

SURVEYING FOR *PHYTOPHTHORA* SPECIES IN WATER AND SOIL IN GEORGIA

by

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(Under the Direction of Jean L. Williams–Woodward)

ABSTRACT

Phytophthora species are water and soil-borne saprophytes and plant pathogens. The introduction of *P. ramorum*, the causal agent of sudden oak death (SOD) into Georgia has created an interest in identifying *Phytophthora* sp. occurring in ornamental plant nurseries and natural forests in Georgia. *Phytophthora* sp. can be identified from water and soil using host plant tissue bait surveys. In this study, water and soil surveys were conducted to identify *Phytophthora* sp. and determine the spread of *P. ramorum* from sites of introduction in ornamental nurseries into surrounding natural areas. The results of this study showed that *Phytophthora* sp. can be recovered from forest and suburban streams and ornamental nursery retention ponds. *Phytophthora ramorum* was recovered from soil in one retail nursery several times over the course of a year, including areas away from the site of initial introduction.

INDEX WORDS: Forest pathology, ornamental pathology, plant nursery, water-borne, waterborne, soilborne

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DEDICATION

To my family and friends that kept me motivated. To my mom, who I knew I could always call. To Susan, who threatened me into grad school and I'm grateful for the threat. To Ms. Donna for all her help and advice. To Johanna, for adopting me. To Adam, for keeping me sane and happy.

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CHAPTER 1

INTRODUCTION

An interest in identifying existing species of *Phytophthora* sp. in ornamental nurseries and forested ecosystems developed as a result of the 2004 introduction of *Phytophthora ramorum* into Georgia ornamental plant nurseries and home landscapes on infected ornamental plants. Knowledge of native and/or naturally occurring *Phytophthora* sp., and their lifecycles within these systems can aid in monitoring species shifts and new species introduction.

Phytophthora species are distributed worldwide and are responsible for major disease epidemics such as late blight of potato, Jarrah dieback in Australia, and sudden oak death (SOD) in the western USA. The host range of *Phytophthora* sp. can be extensive. For example, the host range of *P. cinnamomi* is approximately 1,000 plant species (4). Although the pathogen is not native to the USA, it is now endemic in the southeastern United States and it is the causal agent of littleleaf disease of shortleaf pine (*Pinus echinata*) (4, 7). In contrast, other *Phytophthora* species, such as *P. infestans*, the cause of late blight of potato and tomato, have evolved closely with their hosts, and hence their host range is limited to a specific plant order or family (1).

One of the more recent *Phytophthora*-induced disease epidemics is sudden oak death (SOD), caused by *P. ramorum*. The disease has warranted global concern due to the high mortality of tanoaks (*Lithocarpus densiflora*) and several oak (*Quercus*) species in California and Oregon (5, 8), as well as oak and European beech (*Fagus sylvatica*) death in Europe due to

bleeding stem cankers (2). The pathogen also causes a non-lethal foliage blight on ornamental and forest under-story plants in the USA and Europe (3, 10). It is hypothesized that *P. ramorum* was introduced at least three separate times into the United States on ornamental nursery stock (6). As of March 2008, there are 45 plant species on the USDA APHIS list of regulated hosts (i.e. plants in which Koch's postulates were successfully completed and documented) and 70 plants associated with *P. ramorum* (i.e. plants that have been found naturally-infected, but Koch's postulates have yet to be performed and documented) (9).

Phytophthora species are important plant pathogens worldwide. With the introduction of *P. ramorum* into Georgia on ornamental nursery stock, it is important to know which *Phytophthora* sp. occur in Georgia to help determine spread and possible establishment of alien species. The introduction of *Phytophthora* sp. into waterways has the potential for long distance spread in natural environments. In nursery retention ponds, the pathogen can be spread to uninfected plants by irrigation. The survival of *Phytophthora* sp. in soil increases the potential of establishment and spread. The purpose of this study was to identify what *Phytophthora* sp. are present in water sources in Georgia, and to determine the survival and spread of *P. ramorum* within retail ornamental nurseries.

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CHAPTER 2

LITERATURE REVIEW

Epidemics caused by *Phytophthora* species. Aside from late blight of tomato and potato, caused by *P. infestans*, several introduced *Phytophthora* sp. have caused widespread natural forest plant epidemics (27). Even before the discovery of *P. ramorum*, *Phytophthora* sp. have been responsible for large-scale tree decline in Europe, Australia and the United States. Some of these epidemics include littleleaf disease (*P. cinnamomi*), alder die back (*P. alni*), jarrah (*Eucalyptus marginata*) dieback (*P. cinnamomi*), and Port Orford cedar (*Chamaecyparis lawsoniana*) decline (*P. lateralis*). Other than different hosts and the pathogen species, the diseases are similar because *Phytophthora*-caused diseases are strongly associated with water and soil movement in the areas of decline and cause root and crown infections.

Jarrah dieback. Extensive ecological changes occurred after the introduction of *P. cinnamomi* to Western Australia (53). The number of susceptible plant species present in a niche can affect disease intensity at forest sites. In areas densely populated with susceptible hosts, entire plant communities have been destroyed (60). McDougall *et al.* (52) found that overall species diversity found in healthy and infested forest sites were similar, but the populations of specific genera of susceptible plants were reduced after the introduction of a pathogen.

In Western Australia, the predominate overstory and economically important tree is jarrah (*E. marginata*) (60). Jarrah infected with *P. cinnamomi* decline relatively slowly. Symptoms such as crown decline, chlorosis, small branch dieback and growth of epicormic

shoots can progress up to four years before tree death (60). In contrast, infected under-story plants experience a more rapid decline and death (60).

In sites where *P. cinnamomi* was introduced at least 50 years ago, ecologically important jarrah-forest plants such as *Banksia grandis* and *Tetrathecia hirsute* are no longer found (53). These two species are important components of the small tree and shrub under-story in the forests. McDougall *et al.* (52) hypothesized that susceptible species still present several years after pathogen introduction were the result of high seed set or seed survival in seed banks.

Jarrah forests infested with *P. cinnamomi* have been surveyed to determine pathogen survival in the environment (79). Survival of *P. cinnamomi* has been verified for at least six years after introduction in soils collected from non-infested forest sites at varying water potentials (79). Unfortunately, due to the seasonality of *P. cinnamomi* in the Mediterranean jarrah forest environments, surveying soils and roots for the pathogen can be difficult due to unreliable soil- and root-baiting assays (52). Assays used to detect *P. cinnamomi* included growing containerized plants in naturally-infested soils and plating roots for pathogen detection (82). Therefore, large numbers of samples must be collected to verify pathogen persistence in forest soils and roots (53). Similar seasonal results were reported in Oregon after the introduction of *P. lateralis* into Port Orford cedar stands. Hansen and Hamm (29) found that *P. lateralis* is capable of surviving in natural environments for up to seven years after artificial inoculation. Growth of the pathogen is greatest during cool, wet seasons. Although, dry weather with temperatures of 30-40°C can be fatal to the organism, it can survive if it is located deeper in the soil profile where temperatures are cooler (29).

Port Orford cedar dieback. Death of Port Orford cedar (*C. lawsoniana*) due to *P. lateralis* in the Pacific Northwest of the USA was first noted in 1938 (65). The introduction of

P. lateralis resulted in *C. lawsoniana* natural stand decline and decimated the *C. lawsoniana* ornamental industry in the region (28, 65). Unlike *P. cinnamomi*, *P. lateralis* is not a strong soil inhabitant and viability declines as host roots decompose (29). However, a major concern for the spread and development of this disease is the introduction of *P. lateralis* into watersheds.

C. lawsoniana adjacent to infested waterways undergo more rapid decline and death than trees not directly exposed to waterways (28). Death of *C. lawsoniana* due to *P. lateralis* depends on the age of the tree. Seedling death can occur within weeks of first exposure, while mature trees die within one year after the first crown symptoms appear (28). Similar tree death patterns have been observed in Europe with the decline of alder trees after the introduction of *P. alni* (5, 44).

Inoculum spread via natural waterways. Infested alder plantations that drain into natural waterways have been identified as the source of waterway inoculum (19) that result in downstream tree death (44). There are no feasible means to eradicate *P. lateralis* or *P. alni* after they are introduced into a waterway (28). Water surveys for *P. alni* also have detected other *Phytophthora* sp., including *P. cambivora*, *P. cactorum*, *P. citricola*, *P. megasperma* and *P. quercina*, which are pathogenic on other forest tree species (44). Jung and Blaschke (44) determined that movement of propagules in waterways resulted in local or regional spread of the pathogen; while movement of planting stock is responsible for long distance dispersal. Similar spatial distribution patterns have been observed for *P. ramorum* in California (10).

Similarities to other *Phytophthora*-caused epidemics in forested areas have lead to concerns for the introduction of *P. ramorum*. The closest known related species to *P. ramorum* is *P. lateralis* (78). Like *P. lateralis*, *P. ramorum* is believed to have been introduced and spread via ornamental nursery stock (28, 41, 42). Isolates of *P. ramorum* have been recovered from

nursery irrigation water, soil and ornamental nursery stock. Currently, the transportation of infected ornamental nursery stock is responsible for long distance spread of *P. ramorum* (69), including into Georgia.

Sudden oak death (SOD). In California, McPherson *et al.* (54) described a three-step symptom progression on oak (*Quercus* sp.) and tanoak (*Lithocarpus densiflorus*). First, bleeding cankers were observed on the trunks, followed by the colonization of the tree by ambrosia beetles, and finally the saprophytic colonization of the tree by *Hypoxylon thouarsianum*. The infected tree fails and dies shortly thereafter. This disease has affected approximately 600 km of forested lands in California and is currently described to be at epidemic proportions in the region (63).

Under-story plant symptoms of *P. ramorum*, referred to as ramorum blight, have been found only on foliar and stem tissues (9). The pathogen has been most readily isolated from *Viburnum* sp., *Kalmia latifolia*, *Pieris japonica*, *Rhododendron* sp. and *Camellia* sp. (69, 75, 78). On these five highly susceptible hosts, *P. ramorum* produces abundant caducous sporangia and chlamydospores, making them an epidemiologically significant source of inoculum (75).

Susceptibility trials conducted by Tooley *et al.* (75) determined that species such as *K. latifolia*, *P. japonica* and *Rhododendron* sp. may be at highest risk for spreading *P. ramorum* in the southeastern United States due to their use as ornamentals and natural stands in the region. Since 2004, *Camellia* plants also have been considered high-risk vectors of the pathogen due to the large shipments of infected plants to 200 nurseries across the United States by Monrovia Nurseries in Azusa, California (69).

***Phytophthora* in Georgia.** With the introduction of *P. ramorum* into Georgia, there has been an effort to determine the native *Phytophthora* sp. in ornamental nurseries and forests in the

state. Currently, there are no reported surveys of *Phytophthora* sp. in forest soil, water or plants in Georgia, and only one reported nursery irrigation water survey in which *Phytophthora* sp. was detected (67). Knowing whether a particular pathogen species is introduced or indigenous when studying epidemics is important when anticipating the host range of a particular pathogen (22). Introduced pathogen species can result in high mortality rates of numerous host genera, while pathogens that have co-evolved with a host plant in an area exhibit less disease and have a smaller host range (23).

***Phytophthora* species biology.** *Phytophthora* species are facultative saprophytes. They are classified in the kingdom Chromista, Class Oomycota, Order Peronosporales and Family Pythiaceae (1). Organisms in Family Pythiaceae are typically soil and water inhabitants, preferring cool, moist conditions for sexual and asexual reproduction.

Since the mid 1960s, researchers have reported that species belonging to Oomycota are not related to true fungi (18). Instead, they are more closely related to golden-brown algae. Morphological characteristics that set the Oomycota apart from true fungi include cellulosic cell walls, diploid life cycle, inability to synthesize sterols, inability to deposit polyphosphate as metachromatic granules, tubular cristae within mitochondria, heterokont zoospores, and small-subunit ribosomal DNA sequences (26). There are approximately 60 identified *Phytophthora* species (15). However, this number has recently increased and this group of organisms has been found to be more diverse using molecular analysis (3, 4, 11, 24).

Sexual reproduction. *Phytophthora* sp. may be self-fertile (homothallic) or may require separate mating types (heterothallic). Sexual reproduction involves contact between a haploid female oogonium and a haploid male antheridium followed by plasmogamy and karyogamy (1). The result of this fusion is a diploid oospore that has thick cell walls and is believed to be a long-

term survival spore. Oospores are capable of surviving from at least one month up to years in naturally-infested soils (12, 13, 82). One interesting difference between *Phytophthora* and the true fungi is that meiosis occurs in the oogonium and the antheridium so that the gamete nuclei are the only haploid nuclei in the diploid lifecycle (15).

Asexual reproduction. A common asexual survival spore produced by some *Phytophthora* sp. is the chlamydospore. It is a thick-walled, tan to dark brown spore that is considered to be a primary hibernation structure for heterothallic species (15). There are two types of chlamydospores, thin- and thick-walled, and *P. cinnamomi* produces both (51). The environmental requirements for the two types of chlamydospores to germinate are different regardless of the *Phytophthora* species. Thin-walled chlamydospores of *P. palmivora* will not germinate under sterile conditions, and their nutritional requirements differ to those of thick-walled chlamydospores (45). Chlamydospores of *P. cinnamomi* are produced over a wide range of water and temperature regimes (61). Chlamydospores may be produced at a hyphal tip or intercalary (below the hyphal tip in a continuous strand of hyphae) (15). All spores produced by *Phytophthora* sp. can be transported on infected foliage and rootstocks, as well as in soil and water.

For *Phytophthora*, the most common asexual structure is the sporangium, which can germinate directly by forming a germ tube or develop zoospores (15). The sporangia are lightly pigmented and vary in shape and size. Sporangial shape is distinctive for each species, but can range from spherical, subspherical, ovoid, obovoid, ellipsoid, limoniform, pyriform, obpyriform, turbinate, to obturbinate (15). In some cases sporangia may be easily detached from the sporangiophore (caducous); however, there are instances where detachment is more difficult (non-caducous) (15). Caducous sporangia may be dispersed by wind or water.

At the sporangial tips of many species, a papilla is formed between the cytoplasm and the outer wall (20). The papilla acts as a plug and aids in the release of the zoospores from the sporangia. Prior to zoospore release osmotic pressure increases inside the sporangium and the papilla swells as the discharge vesicle enlarges (21).

The zoospores of *Phytophthora* cleave inside the sporangium and are then released into the vesicle, from which they quickly disperse. This is not the case with the closely related genus *Pythium*, where the zoospores cleave inside the discharge vesicle after the protoplast is released from the sporangium (21). The rate at which zoospores are released from the sporangium depends on the amount of cytoplasm remaining after zoospore cleavage and the water potential outside the sporangium (20). Greater amounts of cytoplasm surrounding the zoospores reduces the zoospore release rate (20) and zoospore release can be stopped if the zoosporangium is transferred to an isotonic solution (21). Zoospores require an aqueous substrate for release, and while temperature is only a moderate variable, chilling sporangia that contain zoospores lead to uniform release (20). Up to 50 reniform, heterokont zoospores can be released from a sporangium and are capable of swimming for hours (74). The primary role of these motile spores is short distance dispersal. During unfavorable conditions, zoospores encyst by secreting a wall and shedding their flagella (31). Encystment also occurs naturally when zoospores collide with soil particles and can be induced by agitating a suspension of zoospores (15).

Zoospore biology. For *Phytophthora* sp. directional zoospore movement involves chemotaxis in response to root exudate gradients (40). Chemotaxis may explain short distance dispersal in aqueous conditions. The chemotaxis in zoospores varies depending on the species and chemicals in the environment and can be used to disrupt attraction and encystment on non-host plants (40).

Once the zoospore makes contact with host tissue the spore secretes an adhesive material that affixes it to the host and within 1 to 2 minutes adhesion is complete (32). Subsequently, flagella are shed or absorbed and the spore encysts (31). The encystment triggers the excretion of an anti-desiccation material and an adhesive protein (31). Once encystment and attachment are complete the germ tube emerges for penetration of the host (31). Depending on the *Phytophthora* sp., penetration may be either inter- or intra-cellular (31).

Penetration of host tissue by *Phytophthora* can occur either directly or via appressorium production. Most soil and root-infecting *Phytophthora* species, such as *P. sojae* and *P. medicaginis* penetrate host tissues directly while foliar-infecting species, such as *P. infestans*, produce appressoria before penetration (58).

***Phytophthora* identification.** Classical identification of *Phytophthora* species is based on morphological characteristics on artificial growth media. Distinguishing species based on morphology can be difficult because differences can be extremely small and subjective (15). Polymerase chain reaction (PCR)-based molecular methods (7, 42, 48) have been used to aid in identification and confirmation of *Phytophthora* sp. from plant tissues and soil- and water-baiting samples. Culturing on artificial media and molecular methods are often combined to verify species identity (56).

Classical identification of *Phytophthora* sp. has been largely based on the morphological groupings of Waterhouse (68). Differential characteristics include the production of oospores (homothallic or heterothallic), the attachment position of antheridia, the shape of the sporangium, prominence of the papillum on the sporangium, spore size, the production and nature of the chlamydospores, the source of the isolate (host species, soil or water), and growth habits on different types of artificial media (15).

The polymerase chain reaction (PCR) consists of a number of cycles of denaturing, annealing and elongating target DNA sequences (66). Using PCR, *Phytophthora* sp. can be differentiated by amplifying and sequencing internal transcribed spacer genes (ITS), amplified fragment length polymorphism (AFLP), and/or single-strand confirmation polymorphism (SSCP) (7, 8, 42, 48).

