EFFECT OF MULCHING, IRRIGATION AND CULTIVAR ON GROWTH AND DISEASE INCIDENCE OF BOXWOOD

by

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(Under the Direction of Tim Smalley)

ABSTRACT

Boxwood (*Buxus* spp.) are valuable landscape plants that are currently suffering from decline caused by plant pathogens such as *Phytophthora* species. High soil moisture is associated with boxwood decline. These experiments were designed to evaluate the effect of mulching and irrigation on *Buxus sempervirens* 'Suffruticosa' and to evaluate eight *Buxus* cultivars for susceptibility to these pathogens. Mulching did not increase growth and may promote the development of disease when *Phytophthora nicotianae* Breda de Haan was present in the soil. Additional irrigation did not have significant effects on growth of 'Suffruticosa'. Only 'Suffruticosa' inoculated with *P. nicotianae* showed significant reduction in growth, and they gradually developed symptoms of *Phytophthora* root rot. The *B. sinica* cultivars and the *B. sempervirens* cultivars were susceptible to *P. nicotianae*, while the *B. microphylla* cultivars were less sensitive to *P. nicotianae*. All the cultivars were less sensitive to *Phytophthora cinnamomi* Rands.

INDEX WORDS: *Buxus sempervirens* 'Suffruticosa', dwarf boxwood, *Phytophthora nicotianae*, irrigation, mulching, growth, disease incidence, *Buxus*, boxwood, cultivar, *Phytophthora cinnamomi*

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DEDICATION

At the age of 21, I went to the United States, which is the 20th country I have lived in or visited. I would like to dedicate this thesis to my mother Prof. Yiping Shao, and my father Prof. Yingbiao Yang. Because of their unconditional love and support, I have seen more of the world.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Plant Descriptions

Buxus in the family Buxaceae, contains about 90 species and over 365 different cultivars known to exhibit a wide variety of forms and foliage (American Boxwood Society, 2016). According to a landscape survey conducted by *Nursery Management* (Anon., 2011), boxwood has become the No.1 plant purchased by consumers among all woody ornamentals. Two kinds of boxwood are the most popular for the landscape (American Boxwood Society, 2016); *Buxus sempervirens* L. (American or common) and *Buxus sempervirens* 'Suffruticosa' (English or dwarf).

Buxus sempervirens L. (Buxaceae) is a broad-leaved evergreen small shrub and tree native to western and southern Europe, northern Africa, and southwest Asia (Krüssmann et al., 1984). It has numerous cultivars, including *Buxus sempervirens* 'Suffruticosa', a cultivar grown in Europe and near the eastern coast of North America (Dirr, 2009).

For centuries, *Buxus sempervirens* 'Suffruticosa' has been one of the most popular boxwood. It is known by a variety of common names such as dwarf boxwood and English boxwood (Batdorf, 2004). It is a multiple-branched, slow-growing cultivar and matures at 10' (3 m) tall. Requiring two or more centuries to achieve its full size, 'Suffruticosa' distinguishes itself with its a dwarf habit and small leaves. In reality, it is

often maintained as an edging plant constantly sheared to keep it about 1' (30 cm) tall or less (Batdorf, 2005).

Currently approximately 217 cultivars of *Buxus* are registered. More than 100 cultivars and species can be obtained commercially (American Boxwood Society, 2016). Among these available boxwood, the following are most frequently found in the United States: *B. harlandii*, *B. microphylla* 'Compacta', *B. microphylla* var. *japonica*, *B. sempervirens* 'Elegantissima', *B. sempervirens* 'Graham Blandy', *B. sempervirens* 'Suffruticosa', *B. sempervirens* 'Vardar Valley', *B. sinica* var. *insularis* 'Justin Brouwers', *B. sinica* var. *insularis* 'Winter Beauty', *B. sinica* var. *insularis* 'Wintergreen', and *Buxus* 'Green Mountain'.

History of Cultivation

The earliest fossil forms of boxwood have been found in the Pliocene deposits of France (Colby et al., 1911). For centuries, boxwood have been popular ornamentals for landscape use. Known as "man's oldest garden ornamental", the earliest use of boxwood was in approximately 4000 B.C, the garden of an Egyptian nobleman (McCarty, 1950). Subsequently, boxwood were widely spread around Europe.

Boxwood were introduced into North America by the early colonists from Europe and reached its peak popularity in the early 19th century (Batdorf, 2004). The first planting in America occurred about 1653 at Sylvester Manor on Long Island in New York (American Boxwood Society, 2016). Dwarf boxwood were available to southern gardeners of the U.S in the middle of the 18th century (Cothran, 2003).

Historically, many gardens were established in the southern U.S. colonies during the colonial period, of which pleasure gardens were one of the distinct types. These gardens featured the extensive use of parterres, which were versions of early seventeenth and eighteenth-century European designs. Parterres consist of a variety of geometric patterns, and dwarf boxwood were traditionally used as borders along sand or gravel paths (Coleman, 2005).

Hedges played an important role in the antebellum South for ornamental uses, as well as for a variety of practical purposes (Cothran, 2003). For instance, gardens located in Williamsburg, Virginia, have represented some of the best examples of Anglo-Dutch gardens in the colonies, which were characterized by geometric symmetry within an enclosed space, common in England in the late seventeenth and early eighteenth century (Brinkley and Chappell, 1995). To Virginians, a garden was nature tamed, trimmed, and enclosed within a fence or hedge. Tall hedges consisting of boxwood can serve as a living privacy fence to provide privacy from outside environment, and a barrier to reduce noise.

Historically, the planting of boxwood in a formal European ornamental garden was served as a device to exhibit the wealth, education and prestige of its owner. Today, planting of boxwood is still dominated by dwarf boxwood (Batdorf, 2004). It can be found at both residential and public gardens throughout the United States.

Mulch Studies

Mulching is a simple and beneficial practice used in agriculture and horticulture. Mulch is a layer of a material spread on the top of soil, which affects soil and plants. It is also commonly used within landscaping to provide a pleasing visual benefit.

Organic mulches are derived mainly from plant materials. The most common organic mulches include wood products, compost, lawn clippings, leaf mold and straw. With organic mulches, decomposition does occur, and they will have physical, chemical and biological effects on the soil and plants.

Decomposition is a good measure of how long mulches will last (Duryea et al., 1999). It can also be a parameter to measure the conservation of soil moisture and synchronization between N release from the mulch and its demand by crops (Seneviratne et al., 1997). However, decomposition may cause a series of problems when considering physical and chemical effects on soil and plants. If a mulch has not been decomposed yet, it may promote soil granulation. When decomposing, it will release heat, which may hurt the roots under the soil (Penn State Cooperative Extension, 2006).

Various studies have indicated that mulching improves soil moisture status (Lemon, 1956; Mulumba and Lal, 2008). One big advantage of mulching is that mulches reduce soil moisture loss through evaporation. Mulches also reduce the soil's exposure to wind and hot sun, which in turn prevent water from evaporating.

In landscape plantings, mulches facilitate landscape maintenance and provide aesthetical benefits as well (Beck et al., 1998). Studies have evaluated and compared different mulches applying in landscape. Significant differences were detected in soil moisture, soil temperature, weed control, and subsidence among fifteen organic mulches, and people preferred cypress mulch and woodchips according to a survey (Stinson et al., 1990). Hay, black plastic and Turface mulches promoted the largest percentage increases in growth of green ash (*Fraxinus pennsylvanica* Marsh.) (Litzow and Pellett, 1983).

However, mulching of trees and shrubs in landscapes can have negative effects on plants, and can even cause death. Soil pH may be changed by the continuous use of the same type of mulch (Smith-Fiola, 2000). Some mulches acidify soils, such as pine bark, making some nutrients unavailable to plants and causing micronutrient toxicity. On the other hand, some mulches cause soils to become too alkaline, such as hardwood bark, causing acid loving plants to decline.

Trees and shrubs with shallow roots can be severely damaged by heavy and repeated applications of mulch, which may suffocate their roots and lead to oxygen starvation (Gouin, 1983). Piles of mulch being placed directly against the stems or trunks of trees and shrubs, can cause inner bark (phloem) death, which make roots malnourished and weakened (Smith-Fiola, 2000). Effects are more severe when wet conditions persist. Some biotic diseases are also associated with heavy applications of mulch. Most biotic causal agents, such as fungi, fungus-like organisms and bacteria, require moisture to spread and reproduce. Over mulching may provide perfect conditions for these causal agents to thrive, by promoting high soil moisture levels.

Water and Irrigation Studies

Flooding and submergence are major abiotic stresses that profoundly influence the quality of soil and plant growth. While plants require sufficient water to satisfy the needs of growth and evapotranspiration, they require oxygen for sufficient gaseous exchange. The depletion of soil oxygen may make plants hypoxic or anoxic. In these conditions, plants cannot maintain the aerobic metabolism, resulting in reduced absorption and transport of water and nutrients (Jackson and Drew, 1984). Thus, excess

soil water leads to stress by slowing gaseous diffusion and altering plant metabolism, thereby inhibiting growth. Edema or corky-like swelling, is another abiotic disorder caused by waterlogging, which usually occurs on the underside of leaves with blister-like swelling (Kennelly et al., 2012).

Excessive irrigation may cause soil-aeration problems by introducing excess water. Excessive irrigation results in saturated water in soil, especially in soil with poor drainage. Excess water in soil decreases growth rate by reduction of root growth. Root elongation is inhibited by hypoxia. The growth of existing roots, formation of new roots, and root viability are usually sensitive to availability of oxygen (Sena Gomes and Kozlowski, 1980; Webb and Armstrong, 1983). The degrees of adaptation to waterlogging vary in different plant species. Woody roots are usually much more tolerant than non-woody roots (Kozlowski, 1984). Tolerance to excess moisture is possibly related to root porosity, oxygen transport and root respiration, and other mechanisms (Yu et al., 1969; Coutts and Philipson, 1978).

