

Evaluating the invasive potential of an exotic scale insect associated with annual Christmas tree harvest and distribution in the southeastern U.S.

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ABSTRACT

The movement of invasive species is a global threat to ecosystems and economies. Scale insects (Hemiptera: Coccoidea) are particularly well-suited to avoid detection, invade new habitats, and escape control efforts. In countries that celebrate Christmas, the annual movement of Christmas trees has in at least one instance been associated with the invasion of a scale insect pest and subsequent devastation of indigenous forest species. In the eastern United States, except for Florida, *Fiorinia externa* is a well-established exotic scale insect pest of keystone hemlock species and Fraser fir Christmas trees. Annually, several hundred thousand Fraser firs are harvested and shipped into Florida, USA for sale to homeowners and businesses. There is concern that this insect may disperse from Christmas trees and establish on Florida conifers of economic and conservation interest. Here, we investigate the invasive potential of *F. externa* on sixteen conifer species by quantifying the reproductive potential of this insect pest and its ability to establish, reproduce, and damage these plants. We find that small amounts of heavily infested Fraser fir plant material can release several hundred juvenile *F. externa* for over a month. Similar to other case studies, we find evidence that host susceptibility may in part be linked to phylogenetic relatedness. Encouragingly, only six of sixteen species evaluated were susceptible to attack. Our results provide new insights into methodology for evaluating scale insect dispersal and host susceptibility. We also provide guidance for future studies investigating scale insect reproduction, dispersal, and risk for plant species of unknown susceptibility to other exotic insect pests.

1. Introduction

Invasive species are major global environmental and economic threats that alter ecosystems (Crooks 2002), affect human health (Donovan et al., 2013), and redefine or eliminate industries (Pimentel et al., 2005; Kenis et al., 2008). It is estimated that in the United States (U.S.) alone, invasive species inflict \$120 billion in losses and mitigation efforts annually (Pimentel et al., 2005). Insects in particular are highly successful invaders due to their ability to avoid detection, rapidly reproduce, and easily disperse (Liebhold et al., 2016). There are several examples of major invasive insect species, particularly in forest systems, that have caused global negative economic impacts (Liebhold et al., 2017). For instance, the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), is credited with killing hundreds of millions of ash trees in North America over the past 20 years and has been linked to billions of dollars in economic losses and reduced human health (Donovan et al., 2013; Herms and McCullough 2014). Other invasive organisms, like Dutch elm disease, chestnut blight, and beech

bark disease have also caused widespread, irreversible ecological and economic losses in North America (Loo 2008). Despite increased screening and sanitation measures (Meyerson and Reaser 2002; Mehta et al., 2007; Sanchirico et al., 2009), the international movement of people and goods is predicted to double by 2035 (IATA 2017), which will likely coincide with higher incidence of exotic invasions (Meurisse et al., 2018).

In the U.S., there are an estimated 50,000 exotic invasive species (Pimentel et al., 2005). Data suggest that Florida receives and harbors more exotic species than any other state in the U.S. (Paini et al., 2010), largely due to the state's tourism industry, trade, and climate. Importantly, the U.S. is not only highly vulnerable to exotic invasions, but is among the top potential sources of exotic invaders that threaten agricultural industries (Paini et al., 2016). Among the most common routes of exotic or invasive forest insect movement is the trade of live plants (Meurisse et al., 2018). In the U.S. and many other countries, cut Christmas tree production, sale, and distribution is culturally ubiquitous and associated with the mass distribution of plant material. The most common U.S. Christmas tree species are Fraser fir (*Abies fraseri*) and Douglas

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fir (*Pseudotsuga menziesii*), of which, North Carolina and Oregon are the largest state producers, respectively (NASS 2019). Although only 30% of consumers who celebrate Christmas use real trees (CTPB 2017), millions of recently harvested trees are annually shipped throughout the continental U.S. and surrounding territories, purchased by consumers, and installed in businesses and homes where they are decorated and maintained for several weeks. Since Christmas trees are typically grown outdoors for 6–10 years or more before harvest (McKinley and Hazel 2019), many trees are shipped along with their resident organisms, primarily arthropods.

Once a tree is cut, it has a limited post-harvest lifespan, thus, cut trees are distributed quickly so that consumers have high quality products. Any obligate parasites associated with a tree have a limited window of opportunity to move to a viable host. In at least one instance, the distribution and sale of U.S. Christmas trees has been associated with the invasion of an exotic insect pest. In 2005, the pine tortoise scale, *Toumeyella parvicornis* (Hemiptera: Coccidae), was first observed on Caribbean pine trees (*Pinus caribaea* var. *bahamensis*) on the Turks and Caicos Islands (Corcoran et al., 2013). These insects are believed to have been introduced from Scots pine (*Pinus sylvestris*) Christmas trees imported from the U.S. Since its introduction, *T. parvicornis* has killed over 95% of *P. caribaea* in the Turks and Caicos Islands, drastically altering its ecosystems (Corcoran et al., 2013). Due to their obscure appearance and behavior, ability to persist and withstand pest control measures, and growing evidence of success under global change (Youngsteadt et al., 2015; Frank and Just 2020), scale insects (Hemiptera: Coccoidea) are excellent candidates for invasion via the movement of plant material.

