial interactions were not significant, data from the two trials were combined for analysis, and means for the combined trials are reported. When the effect of treatments was significant in an analysis, means were separated by individual pairwise comparisons ($P < 0.05$).

Results

This study examined the pathogenicity of nine species of *Phytophthora* to four commercially important *Lavandula* species. Multiple experiments were conducted over the course of a 3-year span as pathogens were isolated and identified and greenhouse space and lavender plants became available. Six experiments successfully demonstrated pathogenicity (Table 3.2), and six others were not successful, at least initially. In two host-pathogen combinations, pathogenicity could not be demonstrated: *P. nicotianae* on *L. stoechas* (two experiments) and *P. cactorum* on *L. ×intermedia* (two experiments). The other two initially unsuccessful experiments were repeated with successful results (i.e., Experiments 7 and 11, Table 3.2). Of the four disease parameters analyzed for each experiment, the overwhelming majority lacked a treatment by trial interaction, signifying consistency among treatments in repeated trials. Occasionally trial effects were significant, which may be because plants were of different ages and conditions in the greenhouse varied slightly between trials. In some experiments, there was not a significant treatment effect for one of the disease parameters, but consistent isolation of the pathogens from inoculated and symptomatic plants confirmed pathogenicity.

Experiment 1. This experiment tested the pathogenicity of two isolates of *P. nicotianae* and one isolate each of *P. palmivora* and *P. tropicalis* to hybrid lavender, *L. ×intermedia*. All results from this trial are summarized in Table 3.3. For final foliage symptom severity, the three-way interaction was significant at $P = 0.0497$, but the two-way interactions were not significant. Therefore, main effects for trials combined were evaluated. There were significant differences among treatments with foliage symptom severity greater on plants inoculated with each of the

four isolates than on non-inoculated plants; *P. palmivora* was significantly more virulent than the other two species.

For AUDPC, a significant treatment by trial interaction occurred in the three-way ANOVA, so results from individual trials were examined by two-way ANOVA. In each trial, there was a highly significant cultivar effect because disease progress on ‘Grosso’ plants occurred more quickly than on ‘Phenomenal’ plants (AUDPC values for trials combined = 389.8 and 266.3, respectfully; data not shown); however, treatment by cultivar interactions were not significant, so cultivar did not differentially affect isolate performance. In Trial 1, disease progress was greater on plants inoculated with each of the four isolates compared to the non-inoculated control plants, with *P. nicotianae* 15-0450 and *P. palmivora* being most virulent. In Trial 2, the two isolates of *P. nicotianae* and the isolate of *P. palmivora* caused significant disease, and disease was greatest on plants inoculated with *P. nicotianae* 15-1123.B and *P. palmivora*.

There also was a significant treatment by trial interaction in the three-way ANOVA for fresh plant mass, and, again, the treatment by cultivar interactions in two-way ANOVAs were not significant. All four isolates significantly reduced plant mass compared to control plants in Trial 1, with *P. nicotianae* 15-0450 and *P. palmivora* causing the greatest reductions. In Trial 2, only *P. palmivora* and *P. nicotianae* 15-1123.B caused significant reductions in plant mass. Pathogens were isolated from 100% of the inoculated plants and from 8.3% of non-inoculated control plants. There was one instance of growth of a *Phytophthora* species on a root bundle from a non-inoculated control plant, suggesting splash contamination during the experiment or cross contamination during harvesting.

Experiment 2. In this experiment, we examined the pathogenicity of *P. palmivora* (two isolates) to English lavender, *L. angustifolia*, and confirmed pathogenicity of *P. nicotianae* (one isolate) on this host. The results from this experiment are summarized in Table 3.4. The only disease parameter with a significant treatment by trial interaction was plant mass, so trials were analyzed separately for this disease parameter. For the other three disease parameters, trials were combined for analyses. For the four disease parameters assessed, all three isolates caused significant levels of disease compared to the non-inoculated control plants. The three isolates were equally virulent except in Trial 1 for plant mass where one isolate of *P. palmivora* was more virulent than the isolate of *P. nicotianae*. Pathogens again were recovered from all inoculated plants. As in Experiment 1, a *Phytophthora* species was also isolated from one non-inoculated control plant presumably from contamination during the experiment or at harvest.

Experiment 3. In this experiment, the pathogenicity of two isolates of *P. nicotianae* on sweet lavender, *L. heterophylla*, was examined, and all results from the experiment are summarized in Table 3.5. In this experiment, both isolates were pathogenic and equally virulent based on final foliage symptom severity. There was a significant treatment by trial interaction for AUDPC, so trial means were examined individually. Based on AUDPC, the two isolates of *P. nicotianae* were each pathogenic—one in Trial 1 and one in Trial 2. Isolates had no significant effect on

total plant mass, but the pathogens were isolated from all inoculated plants and were not isolated from any of the control plants. In Trial 2, two non-inoculated control plants were determined to be outliers, so they were excluded from all statistical analyses based on advice from a statistician (W. C. Bridges, *personal communication*).

Experiment 5. The pathogenicity of *P. citrophthora* to English lavender was examined in this experiment (Table 3.6). There was a significant treatment by trial interaction for final foliage symptom severity but not for the other three disease parameters. *P. citrophthora* caused a significant amount of foliage symptoms in Trial 1 but not in Trial 2. When disease was assessed by AUDPC and total plant mass, *P. citrophthora* was significantly pathogenic. This pathogen was isolated from all but one of the eight inoculated plants and from 34 out of 35 of the root bundles from the seven infected plants. *P. citrophthora* was not isolated from any of the non-inoculated plants.

Experiment 7. The pathogenicity of *P. cinnamomi* to hybrid lavender was tested in this experiment (Table 3.7). All four disease parameters confirmed *P. cinnamomi* as pathogenic with inoculated plants having significantly more disease than non-inoculated control plants. There was a treatment by trial interaction in isolation of the pathogen from roots bundles, but only due to a lower isolation percentage in Trial 2 compared to Trial 1. Across both trials, *P. cinnamomi* was isolated from all inoculated plants and was not isolated from any of the non-inoculated control plants.

Experiment 11. In this experiment, three species of *Phytophthora*—*P. cryptogea*, *P. drechsleri*, and *P. megasperma*—that were infrequently recovered from field samples were tested for pathogenicity on English lavender, and all results are summarized in Table 3.8. Significant treatment by trial interactions occurred for final foliage symptom severity and total plant mass, so data from individual trials were analyzed for these two disease parameters. For final foliage symptom severity, none of the three species caused a significant amount of foliage symptoms by the end of Trial 1, but a significant amount of foliage symptoms occurred on plants inoculated with both *P. cryptogea* and *P. drechsleri* in Trial 2. When the progress of foliage

symptom development was recorded over time, both *P. cryptogea* and *P. drechsleri* had AUDPC values that were significantly greater than those for *P. megasperma* and the control treatment. However, total plant mass was not a useful parameter for determining pathogenicity in this experiment. There was no significant treatment effect in Trial 1, and, in Trial 2, plants inoculated with *P. megasperma* had significantly greater mass than the plants in the other treatments. Only *P. cryptogea* significantly colonized roots on inoculated plants based on isolation from root bundles. However, all three species of *Phytophthora* were isolated from some of the 12 plants inoculated with each species: *P. cryptogea* from 11 plants, *P. drechsleri* from six plants, and *P. megasperma* from three plants. Pathogens were not isolated from control plants.

Discussion

This study consisted of multiple experiments, each with repeated trials and conducted over a 3-year period, that documented the pathogenicity of seven species of *Phytophthora* to three commercially important lavender species. Both *P. palmivora* and *P. citrophthora* were shown to be pathogenic on *L. angustifolia*, and, therefore, to the best of our knowledge, this represents the first documented report of these two host-pathogen relationships in the United States. Previously, *P. palmivora* was shown to be pathogenic to *L. angustifolia* in both Italy (Davino et al. 2002) and Turkey (Dervis et al. 2011). In our study, *P. palmivora* was as virulent as *P. nicotianae* on *L. angustifolia* ‘Hidcote’ plants, demonstrating the potential this pathogen has at becoming a major obstacle to commercial lavender production in the United States. *P. citrophthora* was first documented as pathogenic to *L. angustifolia* in Hungary (Józsa et al. 2011). It also was found associated with *L. angustifolia* plants in the United States and several

European countries (Camacho 2009; Jung et al. 2016; Molnar et al. 2020), but this is the first report documenting pathogenicity of *P. citrophthora* to English lavender in the U.S.

This study successfully demonstrated for the first time pathogenicity, by following Koch's Postulates, of *P. nicotianae*, *P. palmivora*, and *P. cinnamomi* to hybrid lavender, *L. ×intermedia*. In fact, this is the first report of pathogenicity by any species of *Phytophthora* on hybrid lavender. Previous associations of *P. nicotianae* with hybrid lavender had been reported but without documenting pathogenicity (Jung et al. 2016). Pathogenicity of *P. tropicalis* to *L. ×intermedia* cannot yet be confirmed as Koch's Postulates were not fulfilled because the isolate was obtained from soil associated with the roots of diseased plants and not directly from root tissue. However, our results indicate a strong potential for pathogenicity of *P. tropicalis* to *L. ×intermedia*.

Our results documented first reports of other host-pathogen relationships. We proved that *P. nicotianae* can be pathogenic to sweet lavender, *L. heterophylla*. Previously, there were no reports or associations of *P. nicotianae* with this species of lavender, so this expands the host range of *P. nicotianae*. We also proved that *P. cryptogea* and *P. drechsleri* can be pathogens of English lavender, *L. angustifolia*. Both pathogens caused significant disease on inoculated plants as measured by several parameters, and they were readily isolated from roots of diseased plants upon completion of the trials. Previously, *P. cryptogea* and *P. drechsleri* have only been found associated with lavender plants (Cho and Shin 2004; Jung et al. 2016). Under the experimental conditions used in this study, pathogenicity of *P. megasperma* to *L. angustifolia* was not demonstrated even though it was isolated from a few of the inoculated plants. This pathogen has been isolated from diseased lavender plants in Oregon and Washington

(<https://pnwhandbooks.org/node/2979/print>) has been found associated with hybrid lavender in Croatia (Jung et al. 2016).

In addition to the above-mentioned host-pathogen relationships, there were two more experiments we attempted multiple times to document pathogenicity that did not yield success. *P. cactorum* was found associated with the roots of a hybrid lavender plant, which was submitted from a grower in Kansas in 2016. This pathogen had been documented as pathogenic to *L. angustifolia* in China (Chen et al. 2017) and has been reported to cause disease to field-grown lavender plants in Ontario, Canada (Westerveld 2015). *P. cactorum* typically is found in cool, temperate climates (Erwin and Ribeiro 1996), so the environmental conditions in the greenhouse used in this study may not have been conducive to disease development. The other host-pathogen relationship that could not be documented was *P. nicotianae* on Spanish lavender, *L. stoechas*, using an isolate originally recovered from a diseased plant in South Carolina in 2014. Previously, that host-pathogen association had only been reported in Italy (Jung et al. 2016).

In addition to the pathogenic relationships mentioned above, results from this study identified issues that merit further investigation. In Experiment 1, there were significant differences in disease progress on two cultivars of hybrid lavender, which suggests there may be differences in susceptibility between these cultivars. Identifying differences in susceptibility among cultivars of *L. angustifolia* and *L. ×intermedia* would be very beneficial to the lavender industry. In experiments using multiple isolates, differences in virulence were sometimes reported. Therefore, it would be important to determine which species of *Phytophthora* are more virulent to the popular commercial species and cultivars of lavender and if isolates within a species vary in virulence—particularly if lavender species and cultivars are to be screened for resistance.

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Table 3.1. Dates, durations, and environmental conditions for six experiments, each with two independent trials, conducted in a greenhouse to evaluate the pathogenicity of eight species of *Phytophthora* to three species of lavender over a 3-year period, 2017-2019

Experiment	Trial	Trial Dates ^a		Trial duration (weeks) ^b	Temperature (°C) ^c				Relative humidity (%) ^c			
		Start	End		Mean	SD	Min	Max	Mean	SD	Min	Max
1	1	09/15/2017	11/30/2017	11	22.3	2.7	15.7	28.8	58.2	14.7	18.4	80.3
	2	09/28/2017	12/14/2017	11	21.4	2.4	15.7	28.7	54.3	14.7	18.4	80.3
2	1	09/15/2017	11/16/2017	9	22.7	2.8	15.7	28.8	61.4	13.4	21.7	80.3
	2	09/28/2017	11/30/2017	9	21.7	2.5	15.7	28.7	55.8	14.7	18.4	80.3
3	1	09/21/2017	01/25/2018	18	21.2	2.4	15.7	28.8	51.9	16.3	12.6	80.3
	2	10/5/2017	02/08/2018	18	20.6	2.0	15.7	28.4	49.6	16.1	12.6	80.3
5	1	02/08/2018	05/10/2018	13	20.5	1.5	17.6	25.9	51.0	15.7	10.6	79.9
	2	02/22/2018	05/24/2018	13	21.0	2.0	17.6	27.5	53.1	16.7	10.6	83.5
7	1	07/23/2018	10/10/2018	11	24.2	2.1	18.5	32.1	78.4	7.5	38.6	91.4
	2	08/08/2018	10/24/2018	11	23.7	2.3	18.4	32.1	74.6	12.1	18.4	91.4
11	1	05/02/2019	07/09/2019	10	23.6	2.3	14.9	30.0	71.2	11.5	35.1	91.3
	2	05/14/2019	07/15/2019	9	23.9	2.3	14.9	30.0	72.8	11.1	35.1	91.3

^a Start dates are when plants were inoculated; end dates are when plants were harvested for data collection.

^b Numbers of weeks were counted to the nearest whole week.

^c Temperature and relative humidity during each trial were summarized as the mean, standard deviation (SD), minimum (Min), and maximum (Max) values based on data collected every 15 min.