Plants intolerant to waterlogging may lose part of the original root system by death and decay and do not regenerate new roots. Decay of roots is primarily caused by invasion of *Phytophthora* species in flooded soil (Kozlowski, 1984). The degree of root decay is also significant in water-saturated soils when the fungus-like organism is absent. When soil is not fully saturated, root decay directly results from lack of oxygen and not as a result of the fungus (Stolzy et al., 1967).

The direction of root growth can be changed when soil is saturated with water (Kozlowski, 1984). The changes in orientation enable roots to escape from flooding stress

by growing closer to better-aerated soil surface. Thus, root growth is often restricted to the soil surface in poorly aerated soil.

Inhibition of plant height is always observed when soil is waterlogged. Responses to waterlogging vary from stages of development and species. Soil inundation has a significant negative effect on young seedlings of some species, among which some can recover if soil is well drained after waterlogging. In contrast, waterlogging increases height growth of some flood-tolerant species provided that the soil is not inundated with stagnant water. For example, height growth of *Nyssa aquatica* L. seedlings was greater when the soil was continuously saturated than when the soil was watered to field capacity daily (Dickson et al., 1965).

Waterlogging may also cause a chlorotic condition of the leaves and early senescence. In anaerobic soil, the concentration of nitrate in shoots decreases, particularly within old leaves. Deficiencies in nitrogen and other major nutrients lead to these conditions (Jackson and Drew, 1984), thus leading to reduced chlorophyll content. Studies suggests that inhibition of nitrogen uptake, and the consequent redistribution of nitrogen within the shoot, were important contributory factors in the early senescence of leaves and the retarded growth of shoots in flooded barley (Drew and Sisworo, 1977). The importance of chlorophyll content to photosynthesis is emphasized by decline in both chlorophyll and photosynthesis in mineral-deficient plants. The deficiency of nitrogen and possibly other nutrients, reduce the content and/or activity of rubisco, thus decreasing the photosynthetic rate (Herrera, 2013).

Leaf abscission is also induced in many flooded plants. Whereas the number of leaves of *Betula papyrifera* Marsh. seedlings on unflooded plants had approximately doubled, on flooded plants it decreased more than half (Jackson and Drew, 1984).

Water and Disease Studies

The plant disease triangle is a conceptual model that illustrates the relationships among host, pathogen and environment (Agrios, 2005). A susceptible host, a virulent pathogen, and an environment that favors disease development are required for the development of a plant disease caused by a biotic causal agent. Thus, a biotic disease is prevented by eliminating any one of these three causal components.

Predisposition refers to host disposition to disease prior to infection, which affects susceptibility to biotic and abiotic pathogens (Yarwood, 1976). Soil water may act on the pathogen and the host plant as a favorable environmental factor for root disease development. Under waterlogging conditions, root systems become necrotic due to lack of oxygen. The necrotic tissues lose physical integrity. Such root necrotic is often termed root pruning, which not only provides a vector for pathogen entry, but also impairs physiological recovery by limiting root volume. The mechanical support of the root system is substantially reduced in saturated soil. This significantly predisposes the plant canopy to lodging, increasing the likelihood of inoculation and spread of disease in the plant canopy by bringing plant tissue from adjacent plants in contact with one another and wet soil or floodwater when severe (Stolzy and Sojka, 1984).

Root-infecting microorganisms include fungi, fungus-like organisms, bacteria, nematodes and viruses. The list of diseases caused by fungi and fungus-like organisms is

extensive, and many are associated with saturated soil conditions. For instance, *Phytophthora* root rot in alfalfa (*Medicago sativa* L.) has been associated with excessive rainfall and poorly drained or heavily irrigated soils (Kuan and Erwin, 1980). In woody plants, root rot caused by *Phytophthora cinnamomi* Rands. has caused substantial damage on avocado (*Persea Americana* Mill.) in Eastern and Western Hemisphere. Root diseases are more severe in waterlogged or poorly drained soil (Ploetz and Schaffer, 1987). When jarrah trees (*Eucalyptus marginata* Donn ex Sm.) were waterlogged at the same time or after being inoculated, infected roots significantly increased and more lesions were formed, which was probably due both to the increased attraction of zoospores to anaerobically respiring roots and to increased zoospore motility in flooded soil (Davison and Tay, 1987).

Saturated soil conditions also commonly occur in nursery and landscape plantings. Soil moisture extremes can predispose normally resistant rhododendrons to root and crown rot caused by *Phytophthora cinnamomi* (Blaker and MacDonald, 1981).

***Phytophthora*-caused Diseases**

Phytophthora species have a wide host range (Scott et al., 2013) and are responsible for most of the root and crown rots of woody plants (Erwin and Ribeiro, 1996). *Phytophthora* species, often referred to as water molds, are no longer classified in the kingdom Myceteae (fungi). According to the latest classification, they are oomycetes, belonging in the kingdom Chromista. It means that *Phytophthora* species are fungus-like but have unique features (Rossman and Palm, 2006). Their sexual reproduction is the

most important morphological feature of the oomycetes, by production of oospores after union of two gametangia in which meiosis occurs prior to fertilization.

The inoculum of *Phytophthora* increases very quickly. It only takes a few days or weeks to grow from undetectable levels to high levels (MacKenzie et al., 1983; Weste, 1983). Because of their short generation time and great productive capacity (Dick, 1992), *Phytophthora*-caused diseases are generally considered to be multicyclic (Fry, 1982; MacKenzie et al., 1983). At first, the reproductive capacity of *Phytophthora* species allows it to increase rapidly by formation of sporangia and zoospores. Then, zoospores are moved in soil by flowing irrigation water, rainfall runoff, and movement of soil by any means (Erwin and Ribeiro, 1996).

Like most other pathogens, high water tables or excessive irrigation by flooding and sprinkling has a serious impact (Rotem and Palti, 1969), increasing severity and spread of *Phytophthora*-caused diseases. If environmental conditions are favorable for pathogens, production of sporangia and zoospore from infected plant tissues is rapid. In turn, the increase in inoculum exacerbates the severity of *Phytophthora* root rot.

Also disease resistance of hosts is impaired by environment that favors pathogens (Francl, 2001). Conditions of oxygen deficiency can be injurious to roots, and such stress can predispose plants to infection by *Phytophthora* species (MacDonald, 1982). Soil pores are filled with water rather than air; thus oxygen becomes deficit. The degree of anaerobiosis is affected by soil properties, respiration of roots and microorganisms. Previous study suggested that citrus grown in soil infested with *Phytophthora nicotianae* Breda de Haan and *Phytophthora citrophthora* (R.E. Sm. & E.H. Sm.) and exposed to

conditions of low soil oxygen had higher percentages of root decay than plants exposed to normal levels of soil oxygen (Stolzy et al., 1965).

Extent of soil saturation affects the severity of root disease as well, mainly depending on length of time that the soil remains saturated or near saturation (Kuan and Erwin, 1980). However, there are few recommendations on a field basis about what extent of saturation would constitute a significant hazard. Besides, soil texture and structure are highly variable, and water tables change year by year.

However, the importance of *Phytophthora* species in causing root and crown diseases is often underestimated (Erwin and Ribeiro, 1996). The reasons include that, the symptoms of many *Phytophthora*-caused diseases are similar as damage from other pathogens or abiotic agents, and foliage symptoms can only be detected months to years after root infection for woody plants.

Irrigation and Mulch Application in Boxwood

Boxwood are currently suffering from decline in the United States. *Buxus sempervirens* 'Suffruticosa' decline was perhaps first described in the 1930s and has been attributed to many causal factors, including fungi, fungus-like organisms, nematodes, and weather (Andrus, 1933). Now boxwood are facing physiological and pathological problems.

Excessive irrigation is observed to be one of the most common causes of the decline of boxwood (Niemiera, 2013; Bartlett tree experts, 2016). Boxwood is from the Mediterranean region and adapted well to Mediterranean-type climates of warm, dry summers, and cool, wet winters. However, the climate of the Southeastern United States

is relatively wet all year. As a result, irrigation may be easily excessive compared to its natural growing conditions.

Applying mulch is one of maintenance practices for boxwood. Apart from mulch providing an aesthetic benefit to gardens, it can increase water use efficiency and shoot growth (Tolk et al., 1999). Mulch greatly retards the loss of moisture from the soil by reducing soil evaporation (Bussière and Cellier, 2004; Ramakrishna et al., 2006) and irrigation frequency is reduced (Rao et al., 2013). With mulch, a more favorable and uniform soil moisture regime is maintained. Mulch also provides other functional services, which include reducing weed growth, moderating soil temperature, reducing the effects of soil erosion and enhancing soil fertility (Greenly and Rakow, 1995).

According to Batdorf (2005), boxwood are observed to grow well when mulched. However, because boxwood do not tolerate high soil moistures, mulch may keep soil too moist and inhibit growth or promote disease. This occurs most often in poorly drained soils and in areas where surface water collects, such as in the southeastern U.S., drainage of our piedmont soils.

Excessive irrigation might decrease water use (Sun et al., 2006). Applying mulch too thickly is also a serious hazard for boxwood roots, which may lead to lack of soil air so root growth is inhibited and disease might be promoted (Gouin, 1983).

Diseases in Boxwood

Diseases are one of the most common causes of decline of boxwood. Decline of *Buxus sempervirens* ‘Suffruticosa’ has been reported in the US since 1930s (Andrus, 1933). Symptoms of disease may be difficult to distinguish. For instance, plants

beginning to show light chlorosis were found to have already lost 75-90% of their fibrous roots in nurseries in Virginia (Lambe and Wills, 1975).