Armored scale insects in the genus, *Fiorinia* (Hemiptera: Diaspididae), include 70 species, several of which are successful exotic invaders of North America, including *F. externa*, *F. phantasma*, and *F. theae* (Miller and Davidson 2005; Ahmed and Miller 2018). Among these, the elongate hemlock scale, *Fiorinia externa* Ferris, is native to eastern Asia and feeds on nutrients obtained from coniferous tree leaf tissue (Ferris 1942). This insect was first documented in the U.S. in 1908 in Queens, New York, and has since spread throughout the eastern region of the country (Talerico et al., 1967; Stimmel 1979; Miller and Davidson 2005; Dale et al., 2019). The impact of *F. externa* can be severe, but varies depending upon the host and local environmental conditions. For example, Gomez et al. (2012) observed minimal to no effect of increased *F. externa* density on hemlock defoliation and basal area growth, while (McClure 1977a) linked infestations to tree mortality under periods of abiotic stress. Nevertheless, as global change, global trade, and the frequency of abiotic stress events (e.g., drought, heat) increases, scale insect pest outbreaks are becoming more problematic and less predictable (Youngsteadt et al., 2015; Dale and Frank 2018).

In North America, *F. externa* primarily attacks a keystone forest species, eastern hemlock (*Tsuga canadensis*). However, *F. externa* is also a key pest of Fraser fir Christmas trees, beginning in Pennsylvania as early as 1980 and moving south to North Carolina Christmas tree farms by 1993 (Miller and Davidson 2005; Sidebottom 2019). Although Fraser fir infested with *F. externa* rarely show damage, they do create an aesthetic and regulatory challenge for growers. North Carolina Fraser fir Christmas tree producers reporting problems with *F. externa* have increased from just 0.3% of growers in 2000, to 2.3% in 2006, and 53% in 2013 (Sidebottom, personal communication). This rapid increase has coincided with changes in insecticide use and a greater dependence on pyrethroids, which are associated with increased scale insect and other key arthropod pest outbreaks (Luck and Dahlsten 1975; Frank and Sadof 2011). Approximately 17% of North Carolina's annual cut Christmas tree harvest is shipped into Florida's retail market, comprising nearly 700,000 trees in 2017 (NASS 2019). Importantly, *F. externa* is not established in Florida, which has led to state and federal regulatory action prohibiting the movement of infested Christmas trees into Florida. This regulation places a labor and economic burden on regulatory agencies, NC growers, and FL retail outlets (Stocks 2016).

Although hemlocks and firs, the principal hosts of *F. externa*, cannot survive in Florida's climate and thus do not occur in the state, there are concerns that *F. externa* imported into the region on cut Fraser fir trees may disperse and establish on other conifer species that do occur in the state. Of specific interest are two endangered native species, Florida torreyia (*Torreya taxiflora*) and Florida yew (*Taxus floridana*), as well as species of economic importance to Florida forestry and Christmas tree industries. Due to these regional and national concerns, the annual influx of *F. externa*, and documented spread of this pest across the eastern U.S., we conducted a host susceptibility study to determine the reproductive potential of *F. externa* originating on Fraser fir and if such populations can establish and reproduce on native and ornamental conifers common to Florida and the southeastern U.S. Results of this work can inform economic and environmental concerns as well as regulatory decisions based on the relative susceptibility of important conifer species to this exotic insect being moved on Fraser fir Christmas trees. Moreover, this evaluation can help inform the reproductive and dispersal potential of similar exotic armored scale insects of concern.

2. Materials and methods

2.1. Host plant selection and study design

Though the principal hosts of *F. externa* in North America are hemlock (*Tsuga* spp.), nine other genera of conifers are reported as viable hosts including: *Abies*, *Cedrus*, *Cupressus*, *Juniperus*, *Thuja*, *Picea*, *Pinus*, *Pseudotsuga*, and *Taxus* (Tao 1999; Miller and Davidson 2005). We selected sixteen native and one exotic host plant species to evaluate for susceptibility to *F. externa* based on host reports, their conservation status, species grown by Florida Christmas tree growers, and important forestry species (Table 1). We obtained Florida ecotypes from nurseries and botanic gardens of all species native to the state. Eastern hemlock and Fraser fir were obtained from North Carolina growers. All plants were maintained in 3 gallon pots within a soilless media of 100% ground pine bark, supplemented with 1.04 kg dolomitic lime and 0.74 kg granular micronutrients (Micromax; The Scotts Co., Marysville, OH) per cubic meter.

Five individuals of each plant species were arranged in a randomized complete block design with 5 replications per block for a total of 25 plants per species. *Fiorinia externa* used in this study were collected from naturally occurring infestations on *A. fraseri* in North Carolina, U.S. We collected twigs heavily infested with *F. externa* and used those to infest plant material with first instar nymphs (crawlers) as in Meineke et al. (2013). This method also roughly mimics conditions that would occur during annual holiday shipments of trees. We initiated *F. externa* inoculations in October 2017, during peak crawler emergence and actively maintained and monitored each plant until October 2018 (50 weeks), at which point we made final evaluations of host susceptibility.

2.2. *Fiorinia externa* inoculations

To evaluate the susceptibility of each plant species, we inundated each plant with *F. externa* nymphs at a magnitude unlikely to occur in reality, but truly testing if a plant species can support this insect species. To determine if *F. externa* can establish and reproduce on each species, we allowed enough time (50 weeks) for three complete generations to occur after initial infestations and confirmed the occurrence of those generations through active monitoring for different life stages.

Each plant was inoculated with *F. externa* on two dates (4 October and 22 November 2017) using a 15–20 cm heavily-infested Fraser fir twig. The first inoculation date was coincident with peak fall *F. externa* crawler emergence and the second inoculation with the annual harvest and shipment of Christmas trees into Florida. Fraser fir cuttings for the first infestation date were collected from multiple trees on a commercial

Table 1
Conifer species selected for *Fiorinia externa* susceptibility evaluation.

