HOST RANGE AND BIOLOGY OF *FUSARIUM TORREYAE* (SP. NOV), CAUSAL AGENT OF CANKER DISEASE OF FLORIDA TORREYA (*TORREYA TAXIFOLIA* ARN.)

By

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To my wife, for her support, patience, and dedication

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Florida torreya is an endangered, endemic conifer with a limited range near the Apalachicola River. The species began to decline in the 1950's and the population has plummeted from an estimated 375,000 to approximately 1,000. Since 1967 many investigations have tried to determine the cause, including pathological and environmental factors. The Torreya Guardians propose recovering the species through assisted migration, where the species would be "re-introduced" into the southern Appalachian Mountains, the potential "historic" range of Florida torreya. In 2010 a previously unknown pathogen was discovered on Florida torreya, which causes stem cankers and stem girdling. The potential host range was investigated via artificial inoculations. Species tested included conifers whose range overlaps with Florida torreya, other species from the *Torreya* genus, and conifers from the southern Appalachian Mountains. One species with an overlapping range, Florida yew (*Taxus floridana*); two other *Torreya* species, California torreya (*Torreya californica)* and Chinese nutmeg yew (*Torreya grandis*), and five species from the Appalachian Mountains, Fraser fir (*Abies fraseri*), Red spruce (*Picea rubens*), White pine (*Pinus*

strobus), Table mountain pine (*Pinus pungens*), and Eastern hemlock(*Tsuga canadensis*) were found to be potentially susceptible. The effect of temperature on the growth, sporulation, and spore dissemination was tested, also. Growth was maximized at 25 C, sporulation was maximized at 20 C, however, there was no significant difference in spore dissemination among the temperatures tested. The necessity of wounds for infection was investigated; it was found that *F. torreyae* cannot infect leaf or stem tissue without the presence of wounds.

CHAPTER 1
DECLINE OF FLORIDA TORREYA

Florida torreya, *Torreya taxifolia* Arn., is a rare endemic species with limited range along a 35 mile stretch of the Apalachicola River in Georgia and Florida. It underwent rapid decline in the early part of the 20th century and was listed as critically endangered under the Federal Endangered Species Act of 1983 (USFWS, 1984).

In 1875, Asa Gray, the renowned botanist, undertook a "pious pilgrimage to…*Torreya taxifolia*" where he observed, "an abundance of trees, of various ages, interspersed among other growth". Dr. Gray noted one individual, "the largest [he] saw, its trunk just above the base, almost four feet in circumference" (Gray, 1889). Likewise, nearly forty years later, the Florida Geological Survey Annual Report noted: "those who have imagined [*T. taxifolia*] in danger of extinction will be interested to learn now that it stands seventh in the list of trees, and seems to constitute about 4 per cent of the forest" (Harper, 1914).In contrast, by the 1960s no adult individuals could be found and the outlook for the species seemed bleak (Alfieri et al., 1967).*Torreya taxifolia* was considered to be "destined for extinction" (Godfrey and Kurz, 1962) with the first known indication of the decline observed around 1938 (Alfieri et al., 1967). The rapid decline of the species was then attributed to an unknown fungal disease, because of the abundance of leaf spots and stem cankers and rapid nature of the decline (Godfrey and Kurz, 1962).Since then, Florida torreya has continued to decline, and the risk of extinction is high (USFWS 2010). In addition to disease, this species has been subjected to changes in hydrology, forest structure, heavy damage from deer (browsing and rubbing) and a loss of reproductive capability (Schwartz and Hermann, 1995). Despite these challenges, Florida torreya rootstocks which have been killed by disease

often re-sprout from the stump or roots in a manner reminiscent of American chestnut following chestnut blight, although seed reproduction of torreya in the wild has been non-existent for decades (Schwartz and Hermann,2000). Estimates show Florida torreya populations have declined 99% since pre-settlement , from an estimated population of 340,000 individuals in 1914 to approximately 1,350 in the 1990s (Schwartz et al., 2000). It is believed that the population has declined further since 2000 to current estimates of 600-1000individuals (T. Spector, unpublished data, 2010; Smith et. al, 2011).

Changes in soil chemistry, drought, global warming, sunlight exposure and fire regime have been hypothesized as possible causes of the decline of Florida torreya (Schwartz et al., 1995). Some of these environmental changes are thought to have occurred following construction of the Woodruff Dam on the Apalachicola River in 1957(Schwartz et al., 1995), and changing land uses in the surrounding areas. However, none of these environmental hypotheses have been demonstrated as a cause of the decline. The rapid nature of the decline during the period of 1938 to 1945 and numerous observations of disease symptoms provide ample evidence to suggest that a pathogen, possibly non-native, was involved (Schwartz et al., 1995).

Some have suggested that the rapid decline of Florida torreya, with almost complete mortality in adult trees, is characteristic of a root pathogen such as *Phytophthora lateralis*, the causal agent of Port Orford cedar root disease (Sinclair, 2005). *Phytophthora lateralis* kills Port Orford cedar [*Chamaecyparis lawsoniana* (A. Murr.) Parl.] rapidly as the result of root system degradation (Sinclair, 2005). However, recent studies have shown no soil borne pathogens evident around remaining Florida

torreya populations (Rivera, personal communication, 2010).Additionally, the frequent basal re-sprouting following stem top-kill and associated canker symptoms (Smith et al., 2011),in a manner reminiscent of chestnut blight, would suggest a stem disease and that roots are not affected.

Despite several attempts to conclusively determine the causal agent, disease etiology was not determined for several decades (Alfieri et al., 1967; El-Gholl, 1985; Alfieri et al., 1987; Lee et al., 1995; and Schwartz et al., 1996). In the first pathology studies conducted on *T. taxifolia*, it was noted that disease symptoms of leaf spots, needle necrosis, defoliation and stem lesions were common (Alfieri et al., 1967). Several pathogens were isolated commonly from symptomatic needles (*Macrophoma* sp., *Rhizoctonia solani*, *Sphaeropsis* sp. and *Sclerotium rolfsii*); however, no pathogens were isolated from cankered stems and Koch's postulates (proof of pathogenicity) were not demonstrated (Alfieri et al., 1967). About 20 years later, El-Gholl (1985) implicated *Fusarium lateritium* as a causal agent by demonstrating this species' capacity to cause leaf spots; however, the causal agent of the canker disease remained unknown. Alfieri et al. (1987) completed more pathogenicity studies with a *Phyllosticta* sp., *Xylocoremium flabelliforme* and *F. lateritium*. *Pestalotiopsis microspora* was implicated as the causal agent of the canker disease (Schwartz et al., 1996), having isolated the pathogen from 56 symptomatic plants and Koch's postulates were completed on 10 stems. However, no information was given on the canker development, morphology or ability to cause mortality. Typically, *Pestalotiopsis* spp. are considered opportunistic pathogens (Sinclair, 2005). Lee et al. (1995) investigated the endophytic and pathogenic chemical attributes of *P. microspora.* Artificial inoculations resulted in stem

canker development; however, again no stem mortality was observed. Subsequent studies by Herman and Schwartz (1997) implicated a *Scytalidium* sp. due to frequent isolation from cultivated and naturally occurring *T. taxifolia*. Inoculation attempts led to small lesions on needles, but cankers were not observed.

It wasn't until 2010 that the cause of the canker was determined (Smith et al, 2011).A novel *Fusarium*, described as *F. torreyae* (Aoki et al., in press), was isolated from stem cankers occurring on 71-100% of *T. taxifolia* growing at eight sites in the wild. Inoculations of healthy torreya plants with *F. torreyae* resulted in canker development and stem death (Smith et al., 2011). Little is known about the biology of *F. torreyae.* Any future management strategies for the recovery of *T. taxifolia* must include management for the canker disease of Florida torreya (CDFT). It is evident that CDFT has significantly impacting the health of wild trees, suppressing population size and virtually eliminating sexual reproduction in remaining natural populations (Smith et al., 2011).

In addition to scientific efforts to explain the cause of Florida torreya's dramatic decline and continued suppression, there is a group of "citizen naturalists" who believe that the issues surrounding Florida torreya's decline are environmental, and most likely anthropogenic in origin (Torreya Guardians, 2012). The Torreya Guardians use a "deep time perspective" to rationalize the movement of *T. taxifolia's range.* In their view, *T. taxifolia* is adapted to the Appalachian Mountains during an interglacial period, and not to Florida (Ghosts of Evolution, 2010). These citizens are attempting to facilitate "assisted migration" of *T. taxifolia* to the southern Appalachian Mountains in order to save the species from extinction. Their rationale for this radical plan is based on the premise that a seed-dispersing animal went extinct; therefore, the species was unable

to retreat northward at the end of the last glacial period. They believe that Native Americans may have hunted this animal to extinction (Barlow, personal comm., 2010). The Torreya Guardians not only insist that this movement is necessary, but that it needs to be done as quickly as possible. Barlow states, “there isn’t time for scientists” (Ghosts of Evolution, 2010).

The Torreya Guardians, have put *T. taxifolia* in the forefront of the debate over assisted migration by aggressively moving cultivating and planting individuals near Asheville, North Carolina (Torreya Guardians, 2012). Comments in the journal *Nature* (Shirley and Lamberti, 2011) have cast the re-location of *T. taxifolia* as a positive step in the recovery of the species. Although no scientific analysis has been completed on the appropriateness of the locations selected, the survival rate nor the impact on local species has been completed, the positive publicity the Torreya Guardians receive serves to validate their approach. The re-introduction does not take into consideration evolutionary changes the species has made over thousands of years, micro-climate requirements or possible new disease interactions.