Possible pathogens isolated from boxwood are mostly fungi such as *Phoma* spp., *Fusarium* spp., and *Clonostachys buxi* (J.C. Schmidt ex Link) Schroers (formerly *Paecilomyces buxi* (J.C. Schmidt ex Link) J.L. Bezerra) (Montgomery et al., 1977). Symptoms caused by fungi are mostly leaf blight and root rot. *Pseudonectria buxi* (DC.) Seifert, Gräfenhan & Schroers (formerly *Volutella buxi* (DC.) Berk. and *Nectria rousseliana* Mont.) leads to leaves turning straw-yellow, or light tan colored, and smaller twigs die-back for some distance (Dodge, 1944). *Calonectria pseudonaviculata* (Crous, J.Z. Groenew. & C.F. Hill) L. Lombard, M.J. Wingf. & Crous. (formerly *Cylindrocladium buxicola* Henricot) causes a sudden and severe defoliation (Henricot and Culham, 2002).

Excessive irrigation may directly promote these diseases, as fungi thrive under moist conditions. Montgomery and Wills (1973) suggest that in the greenhouse a combination of inoculation with *Clonostachys buxi* and *Fusarium* spp. produced more infection of root rot than either alone, the incidence being highest with excess moisture. Rooted cuttings inoculated with *Clonostachys buxi* and *Fusarium solani* (Mart.) Sacc. also had more root rot than non-inoculated plants.

Plant-parasitic nematodes such as *Pratylenchus* spp. were reported to cause diseases as reported in the eastern U.S. (Tarjan, 1948). The pathogen is migratory in habit and evacuates dead and almost dead roots. Continuous attack over a number of years causes proliferous lateral root formation above the points of attack, with eventual formation of a shallow, densely interwoven root system in the upper soil layers. Above

ground, symptoms may include defoliation, 'stag-head', a sickly, stunted growth and various types of foliage discoloration. Symptoms of the disease are usually more pronounced during periods of drought or freezing. Report from North Carolina concluded that although the frequency of occurrence and density of nematodes may be some indication of their relevance to decline, proof of pathogenicity has only been established in a few cases (Haasis et al., 1961).

Phytophthora-caused Diseases in Boxwood

Phytophthora species are common pathogens causing boxwood diseases. Both dwarf boxwood (*Buxus sempervirens* 'Suffruticosa') and common boxwood (*Buxus sempervirens*) are susceptible to the *Phytophthora*-caused diseases (Andrus, 1933).

Disease occurring on boxwood caused *by P. nicotianae* was first described by Andrus (1933) on *Buxus sempervirens* and *Buxus sempervirens* 'Suffruticosa', and reported to be the cause of root rot, canker and blight (Haasis, 1961). Root and crown rots are probably the most common manifestations of disease. Boxwood root rot occurs before top symptoms become apparent. Belowground, the feeder roots are first infected, and then the larger primary roots get infected. Aboveground, the base of the main stem often becomes discolored, and leaves then turn bronze or dull yellow and eventually drop. The disease develops rapidly in waterlogged soils, when soils are excessively wet or poorly drained.

Study (Montgomery et al., 1977) supports that boxwood decline, mainly caused by pathogens, has been associated with soil drainage, or soil water content. However, Lambe and Wills (1975) conclude that disease incidence could not be attributed to

consistent environmental patterns and could occur both on poorly-drained and well-drained sites at that time.

Estimating Incidence and Severity of Root Diseases

Disease assessment must be accurate, precise and reproducible, and applicable over a range of conditions, economically and simply (Campbell and Neher, 1994). It is common that several types of symptoms and signs are associated with a single disease, maybe both root and shoot symptoms, and diseases caused by different causal agents may present similar symptoms. It is more challenged and costly to assess root epidemics.

Theoretically, shoot symptom assessment can be utilized for root diseases. Pilot experiments are required to establish relationships between aboveground symptoms and the corresponding root disease (Kranz, 1988). Direct assessment requires to uproot each plant, and actual root volume and causal agents can be determined. But destructive sampling prevents repeated assessments on individual roots or plants over time (Campbell and Neher, 1994). Thus, disease intensity would be underestimated.

Nondestructive sampling also has its advantages and disadvantages, by rating shoot symptoms that are associated with corresponding root disease (Campbell and Neher, 1994). Although plants remain intact and repeated assessment can be conducted, physiological, and environmental relationships between aboveground symptoms and root development are not well understood. A time lag often occurs between root infection and symptom expressions on shoots. Aboveground symptoms may appear months to years after root infection.

As a result, shoot symptoms should not be rated in isolation of root symptoms for root diseases. However, it may assist to indirectly assess symptoms of root disease by observing aboveground symptoms for plants with predictable patterns of symptom development on aboveground plant parts. Repeated research observations need to be conducted to confirm those associations. These aboveground symptoms include plant growth index, biomass, color, leaf numbers, canopy density and volume, respiration and so on (Campbell and Neher, 1994). For instance, reduction in plant size indicates water deficiency in the field and is evident as an early symptom of *Phytophthora* root rots. The volume of roots decreases due to *Phytophthora* spp. infection, and absorption of water and nutrients is reduced. Some yellowing of the foliage and possibly limb dieback may also occur in early stage of the disease (Hagan and Mullen, 2000).

Evaluators from various aspects and assessment device errors inevitably lead to variability and inaccuracy over estimation. Several countries have established certain committee to investigate ways to measure diseases. They have developed rating scales for various crops, which were deemed useful to plant disease assessment. These rating scales can be categorized as nominal or descriptive scales, ordinal rating scale, interval (category) scales (with or without standard area diagrams, SADs) and field keys, and ratio scales (with or without SADs) (Bock et al., 2010). However, rating is subjective and disease development varies from host to host, from pathogen to pathogen. Also no empirical test has been performed to quantify the visual assessment methods for reliability or agreement, or the ramifications of using scale-based data in analysis. It is tempting to develop more objective methods to assess root disease.

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CHAPTER 2

EFFECT OF MULCHING AND IRRIGATION ON GROWTH AND DISEASE INCIDENCE OF DWARF BOXWOOD [1]

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Abstract

Dwarf boxwood (*Buxus sempervirens* 'Suffruticosa') is a popular ornamental for landscape use that is currently suffering from decline in the United States caused by *Phytophthora* species. High soil moisture is associated with boxwood decline. In this study, effects of mulch, irrigation and inoculation with *Phytophthora nicotianae* Breda de Haan on dwarf boxwood were examined. Plants were either mulched with pine bark (5 cm depth) or left unmulched, treated with three irrigation levels (no irrigation, 2.5 cm per week, and 5 cm per week), and half of plants were inoculated. Mulching did not increase growth and may promote the development of disease when *P. nicotianae* was present in the soil. Additional irrigation did not have significant effects on growth of dwarf boxwood, which was possibly caused by unusually high precipitation in the growing season in 2015. Only plants inoculated with *P. nicotianae* showed significant reduction in growth, and they gradually developed symptoms of *Phytophthora* root rot. Dwarf boxwood that showed foliar symptoms had low to zero PSII quantum yield and eventually died. *P. nicotianae* was recovered from crowns and roots of symptomatic plants.

Introduction

Boxwood have been rated the most popular woody ornamental in surveys of landscapers (Anon., 2011). *Buxus sempervirens* L. (American or common boxood) and *Buxus sempervirens* 'Suffruticosa' (English or dwarf boxwood) are the two most common types of boxwood. *Buxus sempervirens* L. is a broad-leaved evergreen small shrub and tree species, which has about 185 cultivars named including dwarf boxwood (*Buxus sempervirens* 'Suffruticosa'), a multiple-branched, slow-growing cultivar widely grown in Europe and North America (Krüssmann et al., 1984; Batdorf, 2004). Dwarf boxwood distinguishes itself with its dwarf habit and small leaves. It matures at 10' (3 m) tall, but it is often maintained as an edging plant, constantly sheared to keep it about 1' (30 cm) tall or less (Batdorf, 2005).

Dwarf boxwood was first introduced into North America by the early colonists and used by southern gardeners of the U.S.A. beginning in the middle of the eighteenth century (Cothran, 2003). Dwarf boxwood were used in parterres and hedges, which were essential features of formal European ornamental gardens. These gardens built in the U.S. served as a means to exhibit the wealth, education and prestige of their owners (Coleman, 2005).

Boxwood decline in the United States was first described in the 1930s. The decline has been attributed to many causal factors including fungi, fungus-like organisms, nematodes, and weather (Andrus, 1933). Excessive irrigation is considered one of the most common causes of decline of dwarf boxwood (Niemiera, 2013; Anon., 2016). Dwarf boxwood is originally from the Mediterranean region and adapted to warm, dry summers and cool, wet winters. With boxwood being adapted to lower water levels in the

Mediterranean summers, typical irrigation schedules in the summer in a southeastern U.S. landscape may lead to boxwood decline. Excessive irrigation results in saturated soil, especially soil with poor drainage as is prevalent in the Piedmont of the southeastern U.S. In saturated conditions, plants cannot maintain aerobic metabolism resulting in reduced absorption and transport of water and nutrients (Jackson and Drew, 1984). Excess water in soil normally decreases growth rate, particularly in roots (García et al., 2008).

Diseases are also a common cause of decline of boxwood. Excessive irrigation may directly promote disease, as fungal and oomycete pathogens thrive under moist conditions. *Phytophthora nicotianae* Breda de Haan, an oomycete, was described as a potential causal agent associated with boxwood decline and causing a root rot, canker and blight of boxwood (Andrus, 1933; Haasis, 1961). However, the importance of *Phytophthora* species in causing root and crown diseases is often underestimated (Erwin and Ribeiro, 1996); when foliage symptoms are seen, root infection may already have occurred months to years before.

Whether boxwood decline is associated with excessive soil water and increasing incidence of *Phytophthora* species is still unknown. In one study, incidence of *Phytophthora*-caused disease could not be attributed to consistent environmental patterns and could occur both on poorly-drained and well-drained sites at that time (Lambe and Wills, 1975). Another report indicated that boxwood decline has been observed to be associated with poor soil drainage or excess soil water content (Montgomery et al., 1977).