Table 3.2. Sources of 13 isolates of eight species of *Phytophthora* used in six pathogenicity experiments and the rationale for using these species and conducting the experiments

Experi- ment	Isolate source ^a				Original host plant			
	Species	Isolate no.	State ^b	Year ^c	Species	Cultivar	Substrate ^d	Rationale
1	<i>P. nicotianae</i>	15-0450	SC	2015	<i>L. × intermedia</i>	Phenomenal	Root	First report: <i>P. nicotianae</i> on <i>L. × intermedia</i>
		15-1123.B	NJ	2015	<i>L. × intermedia</i>	Grosso	Root	
	<i>P. palmivora</i>	17-0099	TN	2017	<i>L. × intermedia</i>	Provence	Root	First report: <i>P. palmivora</i> on <i>L. × intermedia</i>
	<i>P. tropicalis</i>	15-1194.D	TN	2015	<i>L. × intermedia</i>	Phenomenal	Soil	Potential pathogenicity: <i>P. tropicalis</i> on <i>L. × intermedia</i>
2	<i>P. nicotianae</i>	16-0718	PA	2016	<i>L. angustifolia</i>	Hidcote	Root	Known pathogen for comparison
	<i>P. palmivora</i>	15-1125.R1	NJ	2015	<i>L. angustifolia</i>	Hidcote	Root	First report in USA: <i>P. palmivora</i> on <i>L. angustifolia</i>
		16-1107	SC	2016	<i>L. angustifolia</i>	Super Blue	Root	
3	<i>P. nicotianae</i>	17-0435	TX	2017	<i>L. heterophylla</i>	Sweet	Root	First report: <i>P. nicotianae</i> on <i>L. heterophylla</i>
		15-0450	SC	2015	<i>L. × intermedia</i>	Phenomenal	Root	Additional isolate for comparison
5	<i>P. citrophthora</i>	PIN1 ^e	NY	2007	<i>L. angustifolia</i>	... ^f	Plant	First report in USA: <i>P. citrophthora</i> on <i>L. angustifolia</i> in USA
7	<i>P. cinnamomi</i>	SC.4308	SC	2018	<i>L. × intermedia</i>	Phenomenal	Root	First report: <i>P. cinnamomi</i> on <i>L. × intermedia</i>

11	<i>P. cryptogea</i>	15-1178	CO	2015	<i>L. angustifolia</i>	Buena Vista	Root	First report: <i>P. cryptogea</i> on <i>L. angustifolia</i>
	<i>P. drechsleri</i>	Purdue 15- 927A ^g	IN	2015	<i>L. angustifolia</i>	... ^f	Plant	First report: <i>P. drechsleri</i> on <i>L. angustifolia</i>
	<i>P. megasperma</i>	16-0236	OH	2016	<i>L. angustifolia</i>	... ^f	Root	First report: <i>P. megasperma</i> on <i>L. angustifolia</i>

^a All isolates were isolated from diseased lavender plant samples: 11 isolates were recovered from samples sent to the Plant and Pest Diagnostic Clinic or the S. N. Jeffers lab at Clemson University; the isolates of *P. citrophthora* and *P. drechsleri* were provided by colleagues.

^b State from which diseased lavender plant originated.

^c Year in which sample was received and isolate was recovered.

^d Isolates were recovered from soil, plant roots, or a part of the plant that was not specified (plant).

^e Isolate provided by Margery Daughtrey at the Cornell University Long Island Horticultural Research and Extension Center.

^f The cultivar for this host plant was not recorded.

^g Isolate provided by Tom Creswell at the Purdue University Plant and Pest Diagnostic Lab.

Table 3.3. Experiment 1: Pathogenicity of three species of *Phytophthora* to two cultivars of hybrid lavender, *Lavandula ×intermedia* 'Phenomenal' and 'Grosso', in the greenhouse based on four disease parameters^u

Treatment		Final foliage symptom severity ^v (%)	Foliage symptom AUDPC ^w			Total plant mass ^x (g)			Root isolation (%): Plants, bundles ^y
Species	Isolate	Trial 1+2	Trial 1+2	Trial 1	Trial 2	Trial 1+2	Trial 1	Trial 2	Trial 1+2
Non-inoculated control									
		27.6 a		99.1 a	174.1 a		136.6 a	96.4 ab	8.3/0.8 a
<i>P. tropicalis</i>	15-1194.D	53.7 b		292.8 b	226.7 ab		79.2 b	100.1 a	100.0/87.5 c
<i>P. nicotianae</i>	15-0450	65.5 bc		544.8 d	290.7 bc		37.3 cd	87.0 abc	100.0/98.3 d
<i>P. nicotianae</i>	15-1123.B	73.0 c		387.9 bc	368.7 cd		57.3 bc	59.8 cd	100.0/75.8 b
<i>P. palmivora</i>	17-0099	86.9 d		432.5 cd	463.5 d		23.6 d	41.3 d	100.0/99.2 d
3-way ANOVA ^z		<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>			<i>P</i> > <i>F</i>			<i>P</i> > <i>F</i>
Treatment		<0.0001	<0.0001			<0.0001			<0.0001
Trial		0.0027	0.0709			0.1202			0.8185
Cultivar		0.0551	<0.0001			0.1425			0.6465
Treatment × trial		0.0507	0.0011			0.0008			0.9947
Treatment × cultivar		0.0981	0.2565			0.6729			0.1487
Trial × cultivar		0.1161	0.1242			0.1632			0.1706

Treatment × trial × cultivar	0.0497	0.1701		0.1826		0.9456
2-way ANOVA ^z		<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>		<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>
Treatment		<0.0001	<0.0001		<0.0001	0.0005
Cultivar		<0.0001	0.0204		0.9582	0.0501
Treatment × cultivar		0.3763	0.1138		0.5596	0.2467

^u Two independent trials were conducted with 12 replicate plants ($n = 12$) per treatment in each trial; $n = 24$ when trials were combined. Differences between cultivars, when present, are not shown--see text.

^v Final foliage symptom severity was assessed as the percentage of the foliage on each plant with disease symptoms at the end of a trial.

^w Foliage symptoms were assessed weekly for 11 weeks, and then the area under the disease progress curve (AUDPC) was calculated.

^x The fresh mass of each plant was measured at the end of a trial.

^y At the end of each trial, isolation of the pathogen was attempted by placing five bundles of 5-10 root segments from each plant on selective medium. Data are mean percentages of plants and bundles that were positive for the pathogen; only data for bundles were analyzed statistically.

^z Two- and three-way analyses of variance (ANOVA); $P > F$: The probability of a greater F statistic occurring. Based on appropriate ANOVAs, means within columns were separated by individual pairwise comparisons ($P = 0.05$); means with the same letter are not significantly different.

Table 3.4. Experiment 2: Pathogenicity of two species of *Phytophthora* to English lavender, *Lavandula angustifolia*, in the greenhouse based on four disease parameters^u

Treatment		Final foliage symptom severity ^v (%)	Foliage symptom AUDPC ^w	Total plant mass ^x (g)			Root isolation (%): Plants, bundles ^y
Species	Isolate	Trial 1+2	Trial 1+2	Trial 1+2	Trial 1	Trial 2	Trial 1+2
Non-inoculated control		25.5 a	87.2 a	61.1 a	38.1 a	7.1/7.1 a	
<i>P. nicotianae</i>	16-0718	95.0 b	422.4 b	27.1 b	13.7 b	100.0/97.1 b	
<i>P. palmivora</i>	16-1107	97.9 b	425.8 b	25.7 bc	13.1 b	100.0/90.7 b	
<i>P. palmivora</i>	15-1125.R1	97.9 b	449.3 b	20.3 c	13.2 b	100.0/95.7 b	
2-way ANOVA ^z		<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>		<i>P</i> > <i>F</i>	
Treatment		<0.0001	<0.0001	<0.0001		<0.0001	
Trial		0.5779	0.0867	<0.0001		0.9406	
Treatment × trial		0.3497	0.171	0.0016		0.4181	
1-way ANOVA ^z				<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>		
Treatment				<0.0001	<0.0001		

^u Two independent trials were conducted with seven replicate plants ($n = 7$) per treatment in each trial; $n = 14$ when trials were combined.

- ^v Final foliage symptom severity was assessed as the percentage of the foliage on each plant with disease symptoms at the end of a trial.
- ^w Foliage symptoms were assessed weekly for 11 weeks, and then the area under the disease progress curve (AUDPC) was calculated.
- ^x The fresh mass of each plant was measured at the end of a trial.
- ^y At the end of each trial, isolation of the pathogen was attempted by placing five bundles of 5-10 root segments from each plant on selective medium. Data are mean percentages of plants and bundles that were positive for the pathogen; only data for bundles were analyzed statistically.
- ^z One- and two-way analyses of variance (ANOVA); $P > F$: The probability of a greater F statistic occurring. Based on appropriate ANOVAs, means within columns were separated by individual pairwise comparisons ($P = 0.05$); means with the same letter are not significantly different.

Table 3.5. Experiment 3: Pathogenicity of *Phytophthora nicotianae* to sweet lavender, *Lavandula heterophylla*, in the greenhouse based on four disease parameters^u

Treatment		Final foliage symptom severity ^v (%)	Foliage symptom AUDPC ^w			Total plant mass ^x (g)	Root isolation (%): Plants, bundles ^y
Species	Isolate	Trial 1+2	Trial 1+2	Trial 1	Trial 2	Trial 1+2	Trial 1+2
Non-inoculated							
control		13.8 a		103.0 a	109.5 a	24.3	0.0/0.0 a
<i>P. nicotianae</i>	15-0450	54.2 b		449.8 b	403.6 ab	17.6	100.0/92.5 b
<i>P. nicotianae</i>	17-0435	57.2 b		345.2 ab	916.5 b	14.6	100.0/95.0 b
2-way ANOVA ^z		<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>			<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>
Treatment		0.0247	0.0065			0.1291	<0.0001
Trial		0.7329	0.1171			0.3042	0.6806
Treatment × trial		0.1118	0.0447			0.1325	0.8269
1-way ANOVA ^z				<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>		
Treatment				0.0346	0.0456		

^u Two independent trials were conducted: In Trial 1, there were four replicate plants ($n = 4$) for each treatment; in Trial 2, $n = 4$ for the two inoculated treatments and $n = 2$ for the control; when trials were combined, $n = 8$ for the two inoculated treatments and $n = 6$ for the control.

^v Final foliage symptom severity was assessed as the percentage of the foliage on each plant with disease symptoms at the end of a trial.

- ^w Foliage symptoms were assessed weekly for 11 weeks, and then the area under the disease progress curve (AUDPC) was calculated.
- ^x The fresh mass of each plant was measured at the end of a trial.
- ^y At the end of each trial, isolation of the pathogen was attempted by placing five bundles of 5-10 root segments from each plant on selective medium. Data are mean percentages of plants and bundles that were positive for the pathogen; only data for bundles were analyzed statistically.
- ^z One-and two-way analyses of variance (ANOVA); $P > F$: The probability of a greater F statistic occurring. Based on appropriate ANOVAs, means within columns were separated by individual pairwise comparisons ($P = 0.05$); means with the same letter are not significantly different.

Table 3.6. Experiment 5: Pathogenicity of *Phytophthora citrophthora* to English lavender, *Lavandula angustifolia*, in the greenhouse based on four disease parameters^u

Treatment	Final foliage symptom severity ^v (%)			Foliage symptom	Total plant mass ^x (g)	Root isolation (%):
	Trial 1+2	Trial 1	Trial 2	AUDPC ^w	Trial 1+2	Plants, bundles ^y
				Trial 1+2	Trial 1+2	Trial 1+2
Non-inoculated control		4.1 a	60.5	136.5 a	80.3 a	0.0/0.0 a
<i>P. citrophthora</i>		91.4 b	72.6	507.8 b	34.3 b	87.5/85.0 b
2-way ANOVA ^z	$P > F$			$P > F$	$P > F$	$P > F$
Treatment	0.0021			0.0066	0.0096	<0.0001
Trial	0.1665			0.1962	0.8341	0.2315
Treatment × trial	0.0123			0.7685	0.7736	0.2315
1-way ANOVA ^z		$P > F$	$P > F$			
Treatment		<0.0001	0.6384			

^u Two independent trials were conducted with four replicate plants ($n = 4$) per treatment in each trial; $n = 8$ when trials were combined.

^v Final foliage symptom severity was assessed as the percentage of the foliage on each plant with disease symptoms at the end of a trial.

^w Foliage symptoms were assessed weekly for 11 weeks, and then the area under the disease progress curve (AUDPC) was calculated.

^x The fresh mass of each plant was measured at the end of a trial.

^y At the end of each trial, isolation of the pathogen was attempted by placing five bundles of 5-10 root segments from each plant on selective medium. Data are mean percentages of plants and bundles that were positive for the pathogen; only data for bundles were analyzed statistically.

^z One-and two-way analyses of variance (ANOVA); $P > F$: The probability of a greater F statistic occurring. Based on appropriate ANOVAs, means within columns were separated by individual pairwise comparisons ($P = 0.05$); means with the same letter are not significantly different.

Table 3.7. Experiment 7: Pathogenicity of *Phytophthora cinnamomi* to hybrid lavender, *Lavandula ×intermedia*, in the greenhouse based on four disease parameters^u

Treatment	Final foliage symptom severity ^v (%)	Foliage symptom AUDPC ^w	Total plant mass ^x (g)	Root isolation (%): Plants, bundles ^y		
	Trial 1+2	Trial 1+2	Trial 1+2	Trial 1+2	Trial 1	Trial 2
Non-inoculated control	0.0 a	0.0 a	176.2 a	0.0/0.0 a	0.0/0.0 a	
<i>P. cinnamomi</i>	96.9 b	656.1 b	11.5 b	100.0/90.0 b	100.0/40.0 b	
2-way ANOVA ^z	$P > F$	$P > F$	$P > F$	$P > F$		
Treatment	<0.0001	<0.0001	<0.0001	<0.0001		
Trial	0.525	0.5577	0.2322	0.0003		
Treatment × trial	0.525	0.5577	0.2094	0.0003		
1-way ANOVA ^z					$P > F$	$P > F$
Treatment					<0.0001	0.0027

^u Two independent trials were conducted with four replicate plants ($n = 4$) per treatment in each trial; $n = 8$ when trials were combined.

^v Final foliage symptom severity was assessed as the percentage of the foliage on each plant with disease symptoms at the end of a trial.

^w Foliage symptoms were assessed weekly for 11 weeks, and then the area under the disease progress curve (AUDPC) was calculated.

^x The fresh mass of each plant was measured at the end of a trial.

^y At the end of each trial, isolation of the pathogen was attempted by placing five bundles of 5-10 root segments from each plant on selective medium. Data are mean percentages of plants and bundles that were positive for the pathogen; only data for bundles were analyzed statistically.