***Phytophthora* species in the southeastern United States.** In the southeastern U.S., *Phytophthora* sp. have been reported from a wide range of hosts and environments (16). Most have been detected from irrigation water, ornamental nursery plants or vegetable crops. There are few reports of *Phytophthora* sp. in forested areas in Georgia and the southeastern United States. Little-leaf disease of pine, caused by *P. cinnamomi*, is an exception and occurs throughout the Piedmont region (33, 55). Recently, Zwart *et al.* (83) were able to isolate *P. cinnamomi* and *P. heveae* from soil samples from the southern Appalachian Mountains using camellia and hemlock leaf tissue baits.

***Phytophthora* species in natural waterways.** Stream baiting has been used to detect introduced *Phytophthora* sp. (70, 76). Surveys targeting specific *Phytophthora* sp. generally do not speciate non-target *Phytophthora* isolates. In addition, seasonal fluctuations in spore production can bias surveys. Surveys by Hwang *et al.* (38, 39) showed monthly fluctuations of *Phytophthora* sp. in streams. The most commonly reported *Phytophthora* species in North American and European waterways is *P. gonapodyides* or a *P. gonapodyides*-like species (27, 70). Ecological studies of existing *Phytophthora* sp. in Georgia have not been conducted, so determining native species can be difficult.

Recirculating irrigation water. Recirculating irrigation water in ornamental production nurseries became a common practice in the early 1970s, particularly in arid regions of the

southwestern United States (74). Recirculating irrigation water is a way to decrease irrigation expenses and minimize water loss. In addition, recirculating irrigation water at production sites helps minimize external pollution and water quality issues from run-off.

Nursery irrigation water management is important because of the high volume of water used during the growing season. It has been estimated that as much as 35% of overhead irrigation water is recirculated in European nurseries (72). No information is available for the U.S. ornamental industry. The percentage of run-off varies depending on plant container spacing, such that the greater the distance between containers, the more irrigation water contacts the ground and runs off. Although water is lost at each irrigation period, recirculating irrigation water increases the number of propagules because of sediment and propagules being washed out of pots and collecting in retention ponds (50). The time of year also affects the concentration of pathogen species and diversity detected in irrigation water either because of *Phytophthora* biology or from irrigation run-off. During times of high irrigation, *Phytophthora* sp. populations may fluctuate because of the change in container sediment run-off (50). The temperature of the retention pond also may affect the activity of different *Phytophthora* sp.

Plant pathogens within recirculating irrigation systems. Recirculating irrigation water also may cycle plant pathogens through irrigation systems (35). Genera found in recirculated irrigation water and bottom sediment in Georgia include: *Alternaria*, *Ascochyta*, *Aspergillus*, *Cladosporium*, *Diplodia*, *Fusarium*, *Macrophomina*, *Phoma*, *Pythium*, *Rhizoctonia*, *Rhizopus* and *Trichoderma* (67).

Pythiaceae species are well adapted for retention pond survival and several *Phytophthora* species have been isolated from recirculated retention ponds, irrigation water and tailwater using filtration and baiting methods, including *P. cactorum*, *P. capsici*, *P. citricola*, *P.*

citrophthora, *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. nicotianae* (syn. *P. parasitica*), *P. gonapodyides*, *P. megasperma*, and *P. syringae* (36, 47, 59, 72, 80). The diversity of *Phytophthora* species found in retention ponds in ornamental nurseries may be due to adaptations for survival within aquatic environments.

Species most frequently found in retention ponds could be examples of highly adapted saprophytes and parasites on aquatic plants or sample collection may have coincided with periods of high influx of propagules from *Phytophthora*-infected host plants (71). Other *Phytophthora* species may be inhibited by higher water temperatures and might not be detectable during summer months. Thomson and Allen (73) found that *P. nicotianae* (syn. *P. parasitica*) could be recovered in water at temperatures greater than 20°C, while *P. citrophthora* was not recovered in water at temperatures higher than 23°C. Spread of plant pathogens to non-infested areas via irrigation water can be accomplished by irrigating non-infested plants with contaminated water, irrigation effluent from infested plants draining into a clean body of water or from flooding of surrounding areas. It has been documented that fumigated citrus orchards became re-infested with *Phytophthora* sp. when irrigated with contaminated irrigation water (47). Zoospores are vital for inoculation by irrigation because they make up more than 94% of the *Phytophthora* sp. propagules in recirculated irrigation water (77).

Irrigation water baiting. Common methods to isolate or detect *Phytophthora* sp. from recirculated irrigation systems are host tissue baiting, ELISA and filtration (2, 6, 59, 81). Studies to determine detection accuracy indicate that no single method supersedes any of the others (2). The method used to isolate *Phytophthora* sp. from nursery irrigation water was determined by the objectives of each study.

Host-tissue baits. Host tissue baits are an effective way to isolate and transport infected tissue from nurseries to the laboratory without damaging the pathogen (6). Susceptible host leaves, shoots and roots can be used as baits for *Phytophthora* detection (59). Initial methods included whole pear fruits baits (71, 81); however, the availability of fresh fruit without prior fungicide exposure and fruit disappearance due to water inhabiting reptiles and amphibians became a problem. This led to the use of leaves from susceptible hosts (25, 73). The plant species used is dependent on the target *Phytophthora* sp. to be detected in a given ornamental nursery. Seedlings of blue lupine (*Lupinus perennis*), tomato (*Solanum lycopersicum*), oak (*Quercus*), alder (*Alnus*) shoots, pine (*Pinus*) needles, cedar (*Cedrus*) needles, pear (*Pyrus*) fruit, and leaf disks from numerous plants, including citrus (members of Rutaceae), rhododendron (*Rhododendron*), camellia (*Camellia*) and holly (*Ilex*) have been used to detect *Phytophthora* sp. in nursery irrigation water (6, 37, 57, 70, 72, 81). Host tissues may vary in the effectiveness of recovery of *Phytophthora* species (17). Streito *et al.* (70) used alder shoots that were left for one week in the summer and four weeks in the winter in natural rivers in France. However, alder shoot baiting displayed a relatively low success rate of isolating the targeted ‘alder *Phytophthora*’ in France (<1%) (70). The most prominent species isolated by this method was a *P. gonapodyides*-like species (70).

Tissue samples used as *Phytophthora* baits can be plated on a selective medium for morphological identification or used in ELISA for *Phytophthora* sp. detection (2, 72). Baiting experiments have exposed baits in bodies of water for 24, 36 or 48 hours in nursery retention ponds and for one to four weeks in natural forest rivers (6, 57, 68, 70). Once the baits are collected they are rinsed with sterile deionized water and plated onto selective or semi-selective

media (17, 59, 70). The plates are then incubated at 20°C for 12 h to 10 days and the cultures are observed for characteristic morphological traits (55, 57, 59).

Baiting *Phytophthora* sp. from recirculated irrigation water has yielded more *Phytophthora* sp. than baiting from the sediment on the banks of recirculated retention ponds in ornamental nurseries (72). Bush *et al.* (6) and Kiziewicz (46) found that *Pythium* sp. were more frequently isolated than *Phytophthora* species from nursery irrigation water when using baiting and filtration methods. The most frequently isolated *Phytophthora* sp. reported by Bush *et al.* (6) were *P. cryptogea* and *P. dreschleri*. Warmer temperatures in June and July yielded more *Phytophthora* sp. and *Mortierella* sp., a common contaminant (57). Using feather-cut and unwounded rhododendron baits, Hwang *et al.* (39) isolated *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. gonapodyides*, *P. heveae*, and *P. pseudosyringae* from natural forest streams in North Carolina. Hwang *et al.* (39) also reported that species diversity varied by month; 11 species were reported in July, while only one in February.

Enzyme-Linked Immunosorbent Assay (ELISA). ELISA has been used to detect *Phytophthora* sp. within host tissues, and to a limited extent in nursery irrigation water and soil (2). Agdia Inc. (Elkhart, IN) produces a double antibody sandwich (DAS) ELISA kit for *Phytophthora* detection that is used as part of the United States Department of Agriculture (USDA) *P. ramorum* detection protocol (43). Potential problems with commercial ELISA kits include the inability to delineate *Phytophthora* species, detection of non-viable propagules and potential false results (2, 50). The color-change detection of ELISA may also lead to ambiguity of test results and raise questions of the quality of the product, but it has been found to be adequate in *P. ramorum* detection (49).

Water filtration. Passing water samples through filter paper and plating the captured propagules on selective media is commonly used to quantify *Phytophthora* and *Pythium* species in recirculated irrigation water (6). The method used by Bush *et al.* (6) required taking a 1-liter sample in three aliquots over a 15 min period. Subsamples (50 ml) were filtered through 47-mm Nucleopore filters with 3.0- μ m pores (Whatman Cop., Ann Arbor, MI) and 47-mm Durapore filters with 5.0- μ m pores (Millipore Corp., Bedford, MA). The filter was then placed into a test tube containing 6 ml of 0.09% agar suspension and aliquots of 1 ml were transferred to an amended selective medium P₅ARP+B and P₅ARP+B+H recipe for PARP (media was amended by adding 50 ppm hymexazol [Tachigaren, 70% a.i.; Sankyo Co., Tokyo] plus 10 mg/L benomyl [Benlate 50WP, DuPont Corp., Wilmington, DE]) (6). In other studies, the filter was placed, filtration side down, onto selective agar media; NVP (vegetable-oatmeal agar and amended with 50 ppm nystatin [Sigma, St. Louis, MO], 100 ppm vancomycin [Sigma, St. Louis, MO] and 10 ppm pentachloronitrobenzene [PCNB; Sigma, St. Louis, MO]) (72), PARP or PARPH (Bacto Agar; PCNB [Terraclor]; Pimaracin; Ampicillin; Rifampicin; with the addition of 70% Hymexazol [Tachigaren] for PARPH in 1 L deionized water) and incubated for various times ranging from 42 to 96 hr (39, 64, 72). The filter was then removed, the culture plates were incubated at 25°C and the plates were inspected daily for at least seven days or after 96 hr for growth (39, 64, 72).

Soil baiting. Baiting soil for *Phytophthora* sp. has become a common practice in orchards, agricultural fields and nursery container mixes (14, 17, 25). Ferguson and Jeffers (17) reported that species detection varied when the same soil was moist or air-dried prior to baiting. More homothallic species were detected if the soil was air-dried and remoistened prior to baiting, while more heterothallic species were detected if the soil was flooded and baited directly (17).

Different host tissues have been used as baits for soil baiting, as with water baiting. Soil baiting generally consists of soil samples being flooded with water and then the host plant tissue baits are floated over or inserted into the flooded soil. Grimm (25) began using citrus leaves instead of citrus fruit due to the lack of year-round fruit availability.

Using a variety of host species tissues for soil baiting is a common practice for *Phytophthora* sp. detection and isolation (62). Ferguson and Jeffers (17) reported that camellia leaf baits yielded the highest number and frequency of *Phytophthora* sp. They also suggested using multiple plant species as baits for each soil sample.

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CHAPTER 3

ISOLATION OF *PHYTOPHTHORA* SPECIES FROM FOREST AND SUBURBAN WATERWAYS AND FROM RETENTION PONDS IN ORNAMENTAL PLANT NURSERIES IN GEORGIA

INTRODUCTION

Phytophthora species are known to cause plant disease epidemics within riparian ecosystems. The effects of *P. cinnamomi*, *P. lateralis*, and *P. alni* infection on forest trees have been well described (2, 17, 19). Although not restricted to riparian ecosystems, *P. ramorum*, the cause of sudden oak death and ramorum blight in the western US, UK, and Europe, and *P. kernoviae*, the cause of beech decline in the UK, have caused considerable forest and landscape tree death (1, 4, 18). Water surveys using leaf baits or filtering methods have been used to detect *P. ramorum* in newly infested areas (9, 27, 28). *Phytophthora ramorum*-infested waterways were identified as a means of pathogen dissemination (5). This finding is consistent with the spread of other *Phytophthora* sp. in water sources. In citrus groves, it was proven that an infested water source can lead to *Phytophthora* infection in areas where the pathogen was not previously found (13). Hansen *et. al.* (6) reported that spread of *P. lateralis*, the cause of Port-Orford cedar dieback, is common along roadways downslope from infested areas and along streams, and that dieback is most prevalent in trees along waterways.

Since the introduction of *P. ramorum* into the US, water and soil surveys have been used to monitor its introduction and spread within infested areas (3, 9, 16, 28). Hwang *et al.* (8) isolated *P. cinnamomi*, *P. citricola*, *P. citrophthora*, *P. gonapodyides* and *P. heveae* from forest waterways in North Carolina. In addition, other *Phytophthora* sp. have been identified in the environment. Although Hwang *et al.* (8) found that water filtration gave the best quantitative results; host tissue leaf baits were comparable.

It is common to isolate non-target *Phytophthora* species while performing surveys for *Phytophthora* pathogens. Jung and Blaschke (12) recovered *P. citricola*, *P. gonapodyides* and *P. pseudosyringae* while surveying for the alder pathogen, *P. alni*. Wamishe *et al.* (28) recovered several species of *Phytophthora*, including *P. gonapodyides*, while surveying suburban waterways for *P. ramorum* in South Carolina.

The introduction of *P. ramorum* into the eastern U.S. on infested ornamental nursery stock could have a devastating impact on eastern forests, particularly within the Appalachian Mountains. *Rhododendron* sp., a common under-story plant in the eastern U.S., is highly susceptible to *P. ramorum* infection (22). Other susceptible native plants include *Kalmia latifolia*, *Viburnum* sp. and *Pieris floribunda* (14, 22). Pathogenicity of *P. ramorum* on *Camellia* varies among species and cultivars, hence, it may be possible for plant breeders to develop cultivars with resistance to ramorum blight (20). *Phytophthora ramorum* could be spread to native forests possibly by homeowners planting infected ornamental plants into their landscapes (29) or possibly from water run-off from ornamental plant nurseries with infected plants (27). Ivors *et al.* (10) reported that the *P. ramorum* populations in U.S. ornamental nurseries and forests fell into three distinct clades using microsatellite markers. It was concluded that the *P.*

ramorum populations found in the U.S. were the result of multiple introductions by the ornamental industry (10).

In 2004, *P. ramorum*-infected *Camellia* plants were detected in 14 retail ornamental plant nurseries and three home landscapes in Georgia (29). The infected plants originated from a large production nursery in California (21). Georgia received over 28,000 potentially-infected camellias since January 2003 from this nursery. Of the 28,000 potentially infected plants, only 8,000 were recovered and destroyed. *Phytophthora ramorum*-infected plants have been repeatedly recovered from retail nurseries in Georgia every year since 2004. The objectives of this study were to 1) assess the recovery rates of *Phytophthora* sp. from forest and suburban streams and nursery retention ponds; 2) to recover and identify *Phytophthora* sp. from these sites and to assess the presence of *P. ramorum* in the three survey location types; and 3) to identify environmental parameters that may affect *Phytophthora* sp. recovery from each location type.

MATERIALS AND METHODS

Forest stream site selection. Ten perennial streams ranging in width from 3 to 9 m, and draining 2-4,000 hectare watersheds in northern Georgia were selected in 2005 (Fig. 3.1). Stream selection was based on potential risk of *P. ramorum* introduction and establishment as noted within the USDA Forest Service SOD Risk/Hazard Map (23). One stream was identified within each of the high-risk hexagons identified on the map. In 2006, eight streams draining 2-4,000 hectare watersheds were selected in northeastern Georgia (Fig. 3.1). Global positioning system (GPS) data points were mapped for each location to assist in sampling (see Appendix C).

Suburban stream site selection. In 2005, four drainage ditches were surveyed in Glynn County, GA near a retail nursery and home landscapes where *P. ramorum* was recovered from infected camellia plants in 2004. Three other stream sites in Forsyth and Fulton Counties, GA

that were directly adjacent to ornamental nurseries where *P. ramorum*-infected plants were recovered were also surveyed in 2005, for a total of seven streams and drainage ditches.

Six perennial streams near or adjacent to retail ornamental plant nurseries in northeastern Georgia were surveyed in 2006 (Fig. 3.1). Five of the six streams were adjacent to nurseries where *P. ramorum*-infected plants were identified in 2004 and 2005. The sixth stream was adjacent to two production nurseries in Clarke County, GA.

Nursery pond site selection. Surface retention ponds within the three largest containerized ornamental production nurseries in Grady and McDuffie Counties, GA, as well as one production nursery in Clarke County, GA were baited in 2006. Additionally, surface retention ponds at two retail nurseries in Fulton and Gwinnett Counties, GA, where *P. ramorum*-infected plants were identified in 2004 and 2005, were also surveyed. A total of 24 pond sites within six nurseries were surveyed from May through September 2006 (Fig. 3.1). Nurseries in Grady, McDuffie, and Fulton Counties were surveyed for two consecutive years (2005 to 2006). All baiting sites were mapped and GPS coordinates were recorded (see Appendix C).

Leaf baiting protocol. Leaf bait cages ($23 \times 30.5 \text{ cm}^2$) for pond sampling were made from plastic mesh (1.27 cm^2 mesh) wrapped at one end over a 30.5 cm sealed PVC pipe (2.54 cm diameter). Leaf bait cages used for stream sampling in 2005 were the same as those used for pond sampling. However, to be consistent with USDA Forest Service stream baiting protocol (24), in 2006 leaf bait cages ($28 \times 33 \text{ cm}^2$) were constructed of 1- x 2-mm mesh screen material that was wrapped at one end over a sealed PVC pipe ($2.5 \times 33 \text{ cm}^2$). The screen material was divided into four compartments, each $8.25 \times 28 \text{ cm}$, by sewing both sides of the material together.