Common name	Scientific name	USDA plant hardiness zone	Relevance
Arizona cypress 'Blue Ice'	<i>Cupressus arizonica</i>	Zone 6 - 9	FL and NC Christmas tree
Arborvitae 'Green Giant'	<i>Thuja x 'Green Giant'</i>	Zone 5 - 7	FL and NC Christmas tree
Atlantic white cedar	<i>Chamaecyparis thyoides</i>	Zone 4 - 9	Native to FL and NC
Blue spruce	<i>Picea pungens</i>	Zone 3 - 7	Reported intermediary host
Deodar cedar	<i>Cedrus deodar</i>	Zone 7 - 9	Reported host, ornamental in NC and FL, western Asia origin
Eastern hemlock	<i>Tsuga canadensis</i>	Zone 3 - 7	Primary host
Florida torreyia	<i>Torreya taxiflora</i>	Zone 6a - 9a	Endangered species native to FL
Florida yew	<i>Taxus floridana</i>	Zone 8a - 9b	Endangered species native to FL
Fraser fir	<i>Abies fraseri</i>	Zone 4 - 7	Known host
Leyland cypress	<i>Cupressus X leylandii</i>	Zone 6 - 10a	NC and FL Christmas tree species
Loblolly pine	<i>Pinus taeda</i>	Zone 6b - 9b	Commercial forest species, native to FL & NC
Sand pine	<i>Pinus clausa</i>	Zone 8 - 9	FL Christmas tree, native to FL
Slash pine	<i>Pinus elliottii</i>	Zone 7 - 11	Commercial forest species, native to FL
Southern eastern red cedar	<i>Juniperus virginiana</i> var. <i>silicicola</i>	Zone 8 - 10	FL Christmas tree, native to FL and NC
Spruce pine	<i>Pinus glabra</i>	Zone 8 - 9	FL Christmas tree, native to FL
Virginia pine	<i>Pinus virginiana</i>	Zone 4 - 8	FL and NC Christmas tree, native to NC

Christmas tree farm in Ashe County, North Carolina and the second infestation date were collected from multiple trees at the North Carolina Upper Mountain Research Station in Laurel Springs, NC (36.393055, -81.305525). Infested cuttings were attached to the canopy of each plant using cable ties and remained in place for 40 days, then removed. The second infestation was made at a different location in the plant's canopy, but followed the same protocol. The inoculation site on each plant was marked by leaving the cable ties loosely in place for the duration of the study.

After inoculations, plants were maintained in a greenhouse at the Mountain Horticultural Crops Research Station in Mills River, NC (35.425261, -82.560835) from October 2017 through May 2018, where the temperature was monitored and maintained above 12 °C to mimic winter conditions in Florida. Plants were watered as needed by hand to the soil surface without wetting plant foliage to reduce risk of interfering with insect establishment. In June 2018, due to logistic constraints, all plants were moved to the North Carolina State University Upper Mountain Research Station, where they were kept until October 2018. There, plants were maintained in a hoop house with 40% shade to reduce heat stress through the summer and watered by both natural precipitation and drip irrigation.

2.3. Insect development and dispersal

Fiorinia externa typically undergoes two generations per year in North Carolina, with peak egg hatch in spring and fall (Lambdin et al., 2007). The only mobile life stage, and thus the only stage that can infest new plant material, is the first instar nymph (or crawler). Although crawler presence periodically coincides with Christmas tree shipments, we have a poor understanding of the timing, duration, and magnitude of crawler emergence, especially after plant material is harvested. To determine the abundance of crawlers dispersing from infested cut plant material over time, using methods similar to (McClure 1977a, 1979), we suspended a heavily-infested Fraser fir branch 6.4 cm over a 7.6 × 12.7 cm yellow sticky card (Sensor® Cards, BASF Corporation) within each of the five blocks of our host study ($n = 5$ per treatment). Infested branches were not provided with water. Cards were deployed with the infested branches on both inoculation dates (October and November) and replaced every 4 to 6 days until crawler emergence ceased. Several natural enemies, including Aphelinid parasitoids, mirid bugs, and lady beetles attack *F. externa* (McClure 1979). Each sticky card was examined under a stereomicroscope to determine crawler abundance and presence of natural enemies. This measure provides evidence that scale insects were dispersing from the plant material in our study, but also provides an estimated post-harvest timeline of insect dispersal and abundance.

2.4. Host plant susceptibility

It typically takes 10 to 12 weeks for a *F. externa* egg to mature into an adult female in the southeastern U.S. (Miller and Davidson 2005; Sidebottom 2019). To monitor *F. externa* establishment and development, we visually inspected each plant for scale insect presence and life stage at 2, 11, and 19-weeks post-infestation for both infestation dates using a hand lens. Although these evaluations did not determine if the insects were alive or dead, they did indicate successful transfer onto new host material and the development of individuals from crawlers to adults. Fifty weeks after the initial inoculation, we destructively sampled each plant to quantify the number of live *F. externa* on each plant after three generations. We inspected each plant, counting the total number of needles per plant with a *F. externa* cover. From the infested needles, we randomly selected 20 needles (or all infested needles if less than 20) to examine under a stereomicroscope and determine if the insects beneath the armored covering were alive or dead and if alive, their life stage and sex. Individuals were considered alive if they actively bled when their body was punctured with a probe. Based on our scale insect counts, we calculated the number of live individuals per plant by multiplying the number of live scale per evaluated needle by the number of needles with a scale insect cover on them. These calculations provide an estimate for the total *F. externa* infestation level per plant for each species.

Five plants of each species were in each randomized block and therefore adjacent to known viable hosts for the duration of the study. Thus, crawlers may have dispersed from viable hosts onto non-hosts at any point during each of three reproductive periods over the 50-week study, which may generate false indications of susceptibility. Since many juveniles that disperse and begin feeding on plants do not survive to reproductive maturity, we also measured the number of gravid females per plant between species. This metric ensures that counted individuals successfully fed, matured, and reproduced on that individual host plant. This metric also provides evidence that a plant species harbors the potential to support *F. externa* populations over time.