^z One-and two-way analyses of variance (ANOVA); $P > F$: The probability of a greater F statistic occurring. Based on appropriate ANOVAs, means within columns were separated by individual pairwise comparisons ($P = 0.05$); means with the same letter are not significantly different.

Table 3.8. Experiment 11: Pathogenicity of three species of *Phytophthora* to English lavender, *Lavandula angustifolia*, in the greenhouse based on four disease parameters^u

Treatment	Final foliage symptom severity ^v (%)			Foliage symptom AUDPC ^w		Total plant mass ^x (g)			Root isolation (%): Plants, bundles ^y	
	Trial 1+2	Trial 1	Trial 2	Trial 1+2	Trial 1+2	Trial 1	Trial 2	Trial 1+2	Trial 1+2	
Non-inoculated control		22.2	18.0 a	88.5 a		12.7	17.0 b		0.0/0.0 a	
<i>P. megasperma</i>		24.7	9.7 a	82.0 a		10.2	30.8 a		25.0/5.0 a	
<i>P. cryptogea</i>		49.4	70.2 b	306.2 b		10.2	8.1 b		91.7/56.7 b	
<i>P. drechsleri</i>		46.3	92.6 b	321.4 b		8.0	5.3 b		50.0/13.3 a	
2-way ANOVA ^z	<i>P</i> > <i>F</i>			<i>P</i> > <i>F</i>		<i>P</i> > <i>F</i>			<i>P</i> > <i>F</i>	
Treatment	<0.0001			0.0003		0.0042			<0.0001	
Trial	0.1447			0.7312		0.0726			0.435	
Treatment × trial	0.0474			0.0566		0.0149			0.8792	
1-way ANOVA ^z		<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>			<i>P</i> > <i>F</i>	<i>P</i> > <i>F</i>			
Treatment		0.3438	<0.0001			0.7232	0.0041			

^u Two independent trials were conducted with six replicate plants ($n = 6$) per treatment in each trial; $n = 12$ when trials were combined.

^v Final foliage symptom severity was assessed as the percentage of the foliage on each plant with disease symptoms at the end of a trial.

^w Foliage symptoms were assessed weekly for 11 weeks, and then the area under the disease progress curve (AUDPC) was calculated.

^x The fresh mass of each plant was measured at the end of a trial.

- ^y At the end of each trial, isolation of the pathogen was attempted by placing five bundles of 5-10 root segments from each plant on selective medium. Data are mean percentages of plants and bundles that were positive for the pathogen; only data for bundles were analyzed statistically.
- ^z One-and two-way analyses of variance (ANOVA); $P > F$: The probability of a greater F statistic occurring. Based on appropriate ANOVAs, means within columns were separated by individual pairwise comparisons ($P = 0.05$); means with the same letter are not significantly different.

CHAPTER 4

AN EVALUATION OF 12 FUNGICIDES FOR MANAGING PHYTOPHTHORA ROOT AND CROWN ROT ON ENGLISH LAVENDER IN THE GREENHOUSE

Abstract

Phytophthora root and crown rot (PRCR) of lavender is an emerging disease that has become one of the most significant threats to the lavender industry worldwide. Primarily caused by *Phytophthora nicotianae*, the disease impacts multiple species of lavender, and the causal agent is spread largely through the nursery trade. This study examined 12 fungicides currently labeled for diseases caused by species of *Phytophthora* and other oomycetes for efficacy against PRCR using artificially inoculated English lavender (*Lavandula angustifolia*) plants in a research greenhouse. Some fungicide treatments significantly reduced disease symptoms while maintaining plant size, but there was considerable variation in efficacy among the fungicide products. The best performing products were phosphonates, and a product containing a relatively new active ingredient, oxathiapiprolin. An industry standard, mefenoxam, the active ingredient in Subdue Maxx, was also effective but to a lesser degree. The results indicate a strong potential to manage PRCR on lavender with commercially available fungicides, particularly phosphonate products.

Introduction

Reports of *Phytophthora nicotianae* attacking nursery-grown English lavender plants (*Lavandula angustifolia*) first occurred in 1991 in Maryland, USA (Putnam 1991). Since that time, the disease, now known as Phytophthora root and crown rot (PRCR), has been found to be

caused by numerous species of *Phytophthora* and to impact several species of lavender (Cho and Shin 2004; Dlugos and Jeffers 2018; Farr and Rossman 2021; Jung et al. 2016). The most common and widely occurring species causing this disease in the United States is *P. nicotianae* (Dlugos and Jeffers 2021).

Lavender is in the genus *Lavandula* and the family Lamiaceae/Labiatae—a family that includes over 6,900 species of herbs, shrubs, and trees (USDA 2021; Zomlefer 1994). Lavender is native to regions with Mediterranean climates—including portions of Europe, Africa, Asia, and the Middle East (Upson 2002). There are 32 species and additional hybrids in the genus (Upson 2002), with English lavender being one of the most common (McCoy and Davis 2021), hardy (Adam 2006), and economically important species (Singh et al. 2007). The economic impact of lavender production in the United States has not been reported, but it is quickly becoming popular for ornamental plantings and farms that focus on cut and dried flowers, production of essential oil, and agritourism (Adam 2006).

Lavender is typically planted as rooted cuttings or young plants, which are produced in greenhouses and nurseries, by means of vegetative propagation (Adam 2006; Naghibi et al. 2005). A concern with this is that nurseries, and the ornamental plant trade in general, have a history of moving plant pathogens in part because a single nursery can cover one to many hectares and contain hundreds of species of plants from various locations (Jones and Baker 2007; Park and Grünwald 2012). This can lead to nurseries being sources of inoculum for *Phytophthora* species, including *P. nicotianae* (Bienapfl and Balci 2014; Schwingle et al. 2007). After introduction to other nurseries, landscapes, or fields, *Phytophthora* species can become established and persist in soils and container mixes (Jeffers et al. 2010), and these pathogens can be disseminated locally, e.g., within a field of lavender, by moving contaminated plant material

and soil or by splashing and flowing water that can move motile zoospores (Erwin and Ribeiro 1996).

P. nicotianae is one of the most studied species of *Phytophthora* (Kamoun et al. 2015). It was first described in 1896 causing a disease on tobacco and now is known to be pathogenic to plants in 255 genera and 90 families, with a cosmopolitan distribution (Cline et al. 2008; Erwin and Ribeiro 1996; Farr and Rossman 2021). In addition to causing problems on English lavender in the United States, it has also been found affecting lavender plants in Spain (Álvarez et al. 2007), Italy (Davino et al. 2002; Faedda et al. 2013), Bulgaria (Nakova 2011), and Greece (Erwin and Ribeiro 1996). Symptoms of infection include grey discoloration and wilting of the foliage, discoloration and rotting of the roots, vascular discoloration, and plant mortality (Putnam 1991).

Managing plant diseases, including those caused by *Phytophthora* species, can be summed up as a four-part plan of pathogen exclusion, avoidance of conducive environmental conditions, pathogen eradication, and plant protection (Agrios 2005; Jarvis 1992; Ludowici et al. 2013; Schumann and D'Arcy 2009). For established pathogens, management often relies, in part, on plant protection, and the most common strategy for diseases caused by fungi and oomycetes, like species of *Phytophthora*, is the application of chemical fungicides (Agrios 2005; Erwin and Ribeiro 1996; Jarvis 1992).

Some fungicides can prevent infection and symptom development and can limit pathogen colonization of host tissue and, therefore, mask pathogen presence and detection (Scott et al. 2013; Shishkoff 2014). The fungicide active ingredients metalaxyl and mefenoxam, commonly used against oomycetes, are actually fungistatic and not fungicidal (Brasier and Jung 2006; Linderman and Davis 2008; Olson et al. 2013). They inhibit pathogen activity without killing

the oomycete pathogens and can result in pathogens being spread in infected but asymptomatic plants and infested soil (Brasier 2005). For this reason, nursery use of oomycete-specific fungicides without proper sanitation leads to a spread of *Phytophthora* species within and from nurseries (Drenth and Guest 2013).

The objective of this study was to examine the efficacy of currently registered and commercially available fungicide products for managing *Phytophthora* root and crown rot on lavender plants in a greenhouse. Information from this study will be used to identify fungicides that should be tested in lavender fields where PRCR has become a serious problem (Dlugos and Jeffers 2018, 2021). Products that specifically target diseases caused by species of *Phytophthora* and that had labels for application to ornamental plants or herbs were selected. Efficacy was evaluated by assessing foliage symptom severity, area under disease progress curves (AUDPC), and fresh plant mass.

Materials and Methods

The greenhouse and plants. Two trials of each of two independent experiments were conducted during the summers of 2018 (Experiment 1) and 2019 (Experiment 2) in a research greenhouse in the Biosystems Research Complex at Clemson University, Clemson, South Carolina. Trial durations varied from 38 to 42 days (Table 4.1). In Experiment 1, there were 4 weeks between initiation of Trials 1 and 2, and there was 1 week between the initiation of the two trials in Experiment 2 (Table 4.1). During the four trials of these two experiments, environmental conditions in the greenhouse, which were controlled and measured by a central computerized system (Argus Controls, Conviron, Winnipeg, Manitoba, Canada), were relatively uniform based on measurements taken every 15 min. The mean greenhouse temperature during

each trial ranged from 23.2°C to 24.4°C with a 9 to 13°C difference between the minimum and maximum temperatures in the four trials (Table 4.1). The mean relative humidity during each trial ranged from 69.2% to 77.9% for each trial; the difference between the minimum and maximum in the four trials ranged from 40 to 56% (Table 4.1). A 16-h photoperiod was maintained throughout all four trials with artificial lighting coming on when outside light energy was below 350 W/m².

English lavender plants (*Lavandula angustifolia*) were used in both experiments. ‘Hidcote Blue’ plants were used in Experiment 1, and ‘Hidcote’ plants were used in Experiment 2 because ‘Hidcote Blue’ plants were not available. However, ‘Hidcote Blue’ is another name sometimes applied to ‘Hidcote’ (Lis-Balchin 2002), so the two cultivars were actually the same. All plants came from the same wholesale nursery (Creek Hill Nursery, Leola, PA). Plants were received as plugs in 72-cell trays; each cell measured 3.8 × 3.8 × 5.7 cm. A subsample of the plugs (approximately 30% to 50%) for each trial were tested for presence of *Phytophthora* species by a non-destructive baiting bioassay to confirm that plugs were not contaminated with these pathogens prior to use. Individual plugs were transplanted into 1.3-liter pots (15 cm top diameter, 11 cm bottom diameter, 11.5 cm tall); each pot contained 1 liter of a soilless peat- and bark-based container mix (Fafard 3B; Sun Gro Horticulture, Agawam, MA). Plants were placed on a bench in the greenhouse, watered overhead by hand as needed, and fertilized weekly with a fertilizer solution delivering 100 ppm of nitrogen (PowerPak 20-20-20 [N-P-K] Soluble Fertilizer with Minor Elements; Southern Agricultural Insecticides, Inc., Hendersonville, NC).

Fungicides and treatments. A total of 11 commercially available fungicides and one experimental formulation of a commercially available fungicide were evaluated in the two experiments; nine fungicides were evaluated in Experiment 1, and five fungicides were evaluated

in Experiment 2 (Table 4.2). Two fungicides were used in both experiments and served as standards. The 11 commercially available fungicides are registered for use on ornamental plants or herbs and labeled to manage diseases caused by species of *Phytophthora* and other oomycetes. These fungicides represented a diverse array of the active ingredients available for managing oomycete diseases—including nine different chemical groups recognized by the Fungicide Resistance Action Committee (<https://www.frac.info/home>) (Table 4.2). In addition to the fungicides, two non-treated control treatments were used in each trial—an inoculated control and a non-inoculated control (see below), so there were 11 and seven treatments in Experiment 1 and Experiment 2, respectively. Six replicate plants were assigned to each treatment in each trial based on size and vigor so that each treatment had a similar assortment of plants; then, plants in all treatments were arranged in a completely randomized design on the greenhouse bench. All products except Reliant Trifecta were applied at label rates by making a single soil drench application to individual pots (Table 4.2). For these 11 products, 3 liters of each fungicide suspension was prepared, and 400 ml of suspension was poured around each plant, which was enough to soak the root zone and container mix in each pot with a slight amount (~10 to 20 ml) of runoff from the bottom of a pot. Plants were not watered for at least 24 h after fungicide application. Reliant Trifecta, an experimental granular formulation, was applied dry to the soil surface at a rate recommended by the manufacturer (1 g per pot; Table 4.2); it then was watered into the container mix by gently pouring 400 ml of water over the surface in each pot.

Isolates and inoculation. All plants were inoculated 4 days after treatments were applied, except for the plants in the non-inoculated control. Three isolates of *P. nicotianae* were used as inoculum: PPC.15-0718, PPC.16-0718, and SC.4284. These were isolated from diseased lavender plants from South Carolina, Pennsylvania, and Virginia, respectively, that had been

submitted for diagnosis to the Clemson Plant and Pest Diagnostic Clinic or the S. N. Jeffers lab at Clemson University. All isolates were recovered from roots of *L. angustifolia* 'Hidcote' plants with typical symptoms of PRCR and now are stored in a permanent collection maintained by S. N. Jeffers at Clemson University. Because some isolates of *P. nicotianae* are known to be resistant to the fungicide mefenoxam, these three isolates were tested in vitro and found to be sensitive to mefenoxam using a standard method (Olson et al. 2013). Inoculum was prepared by independently growing each isolate on sterile vermiculite moistened with 10% V8 juice broth (2:1, v:v) in glass bottles (Jeffers 2015b; Roiger and Jeffers 1991). Bottles were placed in the dark at 25°C for 2 weeks, so each isolate could thoroughly colonize the vermiculate in a bottle. After 10 to 12 days of incubation, a small aliquot (1 to 2 ml) of vermiculite from each bottle was spread on a plate of clarified V8 juice agar (Jeffers 2015b) to ensure purity and uniform colonization. Equal amounts of colonized vermiculite from each of the three isolates were combined and thoroughly mixed to prepare a composite batch of inoculum.