In 2005, two *Camellia japonica* and two *Rhododendron catawbiense* leaves from the previous growing season were used as leaf baits per bait cage per pond or forest stream location. Leaf material was collected from plants within the State Botanical Garden of Georgia, Athens, GA, no more than two days prior to baiting. Leaves were placed in zip-top bags and stored at 5°C until deployment. No fungicides were applied to plants used for leaf collection. On the day of deployment, leaves were surface disinfested in 0.3% sodium hypochlorite for 1 min., then rinsed by submersion in sterile deionized water (SDW) for at least 10 min. Leaves were wounded by cutting slits 1-cm deep at 1-cm intervals along the leaf margins. Leaf baits were secured to the bottom of the cage with metal binder-clips so that they floated 2.5- to 5-cm below the water surface. Cages were then anchored to the pond or stream bank with rope. Leaf baits were retrieved 24 h after deployment at all stream and pond locations. Streams and nursery retention ponds were surveyed twice per location from May to October in 2005. The same leaf baiting protocol used in 2005 was used for nursery retention pond baiting in 2006, except that leaf baits were retrieved 72 h after deployment in an attempt to increase *Phytophthora* sp. recovery.

Subsequent research showed that intact, non-wounded leaves recovered more *Phytophthora* sp. from perennial streams in North Carolina than the feather-cut leaves used previously (8, 9). Therefore, in 2006 for the forest and suburban stream surveys, only the petiole of each leaf was removed using a surface-sterilized razor blade. Leaf margins were not wounded nor surface disinfested as in 2005 in compliance with the USDA Forest Service stream baiting protocol (24). In addition, *R. maximum*, freshly collected from naturally-occurring plants within forested areas near the forest stream sampling sites, and *R. catawbiense*, collected from the State Botanical Garden, were used as leaf baits in 2006 for forest and suburban stream sites,

respectively. Two separate bait cages, each containing four rhododendron leaves, were deployed per forest and suburban stream location. The duplicate leaf bait cage was used in case the first one was lost. Leaves were placed inside the pockets for placement into the waterway. The cages were anchored to the bank with nylon rope so that they floated within the current approximately 1- to 3-cm below the water surface.

Leaf baits were retrieved 2-wk after deployment in May and September, when stream temperature were below 16°C. Leaf baits were deployed for only 1-wk during June to August 2006 when average stream temperature was above 16°C. Pond and stream locations were surveyed for five consecutive months (May to September) in 2006.

Leaf bait processing. All leaf baits were transported to the laboratory on ice and processed within a day of collection. Leaves were gently rinsed in SDW to remove soil or plant debris. In 2005, the feather-cut leaf margins (0.5-1.0 cm²) from all leaves were excised and plated on V8-PARPH medium (15 g Bacto agar [Difco, Sparks, MD]; 50 ml clarified V-8 juice [Campbells, Camden, NJ]; 67 mg 75% PCNB [Terraclor; Chemtura, Middlebury, CT]; 400 µl pimaracin [Sigma-Aldrich, St. Louis, MO]; 250 mg ampicillin [Sigma-Aldrich, St. Louis, MO]; 10 mg rifampicin [Sigma-Aldrich, St. Louis, MO]; 32.5 mg 70% hymexazol [Tachigaren; Sankyo Company, Ltd., Tokyo, Japan] in 1 L deionized water) (11) regardless of the presence of symptoms. In 2006, all tissue showing water-soaking or necrotic symptoms (0.5-1.5 cm²) on the uncut leaves and at least one piece of tissue from the wounded petiole end was plated onto V8-PARPH, between 20 to 119 leaf bait pieces were plated for each sampling time. Samples were incubated at 20-23°C in the dark and *Phytophthora* colonies were transferred onto V8-PARPH, corn meal agar (Difco, Sparks, MD), and V-8 juice agar (15 g Bacto agar; 50 ml clarified V-8 juice in 950 ml deionized water) media for morphological identification and storage.

Environmental data. Environmental data were accessed from the Georgia Automated Environmental Monitoring Network (GAEM) (University of Georgia, Griffin, GA) at locations near the baiting location or on-site. Environmental data collected from GAEM included: cumulative precipitation one and two weeks prior to bait deployment, cumulative precipitation while baits were deployed, air temperature at time of deployment and retrieval, and mean air temperature during deployment. For statistical purposes the cumulative precipitation during bait deployment and one week prior to bait deployment for each baiting period was categorized into three threshold levels (low [< 12.6 mm], medium [12.7 - 25.3 mm] and high [> 25.4 mm]) to determine if quantity of precipitation affected *Phytophthora* recovery rate. Water temperatures for forest streams were taken at the time of leaf bait deployment and retrieval; these temperatures were averaged and used for statistical analysis.

Statistical analysis. Analysis of variance (ANOVA) for 2006 data were conducted using SAS two way ANOVA (v. 8.02; SAS Institute, Cary, N.C) to compare recovery rate of *Phytophthora* species among all survey locations. Regression analysis also was conducted on recovery rate and environmental variables for each sampling site.

RESULTS

The isolation frequency of *Phytophthora* sp. was determined by the percentage of leaf bait pieces from which *Phytophthora* were recovered divided by the total number of pieces plated from each sampling location and month of deployment (Fig. 3.2). The month of baiting had a significant effect on *Phytophthora* sp. isolation frequency at all survey locations (forest streams [$p < 0.001$; $r^2 = 0.40$], suburban streams [$p < 0.001$; $r^2 = 0.76$] and nursery ponds [$p = 0.002$; $r^2 = 0.14$]. The highest mean isolation frequency for all location sites was in September 2006 (Fig. 3.2)

Forest stream baiting. *Phytophthora ramorum* was not recovered from forest streams in 2005 and 2006. In 2005, *P. cryptogea*, *P. gonapodyides* and an unidentified *Phytophthora* species were recovered from four forest streams (Table 3.1). No *Phytophthora* species were isolated from six of the ten stream locations during the sampling periods.

In 2006, nine *Phytophthora* species and three uniquely different unidentified *Phytophthora* species were recovered from forest stream locations (Table 3.2). The *Phytophthora* sp. recovered at each stream location varied, with no species being consistently isolated from all locations. The least number of species recovered was in Rabun County and the most was from the stream in Fannin County. The most common species recovered were *P. cinnamomi*, *P. citricola*, *P. gonapodyides* and *P. nicotianae* (Table 3.2). *P. megasperma* was only recovered from one forest location, and it was not recovered from suburban waterways or nursery retention ponds.

Isolation frequency ranged from 8.8 to 58.7% from May to September 2006, with lowest recovery in July and the highest recovery in September (Fig. 3.3). The percent recovery of *Phytophthora* sp. from leaf baits was statistically higher in baits deployed in September than when baits were deployed May to August (Fig. 3.3).

Of the environmental variables monitored, three had a significant effect on isolation frequency from forest stream locations (Table 3.3). The environmental variables that significantly affected recovery of *Phytophthora* sp. in forest streams were cumulative precipitation one week prior to bait deployment ($p < 0.001$; $r^2 = 0.28$), mean air temperature during leaf bait deployment ($p = 0.016$; $r^2 = 0.14$), mean air temperature during leaf bait deployment and cumulative precipitation one week prior to bait deployment ($p < 0.001$; $r^2 = 0.30$). Less *Phytophthora* was recovered during the warmer summer months than when temperatures were

cooler ($>25^{\circ}\text{C}$ and $<17^{\circ}\text{C}$, respectively). A corresponding increase in *Phytophthora* recovery was seen in September with an increase in cumulative precipitation one week prior to bait deployment (Fig. 3.4). Significantly more *Phytophthora* sp. were recovered when cumulative precipitation one week prior to leaf bait deployment was categorized as high (>25.4 mm) than when less precipitation occurred prior to leaf bait deployment (Fig 3.3).

Suburban stream baiting. *Phytophthora ramorum* was not recovered from suburban streams in 2005 or 2006. In 2005, *P. citrophthora*, *P. cryptogea* and *P. cinnamomi* were isolated from streams in Fulton Co. in July (Table 3.4), but *Phytophthora* sp. were not recovered in October. At the Glynn Co. locations only *P. cryptogea* and *P. gonapodyides* were recovered in May and September (Table 3.4). *Phytophthora* sp. were not recovered from the Forsyth Co. stream in July or October 2005.

In 2006, the isolation frequency of *Phytophthora* sp. ranged from 22.4 to 82.6%, with the highest isolation frequency being in September and the lowest in July (Fig. 3.5). Although about the same number of *Phytophthora* sp. were recovered from each suburban waterway, the species composition varied (Table 3.2). The most common species recovered were *P. gonapodyides* (recovered from all locations), *P. cryptogea* (recovered from 83.3% of the locations) and *P. cinnamomi* (recovered from 66.7% locations). *P. nicotianae* was recovered from 62.5% and 66.7% of the forest and nursery ponds sampled, respectively, but was recovered from 1 suburban waterway (Table 3.2).

Of the environmental data collected from May to September 2006 only cumulative precipitation during bait deployment ($p=0.008$; $r^2=0.23$) was significantly correlated with *Phytophthora* sp. isolation frequency (Table 3.3).

Nursery pond baiting. *Phytophthora ramorum* was not recovered from retention ponds at any nursery in 2005 and 2006. Only *P. cryptogea* was recovered from the retention ponds from the Cairo nursery in 2005 (Table 3.5). *Phytophthora* were recovered from two retention ponds at this location in August 2005. At the Dearing nursery, *P. cryptogea*, *P. citrophthora* and *P. nicotianae* were recovered in April; however, *Phytophthora* sp. were not recovered from ponds at this nursery in August (Table 3.5). *Phytophthora* sp. were not recovered from the retention ponds at the retail nursery in Alpharetta in July. However, both *P. cryptogea* and *P. gonapodyides* were recovered from the ponds in October 2005 (Table 3.5).

The rate of recovery for *Phytophthora* sp. in nursery retention ponds ranged from 24.1 to 48.7% with the highest mean recovery rate in September and the lowest in August (Fig. 3.6). The percent recovery of *Phytophthora* sp. was not statistically different from May to August (Fig. 3.6).

Eleven *Phytophthora* sp. were recovered from nursery retention ponds in 2006; however species composition varied by nursery location (Table 3.2). Of the species recovered *P. citricola* and *P. gonapodyides* were recovered from all nursery locations, *P. cryptogea* was recovered from 88.3% of the locations, *P. nicotianae* from 66.7%, *P. cactorum* from 66.7% and *P. drescherli* was recovered from ponds at three nurseries, but was not recovered from forest or suburban waterways (Table 3.2).

Environmental variables that had a significant effect on the *Phytophthora* sp. isolation frequency from nursery retention ponds were cumulative precipitation one week prior to bait deployment ($p=0.007$; $r^2=0.06$) and average maximum air temperature and cumulative precipitation one week prior to bait deployment ($p=0.014$; $r^2=0.07$) (Table 3.3). Although statistically significant, these two variables accounted for 7% or less of the variability and are

therefore did not contribute greatly to the rate of *Phytophthora* sp. recovery from nursery retention ponds. Precipitation one week prior to leaf bait deployment also had a significant effect, with precipitation between 12.7 to 25.3 mm having a greater effect on *Phytophthora* sp. recovery than low precipitation (<12.6 mm) (Fig. 3.3).

DISCUSSION

During the 2005 and 2006 waterway and retention pond surveys *P. ramorum* was not recovered. Similar to the results of Hwang and Jeffers (7), *P. gonapodyides* was widely distributed among the eight forest survey sites in 2006. During this survey three unique, unidentified *Phytophthora* sp., were isolated from various sites. Hwang and Jeffers (7) also observed unidentified, but distinct species in their surveys. However, unlike the work by Hwang and Jeffers (7) molecular characterization of isolates was not conducted in this study. The species diversity found in this survey was similar to other surveys in the Southeastern U.S. (7, 8, 9, 28) with the exception that *P. heveae*, *P. cambivora* and *P. pseudosyringae* were not identified. This could be due to regional *Phytophthora* sp. introductions or environmental conditions such as climate or soil biology. Hwang *et al.* (8, 9) reported the highest diversity of species and greatest recovery in July in North Carolina, while the lowest isolation frequency was observed in July. Similar observations were made by Hwang *et al.* (8) who reported more diverse species using water filtration compared to leaf baiting in forest streams in North Carolina, although species diversity was comparable. Molecular characterization of isolates from this study may indicate similar species diversity as reported by Hwang *et al.* (8, 9).

From the results of the 2006 survey, the greatest rate of *Phytophthora* sp. recovery from all sampling sites was September (Fig. 3.2), however this is not supported by results from 2005. These differences could be explained by the changes in the baiting methods between 2005 and

2006. In 2006 the leaf baits were deployed for longer periods of time (72 hr in ponds and 1 to 2 weeks in streams) than in 2005, which was only a 24 hr exposure. The leaf baits were deployed for longer periods in 2006 in the suburban and forest streams because they were not feather-cut as in 2005, the overall longer bait deployment could lead to higher *Phytophthora* sp. recovery. This increase could be explained by bait exposure to more *Phytophthora* sp. propagules and/or for a longer period time allowing for *Phytophthora* sp. to penetrate the bait. The host species of leaf bait did not affect the isolation frequency of *Phytophthora* sp. in any of the survey locations. *Phytophthora* recovery was equal for camellia and rhododendron leaf baits used in the surveys. From this study it was determined that the best time to deploy leaf baits was when medium to high volumes (>12.7 mm) of precipitation were forecasted prior to desired sampling time. Precipitation one week prior to baiting could allow *Phytophthora* sp. surviving along waterways time to sporulate and discharge zoospores, which are the primary dissemination propagule.

Results of the 2006 suburban waterway survey indicated that the frequency of *Phytophthora* sp. recovery was higher in August and September than from May to July. *P. gonapodyides* was the most commonly recovered species, while *Phytophthora* sp. were recovered every month. Similar observations were made by Wamishe *et al.* (27), who isolated *P. gonapodyides* from 100% of surveyed sites in 2006 and Hwang *et al.* (7, 8) who reported that *P. gonapodyides* was the most frequently isolated species from forest sites.

The results of this survey may help define the optimum leaf-bait-survey method for *Phytophthora* sp. in Georgia waterways. In order to determine which month results in the highest isolation frequency, the survey should span an entire year. Also, leaf baits should be deployed after periods of medium to high precipitation (> 12.7 mm). From the findings of

Hwang *et al.* (9), possibly filtration methods should be utilized for higher *Phytophthora* sp. recovery rates.

Laboratory procedures can also affect recovery. In this study isolation frequency may have been affected by the time between leaf bait plating and plate examination and subculturing. Delayed evaluation allowed for other organisms to grow, making it more difficult to identify and isolate *Phytophthora* sp. The extended time also could result in *Phytophthora* sp. death. Problems with the growth media used could also affect recovery. After this study was completed it was found that the pH of the V8-based media greatly affected the recovery of *Phytophthora* (data not shown). Hence, future studies should test the pH of the growth media prior to culturing *Phytophthora* sp.

Table 3.1. *Phytophthora* species recovered from rhododendron and camellia leaf baits deployed for 24 hr in perennial streams in forested areas of northern Georgia in 2005.

Forest Stream Location ¹	<i>Phytophthora</i> species recovered from host tissue leaf baits ²				
	May	June	August	September	October
Dade	NB ³	---- ⁴	NB	NB	----
Fannin	NB	----	NB	----	NB
Hart	NB	----	NB	<i>P. cryptogea</i>	NB
Madison	<i>P. cryptogea</i>	NB	<i>P. gonapodyides</i>	NB	NB
Meriweather	NB	----	NB	NB	----
Murray	NB	----	NB	----	NB
Paulding	NB	----	NB	NB	----
Rabun	NB	<i>Phytophthora</i> sp.	NB	----	NB
Walker	NB	<i>P. cryptogea</i>	NB	NB	----
White	NB	----	NB	----	NB

¹ County in northeastern Georgia where perennial streams used for forest survey were located.

² *Phytophthora* species recovered from *Rhododendron catawbiense* and *Camellia japonica* leaf baits plated onto V8-PARPH medium after 24-hr exposure time.

³ Locations were not baited at these times.

⁴ No *Phytophthora* sp. were isolated and identified.

Table 3.2. *Phytophthora* species recovered from host plant leaf baits deployed in perennial streams in forested and suburban areas of northeastern Georgia and from retention ponds from production and retail ornamental plant nurseries from May to September 2006.

Location ¹	<i>P. cactorum</i>	<i>P. cinnamomi</i>	<i>P. citricola</i>	<i>P. citrophthora</i>	<i>P. cryptogea</i>	<i>P. dreschleri</i>	<i>P. gonapodyides</i>	<i>P. megasperma</i>	<i>P. nicotianae</i>	<i>Phytophthora</i> A ²	<i>Phytophthora</i> B	<i>Phytophthora</i> C	<i>Phytophthora</i> sp. ³
Forest Streams													
Dawson		X		X			X			X		X	X
Fannin		X	X	X	X		X		X		X	X	X
Habersham	X	X	X	X			X		X	X			X
Lumpkin	X	X	X				X	X	X		X		X
Murray	X	X	X				X			X	X		X
Rabun			X										X
Stephens		X	X		X		X		X	X			X
White	X	X	X	X					X				X
Suburban Streams													
Clarke		X			X		X			X		X	X
Gwinnett 1		X	X	X	X		X						X
Gwinnett 2			X	X	X		X					X	X
Gwinnett 3	X	X			X		X		X				X
Forsyth	X	X		X	X		X						X
Banks							X			X	X		X
Nursery Retention Ponds													
Grady	X	X	X	X	X		X		X	X			
McDuffie 1		X	X	X	X	X	X		X				X
McDuffie 2	X	X	X	X	X	X	X		X	X			X
Clarke			X		X		X						X
Gwinnett	X		X				X						X
Fulton	X	X	X	X	X	X	X		X		X	X	X

¹ Location by county name in Georgia of surveyed forest and suburban waterways and nursery retention ponds.