*C*urrently, *T. taxifolia* is the only tree known to be affected by the disease, but other trees could become infected, both within the range of Florida torreya and where it is cultivated. The studies reported in chapter two examine whether *F. torreyae* could infect other host species within the range of Florida torreya. Although *F. torreyae* is only known to be pathogenic on one host species, it is likely a generalist, and not biologically limited to *T. taxifolia.* Species closely related to *F. torreyae* are generalists like *Fusarium lateritium* Nees which is a known pathogen on over one hundred species (Farr and Rossman, 2008) *T. taxifolia* individuals that are currently infected are spatially

separated, and root and stump sprouts distant from infected individuals are re-infected. Trees or plants nearby could be infected and “constitute a reservoir…and an important role is therefore ascribed to them as a potential source for disease” (Dinoor, 1974). In addition, the movement of *T. taxifolia* to new locations by the Torreya Guardians and others could potentially expose new host plants to *F. torreyae*. There are only six known species of *Torreya* worldwide. Any pathogen that could affect most or all of them could result in a loss in biodiversity worldwide. In order to examine these possibilities, a host range study was undertaken, in which conifers that occur within the range of *T. taxifolia,* other *Torreya* species, and conifers within the range of the proposed “assisted migration” were inoculated with *F. torreyae* and disease development was measured.

The objectives of studies presented in chapter three were to evaluate the edaphic factors that maximize growth and sporulation of *F. torreyae* and to evaluate the role of wounds as infection sites for *F. torreyae*. The ability of *F. torreyae* to disperse spores aerially was also tested. Several species of *Fusarium*, including *Fusarium circinatum* Nirenberg & O’Donnell, *Fusarium graminearum* Schwabe and *Fusarium lateritium* disperse through aerially disseminated spores, which is important in understanding, modeling and managing disease epidemics. The ability of *F. torreyae* to disperse by airborne spores would increase the likelihood of the pathogen spreading widely. Spore traps placed in a temperature-controlled environment containing diseased *T. taxifolia* plants were used to determine whether spores produced on infected plants could be dispersed aerially. The necessity of wounds for successful infection was also tested. Various inoculation techniques were used to determine if *F. torreyae* could be infectious through plant surfaces, or if wounds were needed. By determining those

conditions that favor *F. torreyae* growth and sporulation and understanding the mechanism of infection management strategies can be developed to mitigate the decline of torreya and foster restoration efforts.

CHAPTER 2
HOST RANGE OF *FUSARIUM TORREYAE*

Introduction

Over the last century and a half North American forests have seen introductions and impacts from hundreds of foreign pests and pathogens. These disease introductions have in some cases affected a very narrow range of species, like chestnut blight [caused by *Cryphonectria parasitica* (Murrill) Barr], while others affect a large number of species, like sudden oak death (caused by *Phytophthora ramorum* Werres et al.). In light of these and other forest diseases that continue to change the composition of our forests, it is clear that in order to preserve our forests it is paramount to limit the spread of new forest diseases and predict which new pests and pathogens are likely to become large scale epidemics. One method of estimating the potential scale of an epidemic is by determining the host range of the pathogen. This chapter investigates the potential host range, in three different spread scenarios, for *Fusarium torreyae*, a newly described fungal pathogen (Aoki et al., in press) that threatens Florida torreya (*Torreya taxifolia*) with extinction (Smith et al., 2011). This new disease, Canker Disease of Florida Torreya (CDFT), girdles the stems of infected individuals, ultimately killing them.

Many *Fusarium* species have broad host ranges and are able to infect hosts across genera and families (Agrios, 2005). Although *F. torreyae* has only been identified as a causal disease agent on *Torreya taxifolia,* it could be or become a pathogen on other plant species. For example, *F. oxysporum* has a host range that includes humans, animals, and plants(Nelson et al., 1994). *Fusarium lateritium*, to which *F. torreyae* is closely related (Aoki et al, in press), is a pathogen of many tree genera, including:

Acacia, Acer, Albizia, Betula, Citrus, Ficus, Fraxinus, Juglans, Malus, Ostrya, Picea, Pinus, Prunus, Quercus, Salix ,and *Ulmus* (Farr and Rossman, 2008).*Fusarium torreyae* may be a generalist, like *F. lateritium,* and be able to infect hosts across several families, or the host range may be confined to only one or two genera. The number and variety of susceptible species is a major factor in how much impact *F. torreyae* will have on forests. Although the pathogen is currently only known on one host, it may spread to new hosts. *Fusarium circinatum* was originally thought to only infect *Pinus* in the southeastern U.S., but when it spread to the western U.S. it became a pathogen on a new genus, *Pseudotsuga* (Vogler et al., 2004).

In order to predict new disease associations for *F. torreyae*, pathogenicity was examined for a wide range of potential host species. The research reported in Chapter 2 investigates three scenarios in which *F. torreyae* may be spread to new hosts. The first scenario is that the pathogen shifts to new hosts in the native range of *T. taxifolia* where the disease already exists. The second scenario is that *F. torreyae* is a pathogen of other species in the same genus, and could cause a similar decline if introduced to areas with *Torreya* species. The third scenario examines the movement of the disease through assisted migration. The Torreya Guardians believe that the decline of *T. taxifolia* is due to climatic changes, and not disease. They are currently planting cultivated *T. taxifolia* in the southern Appalachian Mountains where they believe they will thrive better than in the native range (Torreya Guardians, 2012). It is possible that they will move diseased plants, introducing the pathogen into new areas.

Materials and Methods

Host Range Experiment 1

Species were selected for HRE1 based on the three proposed spread scenarios. The first set of species selected were conifers that have overlapping ranges with *T. taxifolia*. These species may either become infected by *F. torreyae* or may be alternative hosts, and the pathogen could be perpetuated on these hosts enabling it to re-infect any new *T. taxifolia* sprouts or seedlings. If *F. torreyae* has another sporulating host whose range overlaps with *T. taxifolia,* any restoration efforts may be thwarted. The species tested were *Pinus elliottii* Englem.,*Pinus glabra* Walter*, Pinus taeda* L.*, Pinus palustris* Mill.*,* and *Taxus floridana* Nutt. ex Chapm*.*

The second set of species were members of the genus *Torreya.* The genus *Torrey* has only six remaining species and is indigenous to Asia and North American. It is an ancient genus dating back to the Jurassic period (Florin, 1963). The extinction of any of these species would be a loss of biodiversity. Five of the six *Torreya* species were tested: *Torreya californica* Torr.*, Torreya grandis* Fortune ex Lindl.*, Torreya nucifera* (L.) Siebold and Zucc.*,* and *Torreya yunnanensis* Cheng et L. K. Fu; *T. taxifolia* was used as a positive control for HRE1.

The third set of species were conifers from the southern Appalachian Mountains. Due to efforts to move *T. taxifolia* through assisted migration, it may be possible that conifers in this area will become exposed to this new pathogen if diseased individuals are moved. The proposed assisted migration areas are near the natural ranges of several species of conifers, some with a limited or disjunct range. The species tested were *Abies fraseri* (Pursh) Poir., *Picea rubens* Sarg.*, Pinus strobus* L.*, Pinus pungens* Lamb*,* and *Tsuga canadensis* (L.) Carrière.

Inoculation points were created on each plant by making three10 mm X 5 mm vertical flaps under the bark to expose the xylem using a sterile single edge blade. Flaps were made at three positions (upper, middle, and lower stem), with the stem rotated one third of the stem circumference for each wound, so that the inoculation points did not align vertically. A mycelia plug, 5 mm in diameter, containing active *F. torreyae* served as the inoculum, which was inserted into each of the three wounds and then wrapped in Parafilm® “M” (Pechniney Plastic Packaging, Chicago, Il.).For mock-inoculated plants, wounds were made in the same manner, but sterile PDA plugs were used instead of mycelia plugs. Isolate *Fusarium torreyae* NRRL 54152 was used for the inoculations because it was known to be virulent in previous inoculation experiments (Smith et. al 2011), whereas other isolates did not yield consistent lesion lengths (Shin, personal communication, Dec. 2010). The trees were kept in a climate controlled greenhouse between 30 C and 35 Cwith natural lighting, and were hand watered daily or as necessary. The experiment utilized a randomized complete block design, with each block representing a different bench in the greenhouse. Three individuals from each species were randomly placed in each block. Two individuals of each species were inoculated with *F. torreyae* and one served as a mock inoculated experimental control.

After 12 weeks, the inoculation points were exposed by removing the bark, and the total length of stem lesions were recorded to the nearest one-hundredth of a millimeter. The lesion length was defined as the greatest length of necrotic tissue up and down the stem. Samples from around the affected areas were taken for re-isolation of the pathogen and placed on pentachloronitrobenzene media (PCNB), a semi-

selective medium for *Fusarium* species (Summerell et al. 2003). Sub-cultures from each of the isolation plates were taken and morphotyped by examination of conidia using a compound microscope. Morphotypes that exhibited morphological similarities to *Fusarium* species (Summerell et al., 2003) were identified by extracting DNA using the Qiagen DNeasy Plant Mini kit (Qiagen Inc., Valencia, CA), and sequencing the nuclear ribosomal internal transcribed spacer region DNA (ITS rDNA).Polymerase chain reaction conditions utilizing primers ITS1 and ITS4 and sequencing were performed as described previously (Smith et al., 2011). Sequences were then compared to GenBank using BLASTn searches (NIH NCBI, 2012).