Each plant was inoculated by spreading approximately 10 ml of inoculum on the surface of each pot, and then the inoculum was mixed by hand into the upper 1 cm of the container mix. A 1-cm layer of fresh container mix was added to each pot to cover the inoculum, and all pots were gently watered to incorporate the inoculum and prevent desiccation. Plants in the non-inoculated control treatment did not receive inoculum; however, additional container mix was added to each pot, and these pots were also gently watered. After inoculation, each pot was placed in a plastic saucer (14 cm diameter, 3.5 cm deep), and plants were watered from the bottom, by adding water to the plastic saucers, for the remainder of the experiment. This was done to keep the container mix in each pot at or near field capacity throughout the trial, which

promoted disease development and minimized splashing of treated container mix and pathogen propagules among pots.

Data collection and analysis. Each trial was run for a period of approximately 5.5 to 6 weeks post-inoculation (Table 4.1). Plants were evaluated weekly for foliage symptom severity, which was scored on a 0 to 5 scale based on the percentage of foliage showing symptoms of gray discoloration, wilting, or necrosis: 0 (0% of foliage symptomatic; no symptoms), 1 (1 to 10%), 2 (11 to 50%), 3 (51 to 90%), 4 (91 to 99%), and 5 (100% of foliage symptomatic; mortality). At the end of each trial, after each plant was evaluated for final foliage symptom severity, plants were harvested independently and separated into above-ground (shoot) and below-ground (root) material. Roots were washed free of soil and debris and blotted dry, and fresh root and shoot masses were weighed. For all analyses, weekly and final foliage symptom severity scores were converted to the midpoint of each range—e.g., a score of 1 (1 to 10%) was converted to 5.5%. Area under the disease progress curve (AUDPC) was calculated based on weekly and final foliage evaluations using the method reported by Shaner and Finney (1977). AUDPC is a relative measure of the amount of disease over time. To estimate the effect of fungicides on infection, roots from two representative plants from each treatment in each trial were used for isolation after roots were weighed. Five root bundles from each plant were embedded in PARPH-V8 selective medium (Jeffers 2015a) to isolate the pathogen. Root bundles were composed of five to ten segments, approximately 1 to 2 cm in length, of fibrous feeder roots. Isolation plates were held at 25°C in the dark for 7 days and examined regularly for typical hyphae of *P. nicotianae*.

The data initially were examined using one-way analysis of variance (ANOVA) along with a Levene's and Shapiro-Wilk's tests for variance and normality assumptions (JMP Pro; Cary, NC). Results from analyses using data transformations and non-parametric tests were

consistent with standard analyses, so standard parametric analyses were used for all analyses. Trials of each of the two experiments were analyzed together with blocking by trial as a factor. Because there were significant ($P < 0.05$) treatment by trial interactions and changes in the rank ordering of treatment means between trials, trials in Experiment 1 were analyzed separately. However, in Experiment 2, treatment trial by interactions were not significant and the rank ordering of treatment means were consistent between trials, so these two trials were combined and analyzed together. Based on the nature of the response variables, we determined a 1-way ANOVA with a generalized linear model (SAS; Cary, NC) would be the most appropriate analyses for the data and would provide the most accurate and meaningful results. When the effect of treatments was significant ($P < 0.05$) in an analysis, means were separated by individual pairwise comparisons.

Results

In this study, two experiments were conducted to evaluate 12 fungicides, which target diseases caused by *Phytophthora* species, for managing PRCR on lavender (Table 4.2). All products could not be evaluated at one time because of limitations on the number of plants available and greenhouse bench space. Therefore, nine fungicides were evaluated in Experiment 1, including three phosphonate products, and three additional products plus two products from Experiment 1 were evaluated in Experiment 2 (Table 4.2). The two products used in both experiments served as standards to demonstrate consistency between experiments, and these were Reliant and Subdue Maxx. Reliant was the most effective fungicide in both experiments; however, Subdue Maxx was more effective at managing PRCR in Experiment 2 than in

Experiment 1 (Figs 1 and 2), but this does not prevent the results from the two experiments from being compared and interpreted together.

Three disease parameters were used to evaluate efficacy: Final foliage symptom severity, AUDPC based the weekly progress of foliage symptom development, and fresh plant mass at the end of each trial. Although root and shoot masses were weighed separately, these weights were combined for analysis because this gave the best separation of treatments. However, fresh plant mass did not prove to be the most accurate measure of treatment efficacy—perhaps because lavender plants produce relatively short, narrow leaves; therefore, the difference in mass between healthy and diseased leaves would likely not be great.

Experiment 1. In this experiment, environmental conditions in the greenhouse were very conducive to disease development over the course of both trials because plants in the non-inoculated control treatments had 100% of the foliage showing symptoms at the ends of the two trials (Figs. 1A and 2A). Foliage symptoms were not observed on plants in the non-inoculated control treatment in Trial 1, and non-inoculated plants in Trial 2 had only 6% of the foliage showing symptoms at the end of the trial.

The two trials of this experiment were analyzed separately because statistical tests determined the trials should not be combined. The significant treatment by trial interaction and changing rank order between trials may have been due to the difference in age between the plants in Trial 1 and Trial 2. Plants for both trials came from a single shipment of lavender plants, but the trials were started 4 weeks apart (Table 4.1); therefore, plants in Trial 1 were much younger and likely more susceptible than the plants in Trial 2. Data on fresh plant mass indicated plants in Trial 1 were considerably smaller than those in Trial 2 (Figs. 1C and 2C), and AUDPC data suggest disease severity was greater on the younger plants in Trial 1 than on the older plants in

Trial 2 (Figs. 1B and 2B). These differences in plant age and possibly susceptibility provide additional justification for analyzing these two trials independently. It is interesting that plants treated with the three phosphonate fungicides (Reliant, Reliant Trifecta, and Areca) had numerically greater fresh plant mass than plants that were not inoculated in both trials; however, this greater mass was not significant (Figs. 1C and 2C).

In Trial 1, there were significant differences among the 11 treatments in all three disease parameters evaluated based on *F* statistics in one-way ANOVAs (Table 4.3). Treatment means for the three disease parameters in Trial 1 are compared and separated in the graphs in Figure 4.1. Based on final foliage symptom severity and AUDPC, the phosphonate product Reliant provided the best level of disease management by allowing very little development of foliage symptoms. Three other fungicides—Segovis, Areca, and Reliant Trifecta—also provided effective disease management but at a level significantly less than Reliant. Two of these products, Areca and Reliant Trifecta, also are phosphonates. Four of the fungicides—Terrazole, Adorn, Segway O, and Subdue MAXX—provided no significant level of disease management based on final foliage symptom severity and AUDPC because these means were similar to the means for the inoculated, non-treated control plants. The fungicide Micora did provide a moderate level of disease management based on the development of foliage symptoms over time.

Several treatments had plants that died during this trial. Mortality was first observed in week 4 on plants treated with Terrazole and Segway O and on plants that were inoculated and not treated. By week 5, mortality was 100% on plants in the inoculated control treatment and was 83%, 33%, and 17% on plants treated with Terrazole, Segway O, and Adorn, respectively. When roots from representative plants were tested by isolation on PARPH-V8 medium, *P. nicotianae* was not detected on plants in the non-inoculated control treatment or on plants treated

with Segovis and Terrazole, but *P. nicotianae* was detected in the roots of plants treated with the other seven fungicides or not treated but inoculated.

In Trial 2 of this experiment, results overall were similar to those in Trial 1, but rank order of the treatments was different. In this trial, there were significant differences among the 11 treatments in two of the three disease parameters, final foliage symptom severity and AUDPC, based on *F* statistics in one-way ANOVAs (Table 4.3). There was no significant difference ($P = 0.5252$) among treatments for fresh plant mass (Table 4.3). Treatment means for the three disease parameters in this trial are compared and separated in the graphs in Figure 4.2. In this trial, the three phosphonate fungicides (Areca, Reliant, and Reliant Trifecta) provided the best level of disease management with foliage symptom severity and AUDPC values being statistically similar to those for the non-inoculated control treatment. Based on final foliage symptom severity, Segovis also provided a significant level of disease management, but five fungicides—Terrazole, Micora, Subdue Maxx, Adorn, and Segway O—were not effective at managing PRCR because the treatment means for these fungicides were not significantly different from that for the inoculated control. However, when AUDPC was evaluated for these six fungicides, Segovis, Adorn, and Segway O significantly reduced disease progress compared to that on inoculated control plants. In this trial, mortality was observed in only one treatment, presumably because the plants were older and less susceptible than those in Trial 1. In week 5, 17% of the plants treated with Micora were dead. When two representative plants from each treatment were tested for the pathogen by isolation, *P. nicotianae* was detected in roots from all treatments except Segovis and the non-inoculated control.

Experiment 2. As in Experiment 1, greenhouse conditions were very conducive for disease development. On inoculated control plants, 97% of the foliage showed symptoms by the

end of the trials and only 6% of the foliage on non-inoculated plants showed symptoms (Fig. 3A). Trials in this experiment were combined for analysis because of the consistent results between the two trials. There were significant differences among the seven treatments in all three disease parameters based on *F* statistics in one-way ANOVAs (Table 4.3). Treatment means for the three disease parameters in this experiment are compared and separated in the graphs in Figure 4.3. Based on final foliage symptom severity and AUDPC, three fungicides were most effective at managing PRCR on lavender plants—the two phosphonate products, Reliant and Aliette, and Subdue Maxx. The other two fungicides, Orvego and Banol, were not effective at managing this disease because means for these two treatments were not significantly different from those for the inoculated control. When fresh plant mass was weighed, Aliette, Reliant, and Subdue Maxx also produced the largest plants, and their masses were similar to the mass of the plants in the non-inoculated control treatment. Masses of plants treated with Banol and Orvego weighed the least, and these masses were not significantly different from the mass of plants that were inoculated and not treated. However, the mass of plants treated with Subdue Maxx also was not significantly different from the mass of inoculated plants.

Some mortality also was observed on plants in this experiment. Dead plants were first observed in week 4, and, at week 5, 42% of inoculated control plants, 25% of Orvego-treated plants, and 8% of Banol-treated plants had died. Mortality was not observed on plants in any of the other treatments. When two representative plants from each treatment were tested for *P. nicotianae* by isolation, the pathogen was detected in roots from at least one plant in all treatments except the non-inoculated control.

Discussion

Throughout the four trials of both experiments, registered fungicide products were shown to have a significant impact on the development of PRCR on English lavender plants in the greenhouse, with some products consistently performing better than others. Some products were successful both at limiting disease development and at maintaining fresh plant mass, with a trend toward even increasing fresh mass over that of the non-inoculated, non-treated control plants. One product, Terrazole, one of the oldest registered products for managing *Phytophthora* diseases, performed poorly in all trials and consistently had little effect on PRCR development on lavender plants in the greenhouse. In addition, mortality of plants treated with Terrazole was more common than it was on plants treated with any the other product. Only the inoculated control plants had more mortality in these experiments. The observed mortality on Terrazole-treated lavender plants suggested a potential for phytotoxicity on lavender that warrants further investigation. There was no evidence of phytotoxicity with any of the other ten fungicide products used in this study.

By far, the best performing products in this study were what are collectively referred to as phosphonates—Aliette, Areca, Reliant, and Reliant Trifecta (Table 4.2; Landschoot 2016). Reliant Trifecta is an experimental granular formulation of Reliant that was developed to give growers another application option. It can be sprinkled on the soil surface and watered into the soil instead of being applied as a soil drench. Phosphonate products consistently limited disease development and kept symptom severity to a minimum while also showing a trend toward increasing fresh plant mass compared to plants in the non-inoculated control treatment. Phosphonates are known to accumulate in plant tissue and be metabolized, persisting for weeks or even months (Guest and Grant 1991; McDonald et al. 2001; Ouimette and Coffey 1989;

Rohrbach and Schenck 1985; Smillie et al. 1989). The products are systemic with transport in both the xylem and phloem (Guest and Grant 1991; Ouimette and Coffey 1989; Ouimette and Coffey 1990) and are reported to work against oomycetes by multiple modes of action (Guest and Grant 1991; Smillie et al. 1989). It has also been debated whether physiological responses occur in plants to stimulate host defenses (Guest and Grant 1991; Rouhier et al. 1993; Smillie et al. 1989). Regardless of the mechanisms involved, the success of phosphonates is not the same in all pathosystems (Guest and Grant 1991).

It is unknown what caused the trend toward increased fresh plant mass after a single soil application with the phosphonate products. While not statistically significant, in each trial, plants treated with phosphonate products had fresh plant masses that were numerically greater than or equivalent to the fresh mass of control plants that were not inoculated or treated. For example, in Trial 1 of Experiment 1, plants treated with reliant had a mass (29.9 g) that was 1.7× greater than the mass (17.6 g) of the non-inoculated control plants. In addition, the phosphonate-treated plants appeared visually more robust. While phosphonates are often labeled as fertilizers, this use is controversial. Phosphonates were tested as early as the 1930s but were found not to be suitable as fertilizers because only delayed enhanced growth was observed (Guest and Grant 1991; Landschoot 2016). Research has determined that phosphonates are not a suitable source of phosphorous for plants, and any increase in leaf tissue nutrient content was due to increased concentration in what were smaller tissues because of less growth (Ratjen and Gerendás 2009). Positive benefits are still documented, however. In citrus production, foliar applications of potassium phosphite appear to increase fruit yield per tree (Lovatt 1999). Phosphonates have also been reported to increase turf quality without explanation (Landschoot 2016). However, the

reverse has also been demonstrated; Aliette was found to have a negative impact on root and shoot growth in onion (Sukarno et al. 1993).

Subdue Maxx, with the active ingredient mefenoxam, has long been one of the most popular compounds for managing diseases caused by oomycetes, including species of *Phytophthora* (Agrios 2005; Herman et al. 2019; Olson et al. 2013). In this study, the efficacy of Subdue Maxx was inconsistent and varied considerably between the two experiments. In Experiment 1, it did not effectively manage PRCR, but, in Experiment 2, its efficacy was similar to that of the two phosphonate products. This is important information to know for PRCR on lavender because it is known that the effectiveness of fungicides in some host-pathogen relationships does not equally translate to all (Linderman and Davis 2008). Although inoculations were made only four days post-treatment, that should not have impacted the efficacy of the active ingredient. Mefenoxam products are known to be taken up by the roots and are capable of working relatively quickly—for example, a soil drench with Ridomil (a similar product containing the active ingredient mefenoxam) provided protection of tomato plants in 1-liter pots within one hour (Cohen et al. 1979). It also protected the tomato plants when applied two days after inoculation (Cohen et al. 1979). It is also unlikely that the duration of the trial had a negative impact on mefenoxam efficacy because mefenoxam is extremely long lasting with the effectiveness of Subdue Maxx lasting up to 6 weeks (Linderman and Davis 2008). Also, the current label recommends application intervals of at least 1 to 2 months.