² Isolates (A, B, C) had morphological characteristics of *Phytophthora*, but were unidentifiable to species based solely on morphology.

³ Unspecified *Phytophthora* sp. isolated during survey of specified location.

Table 3.3. Analysis of variance and regression analysis for environmental factors and the percentage of *Phytophthora* species recovered from forest and suburban streams and retention ponds at ornamental plant nurseries in Georgia from May to September 2006.

<i>Phytophthora</i> sp. isolation frequency against		Forest Streams	Suburban Streams	Nursery retention ponds
Cumulative rain 1-wk prior to bait deployment	<i>P</i> value ¹	< 0.001	NS	0.007
	<i>r</i> ²	0.28		0.06
Cumulative rain during bait deployment	<i>P</i> value	NS	0.008	NS
	<i>r</i> ²		0.23	
Precipitation thresholds 1-wk prior to leaf bait deployment ²	<i>P</i> value	0.004	NS	0.006
	<i>r</i> ²	0.29		0.10
Mean air temperature during leaf bait deployment	<i>P</i> value	0.016	NS	NS
	<i>r</i> ²	0.14		
Mean maximum air temperature during leaf bait deployment	<i>P</i> value	0.041	NS	NS
	<i>r</i> ²	0.11		
Mean maximum air temperature and cumulative precipitation 1-wk prior to leaf bait deployment	<i>P</i> value	< 0.001	NS	0.014
	<i>r</i> ²	0.28		0.07
Mean minimum air temperature and cumulative precipitation 1-wk prior to leaf bait deployment	<i>P</i> value	0.002	NS	0.028
	<i>r</i> ²	0.28		0.06
All months of deployment mean water temperature taken at deployment and collection	<i>P</i> value	< 0.001	< 0.001	0.002
	<i>r</i> ²	0.40	0.76	0.14

¹ Regression analysis using SAS two way ANOVA (v. 8.02; SAS Institute, Cary, N.C)

² Precipitation thresholds are defined as low (<12.6 mm); medium (12.7-25.3 mm); and high (>25.4 mm).

Table 3.4. *Phytophthora* species recovered from rhododendron and camellia leaf baits deployed for 24 hr in suburban waterways in 2005.

Stream Location ¹	<i>Phytophthora</i> species recovered from host tissue leaf baits ²			
	May	July	September	October
Glynn 1	<i>P. cryptogea</i>	NB ⁴	----	NB
Glynn 2	<i>P. cryptogea</i>	NB	<i>P. cryptogea</i>	NB
Glynn 3	---- ³	NB	<i>P. gonapodyides</i>	NB
Glynn 4	<i>P. cryptogea</i>	NB	----	NB
Forsyth	NB	----	NB	----
Fulton 1	NB	<i>P. citrophthora</i> , <i>P. cryptogea</i>	NB	----
Fulton 2	NB	<i>P. cinnamomi</i> , <i>P. cryptogea</i>	NB	----

¹ Georgia County in which residential drainage ditches and streams used for suburban waterway survey were located.

² *Phytophthora* species recovered from *Rhododendron catawbiense* and *Camellia japonica* leaf baits plated onto V8-PARPH medium after 24-hr exposure time during month of deployment.

³ No *Phytophthora* sp. were isolated and identified.

⁴ Locations were not baited at these times.

Table 3.5. *Phytophthora* species recovered from rhododendron and camellia leaf baits deployed for 24 hr in ornamental nursery retention ponds in Georgia in 2005.

Nursery Pond Location ¹		<i>Phytophthora</i> species recovered from host tissue leaf baits ²				
		April	May	July	August	October
Grady	Pond 1	NB ³	<i>P. cryptogea</i>	NB		NB
	Pond 2	NB	<i>P. cryptogea</i>	NB	<i>P. cryptogea</i>	NB
	Pond 3	NB	---- ⁴	NB	----	NB
	Pond 4	NB	----	NB	----	NB
	Pond 5	NB	<i>P. cryptogea</i>	NB	----	NB
	Pond 6	NB	<i>P. cryptogea</i>	NB	----	NB
	Pond 7	NB	<i>P. cryptogea</i>	NB	----	NB
	Pond 8	NB	<i>P. cryptogea</i>	NB	----	NB
	Pond 9	NB	<i>P. cryptogea</i>	NB	----	NB
	Pond 10	NB	<i>P. cryptogea</i>	NB	----	NB
	Pond 11	NB	<i>P. cryptogea</i>	NB	<i>P. cryptogea</i>	NB
McDuffie	Pond 1	<i>P. cryptogea</i>	NB	NB	----	NB
	Pond 2	<i>P. citrophthora</i>	NB	NB	----	NB
	Pond 3	<i>P. cryptogea</i> , <i>P. citrophthora</i>	NB	NB	----	NB
	Pond 4	<i>P. cryptogea</i> , <i>P. citrophthora</i>	NB	NB	----	NB
	Pond 5	<i>P. citrophthora</i>	NB	NB	----	NB
	Pond 6	----	NB	NB	----	NB
	Pond 7	<i>P. cryptogea</i>	NB	NB	----	NB
	Pond 8	----	NB	NB	----	NB
	Pond 9	<i>P. nicotianae</i> , <i>Phytophthora</i>	NB	NB	----	NB
	Pond 10	----	NB	NB	----	NB
	Pond 11	----	NB	NB	----	NB

Table 3.5. *Phytophthora* species recovered from rhododendron and camellia leaf baits deployed for 24 hr in ornamental nursery retention ponds in Georgia in 2005 (cont'd).

Nursery Pond Location ¹		<i>Phytophthora</i> species recovered from host tissue leaf baits ²				
		April	May	July	August	October
Fulton	Pond 1	NB	NB	----	NB	<i>P. cryptogea</i>
	Pond 2	NB	NB	----	NB	<i>P. gonapodyides</i>

¹ City in which ornamental nursery retention ponds used in the survey were located.

² *Phytophthora* species recovered from wounded *Rhododendron catawbiense* and *Camellia japonica* leaf baits plated onto V8-PARPH medium after 24 hr exposure time.

³ Locations were not baited at these times.

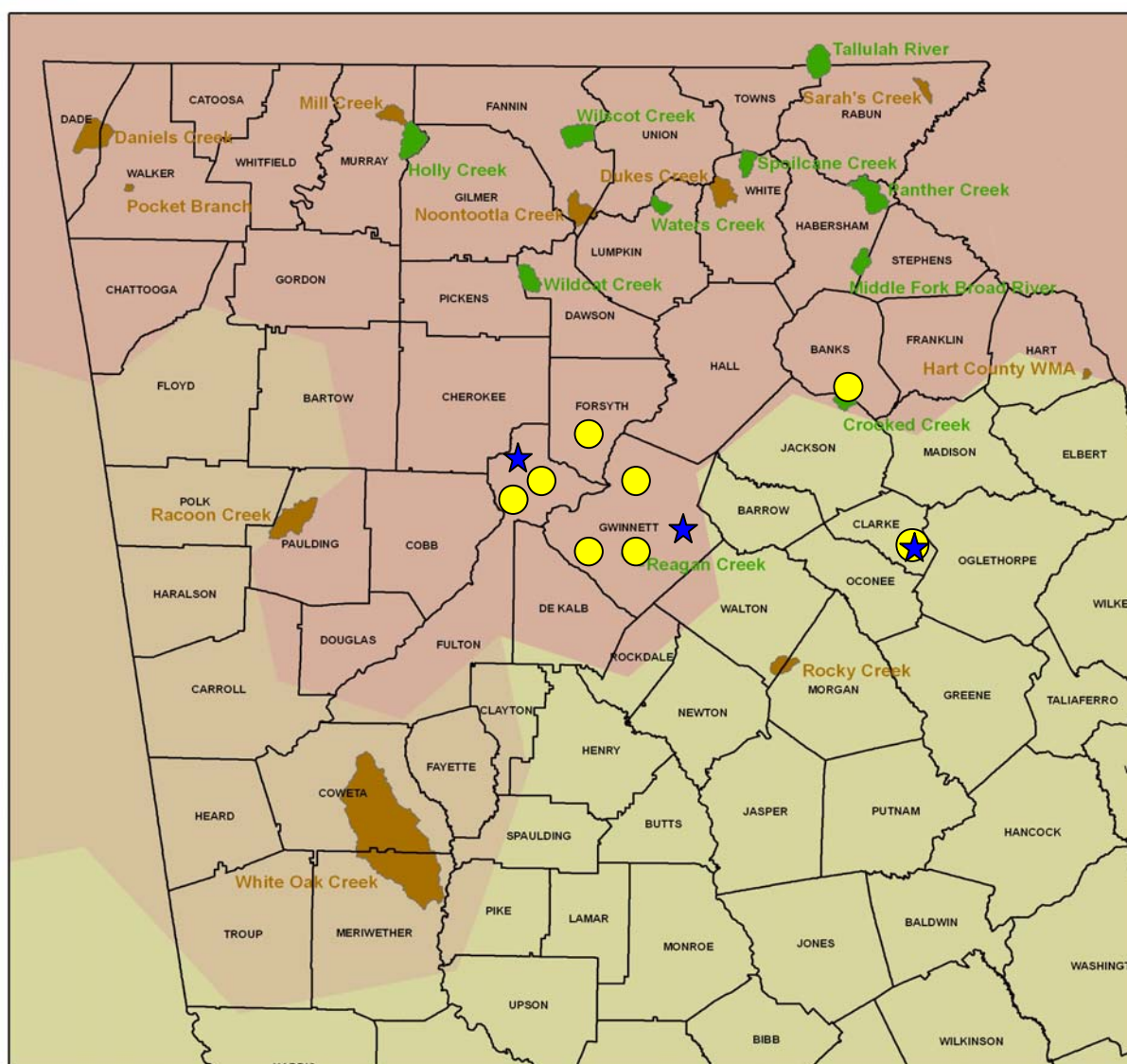
⁴ No *Phytophthora* sp. were isolated and identified.

SOD risk/hazard

High
Moderate
Low

2005 Watersheds
2006 Watersheds
County Boundaries

Data from Georgia GIS Clearhouse,
Georgia Forestry Commission,
USDA-Forest Service.
James Sullivan 11/2007



● Suburban Stream Locations ★ Nursery Pond Locations

Figure 3.1. Forested watersheds, suburban streams and nursery ponds selected and baited in 2005 and 2006 for *Phytophthora* species in Georgia based on the USDA Forest Service SOD Risk/Hazard Map. Not shown in the figure are the nine ponds from McDuffie Co., 11 ponds from Grady Co. and four suburban streams from Glynn Co. in southern Georgia.

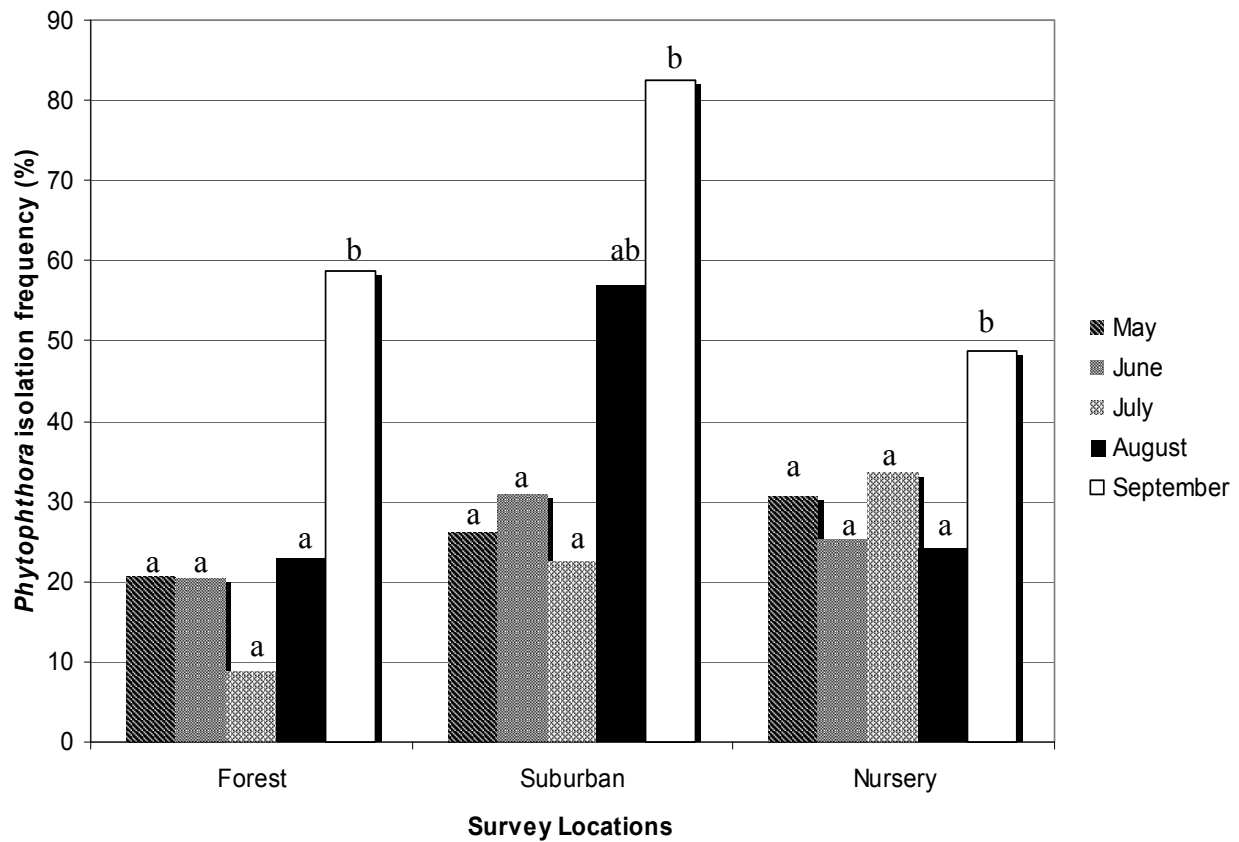


Figure 3.2. Mean isolation frequency of *Phytophthora* species per month from *Rhododendron maximum* for forest streams, *R. catawbiense* for suburban streams, and *R. catawbiense* and *Camellia japonica* for nursery retention ponds out of the number of leaf bait pieces plated onto V8-PARPH medium across location types from May to September 2006. Bars with a common letter are not significantly different ($p= 0.05$) for isolation frequency by month among survey locations.

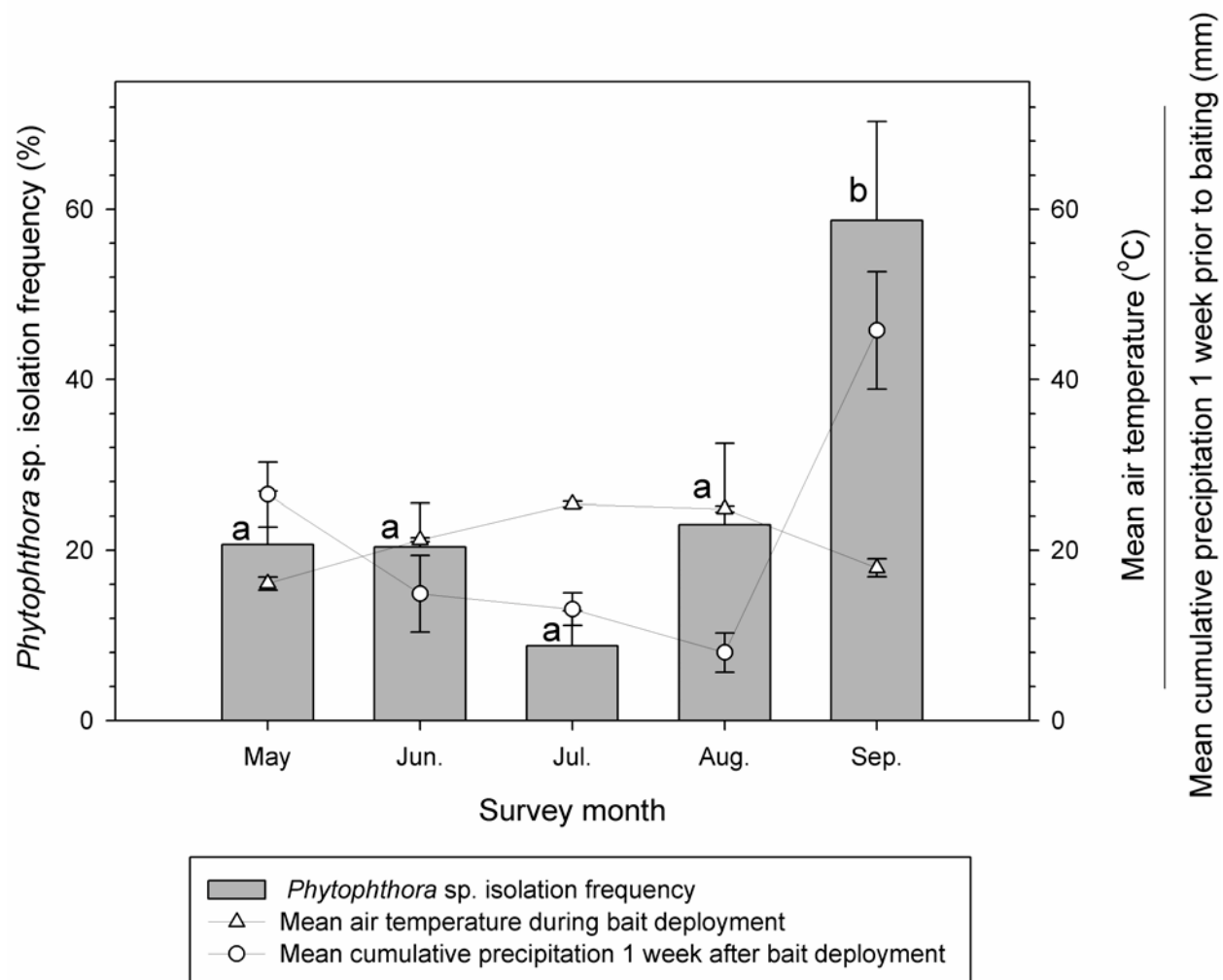


Figure 3.3. Isolation frequency of *Phytophthora* species from rhododendron leaf baits deployed for 7-14 days in streams in forested areas of northeastern Georgia from May to September 2006 with overlay of mean air temperature during bait deployment and cumulative precipitation one week prior to bait deployment. Bars with a common letter are not significantly different ($p=0.05$).