Applying mulch is a common maintenance practice for boxwood. In addition to providing an aesthetic benefit to gardens, it can increase water use efficiency and shoot growth (Greenly and Rakow, 1995; Tolk et al., 1999). Mulch greatly retards the loss of

moisture from the soil by reducing soil evaporation (Bussière and Cellier, 2004; Ramakrishna et al., 2006) and irrigation frequency is reduced (Rao et al., 2013). According to some sources (Batdorf, 2005), boxwood are observed to grow well when mulched. However, for plants such as boxwood that do not tolerate high soil moistures, mulch may keep soil too moist and inhibit growth and promote root disease.

The objectives of this study were to determine whether irrigation and mulching affect boxwood growth and root disease development. We examined the effects of mulching and irrigation levels on boxwood shoot growth, photosynthesis and incidence of disease.

Materials and Methods

Seventy-two 3.8 L container-grown dwarf boxwood were transplanted into three 45 m rows at the University of Georgia Horticulture Farm, Watkinsville, GA, in December 2014. A randomized complete block design was used. Each row was divided as two blocks. The plants were hand watered as needed until irrigation treatments were initiated on 13 June 2015. No fertilizer was applied throughout the whole experiment.

On 24 April 2015, half of the plants in each block were mulched to a depth of 5 cm (2”) of pine bark in a 60 cm × 60 cm (2 ft. × 2 ft.) area at base of each plant. Treatments were irrigated using drip emitters after establishment in June 2015. One third of plants in each block were not irrigated, one third were watered with 2.5 cm per week, and another one third were watered with 5 cm per week. The volume of water applied was calculated by multiplying the surface area of the top of the containerized root at the base of each plant by 2.5 cm or 5 cm; thus, 502.4 mL water was applied for the 2.5 cm

treatment, and 1004.8 mL was applied for the 5 cm treatment. Irrigation treatments were split evenly into twice weekly applications.

One half of the plants were inoculated with *Phytophthora nicotianae* isolate recovered from diseased *Hibiscus syriacus* at the University of Georgia Horticulture Farm, Watkinsville, GA. The isolate was maintained on V8-juice agar (15 g Bacto agar, 100 mL clarified V8 juice, 900 mL deionized water). Inoculum was prepared by autoclaving vermiculite (500 g fine grade vermiculite, 40 g corn meal, 250 mL V8 broth) on 2 consecutive days. Rice grain inoculum was also prepared. 10 agar plugs were added to 500 g autoclaved vermiculite and rice combination, and shaken after 5 days. Inoculum consisted of hyphae, sporangia and chlamydospores of the oomycetes. Inoculum was applied on 5 June 2015. 125 mL inoculum was applied for each plants. Inoculum was placed in four holes around each inoculated plant below the soil surface.

Growth index was used to describe the size of each plant. Heights (mm) and widths (mm) of each plant were measured every month from March 2015 until October 2016.

$$\text{Growth Index} = \frac{\text{Height} + \frac{\text{Width 1}+\text{Width 2}}{2}}{2}$$

The change of growth index from March to October in each year was used to measure the growth of boxwood.

Disease severity was assessed 30 days after inoculation on a disease rating scale of 0 to 10 based on the color of the foliage and survival. 0 = healthy plants with all foliage dark green; 1 – 5 = mostly dark green foliage with a few dead leaves and branches; 6 – 9 = light green foliage to brown foliage with a lot dead leaves and

branches; 10 = no green foliage and dead. A RHS mini color chart (the Royal Horticultural Society, London, England) was used to determine leaf color when rating.

A proxy measure of photosynthesis, PSII quantum yield, was measured with the FluorPen FP 100 (Photon System Instrument, Brno, Czech Republic). Three leaves of each plant were tagged and measured at noon every week from August to October 2015, and the average of quantum yield for each plant was calculated.

Plants were uprooted after death, and root samples were washed under running tap water, blotted dry, and cut into 5 to 10 mm section and embedded into V8-PARPH *Phytophthora*-selective media (15 g Bacto agar, 50 ml clarified V8 juice, 400 μg pimaricin, 250 mg ampicillin, 10 mg rifampicin, 67 mg pentachloronitrobenzene (PCNB), 32.5 mg hymexazol, 950 mL deionized water) to identify the causal agents.

Data were tested for normality and homogeneity of variance before analysis of variance (ANOVA) with the R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). A three-way ANOVA testing the effect of mulching, irrigation and inoculation was conducted for growth. Linear models were fitted to find relationship between PSII quantum yield (estimated photosynthetic capacity) and severity of disease as indicated by the disease rating.

Results and Discussion

We hypothesized that irrigation might inhibit the growth of dwarf boxwood because boxwood are adapted to low water levels, or the irrigation water might aid in establishment. However, mulching and irrigation had no significant influence on growth in both 2015 and 2016 (Table 2.1). Abundant rainfall in 2015 may have confounded the

impact of the additional irrigation on growth and survival of *P. nicotianae*. In 2015, the weekly precipitation from June to October was 2.6 cm, but it was only 1.5 cm in 2016 (University of Georgia Weather Network, 2016). Thus, the growth of plants that were irrigated may not have differed from those with no irrigation in 2015 because of this abundant rainfall (Table 2.1).

In the summer of 2016, the weather was dry and precipitation was as low as that in northwestern Mediterranean area where *Buxus* is endemic. Additionally, the average temperature in the University of Georgia Horticulture Farm was approximately 5 °C higher than that of the native habitat of *Buxus*. High temperature as well as low precipitation could have led to more severe drought in our experimental field. Boxwood might need additional irrigation to compensate for the drought stress. However, plants that were irrigated did not grow more than those with no irrigation in 2016 (Table 2.1).

In 2015 and 2016, inoculation of *Buxus sempervirens* 'Suffruticosa' with *P. nicotianae* had significantly negative effect on growth of dwarf boxwood (Table 2.1). Inoculation of *P. nicotianae* did inhibit dwarf boxwood growth as expected and lasted throughout the experiment (Figure 2.1). This reduction in growth with disease inoculation was suggested by similar reports on azalea plants (Hagan and Mullen, 2000). Reduction of plant growth was found before changes of color were observed, thus slow growth can be an indicator of boxwood decline.

Only inoculated dwarf boxwood plants were infected (Table 2.2). Plants died between 7 days to 30 days after aboveground symptoms were seen. Aboveground symptoms included foliage changing color from green to brown, and wilting. Roots were

dark and sloughing, and discoloration occurred around the crown. *P. nicotianae* was recovered from roots and crown pieces of inoculated plants.

Interaction between mulching and inoculation did significantly affect growth in 2015 ($p = 0.0003$; Appendix A). Plants with mulching but without inoculation grew more than treatments that were mulched and inoculated. Mulching possibly kept soil moist and provided a cooler soil temperature, so it promoted pathogen growing in inoculated plants.

Light energy absorbed by chlorophyll molecules in a leaf will undergo one of three fates: used by photosynthesis (photochemistry), dissipated as heat or reemitted as light which is chlorophyll fluorescence. Photosynthetic capacity can be estimated by measuring fluorescence emission (Maxwell and Johnson, 2000). Infected dwarf boxwood had low to zero quantum yield of photosystem II. Relatively strong negative linear relationships between quantum yield and disease rating were found when disease progressed ($r = -0.79$, Figure 2.2 A; $r = -0.94$, Figure 2.2 B). It indicated that quantum yield could be a potential indicator to assess *Phytophthora* root rot of boxwood while foliage still remained green.

The pattern of color changes after infection were similar to what literature suggested (Lambe and Wills, 1981). However, the literature only listed the names of the colors and no visual pictures were provided. In our experiment, pictures were taken, and codes from the RHS color chart were used to quantify the colors. The change was from normal dark green (136A) to greyish green (137A) to yellow green (146C) to light olive green (145A) to bronze (20A), and finally to brown (163B) before defoliation (Figure 2.3), and disease rating was from 6 to 10 accordingly (Table 2.3). Variation occurred in

different plants. It could be useful to detect *Phytophthora* root rot when foliage colors change is documented to monitor the disease.

Disease symptoms initiated between August and October in both 2015 and 2016. No death occurred between the time when dwarf boxwood underwent dormancy and the summer of the following year. No death occurred among non-inoculated dwarf boxwood (Table 2.2). More death occurred among inoculated plants with mulching than those without mulching in both 2015 and 2016 (Table 2.2). In the first year, plants with more irrigation had more death than those with less irrigation. However, in 2016, many plants with no irrigation died. High precipitation in the summer of 2015 possibly maintained boxwood normal growth, but boxwood underwent drought stress in the summer of 2016, which made roots of inoculated plants potentially more susceptible to *P. nicotianae*, for environmental conditions were unfavorable for host plants.

In terms of boxwood management, this research indicated that mulching was not necessary, as mulching did not significantly increase growth. The data also demonstrated that mulching may promote the development of disease if inoculum is present in the soil, as mulched and inoculated plants grew less than mulched plant that were not inoculated. Because only plants that were inoculated died in the study, dwarf boxwood are not recommended for being planted in a site where *Phytophthora* species are confirmed. Long-term survival chlamydospores produced by *Phytophthora* spp. can infect newly transplanted boxwood.

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Table 2.1 Effect of mulching, irrigation[z] and inoculation with *Phytophthora nicotianae* on the growth index change (mm)[y] of *Buxus sempervirens* 'Suffruticosa' within each year.[x]

Mulching Treatment	Inoculation Treatment	Year	
		2015	2016
Mulching	Inoculation	17.8c[w]	4.5b
No mulching	Inoculation	30.8b	9.5ab
Mulching	No inoculation	42.0a	16.2a
No mulching	No inoculation	33.7ab	15.4ab

[z] Irrigation effect was insignificant so irrigation means were averaged for each mulching and inoculation treatment combinations.

[y] Growth index change (mm) from March to October in each year.

[x] 72 plants assigned to 6 blocks with single plant replicates were measured monthly. Dead plants were included, and the last growth indices when they were alive were used.