Segovis was repeatedly one of the most effective products in both trials of Experiment 1. This new product with the active ingredient oxathiapiprolin, which has a unique mode of action, has been very successful at managing other *Phytophthora* diseases, including black shank on tobacco caused by *P. nicotianae* (Ji et al. 2014) and late blight on tomato caused by *P. infestans*

(Cohen et al. 2018). This product also prevented isolation of *P. nicotianae* from lavender roots on representative plants in one trial. Isolates of *Phytophthora* species that developed insensitivity to mefenoxam were shown to be sensitive to oxathiapiprolin because the two fungicides have different modes of action, and there was with no evidence of cross-resistance between these two fungicides (Bittner and Mila 2016; Cohen et al. 2018). However, Bittner and Mila (2016) suggested resistance to oxathiapiprolin could be possible with overuse of fungicides with this active ingredient.

Unfortunately, the other five fungicides evaluated in this study—Adorn, Banol, Micora, Orvego, and Segway O (each with a different active ingredient)—were not effective at managing PRCR on lavender plants in the greenhouse even though these products are labeled to manage diseases on ornamental crops caused by *Phytophthora* species. Some may be more effective when applied as foliar sprays to manage leaf and stem diseases, or they may need to be applied more frequently. The three isolates of *P. nicotianae* used in this study were tested for sensitivity to mefenoxam, but they were not tested for sensitivity to the active ingredients in these five fungicides. Therefore, fungicide insensitivity could be one reason for the lack of efficacy.

Conclusions and Significance

In this study, we identified commercially available fungicides that were effective at managing *Phytophthora* root and crown rot on English lavender plants (*L. angustifolia* ‘Hidcote Blue’ and ‘Hidcote’) in the greenhouse. Phosphonate products with two different active ingredients (aluminum tris [O-ethyl phosphonate] also known as fosetyl-Al and potassium salts of phosphorous acid) were very effective, and Segovis, with the active ingredient oxathiapiprolin, also was effective. Subdue Maxx, with mefenoxam as the active ingredient, was

effective in one experiment but not in the other one. PRCR is much more of a problem in the field than in the greenhouse (Dlugos and Jeffers 2018, 2021); therefore, based on the results in this greenhouse study, phosphonate products and products with oxathiapiprolin and mefenoxam as active ingredients should be evaluated for efficacy on lavender plants growing in the field.

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Table 4.1. Dates, durations, and environmental conditions of four trials conducted in a greenhouse to evaluate the efficacy of fungicides for managing *Phytophthora nicotianae* on English lavender plants

Experiment no.	Trial dates ^a			Trial duration (days)	Temperature (°C) ^b				Relative humidity (%) ^b			
	Trial	Start	End		Mean	SD	Min	Max	Mean	SD	Min	Max
1	1	7/19/2018	8/30/2018	42	24.4	2.2	19.2	32.1	77.6	8.2	38.6	91.3
	2	8/17/2018	9/25/2018	39	24.3	1.9	19.2	28.3	77.9	7.4	51.0	90.7
2	1	5/13/2019	6/20/2019	38	23.2	2.1	14.9	28.3	69.2	12.0	35.1	91.3
	2	5/20/2019	6/28/2019	39	23.5	2.0	18.3	28.3	71.9	11.4	35.1	91.3

^a Start dates are when plants were treated with fungicides; end dates are when plants were harvested for data collection.

^b Temperature and relative humidity during each trial were summarized as the mean, standard deviation (SD), minimum (Min), and maximum (Max) values based on data collected every 15 min.

Table 4.2. Twelve fungicides that target diseases caused by *Phytophthora* species and other oomycetes were evaluated for efficacy at managing *Phytophthora nicotianae* on English lavender plants in a greenhouse

Experiment no. ^a	Fungicide	Active ingredient	Company	Label rate (per 100 gal) ^b	Use rate (per liter) ^b	FRAC Code ^c	FRAC group name ^c
1	Adorn	Fluopicolide	Valent U.S.A Corporation	4 fl oz	0.31 ml	43	Benzamides
2	Aliette	Aluminum tris (O-ethyl phosphonate)	Bayer Environmental Science	12.8 oz	1.0 g	P07	Phosphonates
1	Areca	Aluminum tris (O-ethyl phosphonate)	OHP, Inc.	12.8 oz	1.0 g	P07	Phosphonates
2	Banol	Propamocarb hydrochloride	Bayer Environmental Science	25 fl oz	1.95 ml	28	Carbamates
1	Micora	Mandipropamid	Syngenta Crop Protection, LLC	8 fl oz	0.62 ml	40	Carboxylic acid amides
2	Orvego	Ametoctradin + dimethomorph	BASF Corporation	14 fl oz	1.09 ml	45 + 40	Quinone outside Inhibitors, stigmatellin binding type + Carboxylic acid amides
1, 2	Reliant	Potassium salts of phosphorous acid	Quest Products	12.8 fl oz	1.0 ml	P07	Phosphonates
1	Reliant Trifecta	Potassium salts of phosphorous acid	Quest Products	... ^d	1.0 g/pot ^d	P07	Phosphonates
1	Segovis	Oxathiapiprolin	Syngenta Crop Protection, LLC	3.2 fl oz	0.25 ml	49	Oxysterol binding protein homologue inhibitors
1	Segway O	Cyazofamid	OHP, Inc.	6 fl oz	0.47 ml	21	Quinone inside inhibitors
1, 2	Subdue MAXX	Mefenoxam	Syngenta Crop Protection, LLC	2 fl oz	0.16 ml	4	Phenylamides
1	Terrazole	Etridiazole	OHP, Inc.	7 fl oz	0.55 ml	14	Heteroaromatics

^a Fungicides were evaluated in two independent experiments, numbered 1 and 2; two trials of each experiment were conducted.

^b Rates are those recommended for soil drench applications, except for Reliant Trifecta.

^c FRAC: Fungicide Resistance Action Committee.

^d Experimental granular formulation applied to the surface of the container mix in each pot.

Table 4.3. Results from one-way analyses of variance (ANOVA) of data for the efficacy of 12 fungicides to manage *Phytophthora nicotianae* on English lavender (*Lavandula angustifolia*) plants in two experiments conducted in a greenhouse^a

Disease parameter ^b	ANOVA statistic ^c	Experiment 1 ^d		Experiment 2 ^e
		Trial 1	Trial 2	Trials 1 + 2
Final foliage symptom severity	<i>F</i> <i>P > F</i> <i>df</i>	14.08 <0.0001 10, 55	8.27 <0.0001 10, 55	10.50 <0.0001 6, 77
AUDPC: Foliage symptom severity	<i>F</i> <i>P > F</i> <i>df</i>	21.89 <0.0001 10, 55	13.63 <0.0001 10, 55	10.08 <0.0001 6, 77
Fresh plant mass	<i>F</i> <i>P > F</i> <i>df</i>	3.39 0.0017 10, 55	0.92 0.5252 10, 55	3.18 0.0077 6, 77

^a Two trials of each of two experiments were conducted, and a different set of fungicides was used in each experiment. ‘Hidcote Blue’ plants and nine fungicides were used in Experiment 1, and ‘Hidcote’ plants and five fungicides were used in Experiment 2. There were six replicate plants used for each treatment in each trial.

^b Three disease parameters were used to evaluate treatment efficacy: Final foliage symptom severity was assessed as the percentage of the foliage on each plant with disease symptoms at the end of a trial; foliage symptoms were assessed weekly for 5 weeks, and then the area under the disease progress curve (AUDPC) was calculated; the fresh mass of each plant was measured at the end of a trial.

^c Summary statistics for treatments when each disease parameter was analyzed by 1-way ANOVA; *F*: The calculated *F* ratio for each disease parameter; *P > F*: The probability of a greater *F* ratio occurring; *df*: Number of degrees of freedom, numerator and denominator.

^d There were significant ($P < 0.05$) trial × treatment interactions in Experiment 1, and treatment rank order varied in the two trials; therefore, data in these trials were analyzed separately.

- ^e There were not significant ($P > 0.05$) trial \times treatment interactions in Experiment 2, and treatment rank order was consistent between trials; therefore, data in these trials were combined and analyzed together.

Figure 4.1. Experiment 1, Trial 1: Three disease parameters were used to evaluate the efficacy of nine fungicides to protect ‘Hidcote Blue’ English lavender plants that were inoculated with *Phytophthora nicotianae* and grown for 42 days in a greenhouse. **A**, Percentage of the foliage showing symptoms of discoloration, wilting, or necrosis at the end of the trial. **B**, Foliage symptoms were assessed weekly and on the last day of the trial, and the area under the disease progress curve (AUDPC) was calculated. **C**, At the end of the trial, fresh mass of each plant was measured. Values in all graphs are means of six replicate plants; error bars are standard errors. In each graph, means with the same letter are not significantly different ($P \geq 0.05$) based on a one-way analysis of variance followed by *t*-tests between all pairs of means.

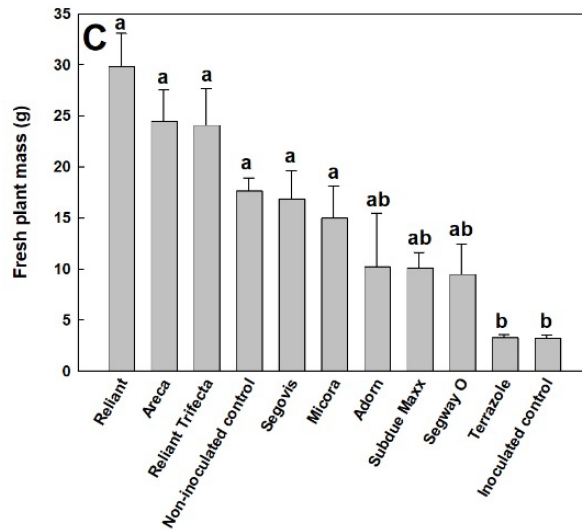
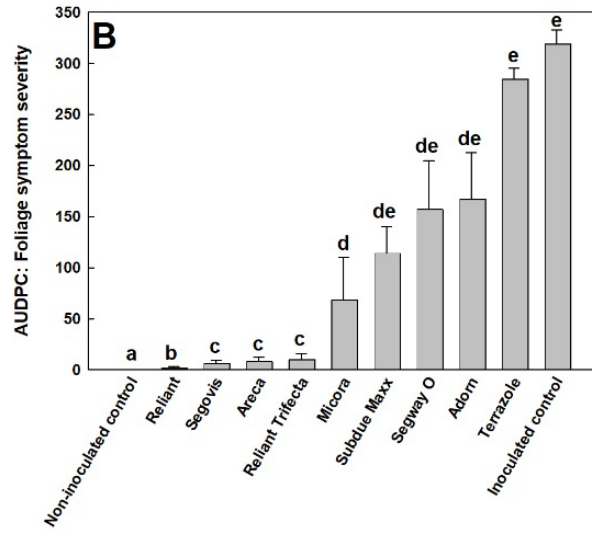
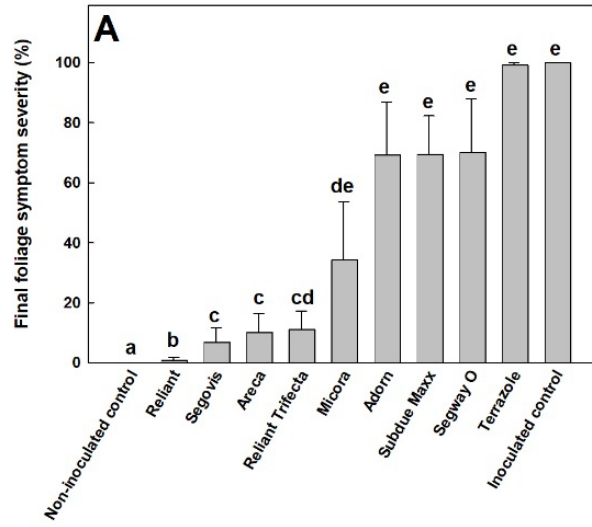


Figure 4.2. Experiment 1, Trial 2: Three disease parameters were used to evaluate the efficacy of nine fungicides to protect ‘Hidcote Blue’ English lavender plants that were inoculated with *Phytophthora nicotianae* and grown for 39 days in a greenhouse. **A**, Percentage of the foliage showing symptoms of discoloration, wilting, or necrosis at the end of the trial. **B**, Foliage symptoms were assessed weekly and on the last day of the trial, and the area under the disease progress curve (AUDPC) was calculated. **C**, At the end of the trial, fresh mass of each plant was measured. Values in all graphs are means of six replicate plants; error bars are standard errors. In each graph, means with the same letter are not significantly different ($P \geq 0.05$) based on a one-way analysis of variance followed by *t*-tests between all pairs of means.