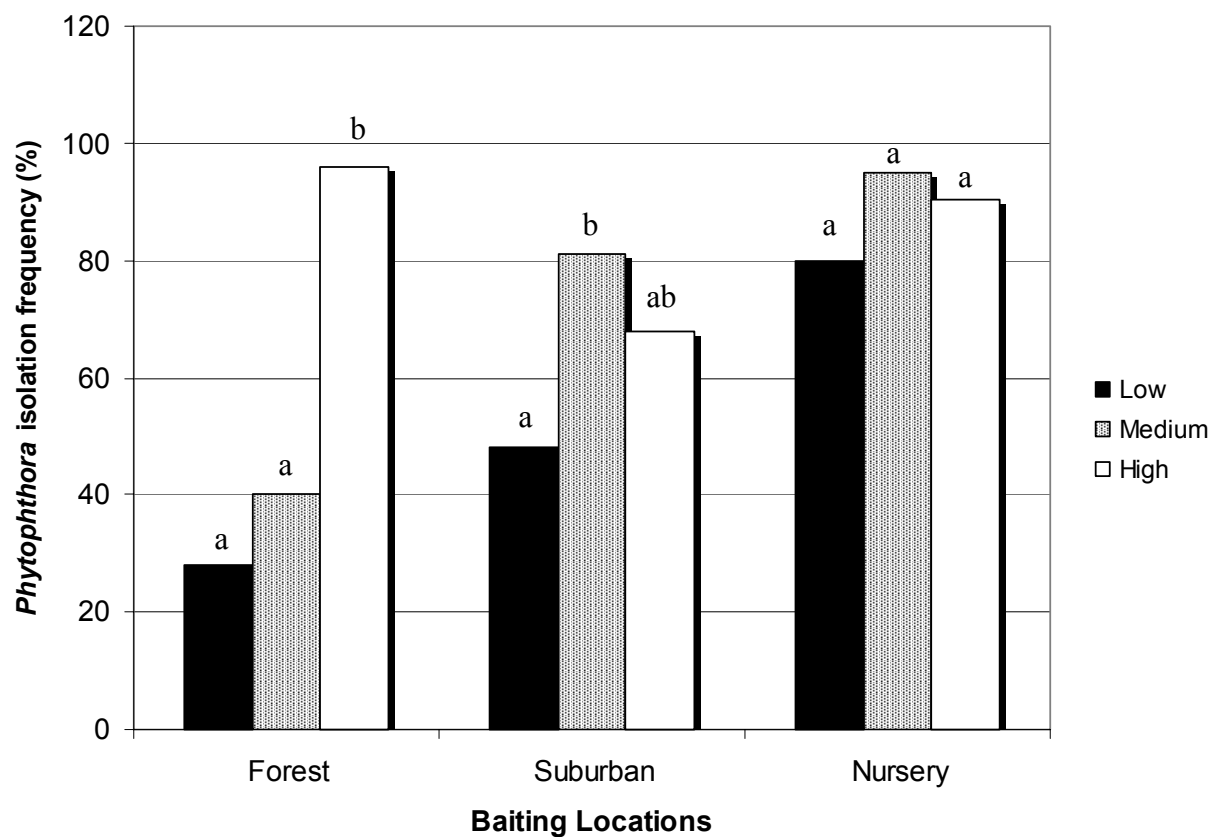


Figure 3.4. Isolation frequency of *Phytophthora* species from all baiting locations against cumulative precipitation one week prior to leaf bait deployment categorized as low (<12.6 mm), medium (12.7-25.3 mm) and high (>25.4 mm) threshold levels. Bars with a common letter are not significantly different ($p = 0.05$) for precipitation thresholds among survey locations.

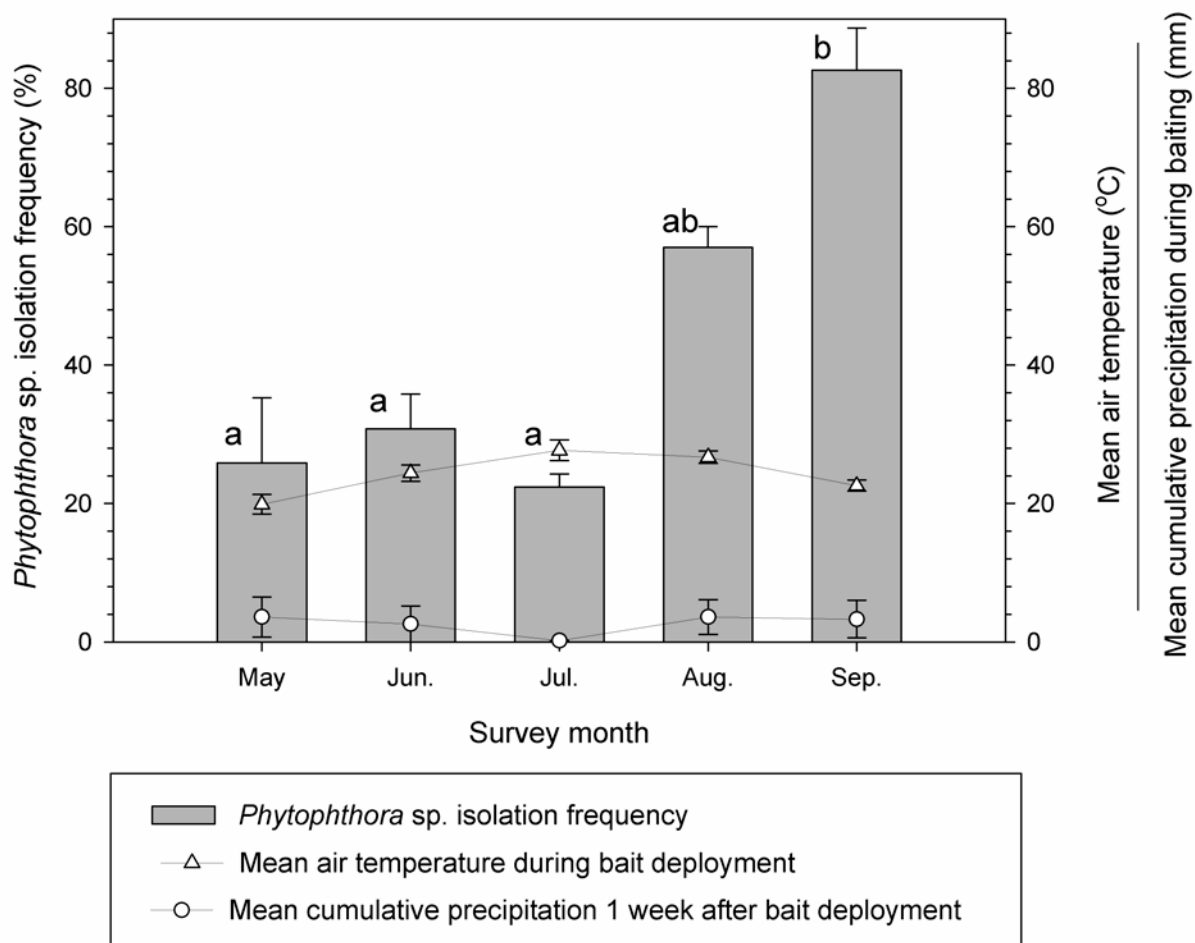


Figure 3.5. Isolation frequency of *Phytophthora* species from rhododendron leaf baits deployed for 7-14 days in suburban waterways near ornamental plant nurseries from May to September 2006 with overlay of mean air temperature and cumulative precipitation during bait deployment. Bars with a common letter are not significantly different ($p = 0.05$) for isolation frequency.

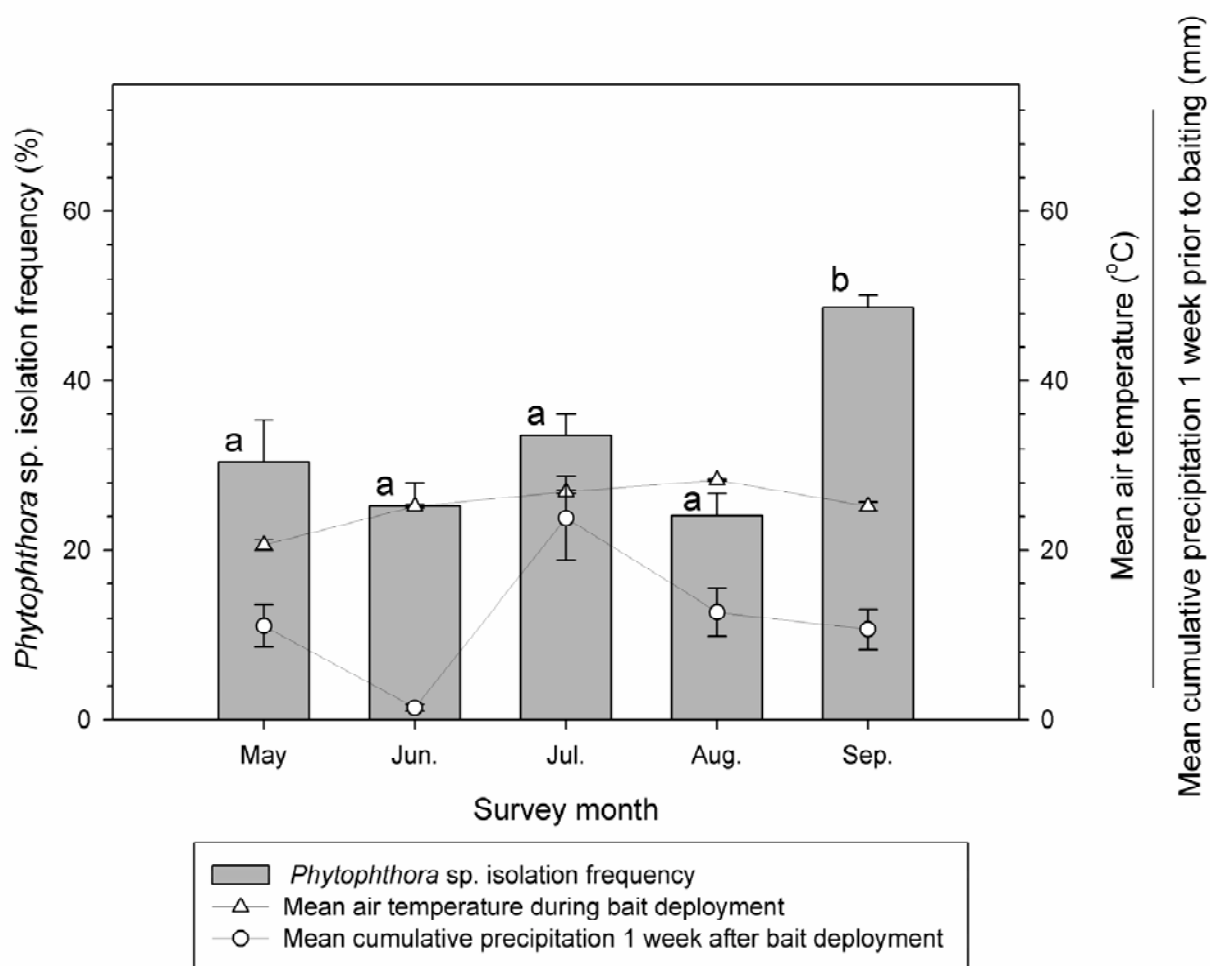


Figure 3.6. Isolation frequency of *Phytophthora* species from rhododendron and camellia leaf baits deployed for 72 hr in retention ponds at four production and two retail ornamental plant nurseries from May to September 2006 and overlay of mean air temperature during bait deployment and cumulative precipitation one week prior to bait deployment. Bars with a common letter are not significantly different ($p = 0.05$) for isolation frequency.

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CHAPTER 4

RECOVERY OF *PHYTOPHTHORA RAMORUM* FROM SOIL WITHIN A RETAIL ORNAMENTAL NURSERY IN GEORGIA

INTRODUCTION

Phytophthora sp. are soil- and water-borne plant pathogens. Most *Phytophthora* sp. are soil inhabitants and infect the root and crowns of host plants. However, some species, including *P. ramorum*, infect the foliage of their hosts (3, 4, 8, 9). Davidson *et al.* (3) found that the spread of *P. ramorum* through the movement of infected plants, soil and water is consistent with other aerial *Phytophthora* species. A major concern of the spread of *P. ramorum* is that the pathogen may infest containerized mixes that non-infected hosts may reside. With the plants not showing symptoms they may be sold and planted, thus spreading the pathogen to new areas away from the nursery. Dart and Chastagner (1) were able to isolate *P. ramorum* from the container mix of a crape myrtle, which is a non-host for the pathogen.

Phytophthora sp. can be spread by the movement of infested soil-less rooting medium from inside plant containers or by water splash during precipitation and irrigation events in ornamental plant nurseries (15). *Phytophthora* sp. have also been able to be recovered from the sides and bottoms of pots, which leads to further spread on asymptomatic plants (1). Water runoff from infested plantations can carry propagules to nearby streams and rivers (11). Recirculated irrigation water also can be a source of inoculum for other host plants irrigated with the infested water (12, 13).

Ornamental nursery beds are often graveled and/or covered with porous ground cloth. The organic matter found in nursery beds is the result of spillage and drainage of soil-less container mixes, which could harbor pathogens such as *P. ramorum* (14). Dart *et al.* (2) reported isolating *P. ramorum* from 5-10 cm depths in nursery ground soil. This could lead to long-term establishment of the pathogen in a nursery because soil treatments to eradicate the pathogen to a depth of 10 cm, which is difficult in densely compacted gravel nursery beds. In addition, *P. ramorum* was recovered from the rooting medium of asymptomatic plants (2). Assaying potentially infected plant tissues for *P. ramorum* could reduce the spread and introduction of this pathogen into new areas.

The objectives of this study were to 1) survey nursery beds for the presence of *P. ramorum* within retail ornamental nurseries in Georgia where *P. ramorum*-infected plants were previously recovered, 2) determine the spread of *P. ramorum* within these nurseries, and 3) to assess the effect of chilling soil samples on the recovery of *P. ramorum* from nursery bed material.

MATERIALS AND METHODS

Sample collection at retail ornamental nurseries. Nursery bed material consisting of native clay loam soil, soil-less bark rooting medium from plant containers, and compacted gravel were collected from four retail ornamental nurseries in Georgia in 2005. The nurseries were located in Glynn, Gwinnett, Forsyth and Fulton counties (Table 4.1). In each nursery, *P. ramorum*-infected plants (camellia and rhododendron cultivars) were recovered in either 2004 or 2005.

Soil or nursery ground bed material (~ 3.79 L) to a depth of 5-10 cm was collected from each sampling location using a hand trowel in a 'W' or 'radiating ray'-pattern within each

sample area in each nursery. After each area was sampled, hand trowels were surface sterilized using 95% ethanol. Samples were placed into separate zip-top bags and transported to the laboratory on ice. Samples were stored at 5°C until processed, which was usually within two days after collection.

Eight 2 L samples were collected from the Glynn County nursery along plant beds where *P. ramorum*-infected plants resided, along drainage patterns between beds, and in a damp drainage ditch on the west-southwest sides of the nursery in May 2005. Four additional 2 L samples were collected from the nursery in Sep 2005 (Table 4.1).

One 2 L sample was collected in Jul 2005 from each of two separate shade-houses (7 x 10 m) from the Gwinnett Co. nursery. One shade-house was re-sampled in Oct 2005. Two 2-L samples were collected in Jul 2005 from the Forsyth Co. nursery. One sample was collected from inside a shade-house (7 x 10 m) where *P. ramorum*-infected plants were observed. The second sample was a cumulative sample taken in a 'ray-pattern' within a 3 m diameter circle around a grated drain adjacent to the shade house and leading to a drainage culvert beneath the nursery beds that eventually drained into nearby streams.