[w] Data were analyzed using a two-way analysis of variance (ANOVA) model for mulching, and inoculation because irrigation effect was not significant (n = 12 for each mulching level and inoculation level combination). Means were separated using Tukey's honestly significant difference (HSD) test (different letters indicate significant differences within each year at $p \leqslant 0.05$).

Table 2.2 Effect of mulching and irrigation on disease incidence[z] of *Buxus sempervirens* 'Suffruticosa' inoculated with *Phytophthora nicotianae*[y] within each year and cumulatively.

Mulching	Irrigation Level (cm per week)		
Treatment	0	2.5	5
	First year: 2015		
Mulching	33.3%	33.3%	50.0%
No mulching	0.0%	16.7%	33.3%
	Second year: 2016		
Mulching	33.3%	33.3%	16.7%
No mulching	33.3%	50.0%	0.0%
	Cumulative		
Mulching	66.7%	66.7%	66.7%
No mulching	33.3%	66.7%	33.3%

[z] Mortality, the proportion of plants that were dead in a population (n = 6 for each treatment combination) within each year and two years cumulatively.

[y] Only inoculated plants developed pathogenic symptoms and died. No data is presented for non-inoculated plants as they did not die.

Data were analyzed using a three-way analysis of variance (ANOVA). No significant differences existed among treatments.

Table 2.3 Foliage symptom ratings[z] as foliage color changed.

Disease Rating	Color Code[y]	Symptom Descriptions
0 – 5	136A	Most foliage were dark green with a few dead leaves and branches.
6	137A	Whole foliage turned greyish green.
7	146C	Whole foliage turned yellow green.
8	145A	Whole foliage turned light olive green.
9	20A	Plant wilted, and bronze branches appeared.
10	163B	Whole foliage turned brown and later defoliated.

[z] A disease rating scale of 0 to 10 based on the color of the foliage and survival.

[y] Code of each color based on RHS mini color chart.

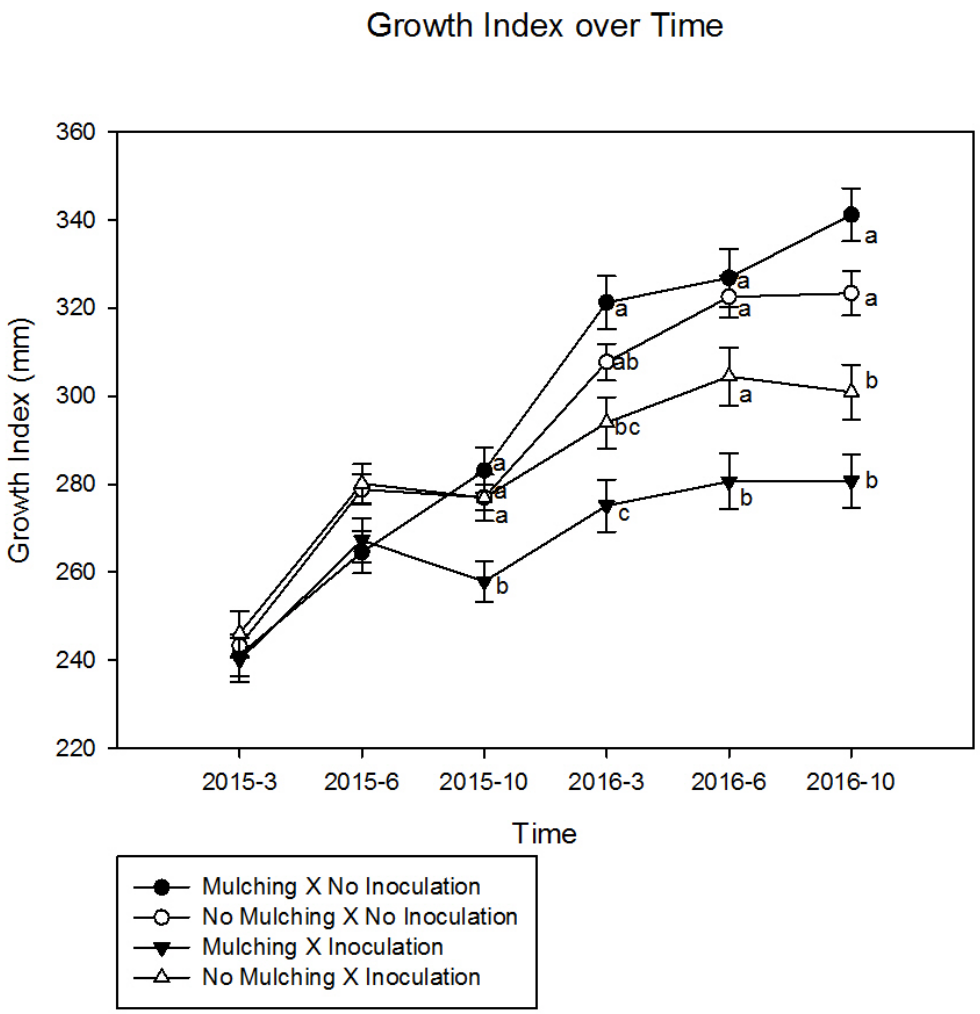
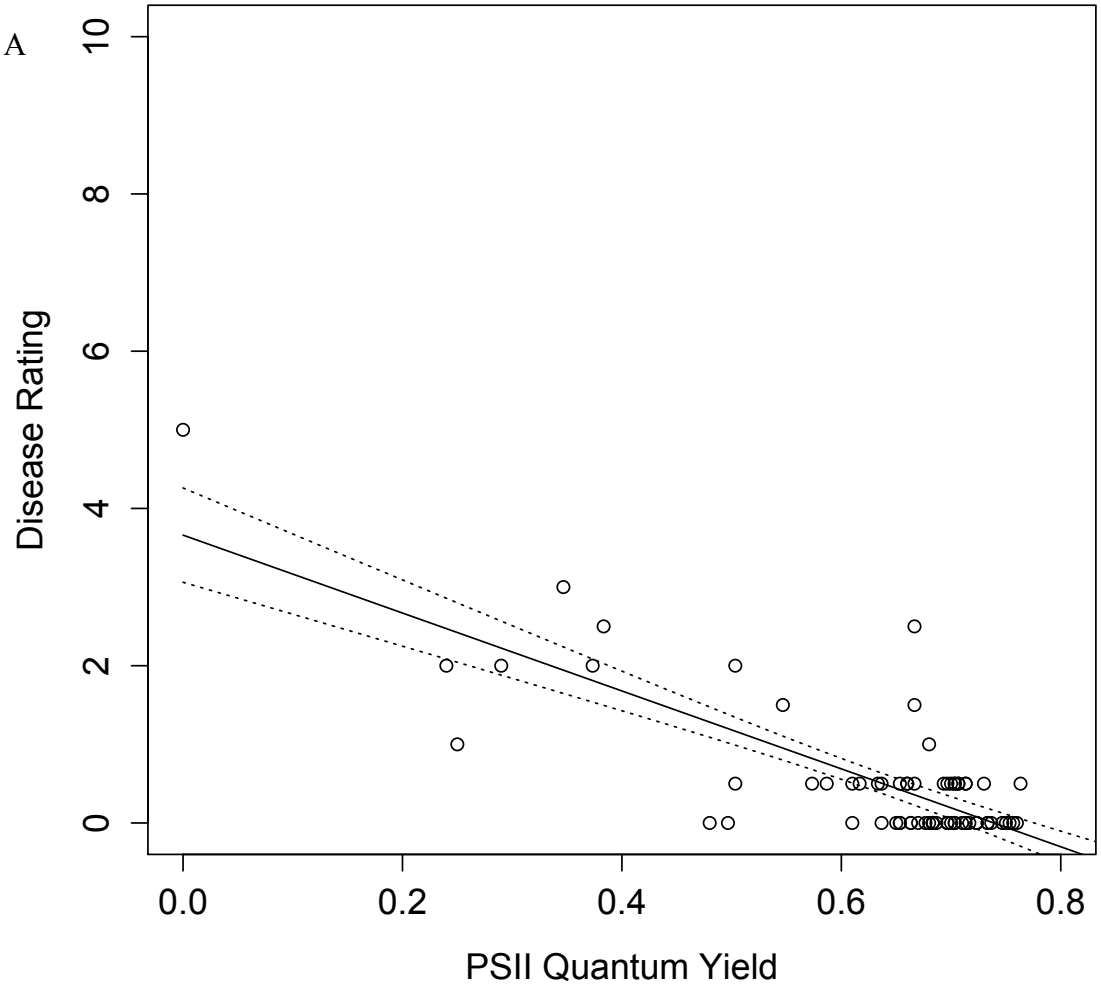