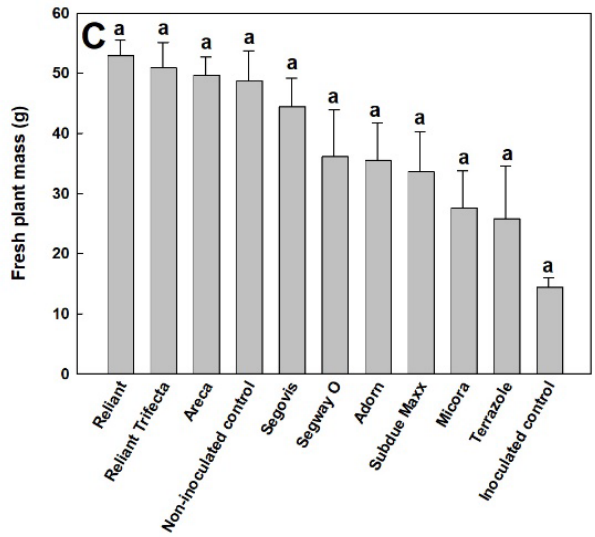
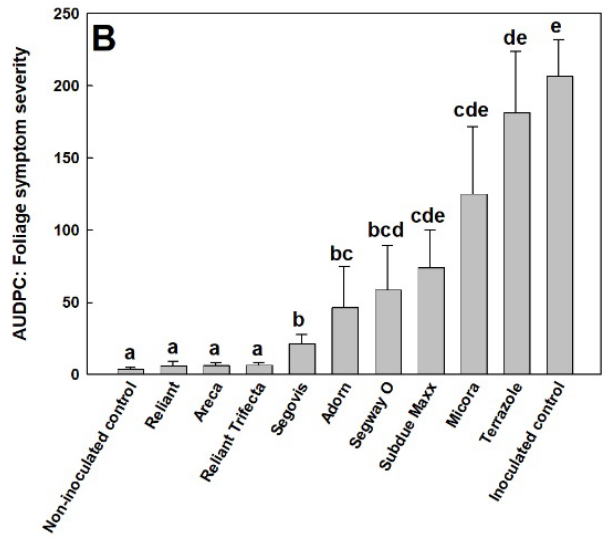
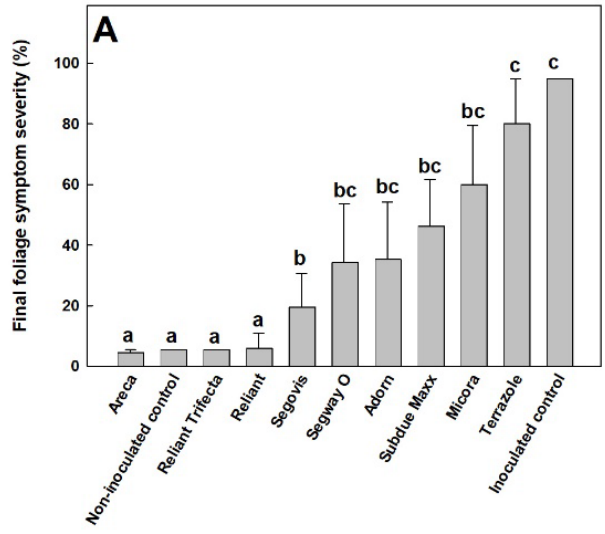
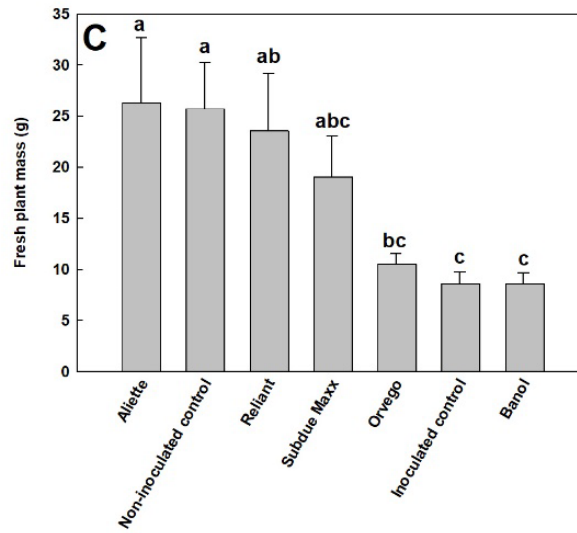
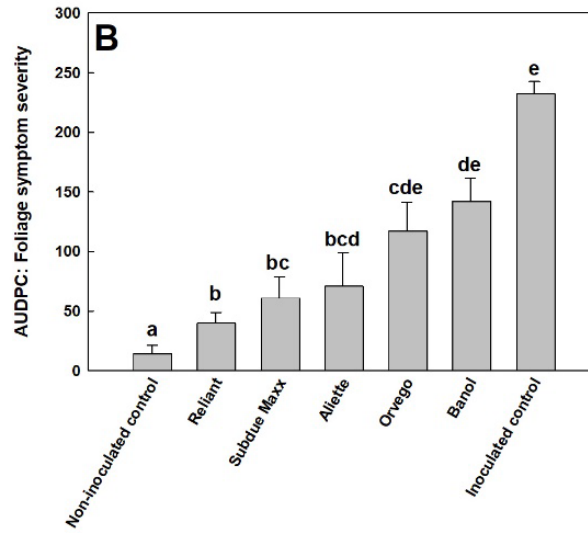
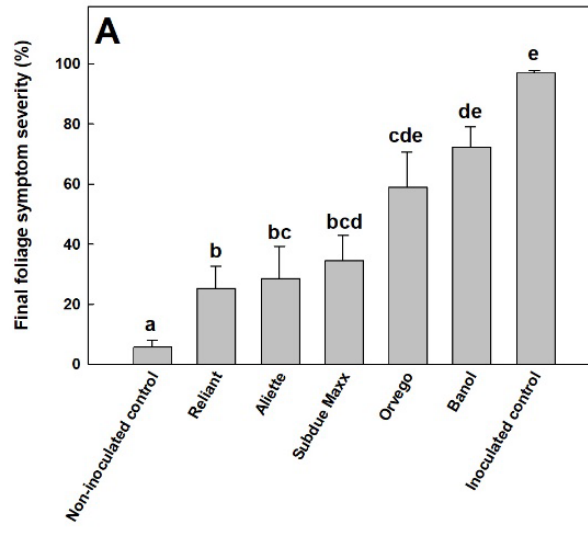


Figure 4.3. Experiment 2, Trials 1 and 2 combined: Three disease parameters were used to evaluate the efficacy of five fungicides to protect ‘Hidcote’ English lavender plants that were inoculated with *Phytophthora nicotianae* and grown for 38 (Trial 1) and 39 days (Trial 2) in a greenhouse. **A**, Percentage of the foliage showing symptoms of discoloration, wilting, or necrosis at the end of the trial. **B**, Foliage symptoms were assessed weekly and on the last day of each trial, and the area under the disease progress curve (AUDPC) was calculated. **C**, At the ends of the trials, fresh mass of each plant was measured. Values in all graphs are means of 12 replicate plants, six in each trial; error bars are standard errors. In each graph, means with the same letter are not significantly different ($P \geq 0.05$) based on a one-way analysis of variance followed by *t*-tests between all pairs of means.



CHAPTER 5

EFFICACY OF A QUATERNARY AMMONIUM PRODUCT TO REMEDIATE SOIL INFESTED WITH THE INTRODUCED PATHOGEN *PHYTOPHTHORA NICOTIANAE*

Abstract

Phytophthora root and crown rot has become a major concern for lavender growers in the United States, and it is primarily caused by *Phytophthora nicotianae*. Since the pathogen is spread on infected or infested plant material, contamination of field soil frequently occurs, and there currently is not a safe and cost-effective means to remediate infested soil. One potential option is the use of quaternary ammonium products to drench soil that has become infested after planting infected plants. Trials using drench applications of the quaternary ammonium product KleenGrow, which contains the active ingredient didecyldimethylammonium chloride, were conducted both in a lavender field and in a greenhouse. In the field, infested field soil was drenched in situ; in the greenhouse, aliquots of infested field soil in containers were drenched. In both settings, only the highest concentration of 15.6 ml/liter of product resulted in significantly less activity of *P. nicotianae* in soil, but even this concentration did not eliminate the pathogen. Pathogen activity, determined by a standard baiting bioassay, remained relatively high in the soil suggesting that *P. nicotianae* still was a potential threat to lavender plants if planted again in the field. In the future, studies might consider combining the use of quaternary ammonium products with other remediation methods.

Introduction

Phytophthora root and crown rot (PRCR) has become a major problem for lavender production in the United States. While there are multiple species of *Phytophthora* that cause PRCR, by far the most common causal agent is *P. nicotianae* (Dlugos and Jeffers 2021). This disease was first documented on English lavender (*Lavandula angustifolia*) in a Maryland nursery (Putnam 1991), and PRCR has since been reported in numerous countries across several continents (Álvarez et al. 2001; Davino et al. 2002; Erwin and Ribeiro 1996; Faedda et al. 2013; Jung et al. 2016; Minuto et al. 2001; Nakova 2011). Additionally, *P. nicotianae* has been found on other species of lavender, including *L. stoechas* and hybrid lavender, *L. ×intermedia* (Dlugos and Jeffers 2018; Jeffers et al. 2016; Jung et al. 2016). *P. nicotianae* was first described in 1896 causing disease on tobacco, and it is now reported to be cosmopolitan with a broad host range—including at least 255 genera in 90 families (Erwin and Ribeiro 1996; Farr and Rossman 2021). *P. nicotianae* also has been ranked as one of the most studied species of *Phytophthora* (Cline et al. 2008; Kamoun et al. 2015).

Lavender (i.e., species of *Lavandula*) is a perennial woody herb in the Lamiaceae/Labiatae family (USDA NRC 2021; Zomlefer 1994), and, in addition to its use as an ornamental plant, it is often planted for agritourism farms featuring activities such as photography, U-pick, and special ceremonies (Adam 2006; Grebenicharski 2016). The plants are propagated primarily in nurseries by vegetative cuttings, which are rooted and sold to farms as transplants (Adam 2006). Often, the plant trade, which frequently involves nurseries, is responsible for movement of pathogens both regionally and globally. This is especially true for the movement of *Phytophthora* species on ornamental plants (Jones and Baker 2007; Migliorini et al. 2015; Webber 2010). Most nurseries grow many different species of ornamental plants that

often have different points of origin, and various species of *Phytophthora* are present, survive, and are spread among the plants in these nurseries (Jung et al. 2016; Parke and Grünwald 2012; Parke et al 2014). These pathogens then may travel undetected with the plants to the purchaser (Brasier 2005) with symptoms present only on roots (Migliorini et al. 2015), or due to the presence of fungicides that inhibit but do not kill the pathogen (Scott et al. 2013). Once *Phytophthora* species are introduced into soil in a landscape or field, they may persist for many years, even in the absence of host plants—through long lived, sexually produced oospores or asexually produced chlamydospores (Erwin and Ribeiro 1996; Webster and Weber 2007).

Remediation of soil contaminated with *Phytophthora* species can be difficult. One of the most efficient ways of eliminating persistent pathogens from field soil was by fumigation with methyl bromide. This product was developed in the 1920s, has been used in agriculture since the 1960s, and became the most widely used chemical for soil fumigation until its use was phased out in the 1990s and 2000s due to concerns of ozone damage (Lodovica Gullino et al. 2003). Some of the current methods of soil disinfestation include other fumigants, steam, solarization, and anaerobic soil treatments (Roskopf et al. 2018). Many of these treatments can be dangerous, especially in suburban areas, or cost prohibitive or may have limited efficacy depending on environmental conditions and climate (Roskopf et al. 2018). However, it may be possible to treat soil with surfactants, which are known to affect the membranes of microorganisms (Hultberg and Alsanus 2014). In fact, surfactants are especially effective on zoospores of species of *Phytophthora*, which lack cell walls, and have an easily ruptured protective membrane (Hultberg and Alsanus 2014; Stanghellini 1997). The class of cationic surfactants known as quaternary ammonium compounds or QACs (Hultberg and Alsanus 2014)

are commonly used for disinfestation in nurseries and greenhouses (e.g., the products Green-Shield II, KleenGrow, and SA-20).

QACs have an extensive history dating back to 1916 when they were discovered to be germicidal at levels not toxic to humans or animals (Rahn and Van Eseltine 1947). Part of the effectiveness of these compounds is due to the positive charge of QAC molecules that bind to the negative charge of cell walls and membranes (Gilbert and Moore 2005). Their ability to serve as bacteriostatic chemicals was due to the adsorption layer that forms and remains on cell surfaces (Rahn and Van Eseltine 1947). They have been used extensively against a diverse array of microorganisms for many decades with no reduction in effectiveness (Gilbert and Moore 2005).

The many uses of QACs include ones for agricultural purposes, particularly in tool and surface sterilization (Copes 2019). QACs have been shown to be significantly more effective than sodium hypochlorite when organic matter is present on surfaces (Nguyen et al. 2017). However, when used against *Pseudomonas savastanoi* pv. *savastanoi*, the causal agent of olive knot, it was not effective as a preventative treatment applied to tree wounds (Nguyen et al. 2017). Therefore, it was registered only as a sanitizer for equipment in California olive orchards in 2015 (Nguyen 2017). It is also recommended by the Florida Department of Agriculture to disinfest inanimate surfaces, skin, and clothing that might be contaminated with the citrus canker pathogen *Xanthomonas citri* subsp. *citri* (Schubert and Sun 2003). When applied to citrus leaves inoculated with the pathogen, it was very effective at reducing bacterium populations on leaf surfaces, but there were ultimately no differences in incidence or severity of disease on the inoculated plants—most likely due to protected sites on the leaf surface that harbored inoculum (Bock et al. 2011). The commercial product KleenGrow (Pace 49 Inc., Delta, British Columbia, Canada) was successfully used to nearly eliminate *Pseudomonas amygdali* pv. *loropetali* on

stainless steel and to significantly reduce it on pressure-treated wood, but KleenGrow did not significantly reduce the pathogen on stems of *Loropetalum chinense* (Copes et al. 2019).

The objective of this study was to examine the efficacy of the QAC product KleenGrow to remediate *P. nicotianae*, a common soilborne plant pathogen, in infested field soil because lavender growers continue to report contamination of fields from planting infected or infested nursery-grown plants. If effective, treatment with a QAC could provide a safe and cost-effective treatment for reducing or eliminating this introduced pathogen in fields used for specialty crops.

Materials and Methods

Field experiment: Site. In late fall 2016 to winter 2017, a field trial was conducted in cooperation with a lavender grower in Greenville County, South Carolina, in a field that had experienced significant losses of lavender plants from PRCLR, caused by *P. nicotianae*, during the two previous growing seasons. Dead and dying plants had been removed by the grower. The field is geographically located in an area with Cecil sandy loam soils (websoilsurvey.nrcs.usda.gov) and had no recent history of agricultural use before lavender was planted. However, the soil had an obvious amount of red clay, which is typical of this region of South Carolina. Founded in 2014, the farm—which is focused on cut flower production, the U-pick market, and event hosting—was planted with several cultivars of both English lavender and hybrid lavender. Plants were planted on raised rows with only one cultivar in each row.

Field experiment: Experimental design. The experiment was planned as a randomized complete block design with rows as blocks and individual plant holes within a row, i.e., obvious depressions in the soil where plants had died, as replicates to be treated. A total of four blocks were arranged across five rows with one block consisting of two side-by-side rows of the same

cultivar to obtain the desired number of infested plant holes. Within a block, individual plant holes were randomly assigned to one of four treatments—see below. There were eight or nine replicate plant holes assigned to each treatment in each block: Two blocks contained nine replicates of each treatment while two blocks contained eight replicates of each treatment.