2.5. Effects of *F. externa* on host plants

Immediately prior to our destructive sampling at the conclusion of the study, we quantified plant growth by measuring the length of the longest shoot and insect damage by documenting the presence or absence of chlorotic mottling on needles. These data were used to document any effects of *F. externa* on each potential host species.

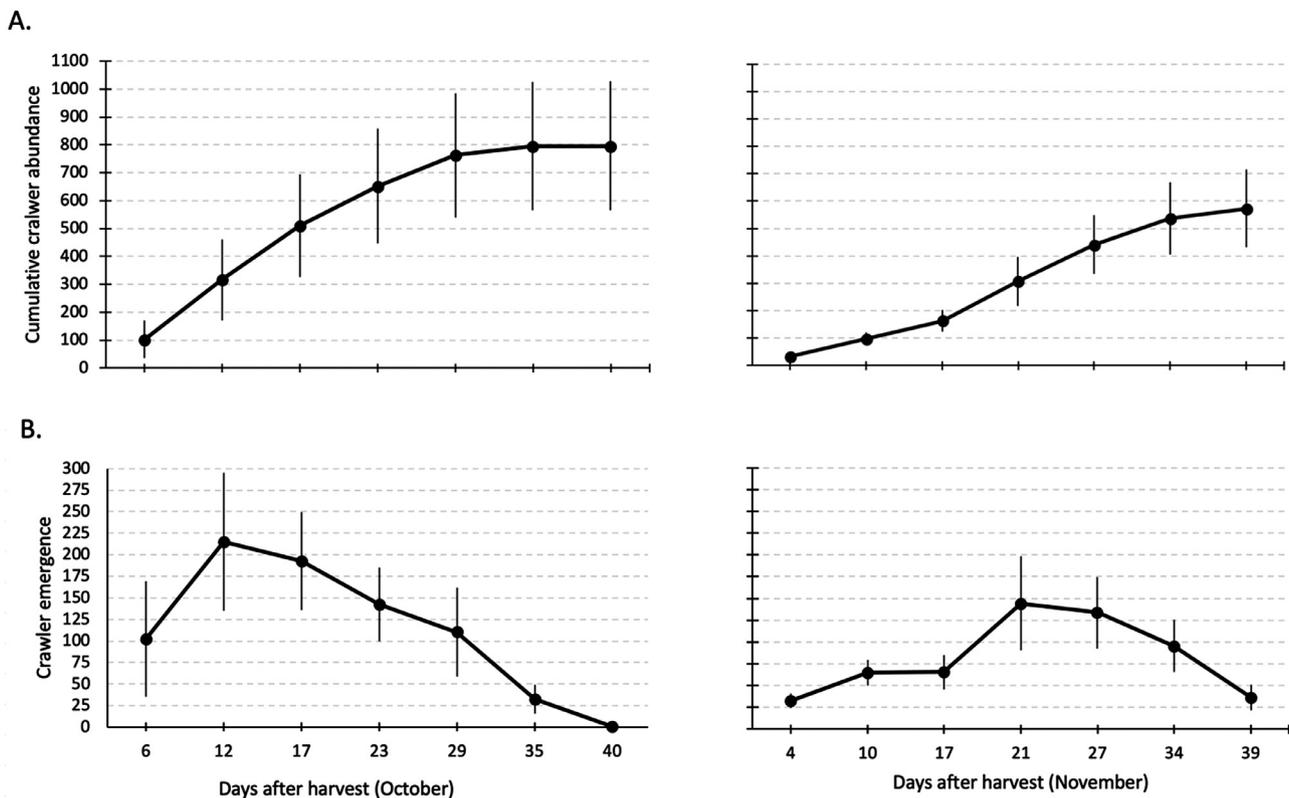


Fig. 1. A) Average cumulative *Fiorinia externa* crawler abundance emerging from each infested Fraser fir twig. B) Average *F. externa* crawler abundance per collection day on yellow sticky cards mounted below infested cut 15–20 cm Fraser fir twigs. Values are the average of five replicates (sticky cards) on each date. The left half of the figure illustrates the first inoculation event (October 2017) and the right half illustrates the second inoculation event (November 2017).

2.6. Statistical analyses

We compared each metric of host susceptibility for all plant species using mixed effect analysis of variance (ANOVA) treating block as a random effect and plant species as the main effect. Scale insect count data were $\log(x + 1)$ transformed to improve the normality of residuals based on a Shapiro Wilk test. If ANOVA detected a significant main effect, we compared means between plant species using Tukey HSD. Although a comparison of *F. externa* abundance between plant species identifies useful interspecific differences in susceptibility, it does not provide a direct measure of regulatory risk associated with this insect and its potential to reproduce, adapt, and establish on a given host. Since the presence of any gravid female on any plant represents the possibility of successful reproduction and establishment, we used Fisher's Exact Test (one-tailed) to determine if the probability of having a gravid female on a given plant species is greater than that of a non-host, or one that supports zero gravid females, based on 25 plants (replicates) of each species. We used Chi-square tests to compare the proportion of plants within each species infested with *F. externa* and to determine if our measure of plant damage caused by *F. externa* differed between host species at the conclusion of the study. All analyses were conducted using JMP Pro-Version 15 (SAS Institute Inc., Cary, North Carolina) and models were considered significant if $P < 0.05$.