A mixed model ANOVA was done using JMP Pro9 software (SAS Institute, Cary, North Carolina) to determine if *F. torreyae* inoculation differed from the mock inoculation control, to determine the significance of inoculation location (upper, middle, lower stem), and to examine the interaction of inoculation treatment and inoculation location on lesion length. A one-tailed Student's t-test was used to determine if mean necrosis length from inoculation treatment was significantly larger than the control for each species.

Host Range Experiment 2

In host range experiment two (HRE2), those species fromHRE1 that are most likely to be exposed to *F. torreyae* because of overlapping ranges, through assisted migration, likely to susceptible because of close phylogenic relation. These tree species are near the assisted migration sites, the southern Appalachian species (*Abies fraseri, Picea rubens, Pinus strobus, Pinus pungens,* and *Tsuga canadensis*), and two North American members of Taxaceae (*Taxus floridana* and *Torreya californica)*. The individuals used were uniform in size and age for *A. fraseri, Pi. rubens, P. strobus* and

Ts. canadensis, and were from three to five years old. Because of difficulty in obtaining *P. pungens, Ta. floridana* and *T. californica,* individuals varied in size and age, but all sizes and ages were included in both the inoculation and control groups. The protocols for inoculation, lesion evaluation, pathogen re-isolation and identification were the same as HRE1. HRE2 was conducted in plant growth chambers with 16-h day length and 25°C (light) and 18°C (dark)conditions for 14 weeks (Smith et. al, 2011).A one-tailed Student's t-test was used to determine if mean necrosis length from inoculation treatment was significantly larger than the control for each species with alpha=0.05. A Student's t-test was used to determine if there was a significant difference between two families represented among species studied with alpha=0.05. The mean lesion lengths for each the species was compared using Tukey's HSD, alpha=0.05. As a post hoc analysis, the lesion expansion rate (mm/week) for the susceptible species in both HRE1 and HRE2 were compared.

Results

Host Range Experiment 1

There was no significant interaction between stem inoculation position and inoculation treatment, nor was the effect of inoculation position found to be significant in any of the species tested. Therefore, within each species *F. torreyae* showed the same lesion length irrespective of the location of the inoculation on the stem.

None of the native Florida *Pinus* spp. had significant inoculated lesion lengths as compared to the control ($p < 0.05$) (Table 2-1), whereas several other genera exhibited significant lesion lengths compared to their control ($p < 0.05$) (*Ta. floridana* (mean lesion length = 18.77 mm),*T. grandis* (11.06mm), *A.fraseri* (14.32 mm), *Pi.rubens* (11.19 mm), *P. strobus* (8.59 mm),and *P. pungens* (12.62 mm). In addition, *T. taxifolia*, the positive

control, had lesions (mean lesion length=11.61) that were significantly larger lesions than the negative control (Table 2-1), but not larger than any of the species that were also susceptible in this study (Figure 2-1).

Isolations from the inoculation points resulted in 30 different morphotypes; three had growth characteristics and macroconidia similar to *F. torreyae.* The ITS rDNA sequences from the three morphotypes had 100% query coverage and 99% identities with *F. torreyae.* All of the species with lesion lengths significantly larger than their control, were represented in the three morphotypes positively identified as *F. torreyae*, thus confirming pathogenicity on these species

Host Range Experiment 2

There was no significant interaction between stem inoculation position and inoculation treatment, nor was position of inoculation found to be significant in any of the species tested. Mean lesion lengths were significantly greater than the mock inoculated control for all species tested, including *T. taxifolia*, the positive control: *Ta. floridana* (24.00 mm), *T. californica* (18.22 mm), *A. fraseri* (19.30 mm), *Pi. rubens* (17.26 mm), *P. strobus* (5.93 mm), *P. pungens*. (18.70 mm), and *Ts. canadensis* (15.83mm); *T. taxifolia*, the positive control had significant lesion expansion (13.02 mm)(Figure 2-2 and Table 2-2).

Isolations from inoculation points resulted in 13 different morphotypes, with two that had growth characteristics and macroconidia similar to *F. torreyae.* The ITS rDNA sequences from the two morphotypes had 100% query coverage and 99% identities with *F. torreyae.* All of the hosts with lesion expansion as compared to the check were represented in the two morphotypes positively identified as *F. torreyae.*

Means comparison by Tukey's HSD showed that *Taxus floridana* and *P. strobus* were significantly different than the other speices, whereas there was no significant difference among the other species (Figure 2-3). Additionally, Student's t-test showed there was no significant difference in mean lesion length between the families represented in HRE2. When the lesion expansion rate (mm/week) was calculated for susceptible species in HRE1 and HRE2, the rate of lesion expansion was higher in HRE2 than HRE1 for every species except *P. strobus* (Table 2-3)

Discussion

The pines with overlapping range with *T. taxifolia* were not susceptible to *F. torreyae*. Although these pines did show some necrosis at the inoculation points, lesion lengths were not significantly different from the mock inoculated controls, nor were the affected tissues consistent with elongated, elliptical reddish-orange to brown lesions characteristic of CDFT symptoms (Figure 2-3). The areas of necrosis were also constrained within the wounded area. The area of necrosis for the inoculation points was larger, on average, than the mock inoculation points (Figure 2-3) which may be from increased resinosis from the tree response to the pathogen attempting to infect the stem. The inability of *F. torreyae* to colonize the *Pinus* hosts suggests that local *Pinus* species are not likely reservoirs for further infection, and that land use changes, including increased pine plantations area, are not likely to have caused the introduction or incidence of CDFT. These results mirror unsuccessful isolation attempts from pines near infected *T. taxifolia* (Trulock, personal communication, Nov. 2010) which also suggests that the *Pinus* species are not asymptomatic hosts.

T. grandis and *Ta. floridana* did show significant lesion expansion as compared to the control in HRE1, and *T. californica* had significant lesion expansion in HRE2, but

not HRE1. However, many of the inoculation points had elliptical, orange lesions characteristic of *F. torreyae*. The inoculated stems that had no lesions develop had very small diameters (less than 1 cm) which made inoculations very difficult and may have led to failed inoculation attempts. Because of this, *T. californica* was included in HRE2. In this study, larger stem diameters were used, and *T. californica* was susceptible as compared to the control (p=0.0004) (Figure 2-3). Eleven of sixteen inoculation points in HRE1 created lesions within the range of the HRE2 lesion lengths. This demonstrates that the inoculations in HRE1 that were successful did create lesions, but that stem diameter may have influenced whether or not the inoculation succeeded. However, since stem diameters were not recorded for HRE1 plants, correlations between stem diameter and susceptibility were not performed. Ultimately, it was shown that the three Taxaceae species tested, including both of the N. American *Torreya* species, are potentially susceptible to *F. torreyae.* The Taxaceae family dates back to the Jurassic period(Florin, 1963), and the genus *Torreya* has only six species still extant, four in Asia and two in N. America. This pathogen is greatly impacting *T. taxifolia,* possibly leading to its extinction. If *F. torreyae* shifts hosts and infects both *Ta. floridana* and *T. californica* a similar scenario could take place. The loss of both of the *Torreya* species in N. America and *Ta. floridana* would constitute both the loss of a genus on the continent, and also a loss of one of ten species in the family (Price, 2003).

Both HRE1 and HRE2 demonstrated that there are conifer species in the southern Appalachian Mountains that may be susceptible to *F. torreyae*. In HRE1 and HRE2, *A. fraseri*, *Pi. rubens*, *P. strobus* and, *P. pungens* all showed significant lesion expansion as compared to controls, additionally in HRE2 *Ts. canadensis* was also

susceptible. If these species are exposed to *F. torreyae* it is possible for the epidemic to spread to new areas and infect new species. Of the species tested, *A. fraseri, Pi. rubens*, and *P. pungens* have ranges that are limited to high elevations in the southern Appalachian Mountains. The Torreya Guardians' effort to move *T. taxifolia* to new areas overlooks the potential threats to native species. If diseased *T. taxifolia* are moved for assisted migration, the species identified as susceptible could be exposed to *F. torreyae.* Exposure to *F. torreyae* could lead to extinction or suppression of potentially susceptible species in these isolated ranges with limited genetic diversity, analogous to the current state of *T. taxifolia*. The likelihood of the Torreya Guardians taking infected plants is unknown, but highly probable given that cultivated plants with CDFT are common and similar pathogens (i.e. *F. circinatum*) are known to be transmitted in seeds (Barnard and Blakeslee, 1987). The majority of *T. taxifolia* clones at the Atlanta Botanical Gardens have canker (Determann, personal communication, Nov. 2010) as do the majority of individuals in the wild (Smith et al., 2011). The mode of infection and dispersal was investigated in Chapter 3. If infected *T. taxifolia* are brought into areas with the tested species, the author believes infection of native plants is likely.