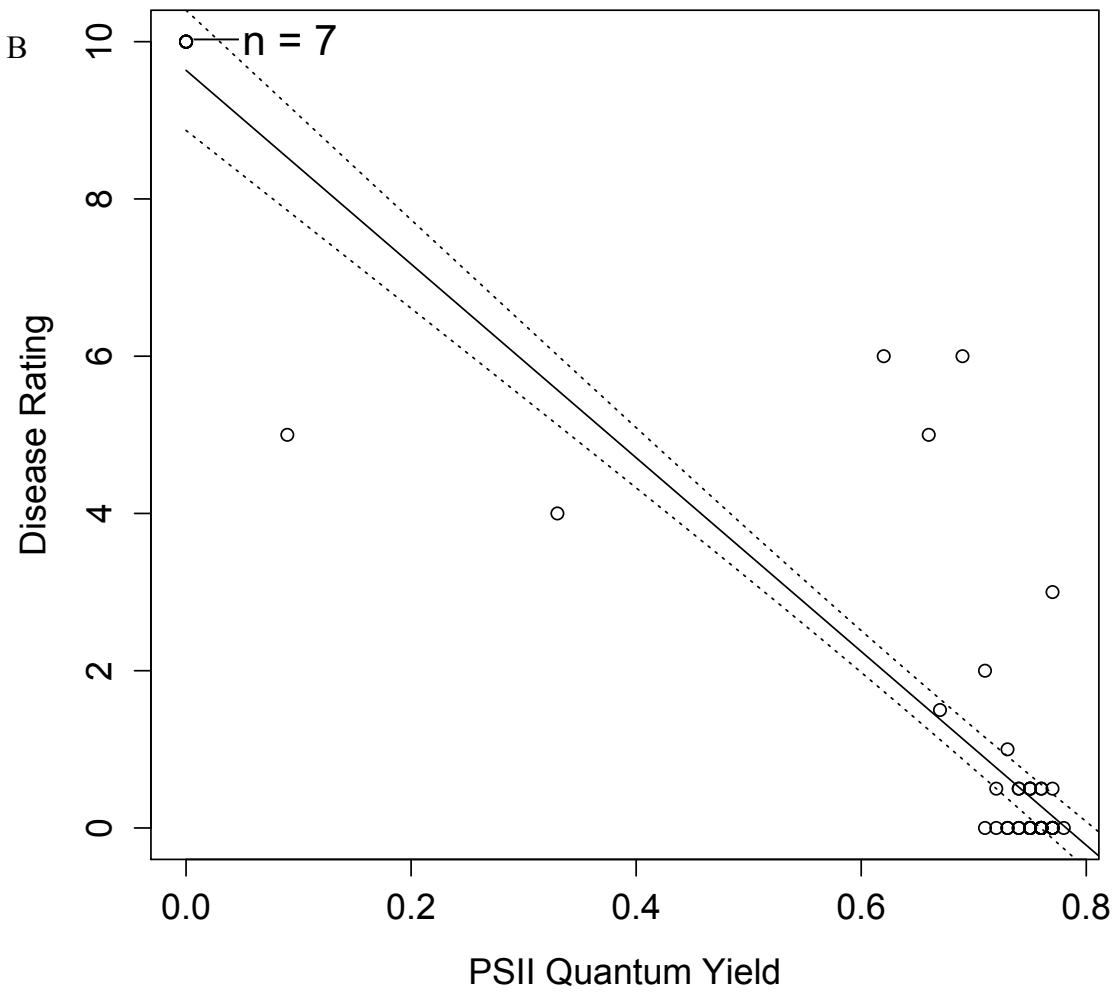
Figure 2.1 Effect of mulching and inoculation with *Phytophthora nicotianae* on growth index of *Buxus sempervirens* 'Suffruticosa' over two consecutive growing seasons.

Data were analyzed using a repeated measurement model accounting for the effect of mulching and inoculation when irrigation effect was not significant throughout 2 years. When significant effect indicated among mulching and inoculation treatments, means were separated using Tukey's honestly significant difference (HSD) test (different letters indicate significant differences within each time point at $p \leqslant 0.05$).

A



Predicted Disease Rating = -5.0 x PSII Quantum Yield + 3.7



Predicted Disease Rating = -12.3 x PSII Quantum Yield + 9.6

Figure 2.2 Relationship between PSII quantum yield and disease rating of *Buxus sempervirens* 'Suffruticosa' inoculated with *Phytophthora nicotianae* in two different days.

Each point referred to one individual plant in one same day. Liner models were fitted for data on (A) 16 August 2015 and (B) 27 September 2015. Data were analyzed using one-way analysis of variance (ANOVA). Disease rating was strongly negatively related to PSII quantum yield (A: $p = 0.0000, r = -0.79$; B: $p = 0.0000;\ r = -0.94$).

Figure 2.3 The pattern of color changes in *Buxus sempervirens* 'Suffruticosa' when infected with *Phytophthora nicotianae*. RHS color codes listed below different stages of diseased plant.

CHAPTER 3

BUXUS CULTIVAR SUSCEPTIBILITY TO *PHYTOPHTHORA* INOCULATION [2]

[2] Yang, S., T.J. Smalley, and J.L. Williams-Woodward. To be submitted to *Plant Health Progress*.

Abstract

Buxus have been reported to be highly susceptible to root rot caused by *Phytophthora* species, but no extensive experimentation has been conducted. The objective of this study was to evaluate eight *Buxus* cultivars for susceptibility to *Phytophthora* species to identify potential substitutes for susceptible cultivars. The cultivars included *Buxus* × 'Green Velvet', *Buxus microphylla* 'Golden Dream ('Peargold')', *Buxus microphylla* var. *japonica* 'Morris Midget', *Buxus microphylla* var. *japonica* 'Wintergreen', *Buxus sinica* var. *insularis* 'Nana', *Buxus sinica* var. *insularis* 'Justin Brouwers', *Buxus sempervirens* 'Elegantissima', and *Buxus sempervirens* 'Suffruticosa'. In two separate experiments, container grown plants were inoculated with 1) *Phytophthora nicotianae* Breda de Haan and *Phytophthora cinnamomi* Rands in October 2015 and April 2016, and 2) *P. cinnamomi* in May 2016. No obvious aboveground symptoms were observed except two deaths of 'Suffruticosa' caused by *P. nicotianae* in September 2016. Root samples were collected in September and October 2016. *P. nicotianae* was recovered from some cultivars, and infected roots were dark and sloughing. In this study, the *B. sinica* cultivars and the *B. sempervirens* cultivars were susceptible to *P. nicotianae*, while the *B. microphylla* cultivars were less sensitive to *P. nicotianae*. All the cultivars were less sensitive to *P. cinnamomi*. Thus, the *B. microphylla* cultivars may be good choices to be used in landscapes to resist *Phytophthora* infection. The *B. sinica* cultivars and the *B. sempervirens* cultivars should not be planted in the sites where *Phytophthora* root rot previously occurred.

Introduction

Boxwood, the genus *Buxus*, contains about 90 species and over 365 different cultivars known to exhibit a wide variety of forms and foliage. Approximately 217 cultivars are registered, and more than 100 cultivars and species can be obtained commercially (Anon., 2016).

Boxwood in the United States currently suffer from decline caused by disease. Disease in boxwood caused by *Phytophthora* species was first described by Andrus (1933) on *Buxus sempervirens* L. and *Buxus sempervirens* 'Suffruticosa'. *Phytophthora nicotianae* Breda de Haan weaken infected boxwood and kill them (Haasis, 1961). The importance of *Phytophthora* species in causing root and crown diseases is often underestimated (Erwin and Ribeiro, 1996). The reasons for this oversight include the symptoms of many *Phytophthora*-caused diseases are similar to damage from other pathogens or abiotic agents and foliage symptoms can only be detected months to years after root infection.

Dwarf boxwood (*Buxus sempervirens* 'Suffruiticosa') is an important component of historical and contemporary landscapes. They were often used as parterres and hedges during the colonial period, resembling early seventeenth and eighteenth-century European gardens, to serve as symbols of the wealth, education and prestige of their owners (Cothran, 2003; Coleman, 2005). Dwarf boxwood is the most difficult to grow among all the boxwood species or cultivars and tends to suffer from root diseases, including *Phytophthora* root rot (Hansen et al., 2009).

Other dwarf boxwood cultivars are used to fulfill the important role of dwarf boxwood in the landscapes. However, little is known about the susceptibility of these

dwarf boxwood substitutes to *Phytophthora* species. Some *Buxus* cultivars are observed to have fewer root problems (Saunders Brothers, 2014). The objective of this study was to evaluate a collection of boxwood cultivars for suscepbility to *Phytophthora* root disease.

Materials and Methods

Eight cultivars of boxwood were grown in 3.8 L containers in University of Georgia Riverbend Greenhouse Complex in Athens, GA. The cultivars included *Buxus* × 'Green Velvet', *Buxus microphylla* 'Golden Dream ('Peargold')', *Buxus microphylla* var. *japonica* 'Morris Midget', *Buxus microphylla* var. *japonica* 'Wintergreen', *Buxus sinica* var. *insularis* 'Nana', *Buxus sinica* var. *insularis* 'Justin Brouwers', *Buxus sempervirens* 'Elegantissima', and *Buxus sempervirens* 'Suffruticosa' (dwarf boxwood). These cultivars were obtained from two different nurseries. Plants from each cultivar were randomly divided into two groups for two experiments.

A randomized complete block design was used in both experiments. For each experiment, eight cultivars were randomly assigned to five blocks with single plant replicates. Half of the plants in each block were inoculated and the other half were not inoculated. Plants were watered by hand once daily.

For the first experiment, both *Phytophthora nicotianae* Breda de Haan and *Phytophthora cinnamomi* Rands were used to inoculate the *Buxus* cultivars. *P. nicotianae* was isolated from hibiscus plants (*Hibiscus syriacus*) at the University of Georgia Horticulture Farm, Watkinsville, GA. The isolate was maintained on V8-juice agar (15 g Bacto agar, 100 mL clarified V8 juice, 900 mL deionized water). Inoculum was prepared

by autoclaving vermiculite (500 g fine grade vermiculite, 40 g corn meal, 250 mL V8 broth) on 2 consecutive days. 10 agar plugs were added to 500 g autoclaved vermiculite combination, and shaken after 5 days. Inoculum consisted of hyphae, sporangia and chlamydospores of the oomycetes. Inoculum was applied on 30 October 2015. 125 mL inoculum was applied for each plants. Inoculum was placed on top of the container of each inoculated plant and covered with a layer of Metro-Mix® 360 (Sun Gro Horticulture Canada Ltd).

These boxwood were reinoculated on 15 April 2016 with *P. cinnamomi* after no obvious aboveground symptoms were observed. Six isolates of *P. cinnamomi* were maintained on V8-juice agar. These isolates were from *Rhododendron* spp. in one nursey in Franklin County, GA. Inoculum was prepared by autoclaving vermiculite on 2 consecutive days. 10 agar plugs were added to 500 g autoclaved vermiculite combination, and shaken after 5 days. Inoculum consisted of hyphae, sporangia and chlamydospores of the oomycetes. 125 mL inoculum was applied for each plants. Inoculum was placed on top of the container of each inoculated plant and covered with a layer of Metro-Mix® 360.