3. Results

3.1. Insect development and dispersal

Fiorinia externa crawlers were observed dispersing from all infested shoots placed above yellow sticky cards within five days of attaching the infested twigs during both inoculation events. The total number of crawlers produced from a single 15–20 cm twig over 40 days was im-

mense, but highly variable with an average (\pm SE) of 796 ± 232 for the first infestation and 573.2 ± 141 for the second infestation (Fig. 1A). In October, crawler dispersal peaked approximately two weeks after inoculation and in November crawler dispersal peaked approximately three weeks after inoculation (Fig. 1B). Based on these data, each of our host plants were inundated with several hundred *F. externa* crawlers over the duration of this study.

Five weeks after the first inoculation, male *F. externa* were observed maturing into later instar nymphs on many of the hosts (indicated by the production of waxy filaments) and by nine weeks, third instar females were observed on host plants. Seventeen weeks after the first inoculation, second generation nymphs were observed on many of the plants at the inoculation site, confirming that individuals had successfully established and reproduced on new plant material.

The parasitoid wasp, *Encarsia citrina* Crawford (Hymenoptera: Aphelinidae), was also observed on sticky cards, which was expected since we used field-collected *F. externa* populations (Abell and Van Driesche 2012). Specifically, during plant inoculations, 40% of sticky cards during the first infestation and 91% of sticky cards during the second infestation captured *E. citrina*, respectively. Drastically more wasps were observed emerging from twigs in the 2nd infestation (45.8 ± 6.0) compared to the first infestation (5.0 ± 2.8) either due to parasitoid phenology or collection site differences. Moreover, 50 weeks later, during the plant susceptibility evaluations, parasitized scale insects were observed on at least one plant from all evaluated species that were infested with *F. externa*. We did not quantify percent parasitism on each host plant, although observations suggested no difference between species.

3.2. Host plant susceptibility

Although highly variable, our initial observations after 19 weeks post-inoculation indicated that *F. externa* crawlers had successfully

Table 2

Percentage of plants (out of 25) within each species infested with some number of live *Fiorinia externa* individuals 19 weeks and 50 weeks after infestation. The far right column provides Fisher's Exact (one-tailed) test results comparing each species to a plant that does not support the development of any gravid females (a non-host) based on 25 plants.

Plant species	19 weeks	50 weeks	P-values, gravid females at 50 weeks
Arizona cypress 'Blue Ice'	2%	0%	–
Arborvitae 'Green Giant'	0%	0%	–
Atlantic white cedar	6%	0%	–
Blue spruce	64%	64%	<0.0001*
Deodar cedar	56%	72%	<0.0001*
Eastern hemlock	100%	100%	<0.0001*
Florida torreyia	90%	3%	0.5614
Florida yew	80%	0%	–
Fraser fir	94%	96%	<0.0001*
Leyland cypress	0%	0%	–
Loblolly pine	95%	27%	0.0069*
Sand pine	65%	0%	–
Slash pine	16%	5%	0.4444
Southern eastern red cedar	57%	3%	0.5455
Spruce pine	85%	9%	0.2029
Virginia pine	85%	41%	0.0004*

* A significant p-value ($P < 0.05$) indicates that the probability of a given species to support gravid female development is greater than that of a non-host.

moved onto 14 of 16 host plant species (Table 2). Our evaluations 50 weeks after inoculation, following three consecutive generations of this insect, showed statistical differences in *F. externa* establishment between host species and confirmed that eastern hemlock and Fraser fir are superior hosts ($df=15$, $X^2=291.64$, $P < 0.0001$). One hundred percent of eastern hemlock and 92% of Fraser fir became infested with live *F. externa*. Deodar cedar and blue spruce were approximately 70% infested with live scale, Virginia pine and loblolly pine were 41% and 27% infested, respectively, and under 9% of all other species were infested 50 weeks after inoculation (Table 2).

Similarly, we found that the number of needles per plant with *F. externa* armored coverings on them were highly variable across species, ranging from 0 to 4028 with a mean of 76.7 (± 15.5). As predicted, there was a statistical difference between plant species ($F_{15,365}=102.41$, $P < 0.0001$) (Fig. 2A). Since armored scale insects often remain on plant material after death and thus appear to be infesting plants but are not feeding, we also determined the number of live individuals (including all life stages) per needle with a *F. externa* armored covering. We also found statistical differences between plant species in live scale per needle ($F_{15,365}=43.29$, $P < 0.0001$) and greater resolution in host viability among the evaluated species (Fig. 2B). Using these data and the number of scale-infested needles per plant, we estimated the number of live *F. externa* per plant, which provided further separation of species and an estimate of infestation severity ($F_{15,365}=100.15$, $P < 0.0001$) (Fig. 2C). These *F. externa* estimates suggest that eastern hemlock, Fraser fir, deodar cedar, and blue spruce were the only species with *F. externa* infestations statistically greater than plants with zero individuals.

To quantify the viability of each plant species to support *F. externa* maturation and reproduction, we also compared the number of gravid adult females on each plant. Average gravid female abundance per infested needle ranged from 0 to 2.3 individuals across all species. Despite the small range in gravid females per infested needle, the range in infested needles per plant was much greater, leading to separation among plant species in gravid female abundance per plant. Analysis of variance detected a significant difference in gravid female abundance between species ($F_{15,365}=70.64$, $P < 0.0001$). Again, the aforementioned susceptible plant species were also the only species with gravid female abundance statistically greater than plants with zero gravid females (Fig. 2D). However, it is worth noting that we did find at least one gravid female on several other plant species. Specifically, Virginia pine (up to 16 on nine plants), loblolly pine (up to seven on six plants), spruce pine (one on two plants), Florida torreyia (two on one plant), and slash pine (one on

one plant). As the presence of one gravid female on a plant presents the possibility of successful reproduction, adaptation, and establishment of a population, these data could have important regulatory implications. Our Fisher's Exact test comparison of each plant species to a non-host using the presence or absence of a gravid female on any plant provides a slightly different picture of host susceptibility than our gravid female abundance data. Specifically, the probability of finding a gravid female on blue spruce, Deodar cedar, eastern hemlock, Fraser fir, loblolly pine, and Virginia pine was statistically greater than the probability of finding a gravid female on a non-host (Table 2).