All species in HRE2 were susceptible to infection, but only *Ta. floridana* and *P. strobus* differed in lesion length(Tukey's HSD, alpha =0.05). Comparing susceptible species in HRE2 by lesion length (Figure 2.3), only *Ta. floridana* and *P. strobus* were significantly different (Tukey's HSD, alpha =0.05). *Ta. floridana* had the greatest lesion length, although *F. torreyae* infection had not been reported for *Ta. floridana* previously and further investigations may show a new association. *Pinus strobus* had the smallest lesions. In both HRE1 and HRE2, *P. strobus* shed copious amounts of resin upon

wounding, as do most pines. The constituents of pine resin have been shown to retard the growth of *F. circinatum* (Friel and Gordon, 2005) and other pathogens. This may partially explain why *P. strobus* had smaller mean lesion lengths than *P. pungens*, which is not known for resin production (Figure 2-3). Likewise, the southern pines tested in HRE1 have historically been used for turpentine production via resin collection and distillation and were not found to be susceptible.

The species in HRE2 are within two families, Taxaceae and Pinaceae. When the lesion lengths of these two families are compared, there is no significant difference between the families (Student's T-test, alpha = 0.05). This suggests that *F. torreyae* is a generalist pathogen and can cause disease across not only genera, but also across families.

There was a noticeable difference in the length of lesions between HRE1 and HRE2 studies. There are two important variables that differ between HRE1 and HRE2, the length of time between inoculation and measurements, and temperature regime. Comparing the two studies qualitatively, it is likely that differences in pathogen performance were affected by temperature; the cooler temperatures of study HRE2, both day and night, appeared more conducive to disease development. An example of a pathogen being active in cool to cold weather and not in warm weather is *Eutypella parasitica* R.W. Davidson & R.C. Lorenz*,* the causal agent of Eutypella canker(Sinclair, 2005). This pathogen is active during the fall and winter months, but latent during the warmer months. Although the difference of two weeks in post inoculation evaluation could impact the length of lesion expansion, it does not seem long enough to explain the difference between HRE1 and HRE2.

Temperature differences could explain why there was a difference in mean lesion length between HRE1 and HRE2 in most species. Qualitatively, more rapid lesion expansion was observed in HRE2 than HRE 1 for every species except *P. strobus* (Table 2-3).Comparing lesion expansion between HRE2 and HRE1, time from inoculation to evaluation is held constant, whereas temperature varies and is thus more likely to explain observed differences in disease development.

The species that are North American members of the family Taxaceae that were shown to be susceptible have different population characteristics. *Torreya californica* has healthy population numbers and is considered a stable population (Howard, 1992). An introduction to this population is less likely due to the vast space between the currently infected species and *T. californica*; however, western *Abies* and *Picea* species could serve as a bridge to *T. californica*. With population numbers that are relatively large compared to *T. taxifolia*, infected individuals may not be identified readily and the disease may be able to become established. Regular monitoring can help identify any disease symptoms in the species and identify any disease introduction. *Ta. floridana,* however, is listed by the State of Florida as a threatened species, and has seen population declines over approximately the same time as *T. taxifolia,* although at a much slower rate (Spector, personal communication, 2010). *Fusarium torreyae* has not been isolated from *Ta. floridana,* but it has not been as extensively surveyed for pathogens as *T. taxifolia*. It seems likely that if *Ta. floridana* is susceptible, and is located within the range of spore dissemination from diseased *T. taxifolia,* that the pathogen should be found. These results may require extensive disease surveys, but could help reveal *F. torreyae* penetration and infection fitness. If only a small number of

Ta. floridana are infected, even when under direct disease pressure, the pathogen may not be very effective at creating new infections.

The Appalachian species that have shown to be susceptible are near the assisted migration areas, and the introduction of diseased *T. taxifolia* has the potential to impact forests already afflicted with Balsam Wooly Adelgid and Hemlock Wooly Adelgid. The introduction of *F. torreyae* could expedite the decline of these forests by compounding a vascular fungal infection with an acute adelgid infestation. *Fusarium torreyae* girdled stems of young *T. taxifolia* seedlings within 12 weeks in previous inoculation experiments (Smith et al., 2011) and several of the inoculated plants from the southern Appalachians in HRE1 were girdled and died within a year of inoculation, including *P. pungens, Pi. rubens* and Ts*. canadensis.* Considering the potential impact of *F. torreyae* on American forests, the assisted migration of *T. taxifolia* and movement of any cultivated plants should be more closely scrutinized.

If assisted migration is continued, it needs to be completed in a well planned program, ensuring the highest likelihood of survival and the least threat to native species. McLachlan et al. (2007), citing the Torreya Guardians as an example of assisted migration, outline three policy positions on assisted migration. The positions are to aggressively pursue assisted migration, avoid assisted migration or constrain assisted migration. McLahlan et al. (2007) acknowledges that assisted migration may be necessary to ameliorate the effects of global warming, however, they recommend scientists and managers form a “consensus that identifies the risks and opportunities…and suggests ecologically sound best-management strategies.”

Perez et al. (2012) have assembled a decision tree for assisted migration. The first level of criteria include: “Have the threatening factors been removed or controlled, or were they absent in release area?” (Perez et al., 2012). Although the initial decline may be disputed, we know that *F. torreyae* is a major factor suppressing *T. taxifolia* currently. Individuals used for assisted migration must be absent of *F. torreya* for successful plant growth. Diseased plants used for assisted migration will eventually die in the field and add nothing to the persistence of the species.

The second level criteria for assisted migration must consider not only the health of the species being moved, but also “[if] there risks for other species or the ecosystem” (Perez et al., 2012). The results presented in Chapter 2 indicate that there are risks for species which already inhabit the southern Appalachian Mountains. Because we know that there is a possible threat to other species other than *T. taxifolia,* the assisted migration efforts should be stopped until the impact on these species can be better assessed. In order for assisted migration to be a successful conservation tool, it must look past the target species and consider all consequences of the movement. Including, both the health of the species being moved, and the impact it will have on the local ecosystem and local people.

Table 2-1. Host species tested and mean lesion length for host range experiment one

			Inoculated			Control		
Common name	Scientific name	Range	N	Mean lesion length (mm)	St. Dev.	N	Mean lesion length (mm)	Std. Dev.
Slash pine	*Pinus ellottii*	FL	18	6.96	8.44	6	3.05	6.11
Spruce pine	*Pinus glabra*	FL	18	9.28	9.18	9	3.60	7.45
Loblolly pine	*Pinus taeda*	FL	18	7.56	8.14	9	3.85	5.86
Longleaf pine	*Pinus palustris*	FL	18	0.67	2.85	9	3.62	5.51
Florida yew	*Taxus floridana*	FL	12	18.77*	11.37	3	5.56	1.12
California torreya	*Torreya californica*	NA	16	8.81	6.88	9	6.73	3.025
Chinese nutmeg yew	*Torreya grandis*	A	18	11.06*	4.87	9	5.56	1.12
Japanese nutmeg yew	*Torreya nucifera*	A	18	8.37	5.59	9	6.32	6.88
Yunnan nutmeg yew	*Torreya yunnanensis*	A	18	9.97	4.96	9	6.70	5.74
Florida torreya†	*Torreya taxifolia*	FL	18	11.61*	4.13	9	5.87	4.58
Fraser fir	*Abies fraseri*	AP	6	14.32*	2.26	6	7.43	8.78
Red spruce	*Picea rubens*	AP	15	11.19*	4.30	9	6.46	5.35
White pine	*Pinus strobes*	AP	18	8.59*	6.26	9	4.16	3.51
Table mountain pine	*Pinus pungens*	AP	18	12.62*	6.55	9	6.47	5.41
Eastern hemlock	*Tsuga canadensis*	AP	18	11.39	5.07	9	10.30	4.53

*Indicates lesion length is significantly different from the mock inoculated control for each species. †Indicates the positive control for the experiment Range: FL: Overlapping with Torreya taxifolia in Florida, CA: California; A: Asia; AP: Appalachian Mountains

Table 2-2. Host species tested and mean lesion length for host range experiment two

			Inoculated			Control		
Common name	Scientific name	Range	N	Mean lesion length (mm)	Std. Dev.	N	Mean lesion length (mm)	Std. Dev.
Florida yew	*Taxus floridana*	FL	9	24.00*	10.27	7	6.57	1.43
California torreya	*Torreya californica*	CA	11	18.22*	4.99	6	5.50	5.65
Florida torreya†	*Torreya taxifolia*	FL	9	13.02*	2.28	9	0.05	0.11
Fraser fir	*Abies fraseri*	AP	18	19.30*	5.34	12	2.80	2.30
Red spruce	*Picea rubens*	AP	20	17.26*	6.63	9	3.82	4.67
White pine	*Pinus strobus*	AP	18	5.93*	7.82	12	0.00	0.00
Table mountain pine	*Pinus pungens*	AP	11	18.7*	8.20	9	0.53	1.58
Eastern hemlock	*Tsuga canadensis*	AP	18.00	15.83*	4.60	11.00	5.81	3.32

*Indicates lesion length is significantly different from the mock inoculated control for each species. †Indicates the positive control for the experiment. Range: FL: Overlapping with Torreya taxifolia in Florida, CA: California, AP: Appalachian Mountains