Field experiment: Pre-treatment sampling. To ensure that plant holes in the field were infested with *P. nicotianae*, soil samples from all plant holes were collected before treatments were applied. On 03 Nov 2016, soil probes (Model LS; Oakfield Apparatus, Oakfield, WI) were used to collect two to three soil cores (approximately 23 cm deep × 2 cm diameter) from each plant hole. Cores from each hole were combined and thoroughly mixed to make a composite soil sample from each plant hole (150 to 200 ml). Samples then were assayed in the lab using a standard baiting bioassay (Bell et al. 2021; Ferguson and Jeffers 1999). In this assay, 100 ml of soil was mixed with 200 ml of deionized water in a 580-ml plastic container (9.6 cm × 9.6 cm × 6.3 cm tall) (Stock no. 4201; Arrow Plastics Mfg., Oak Grove Village, IL). Six leaf pieces (~5 mm in diameter), three from camellia and three from rhododendron, were cut with a standard hole punch and floated on the water surface in each container as baits for zoospores of *P. nicotianae*; containers were held at room temperature (23 to 25°C) for three days. Then, baits were removed, rinsed in deionized water, blotted dry, and embedded in PARPH selective medium (Ferguson and Jeffers 1999; Jeffers 2015). Isolation plates were observed for growth of *P. nicotianae* mycelium for up to 7 days. For the 33 days between pre-treatment sampling and application of treatments, local weather data (www.ncei.noaa.gov) were evaluated. There were seven days of recorded rainfall, with only one of those days in excess of 2.5 cm of rain (2.9 cm). The mean air temperature during this time was 11.4°C, with a maximum of 27.8°C and a minimum of -2.8°C.

Field experiment: Treatments. Treatments consisted of a non-treated control (water only) and three rates of KleenGrow (active ingredient didecyldimethylammonium chloride; Pace 49 Inc., Delta, BC): Low (3.5 ml of product/liter), intermediate (7.8 ml of product/liter), and high (15.6 ml of product/liter). All treatments were applied using a novel application method as soil drenches to individual plant holes on 10 Dec 2016. Ambient temperatures of the soil, air, and water for preparing treatment solutions remained constant at 3°C during application. To apply drenches, a PVC pipe—60 cm in length with an inner diameter of 10 cm and a volume of 5 liters—was inserted several centimeters into the soil at a plant hole; then, 4 liters of treatment solution were poured into the PVC pipe and allowed to slowly percolate into the soil at each plant hole before the PVC pipe was removed and moved to the next plant hole (Figure 5.1).

Field experiment: Post-treatment sampling. Post-treatment samples were collected on 29 Jan 2017, 50 days after drenches were applied in the field, to allow ample time for quaternary ammonium solutions to be effective during the winter season. During this time, local weather data were monitored (www.ncei.noaa.gov). There were 16 days of precipitation, including two snowfall events. Three of the dates consisted of rainfall of 2.5 cm or more, but precipitation did not occur for at least 48 h after treatments were applied. The two consecutive days of snowfall allowed for five consecutive days of snow cover. The mean air temperature during this time was 8.4°C, with a maximum temperature of 25.6°C and a minimum of -8.9°C. Post-treatment sampling and pathogen detection were conducted following the same methods described above for pre-treatment sampling and detection.

Field experiment: Data collection and analysis. Only plant holes that tested positive for *P. nicotianae* before treatment were used for data analysis because treatment efficacy could not be evaluated for plant holes where the pathogen was not initially present and active. Data

collected were numbers of leaf baits plated on PARPH-V8 medium that produced growth of *P. nicotianae* mycelium out of the total number of baits plated. The percentage of baits testing positive for the pathogen was calculated and served as an indicator of pathogen presence and potential activity in the soil at the time samples were collected (Bell et al 2021). The difference in percentage of positive baits for each plant hole was calculated by subtracting the post-treatment positive bait percentage from the pre-treatment positive bait percentage, and the mean difference for each treatment was calculated. A one-way analysis of variance (ANOVA) was used to identify differences among treatment means with blocking as a factor. Assumptions were checked on residuals from the model by using Levene's test for equal variance and Shapiro-Wilks test for normality. Fisher's protected least significant difference (LSD) (i.e., Student's t-test post-hoc test) was used to test for significant differences among treatments ($P \leq 0.05$). These data were analyzed using JMP Pro, ver. 13 (Cary, NC). In addition, the number and percentage of plant holes that showed a change in the presence and potential activity of *P. nicotianae* in soil samples, based on baiting results, between pre- and post-treatment were calculated, and these binomial data were analyzed by chi-square in Excel (Microsoft Office 365; Redmond, WA).

Greenhouse experiment: Experimental design and treatments. The soil used in the greenhouse experiment was collected from the field site described above. The soil was a composite mix of samples collected from plant holes where *P. nicotianae* was confirmed to be present based on previous testing. The experiment was carried out in a greenhouse in the Biosystems Research Complex at Clemson University, Clemson, SC. Two independent trials, separated in time, were conducted for reproducibility. In each trial, the same four treatments used in the field trial were applied as soil drenches: Control (only water) and low (3.5 ml of

product/liter), intermediate (7.8 ml of product/liter), and high (15.6 ml of product/liter) rates of KleenGrow. Each treatment was applied to 10 replicates, for a total of 40 replicates in each trial.

A single replicate consisted of 100 ml of infested soil in a UV-stabilized Ray Leach Cone-tainer cell (“Stubby Cells”, 158 ml; Stuewe & Sons Inc., Tangent, OR); soil was covered with 10 ml of sterilized horticultural-grade vermiculite to prevent soil erosion during treatment application and watering. Fine mesh nylon window screen was placed at the bottom of each cell to retain soil. Cells were arranged in a completely randomized design in RL98 Cone-tainer racks (Stuewe & Sons Inc.) on a greenhouse bench, with control treatments clustered to avoid splash contamination during watering. A single soil drench application of 100 ml of treatment solution per cell was made in each trial—on 14 Mar and 10 May 2017 for trials 1 and 2, respectively. Cells were not watered for 48 h after drenching to allow treatments adequate time to be effective. After that, the cells were watered as needed to maintain field capacity of the soil and simulate natural field conditions.

During the trials, temperature and humidity data were recorded in the greenhouse. In trial 1, the mean daily temperature was $24.4^{\circ}\text{C} \pm 2.4^{\circ}\text{C}$, with a maximum of 28.7°C and a minimum of 20.8°C . Mean daily relative humidity averaged $54.3\% \pm 11.8\%$, with a maximum of 77.6% and minimum of 28.1%. In trial 2, the mean daily temperature was $24.8^{\circ}\text{C} \pm 2.4^{\circ}\text{C}$, with a maximum of 28.8°C and minimum of 16.8°C , and mean daily relative humidity averaged $64.5\% \pm 11.3\%$, with a maximum of 80.0% and a minimum of 31.6%. A 16-h photoperiod, achieved with supplemental lighting, was maintained throughout both trials.

Greenhouse experiment: Data collection and analysis. To determine treatment efficacy, replicate soil samples were removed from cells 16 days after treatment application, which was on 30 Mar and 26 May 2017 for trials 1 and 2, respectively. All of the soil from each

cell was assayed with the standard baiting bioassay as described for pre- and post-treatment soil samples in the field experiment. As in the field trial, the percentage of baits with *P. nicotianae* mycelium on PARPH-V8 medium was used to assess treatment efficacy, and a one-way ANOVA was conducted to identify differences among treatments. Data from the two trials were combined and analyzed together. To ensure validity of this approach, an ANOVA was run with outliers removed and blocking by trial added as a factor, but there was no significant block effect. Assumptions for equal variance and normality were tested by Levene's and Shapiro-Wilks tests, respectively. Fisher's protected LSD was used to test for significant differences between pairs of treatments ($P \leq 0.05$) using JMP Pro, ver. 13.

Results

Field experiment. Because only plant holes where *P. nicotianae* was present and active in pre-treatment samples were used to evaluate treatment efficacy, the number of replicates per treatment used in data analyses varied among treatments and ranged from 34 plant holes for the high rate of KleenGrow to 28 plant holes for the control (Table 5.1). KleenGrow treatments had a significant effect on the presence and activity of *P. nicotianae* in soil where lavender plants had died from PRCR. The treatments significantly ($P = 0.033$) affected the number and percentage of plant holes that showed a change in *P. nicotianae* activity (Table 5.1). The percentage of plant holes with decreased pathogen activity was greatest with the high and intermediate rates of KleenGrow ($P = 0.009$) whereas the low rate of KleenGrow and the water control had the greatest percentage of plant holes in which there was no change in pathogen activity after treatments were applied ($P = 0.016$). There was no significant difference among treatments in the

percentage of plant holes with increased pathogen activity ($P = 0.543$), which was relatively infrequent (3% to 13%).

There also were significant effects on the activity of *P. nicotianae* in soil samples receiving the various KleenGrow treatments as determined by the mean differences in percentages of baits detecting the pathogen in samples collected before and after treatment ($F_{3,9} = 6.45$, $P = 0.0127$). Positive values indicated a greater reduction in the percentages of baits detecting the pathogen after treatment and, therefore, a decrease in *P. nicotianae* presence and activity (Figure 5.2). The greatest changes in pathogen activity from pre- to post-treatment occurred with both high (15.6 ml/liter) and intermediate (7.8 ml/liter) rates of KleenGrow (Figure 5.2). These two treatments reduced pathogen activity equally and had a significantly greater negative effect on pathogen activity than the water control and low rate (3.5 ml/liter) treatments ($P < 0.05$, Figure 5.2). The high and intermediate treatments reduced *P. nicotianae* activity by 46.1% and 46.2%, respectively. The low rate of KleenGrow had no significant effect on reducing pathogen activity compared to the control; reductions in *P. nicotianae* activity by these two treatments were 15.0% and 12.5%, respectively (Figure 5.2).

Greenhouse experiment. Using individual aliquots of infested field soil in a greenhouse, KleenGrow treatments also negatively impacted *P. nicotianae* activity (Figure 5.3). There were significant differences among treatments in percentages of baits testing positive after treatments were applied ($F_{3,76} = 2.84$, $P = 0.0437$). *P. nicotianae* activity, as measured by mean percent positive baits, exhibited an incrementally decreasing trend with higher application rates, from 98% positive baits in the control treatment (water only) to 86% positive baits in the high treatment rate (15.6 ml/liter) (Figure 5.3). Only the high rate of KleenGrow significantly ($P < 0.05$) reduced pathogen activity compared to the control. This treatment also significantly

reduced pathogen activity compared to the low rate (3.5 ml/liter, 96% positive baits), but the high rate and the intermediate rate (7.8 ml/liter, 89% positive baits) had comparable effects on *P. nicotianae* activity in soil aliquots (Figure 5.3).

In addition, 100% of the baits were positive for *P. nicotianae* in 60 out of the 80 soil aliquots treated in both trials of this experiment (Table 5.2). Therefore, in 20 soil aliquots, pathogen presence and activity was reduced significantly ($P = 0.046$) by KleenGrow treatments. Soil aliquots treated with the high and intermediate rates of KleenGrow had the greatest number of replicates with reduced pathogen activity (Table 5.2).

Discussion

In this study, we were able to confirm the presence and potential activity of *P. nicotianae* in soil samples at specific times—i.e., when samples were collected in the field and when soils were treated in the greenhouse—using a standard baiting bioassay with camellia and rhododendron leaf pieces as baits. In the field experiment, a reduction in the percentage of baits colonized by *P. nicotianae* in soil samples collected from individual plant holes and the numbers of plant holes with a decrease in the percentage of baits detecting the pathogen before and after treatment with KleenGrow demonstrated treatment efficacy. Likewise, in the greenhouse experiment, a lower percentage of colonized baits in treated soil aliquots and more soil aliquots with less than 100% of baits colonized also verified treatment efficacy. Therefore, a baiting bioassay can be used for more than just documenting pathogen detection and presence. By using adequate replication and sufficient numbers of leaf piece baits, quantitative estimates of potential pathogen activity in soil were made. Recently, Bell et al. (2021) used a similar baiting bioassay

to measure pathogen activity in aqueous solutions containing zoospores of five species of *Phytophthora*.

As numerous species of *Phytophthora* are being distributed across the nation by means of nursery-grown lavender plants (Dlugos and Jeffers 2021), it is important to identify effective methods of disinfesting field soil that may become contaminated with these pathogens when infested or infected plants are planted. With the phasing out of methyl bromide fumigation (Lodovica Gullino et al. 2003) and the ability of *Phytophthora* species to persist in soil for years (Erwin and Ribeiro 1996), there is a growing importance to find suitable and economical means of disinfesting field soils for specialty crops like lavender. The use of KleenGrow, a QAC containing the active ingredient didecyldimethylammonium chloride, as a soil disinfestant against *P. nicotianae* did result in statistically significant reductions in the presence and potential activity of *P. nicotianae* in both field and greenhouse experiments. The trend in both experiments was for increasing effectiveness with increasing application rate. While statistical significance was encouraging, the results do not merit recommendation as a practical solution for lavender growers. Drenching soil with this QAC product significantly reduced pathogen activity but not to a practical or economically useful level. KleenGrow treatment consistently left populations of *P. nicotianae* that were readily detectable and potentially active in soil samples. Therefore, based on our results, the use of this product alone would not be an effective soil disinfestant treatment for remediation of populations of *P. nicotianae* introduced into field soils. The level of pathogen remaining in the soil post-treatment would likely be high enough to cause a rapid buildup of the pathogen once a suitable host was planted, similar to the situation reported for *P. nicotianae* on tobacco (Kannwischer and Mitchell 1978). Consequently, infection of

lavender plants and subsequent disease development in the field would be likely after this type of treatment.

That the product does significantly decrease pathogen activity is important and provides potential for future opportunities in developing innovative disease management strategies. For example, the solarization method of soil disinfestation has been shown to be most effective at eliminating *Phytophthora* species when soils are moist (Funahashi and Parke 2020). Solarization is effective, primarily, from heat induced membrane disruption (Roskopf et al. 2018). Surfactant disinfestants, like QACs, are reported to have a similar deleterious effect on microorganisms (Hultberg and Alsanius 2014). Therefore, perhaps research could be conducted to determine if there is a synergistic effect of moistening soil with QACs prior to solarization.