For the second experiment, the same six isolates of *P. cinnamomi* used in the first experiment were grown. Inoculum was applied on 20 May 2016.

The difference of growth index from inoculation to root sampling and average of new branch elongation were used to determine the growth of boxwood. Growth index was used to describe the size of each plant. Heights (mm) and widths (mm) of each plant were measured every month after inoculation.

$$\text{Growth Index} = \frac{\text{Height} + \frac{\text{Width 1}+\text{Width 2}}{2}}{2}$$

To quantify elongation of new growth, ten branches were randomly selected and measured on 17 July and 18 July 2016 to determine the length of growth after bud break for all cultivars.

Aboveground symptoms were monitored by unaided eye daily throughout the experiment.

Roots were sampled to determine *Phytophthora* infection for each plant from 11 September to 16 October 2016. Roots were cut from four wedges of each individual plant. Root samples were washed under running tap water, blotted dry, and cut into 5 to 10 mm section and embedded into V8-PARPH *Phytophthora*-selective media (15 g Bacto agar, 50 mL clarified V8 juice, 400 µg pimaricin, 250 mg ampicillin, 10 mg rifampicin, 67 mg pentachloronitrobenzene (PCNB), 32.5 mg hymexazol, 950 mL deionized water). 8 root pieces were plated in one plate of V8-PARPH media, and four plates were used for each plant. Thus, causal agents were identified, and disease incidence was estimated.

Phytophthora species were identified based on morphology and confirmed by DNA ITS sequencing (J.L. Williams-Woodward, personal communication). Disease incidence was determined by the percentage of root sections infected by *Phytophthora* species in each individual plant.

Data were tested for normality and homogeneity of variance before analysis of variance (ANOVA) with the R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Two-way ANOVA testing the effect of cultivar and inoculation were conducted for growth and disease incidence.

Results and Discussion

The amount of growth differed according to cultivar (Table 3.1, Table 3.2). Elongation of new branches better indicated difference in growth than growth index changes. Different cultivars have their own distinct growth habits, so widths and heights vary according to cultivar. Thus, growth index change is not as valuable an indicator of growth as branch elongation. Among eight cultivars, *Buxus sinica* var. *insularis* 'Nana' had the greatest branch elongation, while *Buxus sempervirens* 'Suffruticosa' had the least branch elongation (Table 3.2).

Inoculation did not significantly affect growth among most cultivars (Appendix B; Appendix C). In the first experiment, inoculation only significantly negatively affected growth on 'Suffruticosa' with *P. nicotianae* and *P. cinnamomi* inoculation. However, in the second experiment, growth of 'Suffruticosa' that were inoculated with only *P. cinnamomi* was not inhibited.

In the first experiment, no obvious aboveground symptoms appeared until September 2016, when two plants of 'Suffruticosa' died. In the second experiment, no obvious aboveground symptoms appeared. Growth was possibly unaffected by root infection because established container plants had abundance of roots in dense root systems.

Root samples were taken to estimate any potential incidence of disease. In the first experiment, eight cultivars did differ in response to inoculation (Table 3.3). Only *Phytophthora* infection of the *sempervirens* cultivar 'Suffruticosa' was increased significantly. All of the *microphylla* cultivars, which were 'Golden Dream ('Peargold')', 'Morris Midget' and 'Wintergreen', and the hybrid 'Green Velvet', were infected at a

very low rate. *P. nicotianae* recovered from non-inoculated plants indicated a background level of infection from the source nursery. These infected cultivars at the start of the experiment included the *B. sinica* cultivars 'Nana' and 'Justin Brouwers', and the *B. sempervirens* cultivars 'Suffruticosa' and 'Elegantissima' (Figure 3.1).

In the second experiment, we found inoculation did not cause new infection (data not presented). Similar as the first experiment, the *sinica* cultivars 'Nana' and 'Justin Brouwers', and the *sempervirens* cultivar 'Elegantissima' were infected with *Phytophthora* spp. at a high percentage before inoculum was applied. The other five cultivars were infected at a low percentage.

In both experiments, *P. nicotianae* were identified from infected roots of both non-inoculated individuals and inoculated ones. No infection was discovered by *P. cinnamomi*.

Data indicated that the *sinica* cultivars and the *sempervirens* cultivar were susceptible to *P. nicotianae*, while the *microphylla* cultivars were less sensitive to *P. nicotianae* (Table 3.3).

As indicated by lack of infection by *P. cinnamomi*, this experiment demonstrated that cultivars in this study were less sensitive to *P. cinnamomi*, which contradicts some reports on boxwood in the literature. Infection of *P. cinnamomi* has been listed in plant disease indices in southeastern United States (Grand, 1985; Blake and Williamson, 2005), *Diseases and Pests of Ornamental Plants* (Pirone, 1978) and various university publications (Jacobi et al., 2003). While *P. nicotianae* pathogenic to boxwood has been documented in more scientific publications including *Index of Plant Diseases in the United States* (Anon., 1960) and *Phytophthora Diseases Worldwide* (Erwin and Ribeiro,

1996). Thus, some publications may be repeating assumptions without experimental verification. Further studies need to be conducted to justify the pathogenesis of *P. cinnamomi* in boxwood.

Three cultivars from the same nursery had been infected with *P. nicotianae* when they were in the nursery. Nurseries should be aware of the problem that pathogens can be introduced easily in production processes. *Phytophthora* species has been detected in container mixes from ornamental crop nurseries (Ferguson and Jeffers, 1999). *P. nicotianae* can be also present in irrigation water, which can be a primary source of inoculum for *Phytophthora* diseases in nurseries (Hong et al., 2002; Kong et al., 2003). The sources of contamination should be limited when producing products.

In summary, the *B. microphylla* cultivars are good cultivars to be used in landscapes because they are less susceptible to *Phytophthora* infection. The *B. sinica* cultivars and the *B. sempervirens* cultivars should not be planted in the sites where *Phytophthora* root rot previously occurred.

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Table 3.1 Effect of *Phytophthora* inoculation on growth index change (mm)[z] in eight *Buxus* cultivars[y].

Cultivar	Experiment 1: Inoculated with *P. nicotianae* and *P. cinnamomi*	Experiment 2: Inoculated with *P. cinnamomi*
B. sinica var. *insularis* 'Nana'	79.4a[x]	60.0abc
B. sinica var. *insularis* 'Justin Brouwers'	44.7ab	38.7bc
B. sempervirens 'Suffruticosa'	12.6b	9.1c
B. sempervirens 'Elegantissima'	27.4ab	20.4bc
B. microphylla var. *japonica* 'Morris Midget'	21.1ab	34.3bc
B. microphylla var. *japonica* 'Wintergreen'	44.8ab	103.9a
B. microphylla 'Golden Dream ('Peargold')'	53.4ab	68.5ab
× 'Green Velvet' (*B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*)	57.8ab	10.2c

[z] Growth index change (mm) from inoculation to root sampling in each experiment.

[y] 10 plants from each cultivar assigned to 5 blocks with single plant replicates.

[x] Data were analyzed using a one-way analysis of variance (ANOVA) model for cultivar when inoculation effect was not significant (n = 5). When significant effect indicated among cultivars, means were separated using Tukey's honestly significant difference (HSD) test (different letters indicate significant differences within each experiment for growth index change at $p \leqslant 0.05$).

Table 3.2 Effect of *Phytophthora* inoculation on branch elongation (mm) in eight *Buxus* cultivars.

Cultivar	Experiment 1: Inoculated with *P. nicotianae* and *P. cinnamomi*	Experiment 2: Inoculated with *P. cinnamomi*
B. sinica var. *insularis* 'Nana'	84.9a	84.2a
B. sinica var. *insularis* 'Justin Brouwers'	54.1ab	37.3b
B. sempervirens 'Suffruticosa'	15.0c	7.4c
B. sempervirens 'Elegantissima'	43.6bc	37.4b
B. microphylla var. *japonica* 'Morris Midget'	24.5bc	33.3b
B. microphylla var. *japonica* 'Wintergreen'	47.4bc	59.8ab
B. microphylla 'Golden Dream ('Peargold')'	50.2abc	56.9b
× 'Green Velvet' (*B. sempervirens* 'Suffruticosa' × *B. sinica* var. *insularis*)	45.1bc	38.0b

Data were analyzed using a one-way analysis of variance (ANOVA) model for cultivar when inoculation effect was not significant (n = 5 for each cultivar that were inoculated). When significant effect indicated among cultivars, means were separated using Tukey's honestly significant difference (HSD) test (different letters indicate significant differences within each experiment for new branch elongation at $p \leqslant 0.05$).

Table 3.3 Effect of *Phytophthora* inoculation on disease incidence[z] in eight *Buxus* cultivars.

Cultivar	Experiment 1: Inoculated with *P. nicotianae* and *P. cinnamomi*
B. sinica var. *insularis* 'Nana'	13.1%a[y]
B. sinica var. *insularis* 'Justin Brouwers'	26.9%a
B. sempervirens 'Suffruticosa'	22.5%a
B. sempervirens 'Elegantissima'	11.3%a
B. microphylla var. *japonica* 'Morris Midget'	2.5%[w]
B. microphylla var. *japonica* 'Wintergreen'	2.5%[w]
B. microphylla 'Golden Dream ('Peargold')'	0.0%[w]
× 'Green Velvet' (*B. sempervirens* 'Suffruticosa'	2.5%[w]
× *B. sinica* var. *insularis*)	

[z] Disease incidence was determined by the percentage of root sections infected by *Phytophthora* species in each individual plant.

[y] Data were analyzed using a one-way analysis of variance (ANOVA) model for cultivar when inoculation effect was not significant (n = 5 for each cultivar that were inoculated). When significant effect indicated among cultivars, means were separated using Tukey's honestly significant difference (HSD) test (different letters indicate significant differences for disease incidence at $p \leqslant 0.05$).