Table 2-3. Comparison of lesion expansion rate between HRE1 and HRE2

		Lesion Expansion Rate (mm/week)	
Common name	Species	HRE1	HRE2
Florida yew	*Taxus floridana*	1.56	1.71
California torreya	*Torreya californica*	-	1.30
Fraser fir	*Abies fraseri*	1.19	1.38
Red spruce	*Picea rubens*	0.93	1.23
White pine	*Pinus strobus*	0.72	0.42
Table mountain Pine	*Pinus pungens*	1.05	1.34
Eastern hemlock	*Tsuga canadensis*	-	1.13

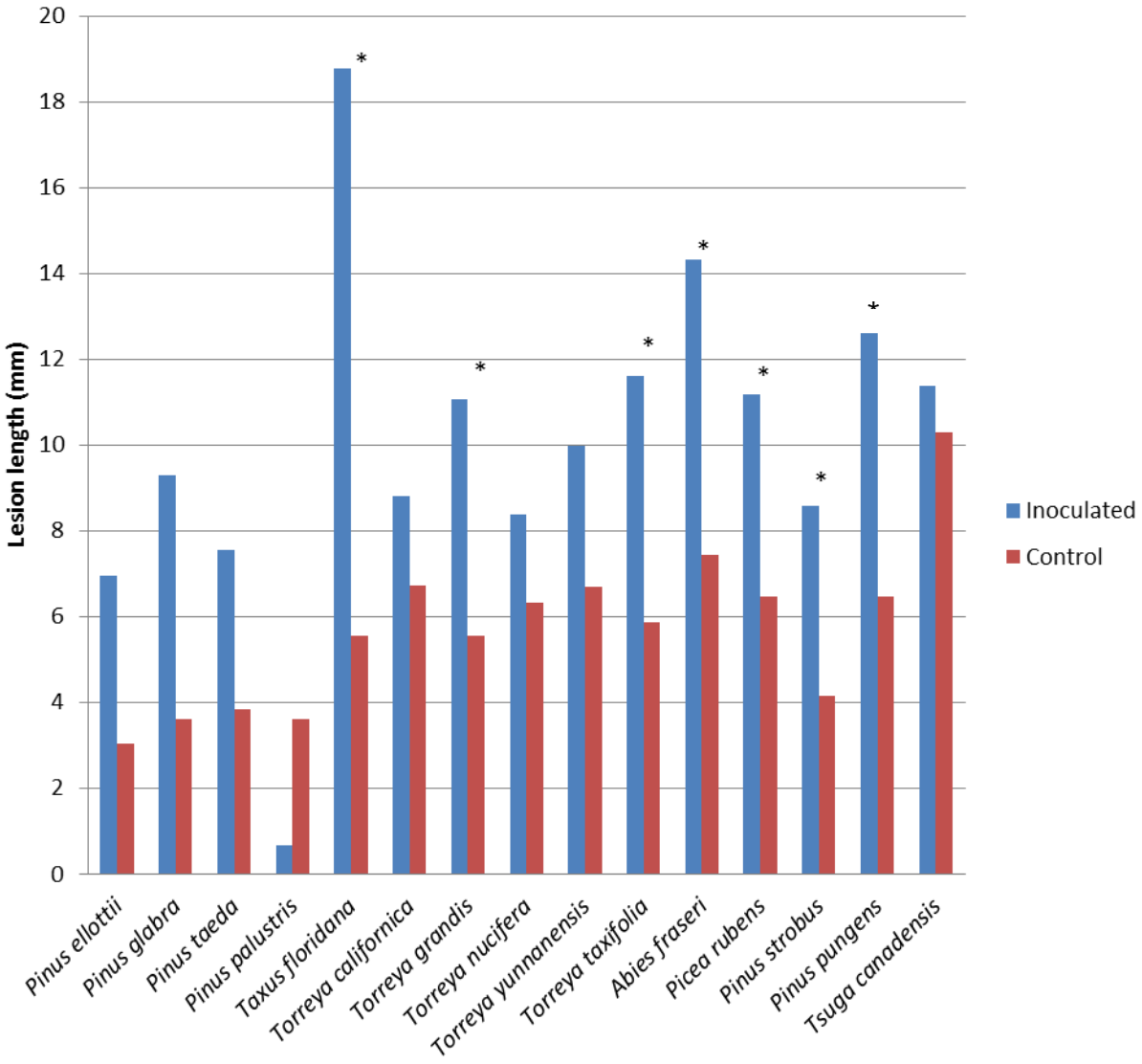

Figure 2-1. Lesion length for host range experiment one *Indicates lesion length is significantly different from the mock inoculated control for each species

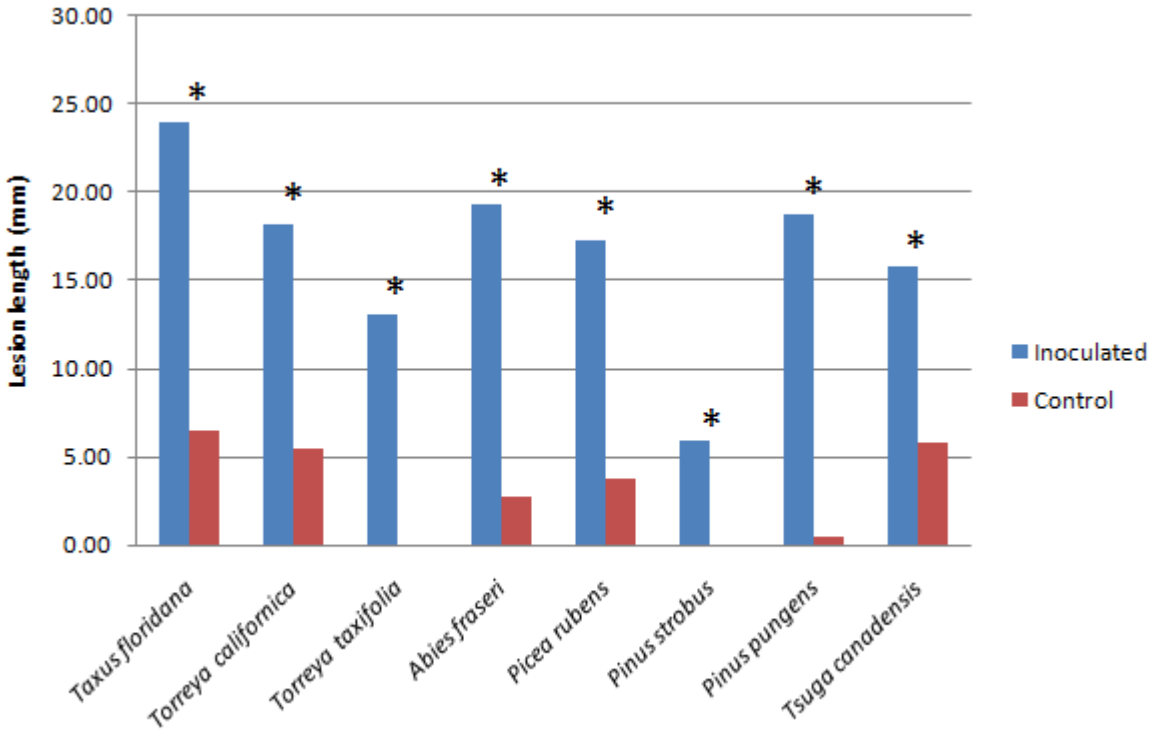

Figure 2-2. Lesion length for host range experiment two *Indicates lesion length is significantly different from the mock inoculated control for each species

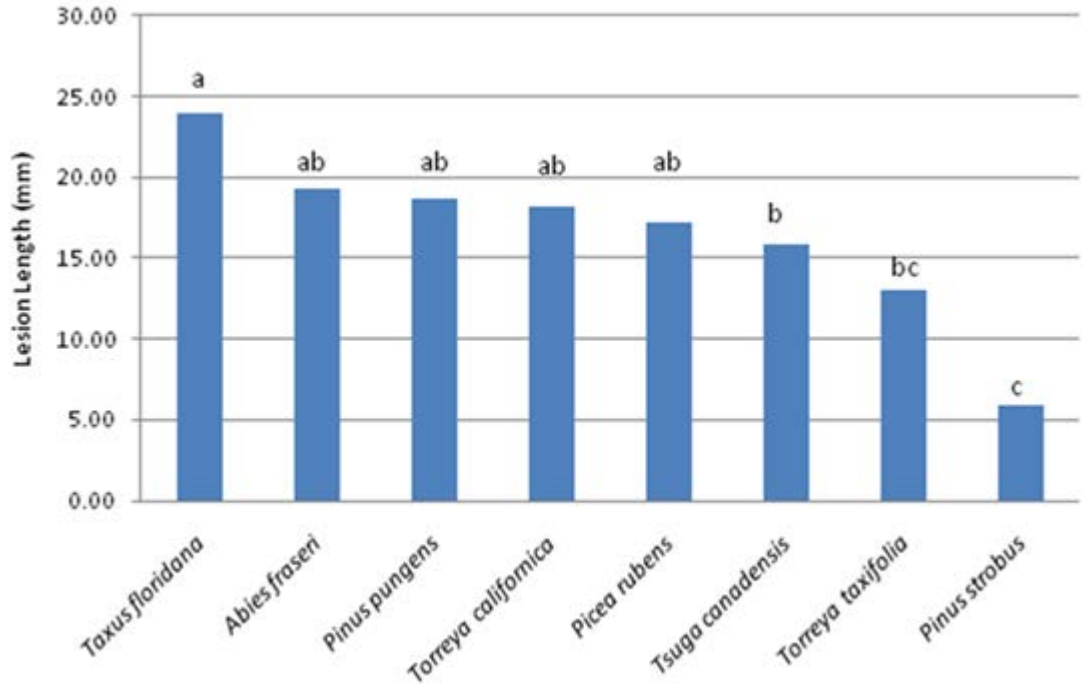

Figure 2-3. Mean lesion lengths for all species in host range experiment two. Different letters represent significantly different values (Tukey's HSD, alpha=0.05)

Figure 2-4. Lesions resulting from inoculationswithHRE1. A) *Taxus floridana,* inoculated, B) *Taxus floridana,* control, C) *Torreya taxifolia*, inoculated, D) *Torreya taxifolia,* control.