3.3. Effects of *F. externa* on plants

All plant species put on new growth over our 50 week study, averaging 18 ± 0.9 cm and ranging from 1.6 to 43 cm per species. Although plant growth varied between species ($F_{15,341}=34.81$, $P < 0.0001$), it was not associated with *F. externa* infestation level ($P > 0.1$) or damage ($P > 0.5$). In general, damage caused by *F. externa* infestations was not severe on any of the evaluated host plant species and never resulted in plant death. However, we did detect differences in the occurrence of insect damage by way of chlorotic mottling between plant species ($df = 15$, $X^2=175.78$, $P < 0.0001$) (Fig. 3). Intuitively, the most severely damaged plants were also those that supported the largest infestations, except for blue spruce, which did not exhibit any damage. Other species, including loblolly pine, spruce pine, and sand pine, had plants with damage although none supported many *F. externa* individuals.

4. Discussion

Invasive species movement is one of the leading and most ubiquitous global threats to natural and anthropogenic ecosystems (Pimentel et al., 2005; Meurisse et al., 2018). Among these, scale insects are one of the most difficult insect pests to detect and control on woody plants (Frank 2019). As such, invasive scale insect species are highly concerning from a regulatory perspective, particularly on plant material shipped frequently across states, continents, or the world (Stocks 2016). Our results suggest that *F. externa* is a prolific reproducer and can easily transfer to pest-free host plants for up to 40 days after harvesting infested plant material. Fortunately, several species in our study were not viable hosts, particularly those of Florida conservation, ornamental, and forestry interest in the genera *Taxus*, *Cupressus*, *Thuja*, and *Chamaecyparis*. Although many factors affect the likelihood of successful

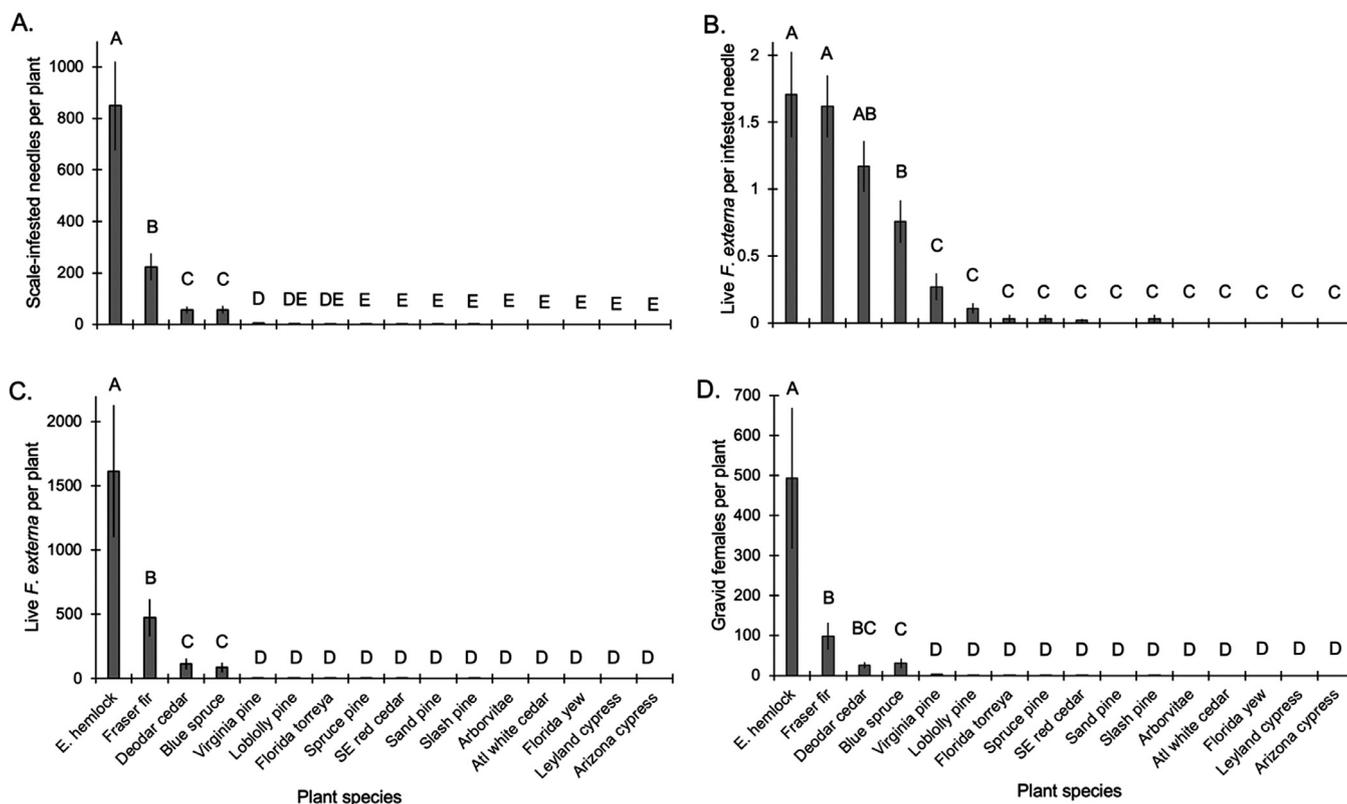


Fig. 2. A) The average number of needles with a *Fiorinia externa* armored covering (live or dead) on them per plant. B) Average number of living *Fiorinia externa* individuals per needle with a scale insect cover on each plant species. C) Average estimates of *Fiorinia externa* abundance per plant for each plant species. D) The average number of gravid adult female *Fiorinia externa* per plant. Different letters indicate statistical differences between means using Tukey’s HSD means comparison ($P < 0.05$).