Site descriptions. The plant beds in the nurseries varied significantly between all locations. Differences included whether ground cover was used on the beds, the amount of organic matter from the plant containers residing on the beds, density of gravel that comprised the beds and the slope of the beds.

The plant beds of the Forsyth and Gwinnett Cos. locations were covered in dense gravel with little to no organic matter. The beds were also sloped, which did not allow for water pooling on the bed surfaces.

The Glynn Co. site used ground cloth on all of its plant beds. Once *P. ramorum*-infected plants were identified at this nursery the ground cloth was changed and the old cloth was disposed. Having the ground cloth under all of the containerized plants made it difficult to take soil samples in areas where potentially-infected plants resided. Soil samples were taken at the edge of the ground cloth and down drainage slopes in the nursery. This location was also sloping, with little water pooling on the bed surface.

The beds of the two shade-houses at the Fulton Co. nursery was covered with sparse gravel and thick, muddy organic matter that had spilled out of the plant containers. The areas outside of shade-house A (SHA) (Figure 4.1) sloped downward toward the northeast. In this area a noticeable drainage pattern could be delineated. The beds were relatively flat and there was water pooling on the bed surfaces. Shade-house B (SHB) had more gravel on the bed surface than SHA.

Fulton County nursery. The Fulton Co. site was sampled several times between 2005 and 2006 (Table 4.2). The first Fulton Co. sample was nursery ground bed material collected in May 2005 by the Georgia Department of Agriculture and it was found to contain *P. ramorum* using the leaf-baiting protocol previously described. This nursery was re-surveyed in July, August, October, and November 2005 and in May 2006 to evaluate survival and spread of *P. ramorum* within this nursery. The area sampled consisted of two shade-houses (10 x 15 m each) (SHA and SHB) and the drainage area and walkway areas between the two shade-houses (Fig. 4.1). Delimitation surveys for *P. ramorum* were conducted in SHA in July, August, October, and November 2005 and May 2006. SHB was surveyed in October 2005 because it was evident that plants from SHA were moved into it after nursery bed material tested positive for *P. ramorum* in SHA.

In July 5 sections were established inside SHA at the Fulton Co. nursery. These five sections were sampled as well as each entrance and the northeast drainage area of the shade-house. At the time of nursery bed material sampling, leaf debris was also collected from the plant beds for ELISA and nested PCR testing (data not shown). The eight nursery bed material samples were processed within one day of collection. Three of the eight nursery bed material samples collected in SHA in July 2005 from the Fulton Co. nursery were baited again after the initial processing did not identify *P. ramorum* within these samples. The July-collected samples were in storage at 5°C for 68 days and then they were transferred to a 10°C incubator for 7 days before baiting again. The remaining five nursery bed material samples were stored at 5°C for 83 days, transferred to 10°C for 8 days, and then to 20°C for 5 days before baiting again.

Because of the *P. ramorum*-positive leaf debris collected in July 2005, the area was re-sampled in Aug 2005. In Aug 2005 10 nursery bed material samples were collected from inside the SHA, and at each entrance and the northeast drainage area. The 13 nursery bed material samples were baited for *P. ramorum* within one day of collection. Five of the 13 nursery bed material samples collected in SHA in August 2005 from the Fulton Co. nursery were stored at 5°C for 40 days and then transferred to 10°C for 8 days prior to baiting again. One additional sample was stored at 5°C for 51 days, transferred to 10°C for 8 days, and 20°C for 5 days prior to baiting again.

Twenty and thirteen nursery bed material samples collected in October and November 2005, respectively, from the Fulton Co. nursery were baited on the day of collection. The October samples were collected from both shade-houses. The November samples were collected from SHA, stored at 5°C and baited after 1 wk, 2 wk and 4 wk intervals.

Sample processing. The nursery bed material was thoroughly mixed prior to subsampling. Three 100 cm³ subsamples per sample were placed into separate 0.47 L sealable plastic freezer containers (11 x 11 x 9 cm), flooded with 200 ml of deionized water, and stirred 10-15 seconds with a surface sterilized spatula. The flooded samples settled for at least 15 minutes prior to baiting. *Rhododendron maximum* and *Camellia japonica* leaf pieces were cut from whole leaves using square (4-mm²) and circular (7-mm diameter) paper-hole punches, respectively. Ten leaf pieces of each plant species were floated on the surface of each flooded sample. Containers were sealed and maintained at room temperature (22-23° C). Five leaf pieces per plant species were removed after 24 h and the remaining five pieces were removed after 72 h of incubation. Bait pieces were gently rinsed in sterile deionized water to remove nursery bed material residue and placed on a sterile filter paper before embedding into plates of V8-PARPH medium (6). Plates were incubated at 20-23°C in the dark for at least five days before observation. If growth of *Phytophthora* sp. colonies were observed on a plate, a subsample was transferred to corn meal agar (Difco; Sparks, MD), V-8 juice (50 ml clarified V-8 juice, 15 g Bacto agar in 1-L deionized water) or V-8PARPH media. Isolates were subcultured until clean isolates were obtained. Species were identified based on visual observation of morphological characteristics (17).

RESULTS

Phytophthora sp. isolated during the 2005 survey of nursery bed material included *P. citricola*, *P. citrophthora*, *P. cryptogea*, *P. gonapodyides*, *P. ramorum* and *Phytophthora* sp. *Phytophthora ramorum* was only isolated from one retail nursery location (Table 4.2). The samples collected from the Fulton Co. nursery in November 2005 had the highest frequency of *P. ramorum* detection (Table 4.2).

Phytophthora sp. isolated from the Glynn Co. retail nursery included *P. citrophthora*, *P.*

cryptogea, *P. citricola*, and *Phytophthora* sp. (Table 4.1). Species isolated from the Fulton Co. nursery SHA bed material collected in July 2005 included *P. cryptogea*, *P. ramorum* and *Phytophthora* sp. when initially processed in July (Table 4.1). However, when these same samples were baited in October (78 to 83 days after collection), *P. ramorum* was recovered from five of the eight samples (Table 4.2). The *Phytophthora* species identified from the nursery SHA bed material samples from the Fulton Co. nursery in August 2005 were *P. gonapodyides* and unidentified *Phytophthora* sp. (Table 4.1). However, *P. ramorum* was recovered from five of the six samples when these samples were baited again 48 to 53 days after collection (Table 4.2).

P. ramorum was recovered from 55% of the nursery bed samples from the Fulton Co. nursery collected in October 2005 (Table 4.2). These included samples from SHA and SHB. Plants from SHA were moved into SHB after *P. ramorum* was recovered from the bed material in SHA (Table 4.2). *P. ramorum* continued to be recovered from the SHA ground bed material samples collected in November 2005 (Table 4.2).

In May 2006 *P. ramorum* was recovered from the walkway area between the two shade-houses and from a SHA drainage area (Fig. 4.1). *P. ramorum* also was recovered from the two shade-houses, and from the bottom of plant containers that were maintained outside of SHB (Fig. 4.1). In May 2006 containerized plants still remained inside SHB, while plants and the shade cloth had been removed from SHA in December 2005 that had been left unused. *P. ramorum* was recovered from almost every section of SHB, while only one section in SHA tested positive: the section where *P. ramorum*-infected plants originally were located in May 2005.

Phytophthora ramorum was not recovered from any plant material collected from containerized plants at this location from July 2005 to May 2006. However, *P. ramorum* was recovered via culturing and nested PCR from fallen leaf litter collected off the nursery bed in

July 2005 (Encore® azalea [*Rhododendron* hybrid], *Hydrangea macrophylla*, *Camellia* sp.) and May 2006 (*Ilex fosterii*).

A SAS two-way analysis of variance (ANOVA) of a split-plot design (v. 8.02; SAS Institute, Cary, N.C) was run to determine if cooling the nursery bed materials has a statistically significant effect on isolation of *P. ramorum* from the nursery bed materials collected in November from the Fulton Co. nursery (Fig. 4.2). The results indicated that cooling did not have a significant effect on isolation. This is likely because *P. ramorum* was readily isolated from the November 2005 samples processed on the day of collection. No subsequent increase in recovery was observed following additional chilling of the samples. In fact, percent recovery following chilling was less than when the samples were assayed the day of collection.

DISCUSSION

The recovery of *P. ramorum* from ground bed material comprised of gravel, native soil, and organic bark rooting medium from areas where *P. ramorum*-infected plants were previously recovered is supported by other research (1, 10). Jeffers (10) successfully recovered *P. ramorum* from soilless mixes within container-grown ornamentals in South Carolina. More recently, Dart and Chastagner (1) recovered *P. ramorum* from nursery bed material under asymptomatic and symptomatic containerized nursery plants in Washington retail nurseries, and suggested that infested rooting medium inside or on the outside of containers could introduce *P. ramorum* into non-infested areas.

Jeffers (10) collected container mixes between May and July 2004 and after bioassays reported that cooling soils at 4°C for several weeks may negatively affect *P. ramorum* detection possibly by inducing dormancy, exhausting the organism or death. *P. ramorum* was readily isolated from 92.3% of the samples when baited the day of collection in November from the

same retail nursery when the mean air temperature was 14 °C. Therefore, subsequent cooling of these samples did not have a significant effect on isolation. However, in this study the effect of cooling on *P. ramorum* recovery was not significant when samples were collected in November. *Phytophthora ramorum* was detected after chilling soils for 6-12 weeks when samples were collected in July and August when air temperatures were above 27 °C. When these samples were baited immediately after collection, *P. ramorum* was initially not detected and lead to a false negative result. *Phytophthora ramorum* was readily isolated from 92.3% of the samples when baited the day of collection in November when mean air temperature was 14 °C. Therefore, subsequent cooling of these samples did not significantly affect isolation. This was supported by Fichtner *et al.* (7) who found that *P. ramorum* could be baited from forest soils in May, but not in August. Additionally, detection from fresh forest leaf litter also decreased between May and August. Rizzo *et al.* (16) hypothesized that climate, especially temperature, has a great effect on the spread and distribution of *P. ramorum*.

The *P. ramorum* isolation patterns from the two Fulton County shade-houses, and their surrounding areas, suggest that spread occurred at that site through forklift or foot-traffic, water movement or movement of containerized plants with infested soil on the exterior of the pots. Water run-off is a concern at this site because drainage is directed toward a recirculated retention pond, which is used to irrigate other areas of the nursery.

The results of this survey show that baiting nursery bed material is an effective way to survey for *P. ramorum* in potentially infected areas. Chilling the soils appears to positively affect detection in nursery bed material collected during warm weather, but further research is needed to increase accuracy. The area in the Fulton Co. nursery where *P. ramorum* was detected was once treated with hydrogen dioxide (ZeroTol; Biosafe Systems, LLC, East Hartford, CT),

yet *P. ramorum* was still detected in the soils. This site was covered with asphalt in January 2007 to prevent further spread and establishment of the pathogen, so no further surveys can be conducted at this site.

Table 4.1. *Phytophthora* species recovered from nursery ground bed material from four retail nurseries in Georgia in 2005.

County Location of retail nursery ¹	Collection Date	Number of samples collected	Isolation frequency of <i>Phytophthora</i> sp. (%) ²	<i>Phytophthora</i> sp. recovered
Forsyth	Jul 2005	2	0.0	
Glynn	May 2005	8	75.0	<i>P. citrophthora</i> , <i>P. cryptogea</i> and <i>P. citricola</i>
Glynn	Sep 2005	4	25.0	<i>Phytophthora</i> sp.
Gwinnett	Jul 2005	2	50.0	<i>Phytophthora</i> sp.
Gwinnett	Oct 2005	2	0.0	
Fulton ³	Jul 2005	8	50.0	<i>P. cryptogea</i> and <i>Phytophthora</i> sp.
Fulton ³	Aug 2005	13	57.1	<i>P. gonapodyides</i> , <i>P. cryptogea</i> and <i>Phytophthora</i> sp.

¹ Nurseries in Glynn, Gwinnett and Fulton Counties were sampled twice.

² Percentage of leaf baits that yielded *P. ramorum* on V8-PARPH medium out of the total number of pieces plated.

Table 4.2. Soil baiting results from the delimitation survey for *Phytophthora ramorum* using camellia and rhododendron leaf baits from soils collected from a Fulton County, Georgia retail nursery in 2005 and 2006.

Collection Date	Number of samples collected	Number of samples baited again in September 2005 ¹	Isolation frequency of <i>P. ramorum</i> (%) ²
May 2005	1		100.0
July 2005	8	8	62.5
August 2005	13	6	83.3
October 2005	20		55.0
November 2005	13		92.3
May 2006	27		53.0

¹ Samples were baited again up to 83 days after collection. *Phytophthora ramorum* was subsequently recovered from some of these soil samples.

² Percentage of leaf baits that yielded *P. ramorum* on V8-PARPH medium out of the total number of pieces plated.

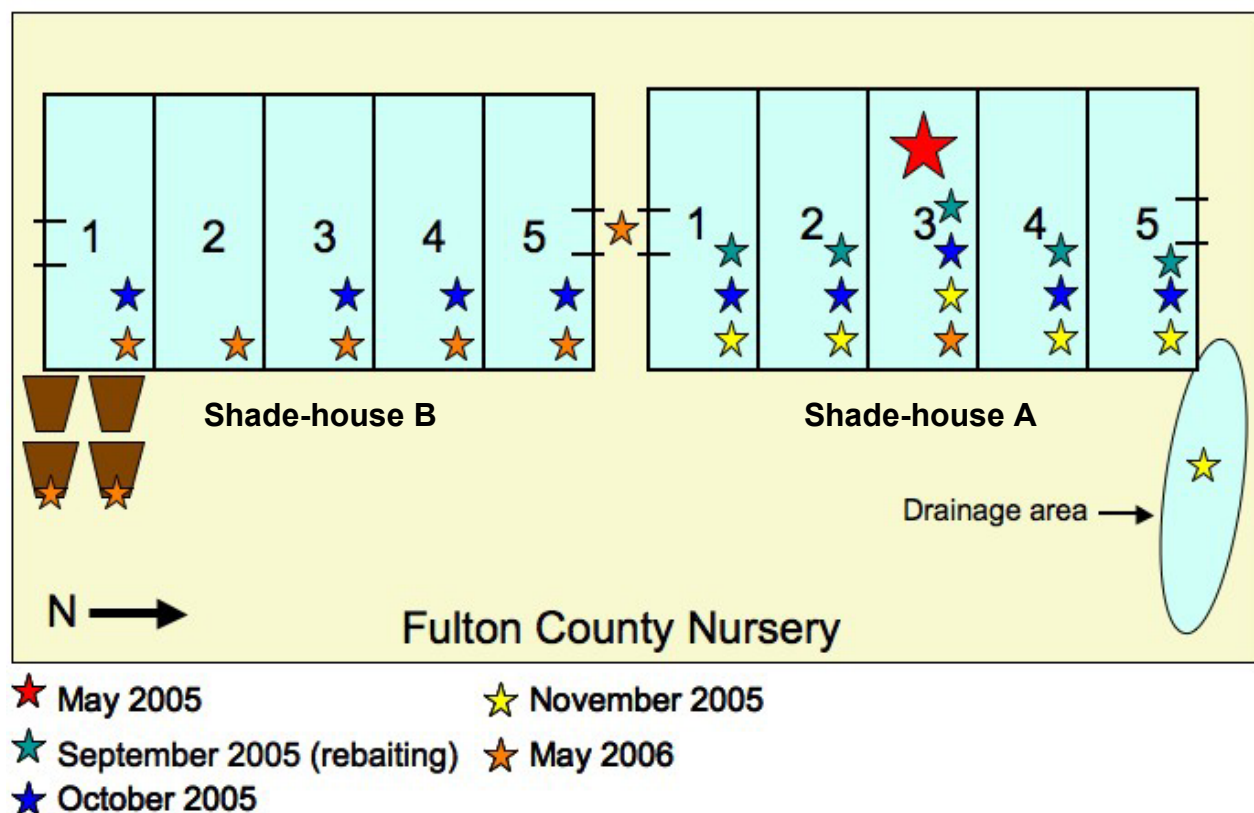


Figure 4.1. Schematic of a Fulton County, Georgia retail nursery showing areas where *Phytophthora ramorum* was recovered from ground bed material during delimitation surveys in 2005 and 2006.