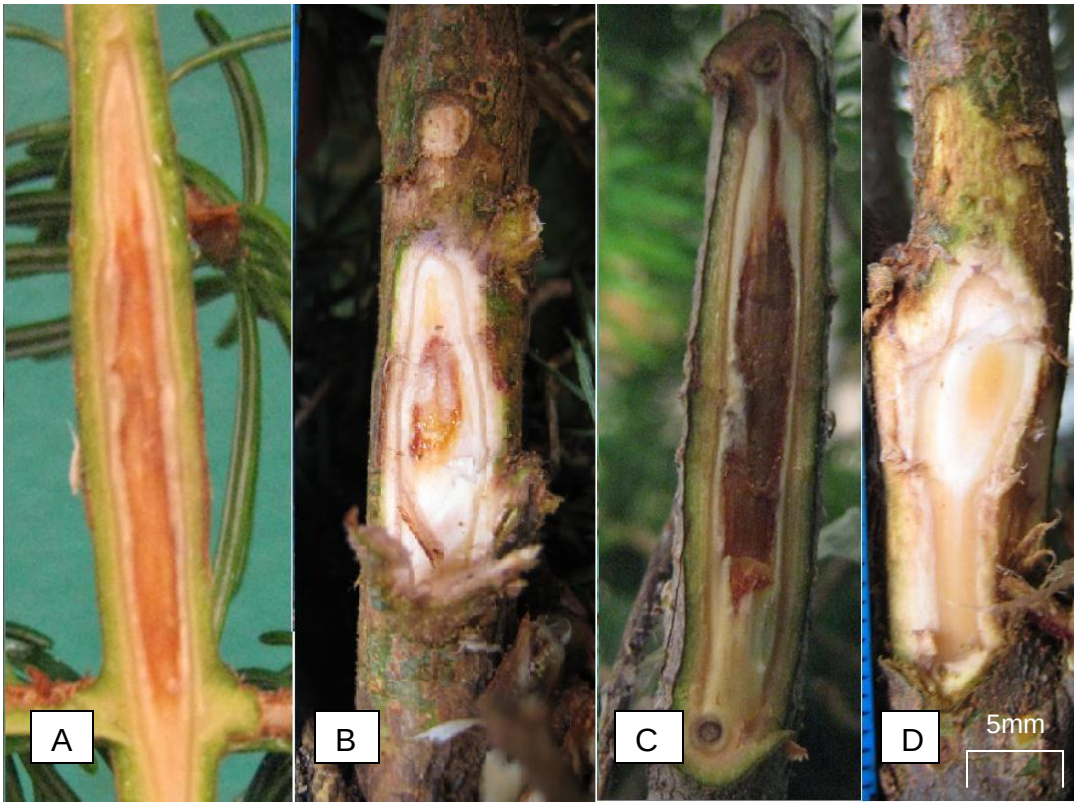

Figure 2-5. Lesions resulting from inoculation with HRE2. A) A*bies fraseri*; inoculated, B) *Abies fraseri,* control, C) *Tsuga canadensis,* inoculated, D) *Tsuga canadensis,* control.

CHAPTER 3
BIOLOGY OF *FUSARIUM TORREYAE*

Introduction

Disease is not the normal condition; it is a phenomenon that occurs only under the proper conditions. In order for disease to occur, there are three conditions that must be met: 1) a virulent pathogen must be present, 2) a susceptible host must be present, and 3) the environment must be conducive to disease development (Agrios, 2005). These three factors form what is known as the disease triangle, and all three sides of the triangle are needed in order for disease to occur. In order understand how disease functions and how it may be managed, we must understand what conditions favor its development. By understanding which conditions favor disease development, we can alter them to avoid or prevent the disease, disrupting the disease triangle.

The lifecycle of a pathogen includes the inoculation, penetration, infection, colonization, reproduction, and dissemination. Inoculation is the initial contact of the pathogen with a susceptible host at a point of possible infection. After the pathogen makes contact with the host, it then attempts to penetrate the host in order to colonize the plant cells and receive nutrition from the plant cells. Not all spores that find a susceptible host successfully penetrate. Penetration may take place either through wounds, natural openings or directly (Agrios, 2005). Fungi can directly penetrate plant tissues through puncturing or degrading plant surfaces and cell walls. Some fungi, like *Magnaporthe grisea* (T.T. Hebert) M.E. Barr or *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, create a specialized structure called an appressorium that aids the fungus in physically puncturing through plant tissues, which may or may not be softened via enzymatic degradation. The method of penetration may not be consistent across a

genus of fungi, for example, *F. oxysporum* and *F. culmorum* (Wm.G. Sm.) Sacc. create an appressorium whereas other *Fusarium,* like *F. avenaceum* R.J. Cook*,* do not(Parry and Pegg, 1985) even though they are attacking similar hosts. *Fusarium circinatum* produces “appresoria-like” structures that penetrate through natural openings and small wounds in the plant tissues (Thoungchaleun et al., 2008). Currently, it is not known how *F. torreyae* penetrates its host (Smith et al., 2011). The ability of *F. torreyae* to penetrate is important for the potential of the epidemic spreading the southern Appalachian Mountains. The findings presented in Chapter 2 showed that several species near the assisted migration areas are susceptible to *F. torreyae.* If the pathogen can take advantage of wounds for infection, *A. fraseri* and *Ts. canadensis* are susceptible infection because of infestation of the Balsam Wooly Adelgid (BWA) and Hemlock Woolly Adelgid (HWA), respectively. Attacks from BWA and HWA create hundreds of small wounds that could serve as penetration points for *F. torreyae.* If a tree is not killed directly from these adelgids, multiple infections by *F. torreyae* could girdle them, killing them more quickly than BWA or HWA alone.

In order for a pathogen to successfully infect, colonize, reproduce and disseminate it must first be able to penetrate the host. The inability of a pathogen to directly penetrate host tissues may decrease the infection rate of the pathogen (i.e. the number of infections per number of spores disseminated). By testing different inoculation methods it is possible to determine if wounds are necessary for penetration and infection, or if *F. torreyae* can directly penetrate plant surfaces.

Colonization, which is the growth of the pathogen in the host, is how a pathogen infects the host plant. Pathogens deteriorate plant tissues in order to obtain nutrition.

The rate of degradation determines how quickly the pathogen kills or injures the plant. Some pathogens grow quickly in warm environments, like *F. circinatum*; however, not all pathogens require warm temperatures. Some, like *Eutypella parasitica*, grow and expand in the host during cool months (Sinclair, 2005). Global climate change may play a large role in plant diseases in the near future. As many parts of the world become warmer, the range for some pathogens will likely spread to new areas, and possibly new hosts. If North America becomes warmer in the coming decades pathogens could spread to previously unavailable hosts because of thermal limitations. Testing the growth of *F. torreyae* across a variety of temperatures is needed to determine at what temperatures the pathogen colonizes hosts.

Dissemination of the pathogen is critical in disease epidemics. The rate, distance and duration of a pathogen's dispersal is a determining factor of how the pathogen spreads, spatially and temporally. It is unknown how *F. torreyae* began to infect *T. taxifolia*, and how it entered the ecosystem. Likewise, it is unknown how *F. torreyae* spreads between trees to an extent that it now infects nearly every living *T. taxifolia* in the wild (Smith et al., 2011). Dissemination of fungal diseases is through the creation and dispersal of sexual or asexual spores of the pathogen. The production of these spores, sporulation, is often based on environmental cues, especially temperature (Leach, 1967).By looking at how the pathogen spreads, and under what conditions it spreads, we may be able to predict if it can spread in new localities. The thermal requirements for sporulation of *F. torreyae* are currently unknown. Knowledge of the thermal requirements for sporulation will make it possible to predict the timing of infection. If we know the timing of infection, management activities, like application of

protective fungicides, may be deployed to prevent important individuals from becoming infected.

Materials and Methods

Research reported in Chapter 2 examined the possibility of multiple susceptible hosts for *F. torreyae*; this chapter examined the environmental conditions required for *F. torreyae* to complete its lifecycle, and if it is plausible for the pathogen to complete its lifecycle in the geographic range of the proposed assisted migration. First, *F. torreyae* was tested *in vitro* to understand the parameters of optimal sporulation and growth. Then, the sporulation of infected plants was measured across the best performing temperatures to estimate the volume of spores disseminated under different conditions. Finally, the necessity of wounds for infection was investigated to determine if *F. torreyae* can directly penetrate host tissues.