An additional point to consider is the potential spread of *Phytophthora* species in field sites through movement of field soil and laterally flowing water from rainfall or irrigation. Diseases from soilborne plant pathogens typically are reported as most severe near primary sources of inoculum (Neher and Duniway 1992). However, water, especially irrigation water, can move *P. nicotianae* through soil, allowing it to pool in certain areas that could include around plants (Café Filho and Duniway 1995). During post-treatment sampling of the lavender field used in this study, samples taken from between both rows and plants tested positive for *P. nicotianae*, suggesting that spread of the pathogen had occurred over time. For a soil drench treatment to be effective against an introduced pathogen—like *P. nicotianae*, the soil where the pathogen was introduced (e.g., the plant hole) should be treated as soon after symptoms on plants are detected to prevent pathogen spread, both laterally and deeper into the soil, and establishment of the pathogen in the field. Obviously, the deeper the pathogen moves down into the soil profile, the more challenging it becomes to effectively remediate the pathogen. In a previous

study on tobacco, *P. parasitica* (now known as *P. nicotianae*) was found at a depth of 15 cm (Flowers and Hendrix 1972); however, the depth to which *P. nicotianae* moves after being introduced into lavender fields has yet to be determined. This information is needed to ensure that remediation efforts are effective to the depth in soil at which the pathogen is present.

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Table 5.1. The number and percentage of holes in a field where lavender plants had died from Phytophthora root and crown rot showed decreased, increased, or no change in the activity of *Phytophthora nicotianae* in soil after a single drench application of KleenGrow (active ingredient: didecyldimethylammonium chloride) at three rates or a drench with water (non-treated control)^a

KleenGrow rate		Total (no.)	Decreased		Increased		No change	
Relative	ml/liter		No.	%	No.	%	No.	%
High	15.6	34	24	70.6	3	8.8	7	20.6
Intermediate	7.8	31	19	61.3	1	3.2	11	35.5
Low	3.5	30	11	36.7	4	13.3	15	50.0
Control	0.0	28	10	35.7	2	7.1	16	57.1
Chi square analysis								
X^2		13.717	11.580		2.144		10.293	
df		6	3		3		3	
P value		0.033	0.009		0.543		0.016	

^a Pathogen activity was determined using a standard baiting bioassay to test soil samples collected from each hole before and after treatment; data were based on the percentages of leaf piece baits (six/replicate) that detected the pathogen before and after treatment.

Table 5.2. Number of soil aliquots with 100% or less than 100% of leaf piece baits that detected *Phytophthora nicotianae* in a baiting bioassay 16 days after drenching aliquots with three rates of KleenGrow (active ingredient: didecyldimethylammonium chloride) in the greenhouse^a

KleenGrow rate		Baits positive for <i>P. nicotianae</i> ^b		Treatment total
Relative	ml/liter	100%	<100%	(no.)
High	15.6	11	9	20
Intermediate	7.8	14	6	20
Low	3.5	17	3	20
Control	0.0	18	2	20
Sample total		60	20	80
Chi square analysis				
		X^2	8.000	
		df	3	
		<i>P</i> value	0.046	

^a Ten replicate aliquots (100 ml each) of infested field soil were drenched with each treatment in each of two trials; control treatment was drenched with water. Soil aliquots then were tested for the presence and activity of the pathogen using a standard baiting bioassay.

^b Six leaf piece baits were used to assay each soil aliquot in each trial; data from the two trials were combined for analysis.

Figure 5.1. The novel application method for applying KleenGrow solutions to field soil in places where lavender plants had been killed by *Phytophthora nicotianae* (i.e., plant holes): **A**, PVC pipes inserted into the soil at plant holes to be treated; **B**, application of 4 liters of KleenGrow solution to a planting hole; **C**, the wetting pattern in the soil around a plant hole after a KleenGrow treatment was applied.



Figure 5.2. Reduction in activity of *Phytophthora nicotianae* in soil was recorded as the mean difference between percentage of leaf piece baits testing positive for the pathogen before and after treatment using a standard baiting bioassay. Mean percentages are based on six baits per replicate and 28 to 34 replicates per treatment. Each replicate consisted of 100 ml of naturally infested soil collected from a place in a field where a lavender plant had died. Treatments were high (15.6 ml/liter, $n = 34$), intermediate (7.8 ml/liter, $n = 31$), and low (3.5 ml/liter, $n = 30$) label rates of KleenGrow (active ingredient: didecyldimethylammonium chloride) and a non-treated control (0 ml/liter, $n = 28$). Treatments were applied as soil drenches to field soil sites from where lavender plants had been killed by *P. nicotianae*. Means were separated by Fisher's protected least significant difference ($P = 0.05$) after a one-way analysis of variance: Means with the same letter are not significantly different; error bars are standard errors.

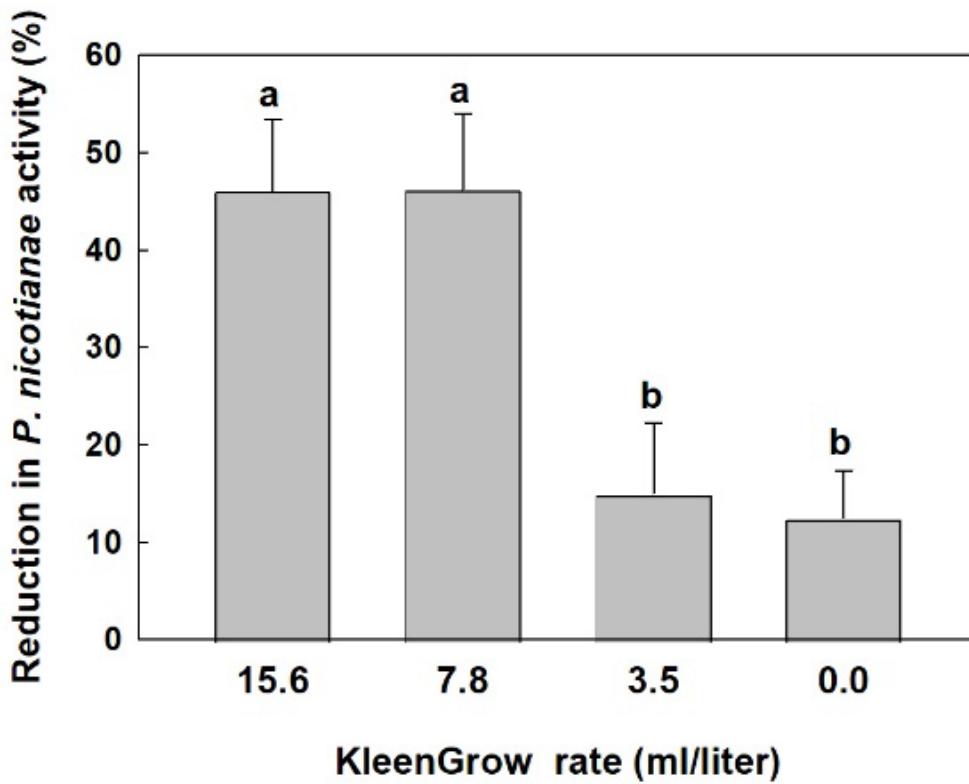
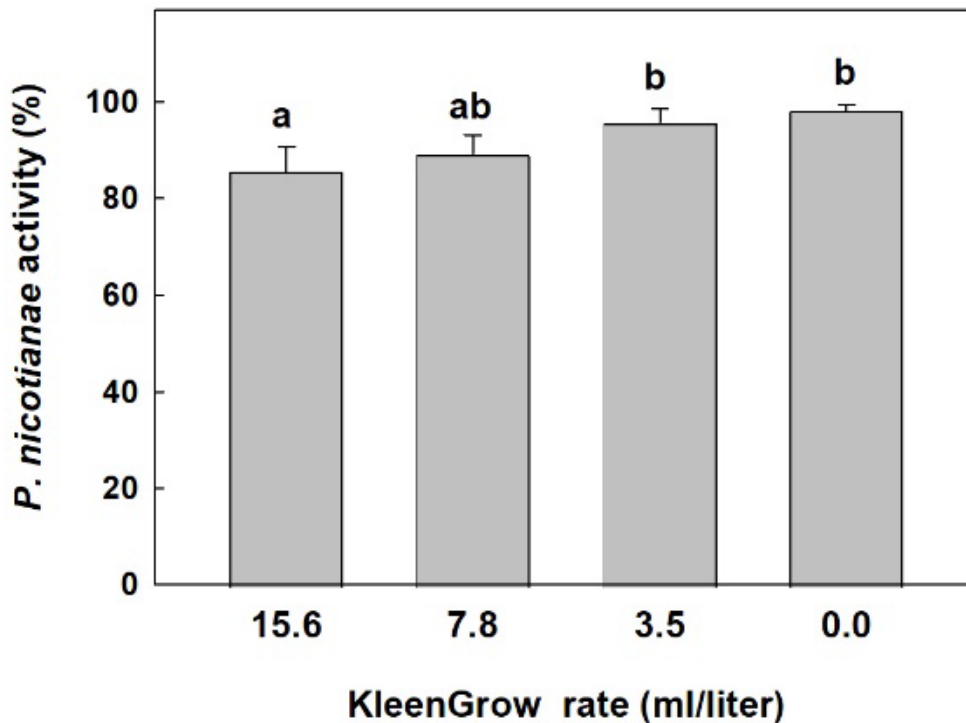


Figure 5.3. Activity of *Phytophthora nicotianae* in soil was recorded as the mean percentage of rhododendron leaf piece baits testing positive for the pathogen in a standard baiting bioassay. Means are based on six baits per replicate and 10 replicates per treatment in each of two trials. A replicate was 100 ml of infested lavender field soil in a cone-tainer. Data from the trials were combined for analysis. Treatments of high (15.6 ml/liter), intermediate (7.8 ml/liter), and low (3.5 ml/liter) label rates of KleenGrow (active ingredient: didecyldimethylammonium chloride) and a non-treated control (0 ml/liter) were drenched on soil aliquots. Means were separated by Fisher's protected least significant difference ($P = 0.05$) after a one-way analysis of variance: Means with the same letter are not significantly different; error bars are standard errors.



APPENDICES

APPENDIX A
A SURVEY OF LAVENDER PRODUCTION IN THE UNITED STATES

A survey conducted by Clemson University in cooperation with the US Lavender Growers Association

This is the complete text of a survey sent to lavender growers in Feb 2019, and growers were allowed to respond until Nov 2019. The survey (Protocol no. IRB2019-024) was reviewed and approved by the Clemson University Institutional Review Board, which is part of the Office of Research Compliance. Results from the survey are reported in Chapter 2 of this dissertation.

Location

Name of farm or grower (TEXT BOX)

Google Map location (MAP)

City, County, State (TEXT BOXES)

Do you grow lavender *Yes* *No* (DROP DOWN OPTIONS) *If you selected no, please submit survey now.

Lavender

Species of lavender (*Lavandula*) grown: **L. angustifolia* – English Lavender* **L. × intermedia* – Hybrid Lavender* **L. stoechas* – Spanish Lavender* **L. heterophylla* – Sweet Lavender* **L. dentata* – French Lavender* *Other* (CHECK BOXES)

Cultivars grown: List the five most widely planted cultivars on your farm for each lavender species (List each species on a separate line followed by 5 text boxes (TEXT BOX))

Original source of plants on your farm: that is, from what nursery or grower did you buy or obtain the plants growing on your farm; include the year plants were purchased; be as specific as possible for each cultivar (TEXT BOX)

Land Use

Which best describes your growing area: *Lavender only* *Multiple crops* (DROP DOWN OPTIONS); if multiple crops, list the three other crops covering the greatest acreage

Total acres of lavender being grown: (TEXT BOX)

Intended use of or market for your lavender crop (check all that apply): *Agritourism/U-Pick* *Nursery/Plant production/Propagation* *Non-Commercial/Personal use* *Farm market/Fresh-

cut flowers/Dried flowers* *Culinary/Edible products* *Oil production/Non-edible products*
Other (CHECK BOXES)

If other, please list (TEXT BOX)

Is the lavender grown organically certified *Yes* *No* (DROP DOWN OPTIONS)

How long has your land been utilized for lavender production (DROP DOWN OPTIONS)

Was another crop previously cultivated where the lavender is now grown *Yes* *No* *Not
Sure* (DROP DOWN OPTIONS)

If so, what was the previous crop cultivated on that land before planting lavender (TEXT BOX)

What is your anticipated annual income from LAVENDER ONLY; this could include admission
fees, U-Pick, lavender products, etc. Optional (DROP DOWN OPTIONS)

Are you looking to expand your lavender production acreage or order new plants in the next five
(5) years? *Yes* *No* *Maybe* (DROP DOWN OPTIONS)

Pest and Disease Issues

Have you sent samples to Clemson University for diagnosis—either to the Plant and Pest
Diagnostic Clinic or to Dr. Jeffers' Lab *Yes* *No* (DROP DOWN OPTIONS)

Have you sent samples to OTHER university or private labs for diagnosis of Phytophthora root
and crown rot *Yes* *No* (DROP DOWN OPTIONS)

If so, where and when were samples sent and were Phytophthora species detected (TEXT BOX)

Have you had documented proof of Phytophthora root and crown rot in your plantings *Yes*
No (DROP DOWN OPTIONS)

Has Phytophthora root and crown rot or the threat thereof influenced your business? *Yes,
greatly* *Yes, to a small extent* *No, it has not* (DROP DOWN OPTIONS)

How confident are you in your suppliers of lavender plants if you were to order more? *Very
confident* *Somewhat confident* *Not at all confident* (DROP DOWN OPTIONS)

Have you had other documented pest or disease problems in your plantings *Yes* *No* (DROP
DOWN OPTIONS)

If so, what were they (TEXT BOXES)

What management techniques do you currently employ for LAVENDER ONLY *None*
Resistant species/cultivar selection *Conventional pesticides (herbicides, fungicides,
insecticides)* *Biocontrol products* *Other* (CHECK BOXES)

Are you willing to employ the use of conventional pesticides (herbicides, fungicides,
insecticides) on your lavender fields *Yes* *No* *Maybe* (DROP DOWN BOX)

Comments

Please provide any additional feedback or comments (TEXT BOX)