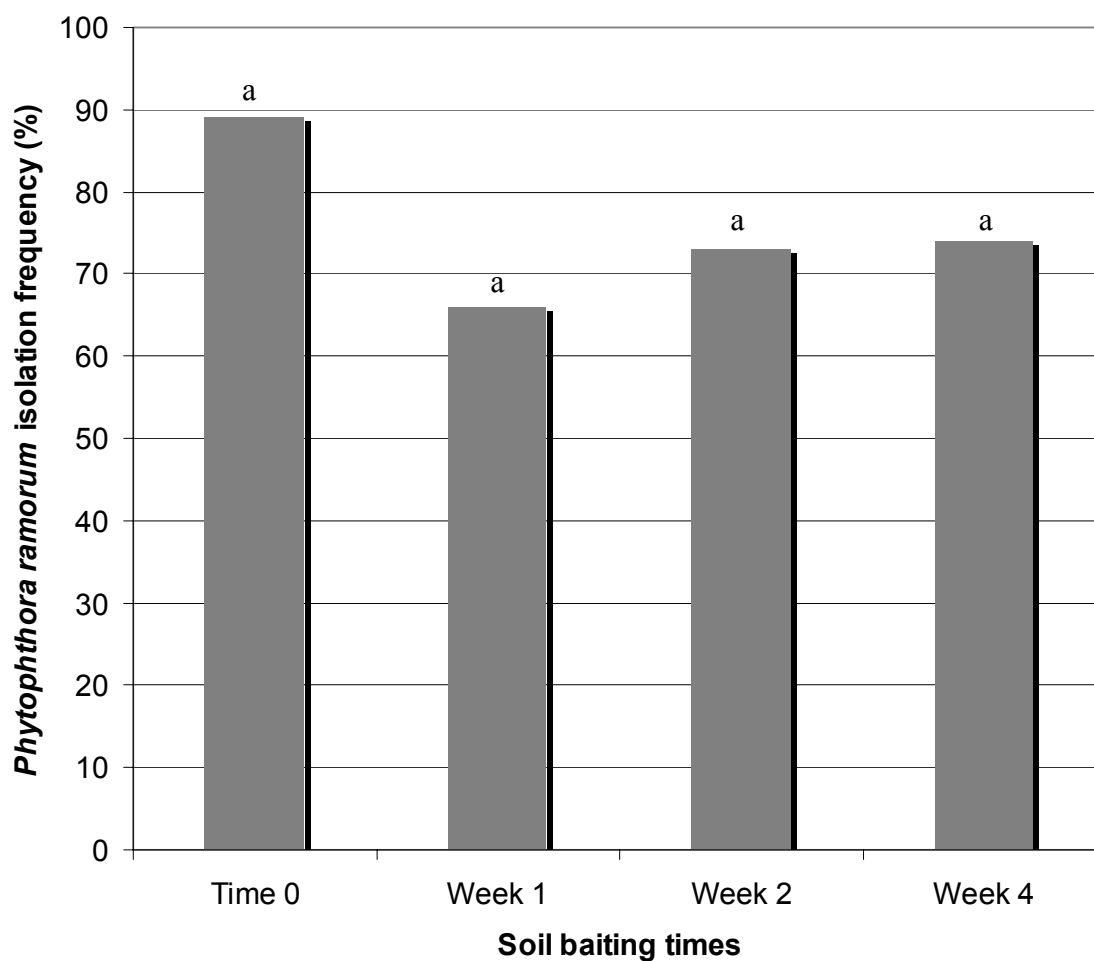


Figure 4.2. *Phytophthora ramorum* recovery from nursery ground bed material collected in November 2005 from a shade-house where *P. ramorum*-infected plants had been identified in a Fulton County, Georgia retail nursery and that were subsequently stored at 5°C prior to rebaiting 1 to 4 wks after collection. Bars with a common letter for each soil baiting time indicate a lack of significance at $p=0.05$.

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CHAPTER 5

CONCLUSIONS

Conducting a water and soil survey for *Phytophthora ramorum* in Georgia may allow the creation of a database for *Phytophthora* sp. populations in different locales. *Phytophthora* sp. are found not only in ornamental production and retail nursery water sources, but also in semi-isolated forest streams. Although more *Phytophthora* sp. were isolated from nursery retention ponds and adjacent streams, there seems to be an underlying native or naturalized population in remote forested areas.

During the surveys in 2005 and 2006, *P. ramorum* was not isolated from any of the water baiting sites. The results indicated a monthly fluctuation in *Phytophthora* recovery in each of the water sources. Further water baiting must be conducted throughout the year in order to determine other periods of seasonal fluctuation as previously reported by Hwang and Jeffers (1) and Hwang *et al.* (2, 3). The results of this study suggest an optimal time to deploy water baits. Based on this study, the best time to survey for *Phytophthora* sp. during the summer months in Georgia is in September. Environmental variables that affect isolation frequency include cumulative precipitation during or one week prior to bait deployment.

The soil baiting results indicate that *P. ramorum* can be isolated from nursery bed materials comprised of gravel and soil-less container medium from containerized ornamental plants. The ability to isolate *P. ramorum* may be affected by the air temperature at time of soil collection and by post sampling treatment of soil samples e.g. chilling treatments. In this study,

P. ramorum was affected by chilling soils, although an optimal increment of time and temperature cannot be specified from this study. The baiting protocol for optimum *P. ramorum* detection may be improved by conducting similar soil surveys for *P. ramorum* throughout the year.

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APPENDICES

APPENDIX A

STATISTICAL ANALYSIS OF ISOLATION OF *PHYTOPHTHORA* SPECIES AGAINST ENVIRONMENTAL FACTORS

Table 1. Analysis of variance for isolation frequency of *Phytophthora* species for all location types against cumulative precipitation two weeks prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.58340	0.58340	9.21	0.0027
Error	186	11.77616	0.06331		
Corrected Total	187	12.35956			
Root MSE	0.25162	R-Square	0.0472		
Dependent Mean	0.33004	Adj R-Sq	0.0421		
Coeff Var	76.23879				

Table 2. Analysis of variance of isolation frequency of *Phytophthora* species in forest streams against cumulative precipitation two weeks prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.17154	0.17154	2.34	0.1269
Error	38	2.67710	0.07045		
Corrected Total	39	2.84864			
Root MSE	0.26542	R-Square	0.0602		
Dependent Mean	0.267710	Adj R-Sq	0.0355		
Coeff Var	100.97607				

Table 3. Analysis of variance of isolation frequency of *Phytophthora* species in nursery retention ponds against cumulative precipitation two weeks prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.19815	0.19815	3.45	0.0659
Error	117	6.72787	0.05750		
Corrected Total	118	6.9262			
Root MSE	0.2398	R-Square	0.0286		
Dependent Mean	0.32465	Adj R-Sq	0.0203		
Coeff Var	73.86402				

Table 4. Analysis of variance of isolation frequency of *Phytophthora* species in adjacent nursery streams against cumulative precipitation two weeks prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.11567	0.11567	1.64	0.2111
Error	27	1.90303	0.07048		
Corrected Total	28	2.01870			
Root MSE	0.26549	R-Square	0.0573		
Dependent Mean	0.44484	Adj R-Sq	0.0224		
Coeff Var	59.68063				

Table 5. Analysis of variance for isolation frequency of *Phytophthora* species for all location types against cumulative precipitation one week prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.71224	0.71224	11.37	0.0009
Error	186	11.64733	0.06262		
Corrected Total	187	12.35956			
Root MSE	0.25024	R-Square	0.0576		
Dependent Mean	0.33004	Adj R-Sq	0.0526		
Coeff Var	75.82061				

Table 6. Analysis of variance of isolation frequency of *Phytophthora* species in forest streams against cumulative precipitation one week prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.79030	0.79030	14.59	0.0005
Error	38	2.05833	0.05417		
Corrected Total	39	2.84864			
Root MSE	0.23274	R-Square	0.2774		
Dependent Mean	0.26286	Adj R-Sq	0.2584		
Coeff Var	88.54098				

Table 7. Analysis of variance of isolation frequency of *Phytophthora* species in nursery retention ponds against cumulative precipitation one week prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.41388	0.41388	7.44	0.0074
Error	117	6.51214	0.05566		
Corrected Total	118	6.92602			
Root MSE	0.23592	R-Square	0.0598		
Dependent Mean	0.32465	Adj R-Sq	0.0517		
Coeff Var	72.67016				

Table 8. Analysis of variance of isolation frequency of *Phytophthora* species in adjacent nursery streams against cumulative precipitation one week prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.001182	0.00182	0.02	0.8772
Error	27	2.01688	0.07470		
Corrected Total	28	2.01870			
Root MSE	0.27331	R-Square	0.0009		
Dependent Mean	0.44484	Adj R-Sq	-0.0361		
Coeff Var	61.43994				

Table 9. Analysis of variance for isolation frequency of *Phytophthora* species for all location types against cumulative precipitation during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00009664	0.00009664	0.00	0.9696
Error	186	12.35947	0.06645		
Corrected Total	187	12.35956			
Root MSE	0.25778	R-Square	0.0000		
Dependent Mean	0.33004	Adj R-Sq	-0.0054		
Coeff Var	78.10413				

Table 10. Analysis of variance of isolation frequency of *Phytophthora* species in forest streams against cumulative precipitation during leaf bait deployment period

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.20947	0.20947	3.02	0.0905
Error	38	2.63916	0.06945		
Corrected Total	39	2.84864			
Root MSE	0.26354	R-Square	0.0735		
Dependent Mean	0.26286	Adj R-Sq	0.0492		
Coeff Var	100.25818				

Table 11. Analysis of variance of isolation frequency of *Phytophthora* species in nursery retention ponds against cumulative precipitation during leaf bait deployment period

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00018847	0.00018847	0.00	0.9551
Error	117	6.92583	0.5920		
Corrected Total	118	6.92602			
Root MSE	0.24330	R-Square	0.0000		
Dependent Mean	0.32465	Adj R-Sq	-0.0085		
Coeff Var	74.94283				

Table 12. Analysis of variance of isolation frequency of *Phytophthora* species in adjacent nursery streams against cumulative precipitation during leaf bait deployment period

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.46461	0.46461	8.07	0.0084
Error	27	1.55408	0.05756		
Corrected Total	28	2.01870			
Root MSE	0.23991	R-Square	0.2302		
Dependent Mean	0.44484	Adj R-Sq	0.2016		
Coeff Var	53.93222				

Table 13. Analysis of variance for isolation frequency of *Phytophthora* species for all location types against mean average soil temperature at 10.16 cm during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.06820	0.06820	1.03	0.3110
Error	186	12.29137	0.06608		
Corrected Total	187	12.35956			
Root MSE	0.25707	R-Square	0.0055		
Dependent Mean	0.33004	Adj R-Sq	0.0002		
Coeff Var	77.88866				

Table 14. Analysis of variance for isolation frequency of *Phytophthora* species for all location types against mean average soil temperature at 5.08 cm during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.13067	0.13067	1.99	0.1603
Error	186	12.22890	0.06575		
Corrected Total	187	12.35956			
Root MSE	0.25641	R-Square	0.0106		
Dependent Mean	0.33004	Adj R-Sq	0.0053		
Coeff Var	77.69048				

Table 15. Analysis of variance of isolation frequency of *Phytophthora* species in forest streams against soil temperature at 5.08 cm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.32179	0.32179	4.84	0.0340
Error	38	2.52685	0.06650		
Corrected Total	39	2.84864			
Root MSE	0.25787	R-Square	0.1130		
Dependent Mean	0.26286	Adj R-Sq	0.0896		
Coeff Var	98.10168				

Table 16. Analysis of variance of isolation frequency of *Phytophthora* species in adjacent nursery streams against soil temperature at 5.08 cm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.03179	0.03179	0.43	0.5166
Error	27	1.98690	0.07359		
Corrected Total	28	2.01870			
Root MSE	0.27127	R-Square	0.0157		
Dependent Mean	0.44484	Adj R-Sq	-0.0207		
Coeff Var	60.98166				

Table 17. Analysis of variance of isolation frequency of *Phytophthora* species in nursery retention ponds against soil temperature at 5.08 cm

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.02188	0.02188	0.37	0.5438
Error	117	6.90414	0.05901		
Corrected Total	118	6.92602			
Root MSE	0.24292	R-Square	0.0032		
Dependent Mean	0.32465	Adj R-Sq	-0.0054		
Coeff Var	74.82538				

Table 18. Analysis of variance for isolation frequency of *Phytophthora* species for all location types against mean average air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.22159	0.22159	3.40	0.0670
Error	186	12.13797	0.06526		
Corrected Total	187	12.35956			
Root MSE	0.25546	R-Square	0.0179		
Dependent Mean	0.33004	Adj R-Sq	0.0126		
Coeff Var	77.40112				

Table 19. Analysis of variance of isolation frequency of *Phytophthora* species in forest streams against mean average air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.40614	0.40614	6.32	0.0163
Error	38	2.44250	0.06428		
Corrected Total	39	2.84864			
Root MSE	0.25353	R-Square	0.1426		
Dependent Mean	0.26286	Adj R-Sq	0.1200		
Coeff Var	96.45035				

Table 20. Analysis of variance of isolation frequency of *Phytophthora* species in nursery retention ponds against mean average air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.08803	0.08803	1.51	0.2222
Error	117	6.83799	0.05844		
Corrected Total	118	6.92602			
Root MSE	0.24175	R-Square	0.0127		
Dependent Mean	0.32465	Adj R-Sq	0.0043		
Coeff Var	74.46604				

Table 21. Analysis of variance of isolation frequency of *Phytophthora* species in adjacent nursery streams against mean average air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.08411	0.08411	1.17	0.2882
Error	27	1.93458	0.07165		
Corrected Total	28	2.01870			
Root MSE	0.26768	R-Square	0.0417		
Dependent Mean	0.44484	Adj R-Sq	0.0062		
Coeff Var	60.17343				

Table 22. Analysis of variance in forest streams to determine if water temperature has an effect on isolation of *Phytophthora* species

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.27572	0.27572	4.07	0.0507
Error	3	2.57292	0.06771		
Corrected Total	39	2.84864			
Root MSE	0.26021	R-Square	0.0968		
Dependent Mean	0.26286	Adj R-Sq	0.0730		
Coeff Var	98.99192				

Table 23. Analysis of variance in nursery retention ponds to determine if water temperature has an effect on isolation of *Phytophthora* species¹

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00005763	0.00005763	0.00	0.9752
Error	21	1.22004	0.05810		
Corrected Total	22	1.22010			
Root MSE	0.24103	R-Square	0.0000		
Dependent Mean	0.44229	Adj R-Sq	-0.0476		
Coeff Var	54.49699				

¹ Temperatures were recorded for August and September only

Table 24. Analysis of variance in adjacent nursery streams to determine if water temperature has an effect on isolation of *Phytophthora* species¹

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00162	0.00162	0.02	0.8979
Error	16	0.52121	0.09508		
Corrected Total	17	1.52283			
Root MSE	0.30834	R-Square	0.0011		
Dependent Mean	0.52006	Adj R-Sq	-0.0614		
Coeff Var	59.28955				

¹ Temperatures were recorded for August and September only

Table 25. Analysis of variance of isolation frequency of *Phytophthora* species in adjacent nursery streams against month of leaf bait deployment (May, Jun, Jul, Aug, Sept)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.52624641	0.38156160	18.60	<0.0001
Error	24	0.49244893	0.02051871		
Corrected Total	28	2.01869534			
Root MSE	0.143244	Coeff Var	32.20089		
Isolation freq Mean	0.444843	R-Square	0.756056		

Table 26. Analysis of variance of isolation frequency of *Phytophthora* species in nursery retention ponds against leaf bait deployment month (May, Jun, Jul, Aug, Sept)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.93641430	0.23410357	4.46	0.0022
Error	114	5.98960731	0.05254041		
Corrected Total	118	6.92602160			
Root MSE	0.229217	Coeff Var	70.60473		
R-Square	0.135202	Isolation freq mean	0.324648		

Table 27. Analysis of variance of isolation frequency of *Phytophthora* species in forest streams against leaf bait deployment month (May, Jun, Jul, Aug, Sept)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.14419277	0.28604819	5.87	0.0010
Error	35	1.70444467	0.04869842		
Corrected Total	39	2.84863743			
Root MSE	0.220677	Coeff Var	83.95292		
R-Square	0.401663	Isolation freq mean	0.262858		

Table 28. Analysis of variance of isolation frequency of *Phytophthora* species from location types against May leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.06029754	0.03014877	0.77	0.4708
Error	35	1.3708701	0.03916706		
Corrected Total	37	1.43114455			
Root MSE	0.197907	Coeff Var	71.43753		
R-Square	0.042132	Isolation freq mean	0.277035		

Table 29. Analysis of variance of isolation frequency of *Phytophthora* species from location types against June leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.03674378	0.01837189	0.72	0.4952
Error	34	0.87067132	0.02560798		
Corrected Total	36	0.90741510			
Root MSE	0.160025	Coeff Var	63.85162		
R-Square	0.040493	Isolation freq mean	0.250620		

Table 30. Analysis of variance of isolation frequency of *Phytophthora* species from location types against July leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.37879163	0.18939581	4.08	0.0257
Error	34	1.57640278	0.04636479		
Corrected Total	36	1.95519441			
Root MSE	0.215325	Coeff Var	80.53918		
R-Square	0.193736	Isolation freq mean	0.267354		

Table 31. Analysis of variance of isolation frequency of *Phytophthora* species from location types against August leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.55982131	0.27991065	5.09	0.0115
Error	35	1.92428061	0.05497945		
Corrected Total	37	2.48410192			
Root MSE	0.234477	Coeff Var	80.83645		
R-Square	0.225362	Isolation freq. mean	0.290063		

Table 32. Analysis of variance of isolation frequency of *Phytophthora* species from location types against September leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.55712483	0.27856242	3.99	0.0275
Error	35	2.44429919	0.06983712		
Corrected Total	37	3.00142402			
Root MSE	0.264267	Coeff Var	47.07299		
R-Square	0.185620	Isolation freq mean	0.561399		

Table 33. Analysis of variance for isolation frequency of *Phytophthora* species for all location types against mean water temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.12073	0.12073	1.48	0.2278
Error	79	6.45546	0.08171		
Corrected Total	80	6.57620			
Root MSE	0.28586	R-Square	0.0184		
Dependent Mean	0.37096	Adj R-Sq	0.0059		
Coeff Var	77.05808				

Table 34. Analysis of variance to determine if air temperature is a good predictor of water temperature at forest stream sites

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	270.48398	270.48398	139.10	<.0001
Error	38	73.89102	1.94450		
Corrected Total	39	344.37500			
Root MSE	1.39445	R-Square	0.7854		
Dependent Mean	18.37500	Adj R-Sq	0.7798		
Coeff Var	7.58886				