Growth of *F. torreyae in vitro*

Five square centimeter plugs from the margins of actively growing *F. torreyae* colonies were plated on potato dextrose agar (PDA) plates and incubated at 15, 20, 25, 30, and 35 C with a 12 hour light/dark cycle. The radial growth of the new colonies was measured at 1,3,5 and 7 days. Five different isolates, USDA Agricultural Research Service Culture Collection (NRRL) numbers 54150, 54151, 54152, 54153, 54154, were used to test across genetic variability previously observed in the species Smith et al. 2011).

Sporulation of *F. torreyae in vitro*

Five square centimeter plugs from the margins of actively growing *F. torreyae* colonies were plated on carnation leaf agar (CLA) and incubated at 20, 25, 30 and 35 C. CLA is a low nutrient medium known to induce sporulation of *Fusarium spp .*“in a

manner similar to that found on a host plant"(Nelson et al., 1994). After 14 days of incubation the plate was washed with two milliliters of sterile water and an the aqueous spore solution was collected. The number of spores per milliliter of solution was measured five times using a hemacytometer and averaged. Five different isolates, NRRL numbers 54150, 54151, 54152, 54153, 54154, were used to test across genetic variability previously described (Smith et al. 2011). The means were compared by Tukey's Honestly Significantly Difference (HSD) test (alpha=0.05).

Sporulation of *F. torreyae in vivo*

Eight infected *T. taxifolia* plants were placed in a temperature controlled growth chamber with a 12 hour light/dark cycle for all of the temperatures tested. Three elevated spore traps made of Whatman filter paper and wetted with 4x TE buffer were placed in the growth chamber between the plants at 50 cm height (Schweigkofler et al, 2004). The temperature was changed every four weeks, with one week between measurement periods. Temperatures tested were: 20, 25, and 30C. The temperatures selected were those which resulted in the highest spore production *in vitro*. The spore traps were replaced every 7 days. The spore traps were washed with 5 milliliters of sterile water and then 0.3 milliliters of the resulting spore suspension was plated on pentachloronitrobenzene medium (PCNB), a semi-selective medium for *Fusarium* species. The resulting colonies were counted, identified through DNA sequencing to verify their identity as *F. torreyae* and the number of spores per square meter was determined. The number of spores per square meter was used to estimate the number of spores disseminated per square meter of forest. The mean number of spores per meter captured per temperature were analyzed using one-way ANOVA and means separated by Tukey's HSD (alpha 0.05).

Necessity of wounds for infection

T. taxifolia plants used in inoculation studies were derived from somatic embryogenesis (Ma et al., 2012) to ensure they were disease free. They were kept in a temperature regulated greenhouse between 25 and 30 C and hand watered. The plants were inoculated on leaves and stems and each was either wounded or left unwounded. Inoculations were completed using a spore suspension of water agar and a mixed isolate *F. torreyae* aqueous spore suspension to create a gel at a final concentration having 1.8 X 10^5 spores/mL (El-Gholl, 1985). Mock inoculations were carried out in the same manner, but sterile water agar was used instead of the spore suspension. Approximately 0.05 ml of gel suspension (calculated to be roughly 9,000 spores) was placed either directly over a fresh wound or on healthy tissue. Wounds were created by puncturing the tissue surface using a sterile dissection needle.

Leaf spot was measured to the nearest 0.01 mm at 7 days post inoculation. Mean leaf spot diameter was compared to non-inoculated controls using Student's t-test (alpha = 0.05). Lesion lengths from the stem inoculation were measured by removing the bark and measuring the resulting lesions to the nearest 0.01 mm. Mean lesion lengths for each treatment were calculated and compared using Student's t-test.

Results

Growth of *F. torreyae in vitro*

Maximal radial growth increased with increasing temperature to 25 C, and then declined. Maximal radial growth over 7 days was observed at 25 C (21.33 mm) (Table 3-1). The average colony growth rates ranked from highest to lowest were at 25 C (21.33 mm), 30 C (18.69 mm), 20 C (16.65 mm), 15 C (13.80 mm) and 35 C (3.21 mm).

There was no significant difference between radial growth 20 C and 30 C , but all other values were significantly different (Tukey's HSD, alpha=0.05, Figure 3-1).

Sporulation of *F. torreyae in vitro*

Maximum sporulation occurred at 20 C (4.7 x 10^6 spores/mL) and decreased with increasing temperature to 35 C. Mean sporulation ranked from highest to lowest at the various temperatures were 20 C, 25 C (3.03 x 10^6 spores/mL), 30 C (1.85 x 10^6 spores/mL), and 35C (3.40 x 10^5 spores/mL) (Table 3-2).Sporulation at 20 C was significantly different from all the other temperatures, 25 and 30C were not significantly different from each other, whereas 30 and 35C were not significantly different, but 25 was significantly different from 35C (Figure 3-3).

Sporulation of *F. torreyae in vivo*

There was no significant difference in the number of spores observed at 20, 25, and 30C (Figure 3-4). However, 20C did produce the most spores per square meter (367.82), followed by 25C and 30C (249.34 and 246.24 spores per square meter, respectively) (Table 3-3).

Necessity of wounds for infection

None of the unwounded tissues, nor the water controls displayed any leaf spots or lesions. All of the wounded leaves and stems both showed disease symptoms, including leaf spots and small lesions. In both cases the leaf spots and lesions were significantly larger than the controls ($p<0.0001$). The wounded leaves developed leaf spots within 3-5 days and averaged 4.04 mm, from which *F. torreyae* was re-isolated. The wounded stems had lesions measured at 14 weeks after inoculation and averaged 3.22 mm; with some lesions coalescing, and the largest being 9.32 mm(Figure 3-5).

Discussion

The disease cycle begins with inoculation and penetration. In the inoculation methods experiment it was found that only wounded tissues became infected with *F. torreyae.* The pathogen did not appear to be able to penetrate the tissue surface, or be able to exploit any natural openings in the stem or leaf surface (lenticels or stomata). Only artificial wounds were tested; natural wounds, like leaf scars, may also serve as penetration sites. Further inoculation experiments could be conducted to see if natural wounds could serve as penetration points. Additionally, while the plants used to conduct these inoculation experiments were disease free plants from embryogenesis, they were small in size and natural openings like bark fissures were not present.

Growth and colonization of the pathogen was only tested *in vitro,* because of the lack of disease free plant material did not allow for an *in vivo* experiment. The lack of plants was because of the difficulty in creating mature clones from the embryo-derived plantlets. The transfer of somatic embryogenesis derived plantlets into soil from growth media is not always successful, and unfortunately many clones were lost in the transfer. Ideally, *in vivo* growth would also be investigated by placing artificially inoculated *F. torreyae* under different temperature regimes. However, in Chapter 2 HRE1 and HRE2 gave an insight as to the potential role temperature may play in disease progression. HRE2 had a lower average temperature than HRE1, and the rate of lesion expansion was greater for every susceptible species, except *P. strobus* in HRE2 (Table 2-3).

In vitro there were significant differences in the number of spores produced at different temperatures; however, *in vivo* there were no significant differences among the three temperatures *in vitro* with the highest spore concentration. There would likely have been significant differences in spore production *in vivo* had a larger range be tested,

away from optimal temperature, the pathogen would be limited and sporulaiton would decrease. The *in vivo* experiment suggests that the pathogen is able to disseminate in cooler temperatures as well as in the warmer temperatures more commonly seen in *T. taxifolia*'s native range. The methodology for this experiment could also be improved in order to have more exact results, and to include trapping *in situ*. The development of taxon specific PCR primers and the use of real time polymerase chain reactions (qPCR) would allow researchers to measure spore levels directly from DNA extractions from spore traps without having to isolate and identify fungal colonies. This method has been shown to increase accuracy and precision (Schweigkofler et al, 2004). The development of taxon specific primers would also facilitate the rapid screening of isolates previously described as *F. lateritium,* El-Gholl (1985) identified *F. lateritium* as a cause of leaf spot disease of *T. taxifolia*; however, recently that isolate has been correctly identified as *F. torreyae*, (O'Donnell, unpublished). If taxon specific primers were developed and used with PCR, large number of *F. lateritium* isolates could be quickly re-evaluated and the origin of *F. torreyae* may become clearer, as well as its lifecycle and host range.

We have identified both that *F. torreyae* can infect tree species in the southern Appalachian Mountains (Chapter 2), and that it both grows well and sporulates in the average summer and fall temperatures. It may even perform better in the conditions common in the southern Appalachian Mountains than in average temperatures in the native range of *T. taxifolia. Fusarium torreyae* can also take advantage of wounds already formed on *A. fraseri* and *Ts. Canadensis* from BWA and HWA infestations. Introducing a vascular canker disease on top of acute adelgid infestations could expiate the demise of these two species.

Fusarium torreyae appears to have the ability to complete its lifecycle if moved to a new, cooler location and would not be limited by the new environment. The movement of infected *T. taxifolia* plants into the southern Appalachian Mountains may spread this pathogen to a new area, to stressed, injured susceptible hosts and create a new epidemic. What is the value in moving one species to potentially imperil others?