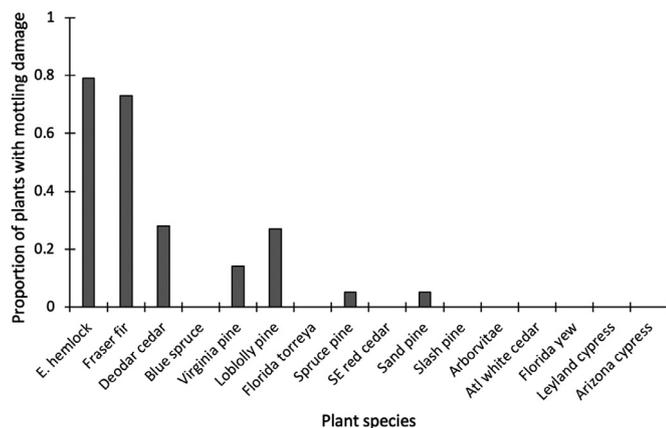


Fig. 3. Proportion of plants within each species exhibiting mottling damage on needles caused by *Fiorinia externa* feeding.

F. externa dispersal and establishment, this study provides insight into which species may be most vulnerable to attack from *F. externa* originating on Fraser fir Christmas trees, the extent to which infested plant material may be a source for founder individuals, and the relative susceptibility of hosts based on provenance and phylogenetic relatedness.

To-date, the primary concern associated with the movement of cut Fraser fir Christmas trees is the dispersal of *F. externa* during transport to retail outlets and homes, or once trees are discarded after the holiday season. Fortunately, heavily-infested Fraser fir trees represent a small percentage of those distributed from farms where they are produced. Thus, the infestation pressure we simulated in this study represents a

worst-case scenario where new hosts are inundated with mobile juveniles. Regardless, we found that heavily-infested Fraser fir plant material is a substantial source of *F. externa* individuals when adult females are present and in their reproductive stage. On average across both of our inoculation events, infested Fraser fir twigs released 685 immature *F. externa* per 15 cm over a 40-day period. Our November inoculation coincides with the beginning of tree harvest season. Based on our data, 54% and 77% of immatures dispersed after 21 and 27 days post-harvest, respectively, suggesting the risk of dispersal is primarily during post-harvest storage, distribution to retail outlets, and sale. It is important to recognize that these data must be interpreted while considering that other external factors like host plant stress, post-harvest tree watering, local abiotic conditions, and *F. externa* infestation severity will affect the duration and quantity of crawler dispersal. For example, our methods may underestimate crawler emergence since our twigs were small, had no access to water, and had minimal resource reserves, whereas branches attached to cut trees will maintain their vigor much longer and may support prolonged gravid female survival and crawler emergence. This question should be pursued in future research.

Interestingly, several plant species in our study supported *F. externa* nymph transfer and initial feeding but did not support insect development. These included Florida yew, Atlantic white cedar, arborvitae, and Arizona cypress. This result is encouraging because it indicates that a species of conservation interest, Florida yew, can tolerate exposure to this exotic insect moving from Fraser fir at high levels and resist insect feeding. A potential explanation for plants supporting only immature life stages highlights a weakness in our study design where plants were randomized into five blocks that each contained five of each plant species. Highly susceptible hosts were likely adjacent to non-hosts and exposing them to dispersing immatures over the duration of the study. Similar future studies should group plants by species after herbivore

inoculations to avoid confounding effects of susceptible neighbors. These results emphasize the importance of quantifying gravid female abundance or fecundity when evaluating armored scale infestations (McClure 1980; McClure 1983; Dale and Frank 2014; Borden and Dale 2020) because the presence of scale insect covers or immatures may not represent true severity of infestation. Several plant species supported *F. externa* feeding, development, and maturation to gravid adult females (Fig. 3), but did not develop populations after three generations. For example, we found at least one gravid female on Virginia pine, loblolly pine, spruce pine, slash pine, and Florida torreyia, but after 50 weeks *F. externa* abundance per plant on each species was no different from that of plants with no *F. externa*. However, these data also indicate that Virginia pine and loblolly pine are more likely to support gravid female development than a non-host. Thus, based on the combination of our measures of host susceptibility, eastern hemlock, Fraser fir, deodar cedar, blue spruce, Virginia pine, and loblolly pine are the only species that supported *F. externa* reproduction and establishment after three reproductive generations. On eastern hemlock, Fraser fir, and deodar cedar, every needle with a scale insect cover had at least one live individual. Despite these results, in western North Carolina where *F. externa* is prevalent, it has not been observed on either deodar cedar nor Virginia pine in areas where infested eastern hemlock or Fraser fir occur (Sidebottom, personal communication).