Table 35. Analysis of variance to determine if air temperature is a good predictor of water temperature at pond sites

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	29.71296	29.71296	6.85	0.0161
Error	21	91.11857	4.33898		
Corrected Total	22	120.83152			
Root MSE	2.08302	R-Square	0.2459		
Dependent Mean	29.54348	Adj R-Sq	0.2100		
Coeff Var	7.05070				

Table 36. Analysis of variance to determine if air temperature is a good predictor of water temperature at adjacent nursery stream sites

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	82.15812	82.15812	35.80	<.0001
Error	16	36.71688	2.29480		
Corrected Total	17	118.87500			
Root MSE	1.51486	R-Square	0.6911		
Dependent Mean	21.41667	Adj R-Sq	0.6718		
Coeff Var	7.07328				

Table 37. Analysis of variance to determine if isolation frequency of *Phytophthora* species at forest sites were affected by the average maximum air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.30019	0.30019	4.48	0.0410
Error	38	2.54844	0.06706		
Corrected Total	39	2.84864			
Root MSE	0.25897	R-Square	0.1054		
Dependent Mean	0.26286	Adj R-Sq	0.0818		
Coeff Var	98.51991				

Table 38. Analysis of variance to determine if isolation frequency of *Phytophthora* species at nursery pond sites were affected by the average maximum air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.15144	0.15144	2.62	0.1085
Error	117	6.77458	0.05790		
Corrected Total	118	6.92602			
Root MSE	0.24063	R-Square	0.0219		
Dependent Mean	0.32465	Adj R-Sq	0.0135		
Coeff Var	74.11997				

Table 39. Analysis of variance to determine if isolation frequency of *Phytophthora* species at adjacent nursery stream sites were affected by the average maximum air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.15549	0.15549	2.25	0.1449
Error	27	1.86320	0.06901		
Corrected Total	28	2.01870			
Root MSE	0.26269	R-Square	0.0770		
Dependent Mean	0.4484	Adj R-Sq	0.0428		
Coeff Var	59.05285				

Table 40. Analysis of variance to determine if isolation frequency of *Phytophthora* species at all sites were affected by the average maximum air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.35595	0.35595	5.52	0.0199
Error	186	12.00361	0.06454		
Corrected Total	187	12.35956			
Root MSE	0.25404	R-Square	0.0288		
Dependent Mean	0.33004	Adj R-Sq	0.0236		
Coeff Var	76.97153				

Table 41. Analysis of variance to determine if isolation frequency of *Phytophthora* species at forest sites were affected by the average minimum air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.26958	0.26958	3.97	0.0535
Error	38	2.57905	0.06787		
Corrected Total	39	2.84864			
Root MSE	0.26052	R-Square	0.0946		
Dependent Mean	0.26286	Adj R-Sq	0.0708		
Coeff Var	99.10985				

Table 42. Analysis of variance to determine if isolation frequency of *Phytophthora* species at nursery pond sites were affected by the average minimum air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.01432	0.01432	0.24	0.6234
Error	117	6.91170	0.05907		
Corrected Total	118	6.92602			
Root MSE	0.24305	R-Square	0.0021		
Dependent Mean	0.32465	Adj R-Sq	-0.0065		
Coeff Var	74.86634				

Table 43. Analysis of variance to determine if isolation frequency of *Phytophthora* species at adjacent nursery streams were affected by the average minimum air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00343	0.00343	0.05	0.8320
Error	27	2.01527	0.07464		
Corrected Total	28	2.01870			
Root MSE	0.27320	R-Square	0.0017		
Dependent Mean	0.44484	Adj R-Sq	-0.0353		
Coeff Var	61.41545				

Table 44. Analysis of variance to determine if isolation frequency of *Phytophthora* species at all sites were affected by the average minimum air temperature during leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.05398	0.05398	0.82	0.3676
Error	186	12.30559	0.06616		
Corrected Total	187	12.35956			
Root MSE	0.25721	R-Square	0.0044		
Dependent Mean	0.33004	Adj R-Sq	-0.0010		
Coeff Var	77.93370				

Table 45. Analysis of variance to determine if isolation frequency of *Phytophthora* species at forest sites was affected by the average mean air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.85781	0.42890	7.79	0.0013
Error	37	1.99083	0.05381		
Corrected Total	39	2.84864			
Root MSE	0.23196	R-Square	0.3011		
Dependent Mean	1.99083	Adj R-Sq	0.2634		
Coeff Var	88.24591				

Table 46. Analysis of variance to determine if isolation frequency of *Phytophthora* species at nursery retention ponds was affected by the average mean air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.44750	0.22375	4.01	0.0208
Error	116	6.47852	0.05585		
Corrected Total	118	6.92602			
Root MSE	0.23632	R-Square	0.0646		
Dependent Mean	0.32465	Adj R-Sq	0.0485		
Coeff Var	72.79407				

Table 47. Analysis of variance to determine if isolation frequency of *Phytophthora* species at adjacent nursery streams was affected by the average mean air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.08788	0.04394	0.59	0.5607
Error	26	1.93082	0.07426		
Corrected Total	28	2.01870			
Root MSE	0.27251	R-Square	0.0435		
Dependent Mean	0.44484	Adj R-Sq	-0.0300		
Coeff Var	61.25995				

Table 48. Analysis of variance to determine if isolation frequency of *Phytophthora* species at forest streams was affected by the average maximum air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.79358	0.39679	7.14	0.0024
Error	37	2.05506	0.05554		
Corrected Total	39	2.84864			
Root MSE	0.23567	R-Square	0.2786		
Dependent Mean	0.26286	Adj R-Sq	0.2396		
Coeff Var	89.65810				

Table 49. Analysis of variance to determine if isolation frequency of *Phytophthora* species at nursery retention ponds was affected by the average maximum air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.48890	0.24445	4.41	0.0143
Error	116	6.43713	0.05549		
Corrected Total	118	6.92602			
Root MSE	0.23557	R-Square	0.0706		
Dependent Mean	0.32465	Adj R-Sq	0.0546		
Coeff Var	72.56113				

Table 50. Analysis of variance to determine if isolation frequency of *Phytophthora* species at adjacent nursery streams was affected by the average maximum air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.17686	0.08843	1.25	0.3036
Error	26	1.84183	0.07084		
Corrected Total	28	2.01870			
Root MSE	0.26616	R-Square	0.0876		
Dependent Mean	0.44484	Adj R-Sq	0.0174		
Coeff Var	59.83169				

Table 51. Analysis of variance to determine if isolation frequency of *Phytophthora* species at forest streams was affected by the average minimum air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.79152	0.39576	7.12	0.0024
Error	37	2.05712	0.05560		
Corrected Total	39	2.84864			
Root MSE	0.23579	R-Square	0.2779		
Dependent Mean	0.26286	Adj R-Sq	0.2388		
Coeff Var	89.70305				

Table 52. Analysis of variance to determine if isolation frequency of *Phytophthora* species at nursery retention ponds was affected by the average minimum air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.41402	0.20701	3.69	0.0280
Error	116	6.51200	0.05614		
Corrected Total	118	6.92602			
Root MSE	0.23693	R-Square	0.0598		
Dependent Mean	0.32465	Adj R-Sq	0.0436		
Coeff Var	72.98192				

Table 53. Analysis of variance to determine if isolation frequency of *Phytophthora* species at adjacent nursery streams was affected by the average minimum air temperature and cumulative precipitation one week before leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.00545	0.00273	0.04	0.9654
Error	26	2.01324	0.07743		
Corrected Total	28	2.01870			
Root MSE	0.27827	R-Square	0.0027		
Dependent Mean	0.44484	Adj R-Sq	-0.0740		
Coeff Var	62.55386				

Table 54. Analysis of variance to determine if isolation frequency of *Phytophthora* species in forest streams was affected by precipitation thresholds¹ one week prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.72673618	0.3336809	6.34	0.0043
Error	37	2.12190125	0.05734868		
Corrected Total	39	2.84863743			
R-Square	0.255117	Root MSE	0.239476		
Coeff Var	91.10455	Isolation Freq.	0.262858		

¹ Precipitation thresholds are defined as: low = 0-12.6 mm; medium = 12.7-25.3 mm; and high = >25.4 mm.

Table 55. Analysis of variance to determine if isolation frequency of *Phytophthora* species in nursery retention ponds was affected by precipitation thresholds¹ one week prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.58627205	0.29313603	5.36	0.0059
Error	116	6.33974955	0.05465301		
Corrected Total	118	6.92602160			

R-Square 0.084648 Root MSE 0.233780

Coeff Var 72.01021 Isolation Freq. 0.324648

¹ Precipitation thresholds are defined as: low = 0-12.6 mm; medium = 12.7-25.3 mm; and high = >25.4 mm.

Table 56. Analysis of variance to determine if isolation frequency of *Phytophthora* species in adjacent nursery streams was effected by precipitation thresholds¹ one week prior to leaf bait deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.03075105	0.01537553	0.20	0.8191
Error	26	1.98794429	0.07645940		
Corrected Total	28	2.01869534			

R-Square 0.015233 Root MSE 0.276513

Coeff Var 62.15962 Isolation Freq. 0.444843

¹ Precipitation thresholds are defined as: low = 0-12.6 mm; medium = 12.7-25.3 mm; and high = >25.4 mm.

Table 57. Analysis of variance of isolation frequency of *Phytophthora* species in forest streams against cumulative precipitation one week prior to leaf bait deployment and date

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00182	0.00182	0.02	0.8772
Error	27	2.01688	0.07470		
Corrected Total	28	2.01870			

Root MSE 0.27331 R-Square 0.0009

Dependent Mean 0.44484 Adj R-Sq -0.0361

Coeff Var 61.43994

Table 58. Analysis of variance of isolation frequency of *Phytophthora* species in nursery retention ponds against cumulative precipitation one week prior to leaf bait deployment and month of deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	0.71121	0.35560	6.64	0.0019
Error	116	6.21481	0.05358		
Corrected Total	118	6.92602			
Root MSE	0.23146	R-Square	0.1027		
Dependent Mean	0.32465	Adj R-Sq	0.0872		
Coeff Var	71.29714				

Table 59. Analysis of variance of isolation frequency of *Phytophthora* species in adjacent nursery streams against cumulative precipitation one week prior to leaf bait deployment and month of deployment

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.18692	0.59346	18.55	<.0001
Error	26	0.83177	0.03199		
Corrected Total	28	2.01870			
Root MSE	0.17866	R-Square	0.5880		
Dependent Mean	0.44484	Adj R-Sq	0.5563		
Coeff Var	40.20759				

APPENDIX B

MEDIA RECIPES

Corn-meal agar (CMA)

1000 mL deionized water
17 g CMA (Sigma)

V8 Agar

50 mL clarified V8 juice *
800 mL deionized water
15 g Bacto agar
Autoclave together

*V8 clarification: add 1 g CaCO₃/100 mL V8 juice then centrifuge at 7000 rpm for 10 minutes;
store in -80°C freezer

V8-PARPH

15 g Bacto Agar
50 mL clarified V8
950 mL dH₂O
67 mg PCNB (Terraclor)
400 µL Pimaracin
250 mg Ampicillin
10 mg Rifampicin
32.5 mg 70% Hymexazol (Tachigaren)
-Autoclave agar, V8, water and PCNB
-Once medium has cooled to 45°C add remaining ingredients

V8-PAR

15 g Bacto Agar
50 mL clarified V8
950 mL dH₂O
400 µL Pimaracin
250 mg Ampicillin
10 mg Rifampicin
-Autoclave agar, V8 and water
-Once medium has cooled to 45°C add remaining ingredients

APPENDIX C

GPS COORDINATES OF BAITING LOCATIONS

Global positioning system (GPS) coordinates of baiting locations for the 2006 *Phytophthora* species Georgia water survey

Nursery Pond Locations				
Location	Address	Description	GPS	
Athens Wholesale	Athens	Pond 1		
Dudley Nursery	Thomson	Pond 1	33.52086 N	-82.50667 W
		Pond 2	33.51865 N	-82.51284 W
		Pond 3	33.51148 N	-82.51790 W
		Pond 4	33.51288 N	-82.51812 W
John Deere	Alpharetta	Pond 1	34.09002 N	-84.19573 W
		Pond 2	34.08930 N	-84.19530 W
McCorkle's Nursery	Dearing	Pond 1	33.35926 N	-82.40213 W
		Pond 2	33.36213 N	-82.37525 W
		Pond 3	33.36442 N	-82.39051 W
		Pond 4	33.36420 N	-82.38963 W
		Pond 5	33.35990 N	-82.39347 W
Monrovia Nursery	Cairo	Pond 1	30.5175 N	-84.13346 W
		Pond 2	30.50528 N	-84.13944 W
		Pond 3	30.51977 N	-84.13708 W
		Pond 4	30.50809 N	-84.13968 W
		Pond 5	30.51135 N	-84.13613 W
		Pond 6	30.51259 N	-84.1356 W
		Pond 7	30.51496 N	-84.1356 W
		Pond 8	30.5147 N	-84.13408 W
		Pond 9	30.51194 N	-84.13819 W
		Pond 10	30.50966 N	-84.13534 W
		Pond 11	30.51879 N	-84.13566 W
Still Lake Nursery	Lawrenceville	Pond 1	33.92097 N	-83.99926 W

Global positioning system (GPS) coordinates of baiting locations for the 2006 *Phytophthora* species Georgia water survey (cont'd).

Forest Stream Locations				
Location	Address	Description	GPS	
Holly Creek		Forest streams	34.81252 N	-84.65771 W
Middle Fork Broad		Forest streams	34.52407 N	-83.43492 W
Panther Creek		Forest streams	34.67329 N	-83.37172 W
Spoilcane Creek		Forest streams	34.74001 N	-83.74999 W
Tallulah River		Forest streams	34.96228 N	-83.55936 W
Water's Creek		Forest streams	34.679 N	-83.93802 W
Wildcat Creek		Forest streams	34.49787 N	-84.28274 W

Adjacent Nursery Stream Locations				
Location	Address	Description	GPS	
Athens Wholesale	Athens	Stream bait	33.87461 N	-83.30081 W
Crooked Creek	n/a	Stream bait	34.25151 N	-83.46093 W
Gwinnett	n/a	Stream bait	33.89656 N	-84.06605 W
John Deere	Alpharetta	Stream bait	33.51287 N	-82.51813 W
John Deere	Lawrenceville	Stream bait	33.89511 N	-84.05535 W
John Deere	Alpharetta	Stream bait	33.51287 N	-82.51813 W
John Deere	Lawrenceville	Stream bait	33.89511 N	-84.05535 W
Skinner Nursery	Cumming	Stream bait	33.94694 N	-84.19656 W

APPENDIX D

2005 AND 2006 FOREST BAITING LOCATION DESCRIPTIONS

Forest watershed descriptions by county where leaf baits were deployed for *Phytophthora* species detection in 2005.

County	Watershed description
Sarah's Creek, Rabun County	All Forest, Chattahoochee National Forest National Forest Campground
Dukes Creek, White County	Rural residential/ permanent and second home Agricultural/mostly pasture Watershed majority forest Chattahoochee National Forest lands
Noontootla Creek, Fannin County	All forest Chattahoochee National Forest lands
Mill Creek, Murray County	All forest Chattahoochee National Forest lands
Daniels Creek, Dade County	Rural subdivisions/permanent and second home Rural residential/ permanent and second home Agricultural/mostly pasture Cloudland Canyon State Park
Pocket Branch, Walker County	All forest Pigeon Mountain Wildlife Management Area
Raccoon Creek, Paulding County	Rural subdivisions/permanent and second home Rural residential/ permanent and second home Agricultural/mostly pasture Paulding Forest Wildlife Management Area
White Oak Creek, Meriwether County	Rural subdivisions/permanent and second home Rural residential/ permanent and second home Agricultural/mostly pasture Joe Kurz Wildlife Management Area

Forest watershed descriptions by county where leaf baits were deployed for *Phytophthora* species detection in 2005 (cont'd).

County	Watershed description
Unnamed Creek, Hart County	Rural residential Agricultural/mostly pasture Hart Wildlife Management Area
Rocky Creek, Morgan County	Rural residential/ permanent and second home Agricultural/mostly pasture Hard Labor Creek State Park

Forest watershed descriptions by county where leaf baits were deployed for *Phytophthora* species detection in 2006.

County	Watershed description
Habersham County	Rural subdivisions/permanent and second home Rural residential/ permanent and second home Agricultural/mostly pasture/poultry Small rural landscape nursery Watershed majority forest Chattahoochee National Forest lands
Stephens County	Rural residential/ permanent and second home Agricultural/mostly pasture/poultry Watershed majority forest Chattahoochee National Forest lands
Rabun County	Rural residential/ permanent and second home Agricultural/mostly pasture Watershed majority forest Chattahoochee and Nantahala National Forest lands
White County	Rural residential/ permanent and second home Agricultural/mostly pasture Large RV campground Watershed majority forest Chattahoochee National Forest lands
Lumpkin County	All Chattahoochee National Forest lands National Forest Campground
Dawson County	Rural subdivisions/permanent and second home Watershed mostly forest Dawson Forest/GADNR
Fannin County	Rural residential/ permanent and second home Agricultural/mostly pasture Watershed majority forest Chattahoochee National Forest lands
Murray County	Rural residential/ permanent and second home Watershed majority forest Chattahoochee National Forest/GADNR lands