[w] Data were not included in statistical analysis because assumption of homogeneity of variance was violated.

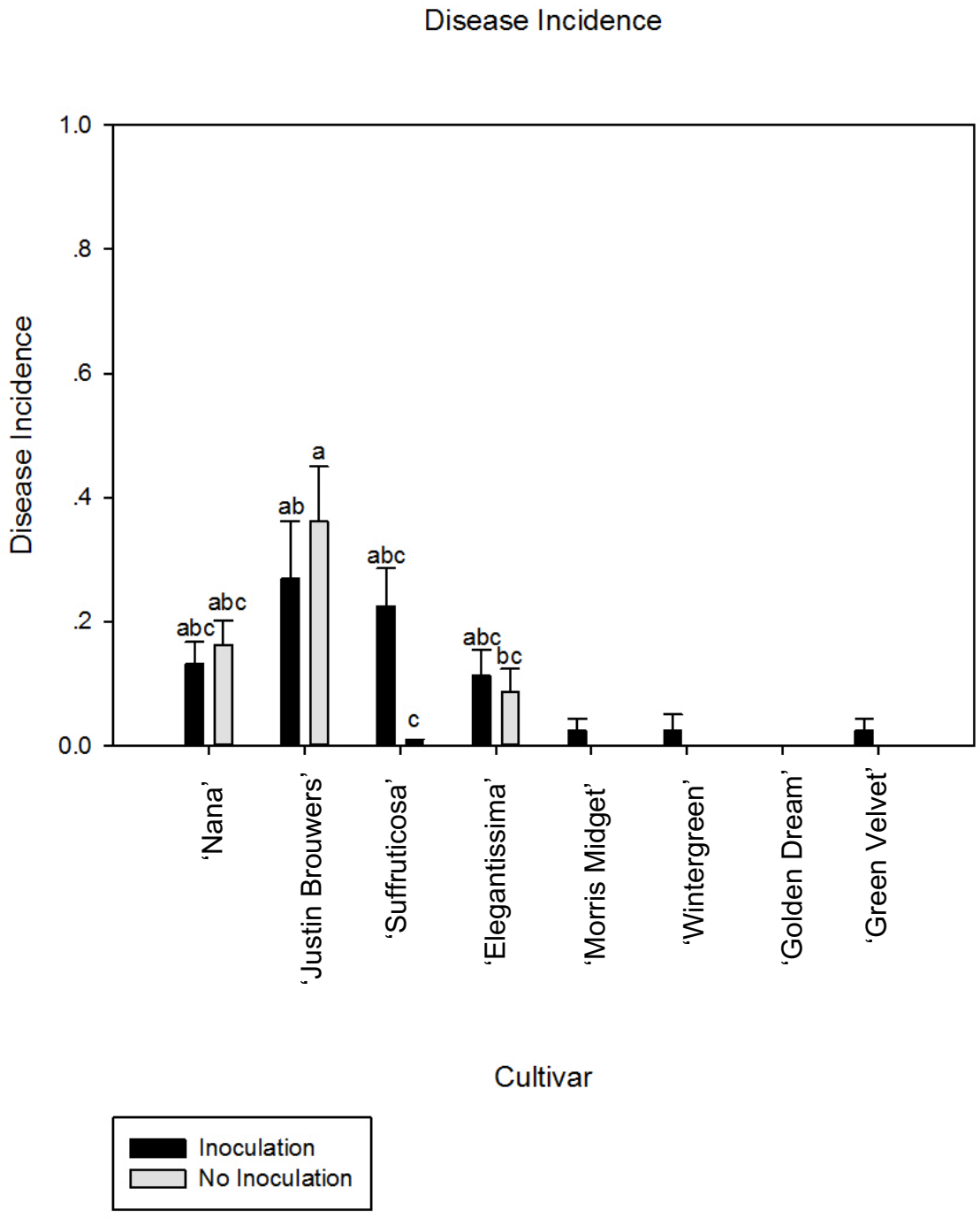

Figure 3.1 Incidence of disease after inoculating eight *Buxus* cultivars with *Phytophthora nicotianae* and *Phytophthora cinnamomi*.

Disease incidence was determined by the percentage of root sections infected by *Phytophthora* species in each individual plant. Data were analyzed using a two-way analysis of variance (ANOVA) model for cultivar and inoculation (n = 5 for each cultivar in two inoculation levels, inoculated or non-inoculated treatments). When significant effect indicated among cultivars and inoculation treatments, means were separated using Tukey's honestly significant difference (HSD) test (different letters indicate significant differences for disease incidence at $p \leqslant 0.05$). Data of 'Morris Midget', 'Wintergreen', 'Golden Dream', 'Green Velvet' were not included in statistical analysis because assumption of homogeneity of variance was violated.

CHAPTER 4

CONCLUSION

Phytophthora nicotianae Breda de Haan limited growth of dwarf boxwood (*Buxus sempervirens* 'Suffruticosa'), and caused rapid death of dwarf boxwood once infection occurred. Mulching did not increase growth and may promote the development of disease when *P. nicotianae* was present in the soil. Additional irrigation did not have significant effects on growth of dwarf boxwood, which was possibly caused by unusually high precipitation in the growing season in the first year. Only plants inoculated with *P. nicotianae* showed significant reduction in growth, and they gradually developed symptoms of *Phytophthora* root rot. Drought may have created unfavorable conditions for host plants, which made them more susceptible to *P. nicotianae*.

Dwarf boxwood that showed pathogenic symptoms had low to zero PSII quantum yield and eventually died. *P. nicotianae* was recovered from crowns and roots of symptomatic plants. Because only plants that were inoculated with *P. nicotianae* died in this study, dwarf boxwood are not recommended for being planted in a site where *Phytophthora* species are confirmed. Some *Phytophthora* species can produce chlamydospores, which are long-term survival spores. Chlamydospores will infect newly transplanted boxwood.

No obvious aboveground symptoms were observed months after inoculation with *Phytophthora* spp., except two plants of 'Suffruticosa' died after inoculation with of *P. nicotianae* of eight *Buxus* cultivars. *P. nicotianae* was recovered from some cultivars, and roots from infected plants were dark and sloughing.

In our research, the *sinica* cultivars and the *sempervirens* cultivars were susceptible to *P. nicotianae*, while the *microphylla* cultivars were less sensitive to *P. nicotianae*. All the cultivars were less sensitive to *Phytophthora cinnamomi* Rands. Thus, the *microphylla* cultivars are better cultivars to be used in landscapes to resist *Phytophthora* infection. The *sinica* cultivars and the *sempervirens* cultivars should not be planted in the sites where *Phytophthora* root rot previously occurred.

Some cultivars had been infected with *P. nicotianae* when they were in the nursery. *Phytophthora* species can be present in container mixes as well as irrigation water, and easily introduced in production processes. The sources of contamination should be limited when producing products.

APPENDICES

A Results of three-way ANOVA testing the effect of mulching, irrigation and inoculation on growth index change (mm) in *Buxus sempervirens* 'Suffruticosa'.

Factor	Degrees of freedom	Sum of squares	Mean square	F value	p-value
			First year: 2015		
A: Mulching level	1	101	101	0.7340	0.3955
B: Irrigation level	2	560	280	2.0330	0.1406
C: Inoculation level	1	3297	3297	23.9620	0.0000***
Block	5	1383	277	2.0090	0.0916
A × B	2	469	235	1.7050	0.1912
A × C	1	2045	2045	14.8630	0.0003***
B × C	2	806	403	2.9280	0.0619
A × B × C	2	25	12	0.0900	0.9140
Error	55	7569	138		
			Second year: 2016		
A: Mulching level	1	39	39	0.2110	0.6481
B: Irrigation level	2	315	158	0.8590	0.4294
C: Inoculation level	1	2378	2378	12.9540	0.0007***
Block	5	1225	245	1.3350	0.2634
A × B	2	158	79	0.4300	0.6524

A × C	1	142	142	0.7760	0.3823
B × C	2	227	114	0.6190	0.5423
A × B × C	2	285	142	0.7760	0.4653
Error	55	10095	184		

*** Significance for $p < 0.001$; ** Significance for $p < 0.01$; * Significance for $p < 0.05$

B Results of two-way ANOVA testing the effect of *Buxus* cultivar and inoculation on growth index change (mm).

Factor	Degrees of freedom	Sum of squares	Mean square	F value	p-value
			Experiment 1		
A: Cultivar	7	23861	3409	4.1950	0.0008***
B: Inoculation level	1	5	5	0.0060	0.9370
Block	4	1493	373	0.4590	0.7652
A × B	7	5881	840	1.0340	0.4173
Error	60	48757	813		
			Experiment 2		
A: Cultivar	7	45700	6529	8.1820	0.0000***
B: Inoculation level	1	770	770	0.9650	0.3300
Block	4	1164	291	0.3650	0.8330
A × B	7	9330	1333	1.6700	0.1340
Error	60	47877	798		

*** Significance for $p < 0.001$; ** Significance for $p < 0.01$; * Significance for $p < 0.05$

C Results of two-way ANOVA testing the effect of *Buxus* cultivar and inoculation on new branch elongation (mm).

Factor	Degrees of freedom	Sum of squares	Mean square	F value	p-value
			Experiment 1		
A: Cultivar	7	20239	2891	9.4570	0.0000***
B: Inoculation level	1	586	586	1.9180	0.1710
Block	4	581	145	0.4750	0.7540
A × B	7	2048	293	0.9570	0.4710
Error	60	18345	306		
			Experiment 2		
A: Cultivar	7	34715	4959	25.0730	0.0000***
B: Inoculation level	1	136	136	0.6860	0.4110
Block	4	849	212	1.0730	0.3780
A × B	7	1100	157	0.7940	0.5950
Error	60	11868	198		

*** Significance for $p < 0.001$; ** Significance for $p < 0.01$; * Significance for $p < 0.05$