Economically and ecologically damaging invasions of exotic insect herbivores are commonly associated with congeneric species of the herbivore's co-evolved hosts. However, this is dependent upon the herbivore's diet breadth, where specialists attack closely related plants and generalist's preferences are less understood. Martinson et al. (2016) investigated a highly polyphagous invasive insect and found that host relatedness was less important than origin in relation to herbivore abundance, but also depended upon the relative abundance of hosts. Conversely, a specialist invasive herbivore, the emerald ash borer, which co-evolved with Asian *Fraxinus* spp. has eliminated millions of *Fraxinus* spp. native to North America while having minimal effect on other plant species (Hermes and McCullough 2014). Research on armored scale insects, which are generally polyphagous, suggests that scale insects, including *F. externa* and others in the same genus, may perform best on their co-evolved hosts (McClure 1983; Borden and Dale 2020). For example, *F. externa* produced over 36% more offspring per female when reared on its Japanese hemlock hosts compared to North American hemlocks (McClure 1983). Moreover, holly (*Ilex*) species native to North America are poor hosts for tea scale, *Fiorinia theae*, while holly and camellia species that share the insect's Asian origin support orders of magnitude more individuals (Borden and Dale 2020). The principal hosts of *F. externa* in its native range are Japanese hemlock, *Tsuga diversifolia* [Maxim.] Mast., and Siebold hemlock, *Tsuga sieboldii* Carriere (Takagi 1963; McClure 1983). Thus, in line with other cases, we found that eastern hemlock, *T. canadensis*, is highly susceptible to *F. externa*. We evaluated plants from 11 genera within four subfamilies and three families of conifers (order Pinales). Although previous reports indicate that species within all 11 evaluated genera are hosts (Tao 1999; Miller and Davidson 2005), our results contradict this and suggest a relationship between phylogenetic relatedness and susceptibility to *F. externa*. Specifically, three of the six species that supported *F. externa* reproduction belong to the subfamily, Abietoideae, while all six species that supported reproduction are in the family, Pinaceae. All four Cupressaceae (family Cupressaceae) species were not viable hosts and both Taxaceae species did not sustain infestations. Based on these results, we suggest that future research and regulatory action associated with *F. externa* originating on Fraser fir should prioritize host plants that are more closely related to the insect's native principal hosts.

Encouragingly, in addition to *F. externa* establishing on select host species, so did the parasitoid wasp, *E. citrina*, which is known to attack *F. externa* and other armored scale species (McClure 1979; Abell and Van Driesche 2012). Over 65% of sticky cards monitoring immature *F. externa* dispersal also captured *E. citrina* during our infestation pe-

riod. Previous work has shown that synchrony between parasitoid emergence and scale insect life stage is critical for successful biological control and that *E. citrina* only attacks second instar females of *F. externa* (McClure 1978; Abell and Van Driesche 2012). Although we did not investigate this synchrony, and are unaware of second instar females present, all plants with live *F. externa* at the conclusion of our study had parasitized individuals on them. *Encarsia citrina* and other natural enemies of *F. externa* are well-established throughout the eastern U.S. where this herbivore remains an economic pest. This parasitoid along with other natural enemies have been shown to be unable to keep *F. externa* below damaging levels, which Abell and Van Driesche (2012) attributed to asynchrony between scales and parasitoids due to overlapping *F. externa* generations. So, although encouraging, the simultaneous movement of the parasitoid may not be sufficient to alleviate concerns, particularly in warmer climates. It is also well-understood that chemical pest control inputs can affect predation and parasitism of scale insects (Luck and Dahlsten 1975; McClure 1977b; Frank 2012). Thus, pre-harvest insect control tactics are important to consider when thinking about the movement of *F. externa* and its natural enemies on Fraser fir trees. Broad-spectrum insecticides (e.g., pyrethroids) commonly used for pre-harvest Fraser fir pest control or post-harvest sanitation do not effectively control armored scales, but instead reduce natural enemy populations and associated biological control (Luck and Dahlsten 1975; McClure 1977b; Raupp et al., 2001; Rebek and Sadof 2003; Frank 2012). Therefore, insecticide applications to Fraser fir that disregard natural enemy conservation and integrated pest management may increase *F. externa* abundance and the risk of movement during Christmas tree harvest and distribution. Current management practices and future research should incorporate the use of insecticides that are compatible with natural enemies to facilitate maximum natural pest regulation during production to limit infestation loads during harvest and shipment.

Although our results provide insights into the potential of several plant species to support *F. externa* populations in the southeastern U.S., there are still many unknowns regarding the movement of this exotic pest and mechanisms behind host plant resistance. Our results indicate that tree species more closely related to hemlocks (Pinaceae: Abietoideae) are more vulnerable to attack than more distantly related species, particularly from *F. externa* adapted to Fraser fir hosts. Previous work has also shown that secondary metabolites that vary among host species (McClure and Hare 1984), and host plant fertilization (McClure 1980) or stress (McClure 1977a) can regulate *F. externa* fecundity and performance. Future investigations may explore the effects of Fraser fir maintenance practices or conditions prior to harvest and defensive compounds within susceptible and resistant hosts. We also found that multiple pine species, particularly Virginia and loblolly, exhibited a fair amount of mottling damage, although they only supported small numbers of *F. externa*. This suggests that juvenile *F. externa* can feed and cause damage. Although these species did not support population growth after three generations, we did find significant gravid female development, which is worth noting and could pose potential challenges over longer time periods. Mechanisms behind this may be worth future investigation.

We evaluated the susceptibility of what we perceived as all potential *F. externa* hosts of economic and ecological importance in the southeastern U.S. based on published literature and host records (Tao 1999; Miller and Davidson 2005). Safeguards against the movement and dispersal of this insect should remain to prevent exposure and risk to susceptible hosts as well as unanticipated hosts. It is worth reiterating that *F. externa* infestation levels used in this study are unlikely to be encountered on harvested and distributed Fraser fir Christmas trees. Therefore, invasion pressure and herbivore transfer and establishment will likely be less severe than we simulated. Since there are many additional scale insect species of regional and global concern, the methods used in this study can be used to evaluate their invasive potential and risk to plant species of interest. Growing evidence indicates that scale insects are among the best insect pests to adapt to conditions associated with global

change and become problematic (Meineke et al., 2013; Dale and Frank 2014; Youngsteadt et al., 2015; Frank and Just 2020). Therefore, efforts must continue to detect, identify, and prevent the spread of potentially damaging species around the globe.

Declaration of Competing Interest

None.

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