Table 3-1. Mean colony radial growth in mm at different temperatures

Temperature	Day	Radial colony growth (mm)
15	1	0.76
	3	6.46
	5	12.21
	7	13.80
20	1	2.60
	3	8.44
	5	13.44
	7	16.65
25	1	2.81
	3	9.81
	5	15.71
	7	21.33
30	1	2.76
	3	8.46
	5	13.92
	7	18.69
35	1	0.46
	3	1.44
	5	1.86
	7	3.21

Table 3-2. Suspension concentration (spores per milliliter) after 14 days of incubation at various temperatures in vitro

Temperature (^{o}C)	Mean spore concentration (spore/mL)
20	4.70×10^{6}
25	3.03×10^{6}
30	1.85×10^{6}
35	3.40×10^{5}

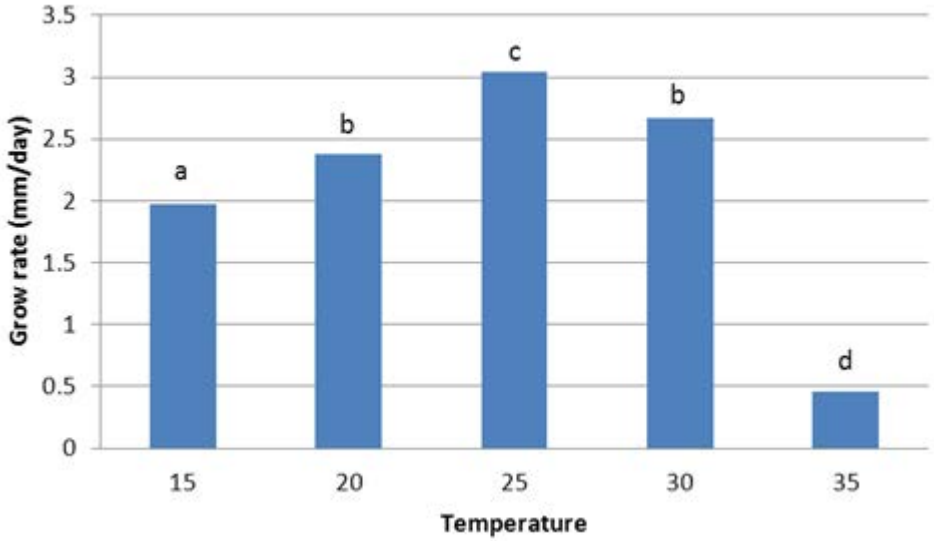

Figure 3-1. Growth rate (mm/day) of *Fusarium torreyae* at various temperatures. Different letters represent significantly different values (Tukey's HSD, alpha=0.05)

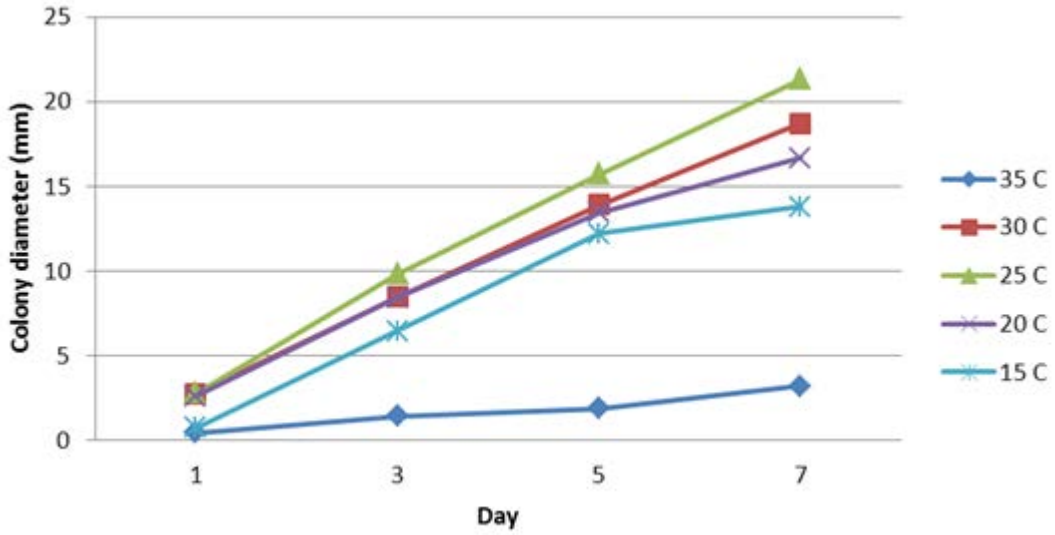

Figure 3-2. Radial growth of *Fusarium torreyae* colonies at five different temperatures for seven days

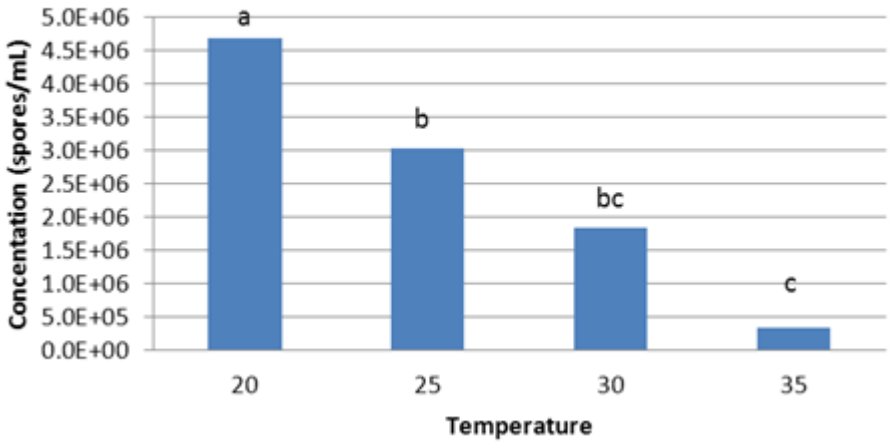

Figure 3-3. Spore concentration (spores per mL) after 14 days of incubation at various temperatures. Different letters represent significantly different values (Tukey's HSD, alpha=0.05)

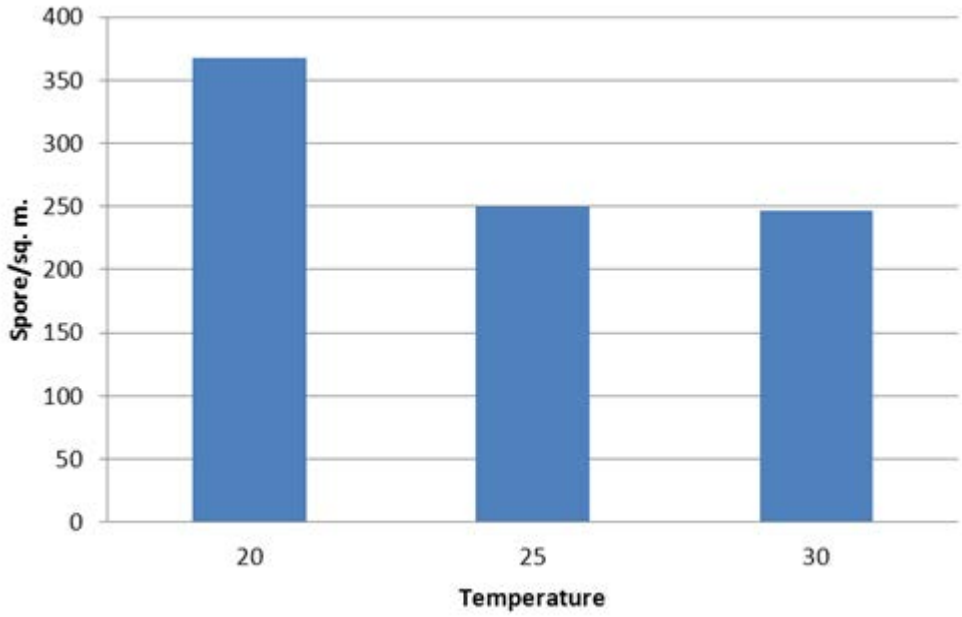

Figure 3-4. Mean spores per square meter captured for three temperatures

Figure 3-5. Example of stem inoculation point via needle wound (A:Inoculated, B:Control)

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BIOGRAPHICAL SKETCH

Aaron Trulock was born in Dunedin, Florida, and grew up in nearby Safety Harbor as a middle child with an older brother, Joshua, and younger sister, Rebecca. Aaron was the third generation in his family to grow up in Safety Harbor, Florida. He graduated from Dunedin High School after attending a magnet program for architecture and engineering. Aaron was a three sport varsity athlete, and was a member of the varsity soccer and cross country teams for three years, as well as four years on the varsity track and field team. In addition to athletic achievements, Aaron co-founded the Dunedin High School Debate Team, was on the Academic Team, and a member of both the National Honor Society and National Spanish Honor Society.

After high school Aaron attended the University of Florida. During his freshman year Aaron changed majors from engineering to forestry, where he became very interested in ecological interactions. He became interested in plant-pathogen interactions after a summer internship between his junior and senior years; in which he spent many hours in the field and saw various disease signs and symptoms. The following semester he enrolled in Forest Health and General Plant Pathology courses to learn more about forest pathology. After graduating a Bachelor of Science in Forest Resources and Conservation degree, he married his college sweetheart, Robyn, and worked in Gainesville until she completed her master's degree. Upon her degree completion Aaron began his master's program with Dr. Jason Smith.